



International Journal of Research in Agronomy

E-ISSN: 2618-0618
P-ISSN: 2618-060X
© Agronomy
NAAS Rating (2026): 5.20
www.agronomyjournals.com
2026; 9(7): 1492-1495
Received: 11-05-2026
Accepted: 14-06-2026

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Effect of ethyl methane sulphonate on germination, survival and quantitative traits in linseed (*Linum usitatissimum* L.)

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DOI: <https://www.doi.org/10.33545/2618060X.2026.v9.i7s.6151>

Abstract

Ethyl Methane Sulphonate (EMS) is one of the most effective chemical mutagens used to induce genetic variability for crop improvement. The present study was undertaken to evaluate the mutagenic effects of different EMS concentrations on germination, survival, mutation frequency and quantitative traits in linseed (*Linum usitatissimum* L.). Four linseed varieties, namely Himpalam Als-2, Himani, Nagarkot and Local collection, were treated with 0.1, 0.3, 0.5 and 0.7% EMS along with an untreated control. Treated seeds were evaluated in the M₁ generation for germination and survival, while the M₂ generation was assessed for viable macromutations and important agronomic traits. Germination and plant survival decreased progressively with increasing EMS concentration, indicating a dose-dependent inhibitory effect of the mutagen. The estimated LD₅₀ values ranged from 0.77 to 0.89%, with Himani exhibiting the highest tolerance and the Local variety showing the greatest sensitivity to EMS treatment. Higher EMS concentrations induced a wider spectrum and greater frequency of viable macromutations, including alterations in plant growth habit, flower shape, stem type, seed colour and seed texture. Moderate EMS doses generated greater variability for quantitative and yield-related traits, whereas higher concentrations caused considerable biological damage and reduced plant performance. The findings demonstrate that EMS is an effective mutagen for generating useful genetic variability in linseed, and concentrations around the estimated LD₅₀ may be considered suitable for mutation breeding programmes aimed at the development of improved genotypes with desirable agronomic characteristics.

Keywords: Ethyl methane sulphonate, EMS, linseed, mutation breeding, LD₅₀, genetic variability, germination, yield traits

Introduction

Linseed (*Linum usitatissimum* L.) is an important oilseed and fibre crop belonging to the family *Linaceae*. It is cultivated for its seed oil, fibre and several industrial uses. Linseed seed contains about 35–45% oil and is a rich source of α -linolenic acid, an important omega-3 fatty acid. The seeds also contain protein, dietary fibre, lignans and antioxidants. Owing to its nutritional value and industrial importance, linseed has received considerable attention in crop improvement programmes.

The available genetic variability in cultivated linseed is limited due to continuous selection and domestication. This narrow genetic base restricts the improvement of important traits such as seed yield, plant architecture, maturity duration and tolerance to biotic and abiotic stresses. Therefore, creation of new genetic variability is essential for effective crop improvement.

Mutation breeding is an important approach for generating new heritable variation in crop plants. Chemical mutagens, particularly ethyl methane sulphonate (EMS), are widely used for inducing point mutations. EMS causes alkylation of DNA bases and mainly produces GC→AT transitions. Such mutations can generate useful variability for qualitative and quantitative traits without causing extensive chromosomal damage.

The response of plants to EMS depends on genotype, mutagen concentration, treatment duration and physiological condition of seeds. Higher EMS concentrations generally reduce seed germination, plant survival and reproductive fertility due to increased biological damage. However, moderate concentrations may induce useful mutations while maintaining sufficient

plant survival and fertility. Therefore, identification of an optimum EMS dose is important for successful mutation breeding.

The present study was undertaken to assess the effect of different EMS concentrations on seed germination, plant survival, pollen fertility, ovule fertility and quantitative traits in four linseed varieties. The study also aimed to identify a suitable EMS concentration for generating useful variability in linseed.

Materials and Methods

The present investigation was conducted at the Experimental Farm and Laboratory of the School of Agriculture, Abhilashi University, Chailchowk, Mandi, Himachal Pradesh, India during the rabi season of 2024–25. Four linseed varieties, namely Himpalam Alsi-2, Himani, Nagarkot and Local collection, were used as experimental material.

Healthy and uniform seeds of each variety were pre-soaked in distilled water for 6 h. The seeds were treated with freshly prepared aqueous solutions of EMS at 0.1, 0.3, 0.5 and 0.7% concentrations for 6 h using phosphate buffer of pH 7.0. After treatment, the seeds were thoroughly washed under running tap water followed by distilled water to remove residual EMS. Untreated seeds served as control.

The treated and untreated seeds were sown in the field Standard agronomic practices were followed during the crop season. Germination percentage and plant survival percentage were recorded in M₁ generation. Pollen fertility was estimated by staining pollen grains with 1% acetocarmine stain. Ovule fertility was calculated on the basis of seed setting per capsule.

Seeds harvested from individual M₁ plants were advanced to M₂ generation. Observations were recorded for qualitative traits such as plant growth habit, flower shape, stem type, seed colour and seed texture. Quantitative observations included days to first pod initiation, days to 75% maturity, plant height, technical height, number of primary branches, capsules per plant, seeds per capsule, seeds per plant, grain yield per plant, biological yield, 1000-seed weight and harvest index. Mean values, coefficient of variation, mutation frequency, mutagenic effectiveness and mutagenic efficiency were calculated using standard statistical procedures.

Results

Results of the present investigation indicated that different concentrations of EMS significantly influenced seed germination, plant survival, pollen fertility, ovule fertility and quantitative traits in all four linseed varieties. The observations are presented in Tables 1–3.

Seed Germination and Plant Survival

Seed germination and plant survival decreased progressively with increase in EMS concentration in all the varieties (Table 1). The untreated control recorded maximum germination and survival, whereas the minimum values were recorded at 0.70% EMS treatment. The reduction was more pronounced at 0.50 and 0.70% EMS concentrations.

The decline in germination and survival at higher EMS concentrations may be due to mutagen-induced disturbance in cellular metabolism, enzyme activity and cell division. The differences among varieties showed that the response to EMS was genotype dependent. Higher EMS doses caused greater biological damage; therefore, moderate concentrations were more suitable for maintaining an adequate plant population.

Pollen and Ovule Fertility

Pollen fertility and ovule fertility decreased with increasing EMS concentration in all the varieties (Table 2). Maximum fertility was observed in the control, while minimum pollen and ovule fertility were recorded at 0.70% EMS treatment. Lower EMS concentrations maintained comparatively higher fertility and seed setting.

The reduction in pollen fertility may be due to mutagen-induced meiotic abnormalities, chromosomal disturbances and abnormal pollen development. Similarly, reduction in ovule fertility may be associated with impaired female gametophyte development and reduced seed setting. The results indicated that higher EMS concentrations caused greater reproductive damage, whereas lower and moderate doses were more suitable for obtaining viable mutant populations.

Effect of EMS on Quantitative Traits

EMS treatments induced considerable variation in M₂ generation for days to first pod initiation, days to 75% maturity, plant height, technical height, number of primary branches, capsules per plant, seeds per capsule, seeds per plant, grain yield per plant, biological yield, 1000-seed weight and harvest index (Table 3).

Table 1: Effect of different doses of EMS on seed germination and plant survival percentage

Treatment	Plant Germination (%)	Plant Survival (%)
Himpalam Alsi-2		
0.1	93	89
0.3	87	82
0.5	79	72
0.7	68	59
Control	97	95
Himani		
0.1	95	91
0.3	89	84
0.5	82	75
0.7	71	63
Control	98	96
Nagarkot		
0.1	92	88
0.3	85	80
0.5	77	70
0.7	66	57
Control	96	94
Local		
0.1	90	86
0.3	83	78
0.5	74	67
0.7	63	54
Control	94	92

The treated populations showed both positive and negative deviations from the control. Moderate EMS concentrations, particularly 0.50%, produced useful variation for plant architecture, maturity and yield-attributing traits. Selected plants under 0.50% EMS showed improvement in number of primary branches, capsules per plant, seeds per plant, grain yield and 1000-seed weight.

Table 2: Effect of Different doses of EMS on pollen and ovule Fertility

Treatment	Pollen fertility (%)	Reduction	Ovule fertility	Reduction
Himpalam Alsi-2				
0.1%	95.8	3.23	94.6	3.47
0.3%	89.4	9.72	88.2	9.98
0.5%	81.6	17.64	80.4	18.11
0.7%	72.2	27.18	71	27.55
Control	99	-	98	-
Himani				
0.1%	94.6	4.44	93.2	4.9
0.3%	87.8	11.31	86.4	11.84
0.5%	79.2	20	77.8	20.61
0.7%	69.4	29.9	68	30.61
Control	99	-	98	-
Nagarkot				
0.1%	93.8	5.25	92.4	5.71
0.3%	86.2	12.93	84.8	13.47
0.5%	77.4	21.82	76	22.45
0.7%	66.8	32.53	65.4	33.27
Control	99	-	98	-
Local				
0.1%	92.6	6.46	91.2	6.94
0.3%	84.2	14.95	82.8	15.51
0.5%	74.6	24.65	73.2	25.31
0.7%	63.2	36.16	61.8	36.94
Control	99	-	98	-

In Local variety, 0.50% EMS recorded 95.60 capsules per plant with 28.69% superiority over the control. Grain yield per plant was also highest under 0.50% EMS, with a mean value of 8.15 g and 32.49% superiority over the control. The 1000-seed weight reached 7.48 g under the same treatment, representing 14.71% improvement over the control.

At 0.70% EMS, plant growth, reproductive fertility and yield performance were generally reduced because of severe biological damage. Although the higher EMS concentration induced greater visible variation, its usefulness was limited due to poor survival and lower seed production.

Conclusion

The present investigation showed that EMS concentration had a marked effect on seed germination, plant survival, pollen fertility, ovule fertility and quantitative traits in linseed. Increasing EMS concentration caused progressive reduction in germination, survival and fertility. The highest concentration of 0.70% EMS caused maximum biological damage and reduced yield performance.

The 0.50% EMS concentration was found to be more useful because it induced considerable variability for plant architecture, maturity and yield traits while maintaining comparatively better survival and fertility. The promising plants identified under 0.50% EMS treatment may be advanced to subsequent generations for confirmation of stability and use in linseed improvement programmes.

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