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Effect of ethyl methane sulphonate on germination, survival, fertility and mutagenic efficiency in Lentil (*Lens culinaris* Medik.)

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Abstract

Lentil (*Lens culinaris* Medik.) is an important pulse crop, but its improvement is often limited by a narrow genetic base. The present study evaluated the effect of ethyl methane sulphonate (EMS) on seed germination, plant survival and reproductive fertility in two lentil varieties, Markanda and Vipasha. Seeds were treated with 0.1, 0.2, 0.3, 0.4 and 0.5% EMS, along with an untreated control. A progressive reduction in germination, survival, pollen fertility and ovule fertility was observed with increasing EMS concentration in both varieties. Under laboratory conditions, germination declined from 96.00% in the control to 58.00% in Markanda and 48.00% in Vipasha at 0.5% EMS. Pollen fertility declined from 99.00% in the control to 77.20% and 68.20% in Markanda and Vipasha, respectively, at the highest EMS dose. The LD₅₀ dose was estimated to be approximately 0.5% EMS for both varieties. Markanda showed comparatively greater tolerance to EMS than Vipasha. Lower and moderate EMS doses were found suitable for inducing useful variability with comparatively lower biological damage. The findings indicate that EMS can be effectively used for generating genetic variability in lentil breeding programmes.

Keywords: *Lens culinaris*, EMS, induced mutagenesis, germination, fertility, lentil breeding

Introduction

Lentil (*Lens culinaris* Medik.) is an important food legume crop cultivated widely in many parts of the world, particularly in South Asia. It is a rich source of dietary protein, carbohydrates, vitamins, minerals and dietary fibre. Lentil also improves soil fertility through biological nitrogen fixation and therefore plays an important role in sustainable cropping systems. In India, lentil is mainly cultivated during the rabi season and contributes significantly to nutritional security, especially among vegetarian populations.

Despite its importance, the productivity of lentil remains comparatively low due to several biotic and abiotic stresses, limited availability of high-yielding varieties and a narrow genetic base in cultivated germplasm. Conventional breeding methods such as selection and hybridization have contributed to lentil improvement; however, the creation of sufficient genetic variability remains a major challenge. Therefore, induced mutagenesis is considered a useful supplementary approach for broadening genetic variability and developing desirable plant types.

Chemical mutagens, particularly ethyl methane sulphonate (EMS), are widely used in plant breeding because they induce point mutations and create heritable variation without drastically disturbing the overall genetic background of an established variety. EMS has been successfully used in several pulse crops to generate variation for traits such as plant height, maturity duration, branching, pod number, seed size, yield and stress tolerance. However, the response to EMS varies according to genotype, concentration and treatment conditions. Higher concentrations may cause excessive biological damage, including poor germination, reduced survival and reduced reproductive fertility.

Therefore, the present investigation was undertaken to study the effect of different EMS concentrations on seed germination, plant survival, pollen fertility and ovule fertility in two lentil varieties, Markanda and Vipasha. The study also aimed to identify an appropriate EMS dose for inducing useful variability with minimum biological damage in lentil.

Materials and Methods

The present investigation was conducted during the Rabi season of 2024–25 at the Research Farm and laboratories of the School of Agriculture, Abhilashi University, Chail Chowk, Mandi, Himachal Pradesh, India. The experimental site is located at approximately 31°33'12" N latitude and 76°59'18" E longitude, at an altitude of about 1450 m above mean sea level.

Two lentil (*Lens culinaris* Medik.) varieties, namely Markanda and Vipasha, were used as experimental material. Healthy, uniform and undamaged seeds of both varieties were selected for mutagenic treatment. Ethyl methane sulphonate (EMS) was used as the chemical mutagen.

Seeds were pre-soaked in distilled water for six hours and then treated with five EMS concentrations: 0.1, 0.2, 0.3, 0.4 and 0.5%. EMS solutions were prepared in phosphate buffer of pH 7.0. The pre-soaked seeds were treated with respective EMS solutions for six hours at room temperature with intermittent stirring. Untreated seeds soaked in distilled water served as the control. After treatment, seeds were thoroughly washed under running water to remove residual EMS.

For laboratory evaluation, 100 seeds from each treatment were placed in Petri dishes lined with moist filter paper. Seed germination percentage was recorded after germination, while plant survival percentage was recorded on the basis of surviving seedlings. For field evaluation, treated and untreated seeds were sown in a randomized block design with three replications. Seed germination and plant survival were recorded in the M₁ generation under field conditions.

Pollen fertility was estimated by staining pollen grains using acetocarmine stain. Well-stained, round and fully developed pollen grains were considered fertile, whereas shrivelled or unstained pollen grains were considered sterile. Ovule fertility was calculated based on the proportion of fertile ovules.

The LD₅₀ dose was estimated from seedling survival percentage using probit analysis. Mutation frequency, mutagenic effectiveness and mutagenic efficiency were calculated in the M₂ generation according to the method suggested by Konzak *et al.* (1965) [1]. The collected data were statistically analysed to determine the effect of EMS concentrations on the studied characters.

Results and Discussion

Effect of EMS on Seed Germination and Plant Survival under Laboratory Conditions

The effect of different concentrations of EMS on seed germination and plant survival of lentil varieties Markanda and Vipasha under laboratory conditions is presented in Table 1. A progressive decline in seed germination and plant survival was observed with increasing EMS concentration in both varieties.

In Markanda, seed germination decreased from 96.00% in the control to 92.00, 86.00, 79.00, 70.00 and 58.00% at 0.1, 0.2, 0.3, 0.4 and 0.5% EMS, respectively. The maximum reduction in germination over control was recorded at 0.5% EMS (39.58%), whereas the minimum reduction was observed at 0.1% EMS (4.17%). Plant survival also declined from 95.00% in the control to 93.68% at 0.1% EMS and 67.37% at 0.5% EMS. The highest reduction in plant survival was observed at 0.5% EMS (29.08%). A similar trend was observed in Vipasha. Seed germination declined from 96.00% in the control to 90.00, 82.00, 73.00, 62.00 and 48.00% at increasing EMS concentrations from 0.1 to 0.5%. The reduction in germination was highest at 0.5% EMS (50.00%). Plant survival in Vipasha decreased from 93.00% in the control to 88.17% at 0.1% EMS and 55.91% at 0.5% EMS. The maximum reduction in plant survival over control was recorded at 0.5% EMS (39.88%).

The results indicated that EMS caused dose-dependent biological damage in both lentil varieties. Higher EMS concentrations adversely affected seed viability, seedling growth and survival, possibly due to mutagen-induced physiological disturbances and chromosomal damage. Vipasha showed greater reduction in germination and survival than Markanda, indicating comparatively higher sensitivity to EMS treatment. Similar reductions in germination and survival with increasing mutagen concentration have been reported in lentil and other pulse crops by Konzak *et al.* (1965) [1], Singh *et al.* (2014) [3], and Sood *et al.* (2023) [2].

Effect of EMS on Seed Germination and Plant Survival under Field Conditions

The effect of different EMS concentrations on seed germination and plant survival of lentil varieties Markanda and Vipasha under field conditions is presented in Table 2. Similar to laboratory observations, seed germination and plant survival decreased progressively with increasing EMS concentration in both varieties.

Table 1: Effect of different EMS concentrations on seed germination and plant survival of lentil varieties under laboratory conditions

Variety	EMS concentration (%)	Seed germination (%)	Reduction over control (%)	Plant survival (%)	Reduction over control (%)
Markanda	0.1	92	4.17	93.68	1.39
Markanda	0.2	86	10.42	89.47	5.82
Markanda	0.3	79	17.71	84.21	11.36
Markanda	0.4	70	27.08	76.84	19.12
Markanda	0.5	58	39.58	67.37	29.08
Markanda	Cont.	96	–	95	–
Vipasha	0.1	90	6.25	88.17	5.19
Vipasha	0.2	82	14.58	82.97	10.78
Vipasha	0.3	73	23.96	75.82	18.47
Vipasha	0.4	62	35.42	66.48	28.52
Vipasha	0.5	48	50	55.91	39.88
Vipasha	Cont.	96	–	93	–

In Markanda, seed germination decreased from 92.00% in the control to 87.00% at 0.1% EMS and further to 49.00% at 0.5% EMS. The maximum reduction in seed germination over control was observed at 0.5% EMS (46.74%), followed by 0.4% EMS (32.61%). Plant survival also declined from 90.40% in the control to 87.70% at 0.1% EMS and 58.48% at 0.5% EMS. The highest

reduction in plant survival was recorded at 0.5% EMS (35.31%). In Vipasha, seed germination declined from 90.00% in the control to 84.00% at 0.1% EMS and 42.00% at 0.5% EMS. The reduction in seed germination over control increased from 6.67% at 0.1% EMS to 53.33% at 0.5% EMS. Plant survival decreased from 85.89% in the control to 81.60% at 0.1% EMS and 47.21% at

0.5% EMS. The maximum reduction in plant survival was observed at 0.5% EMS (45.03%).

The reduction in germination and survival under field conditions was comparatively greater than that observed under laboratory conditions, indicating that treated seedlings were further affected by environmental stress after sowing. Vipasha showed greater sensitivity to EMS than Markanda, as reflected by its lower germination and survival percentages at higher concentrations. The findings confirm that increasing EMS concentration causes greater biological damage, resulting in poor seedling establishment and survival. Similar dose-dependent effects of EMS on germination and survival have been reported in several pulse crops.

Effect of EMS on Pollen and Ovule Fertility in M₁ Generation

The effect of different concentrations of EMS on pollen and ovule fertility in lentil varieties Markanda and Vipasha is presented in Table 3. Pollen fertility and ovule fertility decreased progressively with increasing EMS concentration in both varieties, indicating the adverse effect of EMS on reproductive behaviour.

In Markanda, pollen fertility decreased from 99.00% in the

control to 96.20% at 0.1% EMS and 77.20% at 0.5% EMS. The reduction in pollen fertility over control increased from 2.83% at 0.1% EMS to 22.02% at 0.5% EMS. Ovule fertility followed a similar trend, declining from 98.00% in the control to 95.10% at 0.1% EMS and 76.00% at 0.5% EMS. The maximum reduction in ovule fertility was recorded at 0.5% EMS (22.45%).

In Vipasha, pollen fertility decreased from 99.00% in the control to 94.00% at 0.1% EMS and 68.20% at 0.5% EMS. The maximum reduction in pollen fertility over control was observed at 0.5% EMS (31.11%). Similarly, ovule fertility declined from 97.00% in the control to 92.80% at 0.1% EMS and 66.40% at 0.5% EMS. The reduction in ovule fertility was highest at 0.5% EMS (31.55%).

The reduction in pollen and ovule fertility at higher EMS concentrations may be due to mutagen-induced chromosomal abnormalities, meiotic disturbances and physiological damage to reproductive tissues. The decline was more pronounced in Vipasha than in Markanda, suggesting that Vipasha was comparatively more sensitive to EMS treatment. The results indicate that higher EMS concentrations may reduce reproductive success, whereas lower and moderate concentrations can be used for inducing variability with relatively less fertility loss.

Table 2: Effect of different EMS concentrations on seed germination and plant survival of lentil varieties under field conditions

Variety	EMS concentration (%)	Seed germination (%)	Reduction over control (%)	Plant survival (%)	Reduction over control (%)
Markanda	0.1	87	5.43	87.7	2.99
Markanda	0.2	81	13.04	83.15	8.02
Markanda	0.3	72	21.74	76.82	15.02
Markanda	0.4	62	32.61	68.95	23.73
Markanda	0.5	49	46.74	58.48	35.31
Markanda	Con	92	—	90.4	—
Vipasha	0.1	84	6.67	81.6	4.99
Vipasha	0.2	75	16.67	75.58	12
Vipasha	0.3	66	26.67	67.55	21.35
Vipasha	0.4	55	38.89	57.77	32.74
Vipasha	0.5	42	53.33	47.21	45.03
Vipasha	Con	90	—	85.89	—

Effect of EMS on Mutation Frequency, Mutagenic Effectiveness and Mutagenic Efficiency

The effect of different EMS concentrations on mutation frequency, mutagenic effectiveness and mutagenic efficiency in the M₂ generation of lentil is presented in Table 4. Mutation frequency increased with increasing EMS concentration up to 0.4% and declined at 0.5% EMS in both varieties.

In Markanda, mutation frequency increased from 1.42% at 0.1% EMS to 6.12% at 0.4% EMS, followed by a decline to 5.18% at 0.5% EMS. Mutagenic effectiveness ranged from 10.36 to 16.20,

with the maximum value recorded at 0.3% EMS. Mutagenic efficiency increased from 0.19 at 0.1% EMS to 0.52 at 0.4% EMS and then declined to 0.40 at 0.5% EMS.

In Vipasha, mutation frequency ranged from 1.68% at 0.1% EMS to 6.86% at 0.4% EMS, followed by a decline to 5.62% at 0.5% EMS. The highest mutagenic effectiveness (17.80) was recorded at 0.3% EMS, whereas the maximum mutagenic efficiency (0.54) was observed at 0.4% EMS. At 0.5% EMS, mutagenic effectiveness and efficiency declined to 11.24 and 0.42, respectively.

Table 3: Effect of different EMS concentrations on pollen and ovule fertility in M₁ generation of lentil

Variety	EMS concentration (%)	Pollen fertility (%)	Reduction over control (%)	Ovule fertility (%)	Reduction over control (%)
Markanda	0.1	96.2	2.83	95.1	2.96
Markanda	0.2	92.8	6.26	91.6	6.53
Markanda	0.3	88.6	10.51	87.4	10.82
Markanda	0.4	83.4	15.76	82.2	16.12
Markanda	0.5	77.2	22.02	76	22.45
Markanda	Cont	99	—	98	—
Vipasha	0.1	94	5.05	92.8	4.33
Vipasha	0.2	89.5	9.6	88.2	9.07
Vipasha	0.3	83.2	15.96	81.6	15.88
Vipasha	0.4	76.4	22.83	74.8	22.89
Vipasha	0.5	68.2	31.11	66.4	31.55
Vipasha	Cont	99	—	97	—

The results indicated that intermediate EMS concentrations were more effective for inducing useful mutations than either lower or higher concentrations. Although 0.4% EMS produced the highest mutation frequency and mutagenic efficiency, 0.3% EMS produced the maximum mutagenic effectiveness in both varieties. The decline at 0.5% EMS may be attributed to excessive

biological damage, including reduced survival and fertility. Vipasha recorded higher mutation frequency, mutagenic effectiveness and mutagenic efficiency than Markanda; however, it also showed greater biological sensitivity to EMS. Overall, 0.3–0.4% EMS was found suitable for inducing useful variability in lentil.

Table 4: Effect of different EMS concentrations on mutation frequency, mutagenic effectiveness and mutagenic efficiency in M₂ generation of lentil

Variety	EMS concentration (%)	Mutation frequency (%)	Mutagenic effectiveness	Mutagenic efficiency
Markanda	0.1	1.42	14.2	0.19
Markanda	0.2	2.98	14.9	0.33
Markanda	0.3	4.86	16.2	0.46
Markanda	0.4	6.12	15.3	0.52
Markanda	0.5	5.18	10.36	0.4
Vipasha	0.1	1.68	16.8	0.21
Vipasha	0.2	3.42	17.1	0.36
Vipasha	0.3	5.34	17.8	0.48
Vipasha	0.4	6.86	17.15	0.54
Vipasha	0.5	5.62	11.24	0.42

Mutation frequency and mutagenic efficiency increased up to 0.4% EMS and declined at 0.5% EMS, indicating that excessive mutagen concentration caused greater biological damage than useful genetic variation. Although 0.4% EMS recorded the maximum mutation frequency and mutagenic efficiency, 0.3% EMS showed the highest mutagenic effectiveness in both varieties. Therefore, 0.3–0.4% EMS may be considered an appropriate dose range for generating useful genetic variability in lentil. The mutant populations developed through these treatments can be further screened in subsequent generations for desirable agronomic and yield-related traits.

Conclusion

The present study demonstrated that EMS treatment caused a concentration-dependent reduction in seed germination, plant survival, pollen fertility and ovule fertility in both lentil varieties, Markanda and Vipasha. The highest biological damage was observed at 0.5% EMS, whereas lower concentrations caused comparatively less reduction in germination, survival and reproductive fertility. Markanda showed greater tolerance to EMS treatment than Vipasha.

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