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Study on chemical mutagenesis in cotton (*Gossypium* sp.) using ethyl methane Sulfonate (EMS)

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Abstract
A study was undertaken to investigate the mutagenic effects and optimise the effective mutagenic dose for ethyl methane sulfonate in two cotton varieties MCU5 and MCU12 and to study the nature of induced genetic variability in the seedling related traits. The EMS concentrations ranging from 0.2%, 0.4% and 0.6% were used for inducing mutagenesis at 8 hrs and 14 hrs intervals separately. Mutagen-treated seedlings exhibited reduced growth relative to untreated controls, indicating dose-dependent inhibitory effects. However, out of the twelve mutated treatments studied, treatment T_1 (MCU 5 at 0.2 percent for 8 hrs) successfully induced novel variability, offering valuable scope for the selection of desirable mutants in cotton breeding.

Keywords: Lethal dose 50, genetic variation, ethyl methane sulfonate, cotton

Introduction
Cotton (*Gossypium* spp.), belonging to the *Malvaceae* family, is one of the most important fibre crops globally which thrives in tropical and subtropical regions, requiring warm, dry conditions and sufficient water, often through irrigation. Cotton is an often cross-pollinated crop, predominantly self-pollinated but with 5-50% cross-pollination influenced by species, floral traits, and pollinators. India leads the world in cotton cultivation area, with around 9.6 million hectares, approximately 25% of the global total, and contributes about 16% to global cotton production. Cotton plays a vital role in India's agricultural economy, contributing nearly 30% to the agricultural GDP and supporting millions of farmers and textile workers.
Mutation breeding is favoured over hybridisation for cotton improvement due to its efficiency in creating variability, preserving desirable traits, and overcoming hybridisation barriers (Waghmare *et al.*, 2000 and Oladosu *et al.*, 2016) [11, 12, 7]. Mutation breeding involves altering a plant's DNA sequence, either naturally or artificially, to enhance traits such as disease resistance, yields, or stress tolerance. Chemical mutagenesis, particularly using agents like ethyl methane sulfonate (EMS), is widely applied in agriculture (Waghmare & Koranne, 2000) [11, 12]. EMS is an alkylating agent that causes point mutations by ethylating guanine bases in DNA, leading to GC to AT transitions. It is effective in inducing high-frequency mutations without major chromosomal damage, making it a preferred tool in plant breeding and genetic research (Atiq *et al.*, 2023) [1].
A study was conducted to examine the effects of different concentrations of EMS and to find out the optimum mutagenic dose (LD_{50}) of EMS on seedling and growth related traits in selected cotton varieties. Ultimately, the superior EMS-induced mutant lines with improved germination and early vigour traits were identified with enough variability induction without drastically reducing its viability.

Materials and Methods
Study details: The present research on induced chemical mutagenesis in cotton using Ethyl Methane Sulfonate (EMS) was conducted under *in vitro* conditions. The study aimed to induce genetic variability in two high-yielding cotton varieties, MCU 5 and MCU 12 obtained from Tamil Nadu Agricultural University, Coimbatore. EMS, a potent alkylating agent, was selected as the mutagen for this study.

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It induces point mutations by ethylating guanine, causing G:C to A:T transitions. Though EMS is efficient in inducing mutations, it is highly toxic and requires careful handling under strict safety measures.

Uniform and healthy seeds of MCU 5 and MCU 12 were treated with EMS at concentrations of 0.2%, 0.4%, and 0.6% for two durations of 8 and 14 hours each. Each treatment used 100 seeds per variety. A control set (T_{13} and T_{14}) was soaked in distilled water for 12 hours. Post-treatment, seeds were thoroughly washed and sown in sterilized pot mixtures under controlled conditions.

Treatment details: The experiment included 14 treatments (T_1-T_{14}) and was laid out in three replications of 100 seeds each.

T_1-MCU 5 @ 0.2 percent concentration for 8 hours
T_2-MCU 5 @ 0.2 percent concentration for 14 hours
T_3-MCU 5 @ 0.4 percent concentration for 8 hours
T_4-MCU 5 @ 0.4 percent concentration for 14 hours
T_5-MCU 5 @ 0.6 percent concentration for 8 hours
T_6-MCU 5 @ 0.6 percent concentration for 14 hours
T_7-MCU 12 @ 0.2 percent concentration for 8 hours
T_8-MCU 12 @ 0.2 percent concentration for 14 hours
T_9-MCU 12 @ 0.4 percent concentration for 8 hours
T_{10}-MCU 12 @ 0.4 percent concentration for 14 hours
T_{11}-MCU 12 @ 0.6 percent concentration for 8 hours
T_{12}-MCU 12 @ 0.6 percent concentration for 14 hours
T_{13}-MCU 5 soaked for 12 hrs (Control)
T_{14}-MCU 12 soaked for 12 hrs (Control)

Observations noted: Data were recorded for germination percentage (%), seedling survival rate (%), root length (cm), mean seedling length (cm), seedling fresh and dry weight (mg), and seedling vigour indices I and II. Germination was recorded on the 7th day, and biometric observations followed. Seedling Vigour Index I was calculated by multiplying germination percentage with mean seedling length, while Index II used seedling dry weight.

Statistical approach: In the present experiment, the Completely Randomized Design (CRD) was adopted, as it provides a simple and effective layout where treatments are assigned to experimental units entirely at random. For the statistical analysis of the data obtained under CRD, *INDOSTAT* software was employed. The software facilitates precise computation of various statistical parameters, analysis of variance (ANOVA), and comparison of treatment means, thereby enabling accurate interpretation of the experimental results.

Results and Discussion

Analysis of variance: The mean square values for eight seedling related traits are mentioned in the table 1.

The mean square value of germination percentage (65.821), survival rate (22.453), root length (3.965), shoot length (6.223), fresh weight (0.200), dry weight (0.004), seed vigour index I (119.155) and seed vigour index II (80.80). The maximum mean square value is seed vigour index I (119.155) and the lowest mean square value is dry weight (0.004).

The results of ANOVA revealed a significant treatment effect, indicating that the differences among the treatment means were not due to experimental error but rather to the actual influence of the treatments. This suggests that at least one treatment mean differed significantly from the others (Badigannavar *et al.*, 2000 and Winkler *et al.*, 2023) [2, 13, 14]. It is inferred that the treatments imposed in the experiment had a measurable and meaningful impact on the response variable.

Mean Performance of EMS Treatments: The mean performance of treatments, including treated and untreated, evaluated for eight seedling related traits was represented in Table 2.

In respective of all the traits, the mutagen-treated treatment T_1 (MCU 5 @ 0.2 percent concentration for 8 hours) marked the highest scores for germination percentage (79.74%), seedling survival rate (62.45%), root length (6.67 cm), mean seedling length (14.74 cm), seedling fresh and dry weight (1.51 mg and 0.20 mg respectively), and seedling vigour indices I and II (616.79 and 15.99 respectively) whereas the mutagen-treated treatment T_{12} (MCU 12 @ 0.6 percent concentration for 14 hours) secures the least scores for germination percentage (42.00 %), seedling survival rate (25.54 %), root length (3.91cm), mean seedling length (9.25 cm), seedling fresh and dry weight (0.89 mg and 0.11 mg respectively), and seedling vigour indices I and II (178.55 and 4.77 respectively).

Apart from the treatment T_1, the treatment T_2 (MCU 5 @ 0.2% concentration for 14 hours) exhibited consistently superior performance across all the eight seedling-related traits, recording appreciable improvements without any noticeable deviations, thereby highlighting its effectiveness in enhancing seedling vigour. Among the control, T_{13} (MCU 5 soaked for 12 hrs) recorded the highest overall germination at 97.00% with more survival rate of 92.64%, mean seedling length (17.23 cm) and seedling vigour indices I and II (931.10 and 25.64 respectively). For the traits, root length (8.25 cm), seedling fresh weight (1.85 mg) and seedling dry weight (0.243 mg), T_{14} (MCU 12 soaked for 12 hrs) registered its highest measures.

The present investigation highlights the contrasting responses of MCU 5 and MCU 12 genotypes to EMS mutagenesis. Among the treatments, T_1 (MCU 5 @ 0.2% for 8 hours) consistently recorded higher values for germination percentage, seedling survival, root length, seedling length, and vigour indices, indicating that this treatment represents an optimum mutagenic dose capable of inducing variability without severely affecting physiological processes. Similar observations were reported by Gaul (1964) [3], who emphasized the importance of identifying a balanced mutagenic dose to ensure effectiveness with minimum lethality. Interestingly, T_2 (MCU 5 @ 0.2% for 14 hours) also exhibited superior seedling performance, ranking next to T_1. This highlights the relative tolerance of MCU 5 to prolonged mutagen exposure, particularly at lower concentrations, which aligns with the concept of a biologically effective dose as suggested by Jain (2010) [5].

In contrast, T_{12} (MCU 12 @ 0.6% for 14 hours) resulted in severe reductions in all seedling traits, which may be attributed to the cytotoxic and genotoxic effects of higher EMS concentrations. This is mainly attributed to mitotic arrest and abnormal cell division in meristematic zones, reducing elongation and root architecture development (Muthusamy & Jayabalan, 2001) [6]. At elevated levels, EMS is known to cause excessive chromosomal aberrations and physiological damage. EMS affects protein synthesis; membrane permeability and chlorophyll content of the

cotton seedlings that often show delayed leaf emergence, reduced chlorophyll index and slower seedling vigour. **(**Girija & Dhanavel, 2009) [4]. This clearly demonstrates the dose-and genotype-dependent response of mutagens, wherein MCU 12 exhibited greater sensitivity compared to MCU 5. MCU 12 exhibited high sensitivity to mutagenic stress and would require cautious dosage standardization in future breeding experiments (Winkler *et al.*, 2023) [13].

Among the controls, T_{13} (MCU 5 soaked for 12 hours) displayed maximum germination (97.00%) and vigour indices, confirming the innate vigour of MCU 5 under untreated conditions. Conversely, T_{14} (MCU 12 soaked for 12 hours) recorded maximum values for root length and seedling biomass, indicating inherent differences in genotype-specific growth attributes. It is clearly evident that the untreated seeds recorded the highest results because they were free from the cytotoxic and genotoxic effects of EMS (Shim *et al.*, 2019) [9]. In the absence of mutagenic stress, the physiological and biochemical processes such as enzymatic activation, hormonal regulation, and mobilization of stored reserves proceed normally, ensuring higher germination, better seedling survival, and superior vigour indices (Roychowdhury & Tah, 2011) [8]. Since no DNA alkylation or chromosomal damage occurs in untreated seeds, the genetic stability is maintained and the seedlings are able to express their full inherent growth potential (Atiq *et al.*, 2023) [1].

Variability measures of CRD: The variability factors calculated under CRD such as standard error (SE), critical difference (CD), and coefficient of variation (CV %) provide valuable insights into the reliability and precision of the experiment were demonstrated in the Table 3.

In this study, SE for mean across traits was ranged from 0.007 (dry weight) to 14.865 (vigour index I). Root length had the next lowest SE (0.026). Standard error difference was lowest in dry weight (0.011), followed by fresh weight (0.036), and highest in vigour index I (21.022). Critical difference values ranged from 0.023 (dry weight) to 8.526 (vigour index I). CV was lowest for germination percentage (1.906%), followed by root length (2.496%), suggesting stable expression. The highest CV was noted in seed vigour index II (8.448%).

In this study, the acceptable range of SE and CD values, together with comparatively low CV percentages, indicate that the experimental error was minimal and the experiment achieved strong precision and reliability. The small SE and SE (d) values across most traits, along with moderate CVs and significant CD values, confirm that the observed differences among treatments are genuine and attributable to treatment effects rather than random error (Ul-Allah *et al.*, 2019) [10]. Collectively, these variability measures demonstrate that the experiment not only ensured statistical precision but also generated substantial variability, which can be effectively exploited through appropriate selection strategies for the genetic improvement of cotton.

Table 1: Analysis of variance (ANOVA) for eight seedling related traits in cotton

Source of variation	df	Germination percentage (%)	Survival rate (%)	Root length (cm)	Seedling length (cm)	Fresh weight (mg)	Dry weight (mg)	Vigour index I	Vigour index II
Treatment	13	65.821*	22.453*	3.965*	6.223*	0.200*	0.004*	119.219*	80.459*
Error	14	1.579	1.061	0.020	0.054	0.001	0.000	4.1944	2.1694
Total	27								

** indicates significance at 5 percent probability level

Table 2: Estimation of mean performance for treated as well as untreated in cotton

Treatment	Germination percentage (%)	Survival rate (%)	Root length (cm)	Seedling length (cm)	Fresh weight (mg)	Dry weight (mg)	Vigour index I	Vigour index II
T_1	79.740	62.451	6.670	14.735	1.509	0.201	616.795	15.990
T_2	77.280	60.823	6.490	14.540	1.466	0.191	582.700	14.725
T_3	63.685	48.642	5.465	13.645	1.263	0.163	423.420	10.350
T_4	60.475	47.164	5.555	12.985	1.167	0.148	362.800	8.940
T_5	47.555	30.586	4.120	11.435	0.946	0.121	210.930	5.755
T_6	46.050	29.305	4.050	10.305	0.919	0.117	198.275	5.365
T_7	77.000	55.718	6.495	13.500	1.414	0.230	577.600	17.730
T_8	74.500	54.837	6.250	13.345	1.425	0.180	547.205	13.415
T_9	59.500	39.659	5.300	12.400	1.225	0.159	381.100	9.460
T_{10}	58.000	38.977	5.135	11.300	1.190	0.153	365.450	8.850
T_{11}	44.500	27.895	4.200	10.440	0.935	0.117	197.585	5.204
T_{12}	42.000	25.536	3.905	9.250	0.890	0.114	178.550	4.770
T_{13}	97.000	92.644	8.050	17.230	1.845	0.240	931.100	23.640
T_{14}	96.000	89.855	8.250	16.300	1.845	0.243	892.900	23.290
Grand total	65.9489	50.243	5.7096	12.984	1.2885	0.1697	461.886	11.938

Table 3: Variability measures of CRD for eight seedling related traits in cotton

Variables	Critical difference	Standard error for mean	Standard error difference	Coefficient of variation (%)
Germination percentage (%)	2.721	0.889	1.257	1.906
Survival rate (%)	2.486	0.576	0.922	1.412
Root length (cm)	0.309	0.101	0.142	2.496
Seedling length (cm)	0.502	0.164	0.232	3.536
Fresh weight (mg)	0.079	0.026	0.036	2.816
Dry weight (mg)	0.023	0.007	0.011	6.232
Vigour index I	8.526	4.865	2.022	4.551
Vigour index II	2.184	0.713	1.009	8.448

Conclusion

Overall, it is concluded that the mutagen-treated seedlings showed reduced growth performance compared to untreated controls, highlighting inhibitory effects at higher doses. From these results, it can be inferred that MCU 5 is more amenable to EMS mutagenesis than MCU 12, particularly at lower concentrations and shorter durations. Treatment T_1 (0.2% for 8 hours) may be considered as the optimum/LD_{50} level treatment for MCU 5, where sufficient mutagenic effectiveness was achieved without compromising viability. Excessive mutagen dosage (0.6% for 14 hours) proved detrimental, reinforcing the importance of selecting an effective but safe mutagenic dose. Therefore, MCU 5 emerges as a promising genotype for mutation breeding programmes, and EMS treatment at 0.2% for 8 hours may be recommended for generating variability in seedling vigour-related traits.

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