

Pre-Sowing Seed Treatment with Gamma Rays, Ethyl Methanesulphonate and Sodium Azide in Lentil (*Lens culinaris*) Cultivar Shekhar-4

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(Received February 2025; Revised March 2025; Accepted March 2025)

ABSTRACT: This study tested the effects of pre-sowing treatments—gamma rays, EMS, and SA—on lentil growth and yield. Lentil seeds (cv. Shekhar-4), stored for one season, were treated with various doses and grown under field and lab conditions. Seeds treated with 5 kR gamma rays showed the best growth and yield. Lower doses improved performance, while higher doses reduced it. Overall, gamma irradiation was most effective at lower levels.

Keywords: Lentil seeds, Shekhar-4, Ethyl Methanesulphonate, Sodium Azide, Gamma irradiation.

INTRODUCTION

Lentil (*Lens culinaris* Medik.), member of the legume family Fabaceae, have been cultivated for over 10,000 years, with origins traced to the ancient Near East [1]. Archaeological records show they were staple foods for early civilizations such as the Egyptians, Greeks, and Romans [2]. Their global appeal stems from their culinary versatility—used in soups, stews, salads, and spreads [3].

It is a self-pollinated plant and among India's most important winter legumes, ranking second after chickpea [4]. Nutritionally, lentils are a powerhouse, offering high-quality plant protein, dietary fibre, folate, vitamin B6, and minerals like iron, magnesium, and potassium [5].

Agronomically, lentils contribute to sustainable farming systems. Their symbiotic association with nitrogen-fixing rhizobia enriches soil fertility and reduces the need for synthetic fertilizers, mitigating environmental pollution and greenhouse gas emissions [6].

To enhance productivity, mutagenesis using agents like gamma rays and ethyl methanesulfonate (EMS) has been employed to induce beneficial genetic changes [7]. These treatments improve seed germination, seedling vigor, and key agronomic traits. EMS is a widely used alkylating agent that modifies nucleotides, primarily targeting

guanine bases. These modifications often convert G:C to A:T base pairs, leading to genetic variation [8]. However, EMS is genotoxic and potentially carcinogenic, necessitating cautious application.

Gamma irradiation is another effective method for inducing mutations. It is widely used in agriculture for creating genetic variability and enhancing traits like yield, stress tolerance, and disease resistance [9]. In lentils and other crops, gamma rays offer high penetrative ability and generate a wide range of mutations in a single application [10].

This study explores the effects of mutagenesis on growth, yield, and seedling traits in the lentil variety Shekhar-4, aiming to identify optimal doses of mutagens that can improve crop performance and support higher yields.

MATERIALS AND METHODS

Seeds of the lentil variety Shekhar-4 (K.L.B 345) were treated with different doses of Ethyl Methanesulphonate (EMS), Sodium Azide (SA), and gamma irradiation in NBRI, Lucknow (March 2023), and the seeds were stored under ambient conditions for one planting season. These treated seeds were used for both field and laboratory experiments. The treatments included T0 (untreated control), T1 (EMS @ 0.3% for 3 hours), T2 (EMS @ 0.3% for 6 hours), T3 (SA @ 0.3% for 3 hours), T4 (SA @

0.3% for 6 hours), T5 (gamma irradiation @ 5 kR), T6 (gamma irradiation @ 10 kR), T7 (gamma irradiation @ 15 kR), T8 (gamma irradiation @ 20 kR), T9 (gamma irradiation @ 25 kR), and T10 (gamma irradiation @ 30 kR). Gamma irradiation was conducted at the National Botanical Research Institute (NBRI), Lucknow, using a GIC-1200 gamma irradiator with a Cobalt-60 source. EMS and Sodium Azide solutions were prepared at 0.3% concentration by dissolving the respective chemicals in distilled water with thorough mixing to ensure homogeneity. EMS-treated seeds were exposed for 3 and 6 hours. All treatments were administered under standardized settings to ensure consistency and reliability in the mutagenesis process.

For this research, a mutagenized population of lentil seeds was developed to assess the effects of different mutagenic treatments on growth and development. The experiment included ten treatments—one control and nine exposed to varying doses of Ethyl Methane Sulphonate (EMS), Sodium Azide, and gamma radiation. In the laboratory, 100 seeds per treatment (four replications of 25 seeds) were used. Field trials included three replications with 60 seeds each.

For germination studies, seeds were placed between moist germination papers in a chamber maintained at $25 \pm 1^\circ\text{C}$ and 90% humidity in darkness. Observations over 7–14 days included germination percentage, seedling length, and seed vigor.

Field observations were recorded on five randomly selected plants per replication, covering traits such as field emergence rate, days to 50% flowering, plant height (30, 60, 90 DAS), number of branches, pods per plant, seeds per pod, seed yield (per plant, plot, hectare), biological yield, harvest index, and test weight.

Laboratory parameters such as germination (%), shoot/root/seedling length, seedling fresh and dry weight, and Seed Vigour Index I and II were assessed as per [11, 12]. Statistical analysis followed methods described in [13].

RESULTS AND DISCUSSION

This study investigated the impact of induced mutagens on seedling, growth, and yield parameters on lentil seeds. Seeds treated with a lower dose of gamma radiation (5 kR) showed significantly higher values for all seedling, growth, and yield traits, while higher doses such as 30 kR had negative effects (Table 1 & 2).

Growth and Yield Parameters

Rate of Field Emergence

Field emergence in lentil ranged from 1487.62 to 1016.67. The highest emergence (1487.62) was in T5 (5kR), followed by T6 (10kR) (1437), and the lowest in T10 (30kR) (1016.67). Low-dose gamma radiation enhances emergence through improved enzymatic activity and hormonal balance, with similar results reported in okra [14] and tomato [15].

Days to 50% Flowering

Flowering ranged from 41 to 68.33 days. T5 (5kR) had the earliest flowering (41 days), followed by T6 (10kR) (43 days), and the latest in T10 (30kR) (68.33 days), aligning with findings in chickpea [16].

Plant Height (cm) at 30, 60, and 90 DAS

Plant height ranged from 12.50 to 8.58 (30 DAS), 27.11 to 12.80 (60 DAS), and 36.61 to 20.41 (90 DAS). T5 consistently showed maximum height at all stages, while T10 had the lowest. Low-dose radiation enhances nutrient mobilization, enzyme activity, and stress resistance [17, 18, 19].

Number of Branches per Plant

Branches ranged from 9.87 (T5) to 1.40 (T10). Gamma radiation up to 10kR increased branching due to enhanced enzymatic activity and stress resistance [19].

Days to Maturity

Maturity varied from 110 (T5) to 120 days (T10), with earlier maturity at lower radiation doses.

Number of Pods and Seeds per Plant

Pods ranged from 84 (T5) to 30.47 (T10). Low-dose radiation improved pod numbers by modifying hormonal balances, promoting branching and flowering [19]. Seeds per pod varied from 2.00 (T5) to 0.73 (T10), with higher values at lower doses.

Seed Yield per Plant (g); per Plot (kg) and per Hectare (q)

Yield per plant ranged from 12.23 g (T5) to 4.03 g (T10), showing a clear advantage of low-dose treatments. Plot yields varied from 0.73 kg (T5) to 0.24 kg (T10), highest at 5kR and 10kR. Hectare yield ranged from 18.35 q (T5) to 6.05 q (T10). Improved yields were linked to enhanced reproductive traits and stress resilience, consistent

Table 1. Mean performance of the impact of pre-sowing seed treatments with Gamma rays, Ethyl Methanesulphonate and sodium Azide on growth and yield characteristics of Lentil cultivar Shekhar-4

Treatments	Rate of field emergence	Days to 50% flowering	Plant height 30 DAS (cm)	Plant height 60 DAS (cm)	Plant height 90 DAS (cm)	No. of branches	Days to maturity	No. of pods per plant	No. of seeds per pod	Seed yield per plant (gm)	Seed yield per plot (kg)	Seed yield per hectare (q)	Biological yield per plot (kg)	Harvest index (%)	Test weight (gm)
T0	1393	46.67	10.58	23.71	32.87	8.33	111.7	76.00	1.7	11.15	0.67	16.73	9.26	44.45	4.21
T1	1320	48.33	9.93	21.70	31.25	6.33	113.7	69.87	1.4	9.84	0.59	14.76	8.84	42.90	4.10
T2	1225	53.00	9.58	19.61	29.62	4.33	114.7	63.27	1.5	8.18	0.49	12.27	8.49	32.45	3.85
T3	1263	50.00	9.74	19.95	30.83	4.93	114.3	63.13	1.4	8.63	0.52	12.95	8.73	36.36	4.02
T4	1211	54.00	9.44	18.49	26.17	3.80	115.3	53.00	1.5	7.71	0.46	11.57	8.24	31.34	3.68
T5	1488	41.00	12.50	27.11	36.61	9.87	110.0	84.00	2.0	12.23	0.73	18.35	9.83	49.20	4.69
T6	1437	43.00	11.81	26.06	33.59	9.33	111.3	81.27	1.9	11.79	0.71	17.69	9.70	46.92	4.34
T7	1365	49.00	10.60	22.22	31.91	7.20	112.3	72.67	1.7	10.57	0.63	15.85	9.20	42.80	4.18
T8	1157	60.00	9.11	16.09	24.93	3.13	117.3	46.73	1.1	6.19	0.37	9.29	6.95	30.96	3.60
T9	1105	64.00	8.95	13.79	22.81	1.93	118.7	43.67	1.0	5.51	0.33	8.26	6.61	29.91	3.22
T10	1017	68.33	8.58	12.80	20.41	1.40	120.0	30.47	0.7	4.03	0.24	6.05	6.48	26.47	2.98
Grand	1270.93	52.48	10.07	20.14	29.18	5.51	114.48	62.19	1.44	8.71	0.52	13.07	8.39	37.62	3.90
Mean															
Max.	1487.62	68.33	12.50	27.11	36.61	9.87	120.00	84.00	2.00	12.23	0.73	18.35	9.83	49.20	4.69
Min.	1016.67	41.00	8.58	12.80	20.41	1.40	110.00	30.47	0.73	4.03	0.24	6.05	6.48	26.47	2.98
S Em	8.57	0.53	0.05	0.07	0.10	0.08	0.39	0.22	0.06	0.03	0.01	0.04	0.02	0.13	0.03
S Ed	12.12	0.74	0.07	0.10	0.14	0.11	0.56	0.32	0.08	0.04	0.01	0.05	0.03	0.18	0.05
CD at 5%	43.80	2.69	0.25	0.36	0.50	0.39	2.00	1.14	0.29	0.13	0.01	0.19	0.11	0.67	0.18

T0- Control, T1- EMS (Ethyl Methanesulphonate) 0.3% (3 hrs.), T2- EMS (Ethyl Methanesulphonate) 0.3% (6 hrs.), T3- SA (Sodium Azide) 0.3% (3 hrs), T4- SA (Sodium Azide) 0.3% (6 hrs), T5- 5 kR, T6- 10 kR, T7- 15 kR, T8- 20 kR, T9- 25 kR, T10- 30 kR

Table 2. Mean performance of the impact of pre-sowing seed treatments with Gamma rays, Ethyl Methanesulphonate and sodium Azide on Seedling characteristics of Lentil cultivar Shekhar-4

Treatments	Germination %	Germination Energy	Shoot Length (cm)	Root Length (cm)	Seedling Length (cm)	Fresh weight (gm)	Dry weight (gm)	Seed Vigour Index-I	Seed Vigour Index-II
T0	92.75	50.00	11.71	16.00	27.71	3.13	0.39	2569.11	36.21
T1	87.50	45.50	10.96	15.28	26.24	3.06	0.36	2295.68	31.25
T2	84.00	41.50	10.16	14.19	24.35	2.74	0.33	2045.39	27.68
T3	87.00	44.25	10.63	14.76	25.38	2.94	0.34	2208.53	29.54
T4	83.75	39.50	10.05	13.76	23.80	2.64	0.32	1993.37	26.49
T5	97.25	52.75	13.58	16.79	30.36	4.11	0.43	2952.58	41.48
T6	96.00	51.25	12.94	16.21	29.15	3.50	0.41	2797.92	39.79
T7	89.75	48.25	11.20	15.69	26.89	3.09	0.38	2413.20	34.13
T8	81.00	37.00	9.93	12.71	22.64	2.47	0.30	1833.70	24.62
T9	79.50	35.25	9.68	12.19	21.87	2.25	0.29	1738.75	23.28
T10	77.50	30.50	9.37	11.88	21.25	2.13	0.28	1647.23	21.64
Grand Mean	86.91	43.25	10.93	14.49	25.42	2.91	0.35	2226.86	30.55
S Em	0.34	0.53	0.03	0.03	0.06	0.02	0.01	9.52	0.19
S Ed	0.48	0.74	0.04	0.04	0.08	0.03	0.01	13.46	0.26
CD at 5%	1.97	3.02	0.17	0.16	0.32	0.12	0.009	54.79	1.07

T0- Control, T1- EMS (Ethyl Methanesulphonate) 0.3% (3 hrs.), T2- EMS (Ethyl Methanesulphonate) 0.3% (6 hrs.), T3- SA (Sodium Azide) 0.3% (3 hrs), T4- SA (Sodium Azide) 0.3% (6 hrs), T5- 5 kR, T6- 10 kR, T7- 15 kR, T8- 20 kR, T9- 25 kR, T10- 30 kR

with findings in okra [14], chickpea [16, 17], and wheat [20].

Harvest Index (%)

HI varied from 49.20% (T5) to 26.47% (T10), reflecting better source-sink efficiency at low doses.

Test Weight (g)

TW ranged from 4.69 g (T5) to 2.98 g (T10), indicating improved seed quality under low-dose treatments.

Seedling Parameters

Germination ranged from 97.25% (T5) to 77.50% (T10). Similar trends were seen in, okra [14], chickpea [16], mung bean [18], and tomato [15]. Germination energy varied between 52.75 (T5) and 30.50 (T10), with improvements linked to enhanced enzymatic activity. Shoot length ranged from 13.58 cm (T5) to 9.37 cm (T10). Root length varied from 16.79 cm (T5) to 11.88 cm (T10). Seedling length ranged from 30.36 cm (T5) to 21.25 cm (T10). Fresh weight ranged from 4.11 g (T5) to 2.13 g (T10). Dry weight ranged from 0.43 g (T5) to 0.28 g (T10). Seedling Vigour Index I varied from 2952 (T5) to 1647 (T10). Seedling Vigour Index II ranged from 41.48 (T5) to 21.64 (T10).

This study also underlined the importance of having standardized and validated pre-sowing treatments as a

compulsory component of crop management similar to those reported by [21] in cowpea and [22] in sunflower.

CONCLUSION

Pre-sowing treatments, particularly low-dose gamma irradiation, significantly enhanced seedling vigour, growth, and yield in lentil. The 5kR and 10kR doses consistently outperformed controls across traits, while higher doses (30kR) had adverse effects.

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