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Research Article

Induction of short stature and early maturity in lentil (*Lens culinaris* Medik.) through mutagenesis

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ABSTRACT

Induced mutagenesis is a pivotal tool for enhancing the genetic diversity and achieving targeted trait selection in pulse crops. In M_2 and M_3 generations of lentil (*Lens culinaris* Medik, var. Pant L-406), this study assesses the genetic variability induced in three quantitative traits viz., days to flowering, days to maturity, and plant height. Different concentrations of sodium azide (SA: 0.01%–0.04%), hydrazine hydrate (HZ: 0.01%–0.04%), and ethylmethane sulphonate (EMS: 0.1%–0.4%) were applied to the seeds. The mean values for all the three attributes showed a significant negative change, according to the results. In the M_3 generation, 0.3% EMS reduced the flowering by 5.20 days and maturity by 5.60 days, resulting in the most significant decreases in both. With a negative shift of 5.12 cm in comparison to the control, 0.03% SA was the most effective in reducing the plant height. Phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), broad-sense heritability (h^2), and genetic advance (GA) were all consistently higher in M_2 generation than in the M_3 generation. For flowering in M_2 generation, 0.3% EMS produced the highest h^2 (60.64%). These results imply that in earlier mutant generations, selection for early maturity and decreased plant height is very successful.

Keywords: Lentil, Genetic variability, Early maturity, Shorter plant height, Induced mutagenesis

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INTRODUCTION

Lentil (*Lens culinaris* Medik) is one of the oldest cultivated protein-rich pulses, essential for global food security and often referred to as "poor man's meat" due to its high nutritional value and digestibility. Beyond its role in human diets, lentil serves as high-quality livestock

feed and supports rural economies through its lucrative market value. However, as a winter crop, lentil faces significant production constraints. Its fragile stem and limited root system makes it highly susceptible to lodging at maturity, while extreme drought and elevated temperatures during the pod-filling stage (terminal heat stress) often drastically reduce yields (Tyagi & Khan, 2011). Genetic variation is the prerequisite for any successful crop improvement program. While conventional breeding (hybridization) is the traditional approach, it is often limited in lentil due to a narrow natural genetic base and technical challenges, such as the crop's small floral architecture which makes artificial emasculation and pollination difficult. Furthermore, while marker-assisted selection (MAS) and genomic tools offer precision, they require significant prior genomic information and high costs which are often unavailable for local varieties. In this context, induced mutagenesis serves as a powerful, cost-effective alternative to create novel alleles and expand genetic diversity without disrupting the adapted genetic background of elite varieties (Laskar & Khan, 2017; Wani, 2018; Goyal *et al.*, 2021; Wani, 2024).

Despite the development of 3,443 mutant varieties globally, including 468 pulse varieties, mutation breeding remains underutilized in lentil. Out of only 19 global lentil mutant varieties, just two 'S-256 (Ranjan)' and 'Rajendra Masoor 1' originated in India. This limited application is largely due to biological constraints (self-pollinating nature) and a historical lack of large-scale screening for polygenic traits in this crop. There is a substantial information vacuum regarding the generation of polygenic variability for complex traits like maturity and stature using contemporary chemical mutagens because most of the prior research in lentils has been on chlorophyll mutations or morphological indicators. To close existing gaps, the current study evaluated the relative effectiveness of three chemical mutagens viz., EMS, HZ and SA in inducing genetic variability across M_2 and M_3 generations. The goal was to develop lodging-resistant, shorter-statured plants suitable for high-density planting and early-maturing mutants capable of escaping terminal heat and drought stress. It also estimated important genetic parameters like heritability and genetic advance to guarantee the stability and breeding value of the induced traits for future improvement initiatives (Laskar *et al.*, 2018; Wani *et al.*, 2021).

METHODOLOGY

Seeds of lentil (*Lens culinaris* Medik.) variety Pant L-406 were used in this study. Seeds were pre-soaked in distilled water for 9 h and treated for 6 h with different concentrations of ethyl methanesulphonate (EMS; 0.1–0.4%), hydrazine hydrate (HZ; 0.01–0.04%), and sodium azide (SA; 0.01–0.04%). EMS and HZ solutions were prepared in phosphate buffer (pH 7.0), while SA solutions were prepared in phosphate buffer (pH 3.0). Control seeds were soaked in distilled water for 15 h. After treatment, seeds were thoroughly washed under running tap water.

For raising M_1 generation, 300 seeds per treatment and control were sown in the field following a Complete Randomized Block Design (CRBD) with a spacing of 30 cm × 60 cm. To raise the M_2 generation, 30 healthy seeds from each normal M_1 plant were sown in progeny rows. The M_3 generation was developed by advancing ten selected M_2 progenies showing significant negative deviations for days to flowering, days to maturity, and plant height compared to the control.

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Observations on days to flowering, days to maturity and plant height (cm) were recorded in M_2 and M_3 generations. Data were statistically analyzed following the method of Singh and Chaudhary (1985). Analysis of variance was performed to estimate genotypic and phenotypic variances, genotypic and phenotypic coefficients of variation (GCV and PCV), broad-sense heritability (h^2), and genetic advance (GA) at 1% selection intensity. Treatment means were compared using critical difference (CD) at 5% probability level.

Genotypic variance ($\sigma^2 g$)

The following formula estimated the genotypic variance ($\sigma^2 g$):

$$\sigma^2 g = \frac{(MS_{Bf} - MS_e)}{N} \quad \text{.....Eq.1}$$

where, MS_{Bf} and MSe = Mean sum of squares for between families and within families or error, respectively

N = Number of replications

Genotypic coefficient of variation (GCV)

$$GCV\,(\%) = \frac{\sqrt{\sigma^2 g}}{\bar{X}} \times 100 \quad \text{.....Eq.2}$$

Phenotypic variance ($\sigma^2 p$)

Phenotypic variance was estimated by summing the estimated genotypic variance ($\sigma^2 g$) and the environmental variance (MS_e or $\sigma^2 e$)

$$\sigma^2 p = \sigma^2 g + \sigma^2 e \quad \text{.....Eq3}$$

Phenotypic coefficient of variation (PCV)

$$PCV\,(\%) = \frac{\sqrt{\sigma^2 p}}{\bar{X}} \times 100 \quad \text{.....Eq.4}$$

Heritability (h^2)

It is the ratio of genotypic variance to the total phenotypic variance. The broad-sense heritability (h^2) was estimated by the formula suggested by Johnson *et al.* (1955).

$$h^2\,(\%) = \frac{\sigma^2 g}{\sigma^2 t} \times 100 \quad \text{.....Eq.5}$$

where, $\sigma^2 g$ = Induced genotypic variance

$\sigma^2 t$ = Total phenotypic variance ($\sigma^2 t = \sigma^2 g + \sigma^2 e$) calculated from the treated population

Genetic advance (GA)

The estimates of genetic advance (GA) with 1% selection intensity were based on the formula given below:

$$GA = k.\ \sigma p.\ h^2 \quad \text{.....Eq.6}$$

Where, h^2 = Broad sense heritability

σp = Phenotypic standard deviation of the mean

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$$K = \frac{\text{performance of treated population}}{\text{2.64, constant for 1\% selection intensity}}$$

$$GA\ (\%\ of\ \bar{X}) = \frac{GA}{\bar{X}} \times 100 \ldots\ldots\ldots\ldots Eq.7$$

The data was compiled so that each treatment occupies a column and their replicates were arranged in rows. If the difference between any two treatment means exceeds the CD value obtained at 5% level, the difference between the two means is treated to be significant.

RESULTS AND DISCUSSION

Induced variability for days to flowering in M_2 and M_3 generations

Mutagenic treatments induced a clear negative shift in mean days to flowering across both generations, except at the lowest concentrations of HZ and SA in M_2 generation (Table 1). On the other hand, compared to the control, all treatments in M_3 consistently showed earlier flowering. The greatest reduction was observed with 0.3% EMS, especially in M_3 (–5.20 days), indicating that EMS is more effective at causing earliness. Particularly M_2 treated populations showed better heritability, genetic advance, and phenotypic and genotypic coefficients of variation. 0.3% EMS in M_2 was linked to the highest PCV, GCV, heritability, and genetic progress, suggesting more exploitable genetic variability at this stage. A partial stability of generated variation is suggested by M_3's relatively lower genetic parameters. Mutagenesis successfully steered variability toward early flowering, allowing the selection of attractive mutants, as evidenced by the reported reduction in flowering time and increased diversity. Similar genetic control of early flowering through recessive gene action has been reported earlier in legumes (Gumber & Sarvjeet, 1996).

Table 1: Estimates of mean values ($\bar{X}$), shift in $\bar{X}$ and genetic parameters for days to flowering in M_2 and M_3 generations of lentil*

Treatment	Mean±S.E.	Shift in $\bar{X}$	PCV (%)	GCV (%)	h^2(%)	GA (% of $\bar{X}$)
			M_2 generation			
Control	81.12±0.41	-	5.25	4.79	20.87	5.56
0.1% EMS	80.76±0.75	-0.36	10.73	8.93	47.13	10.19
0.2% EMS	78.91±0.79	-2.21	12.68	9.13	59.35	11.27
0.3% EMS	77.98±0.71	-3.14	14.13	10.65	60.64	11.69
0.4% EMS	79.50±0.69	-1.62	10.15	8.79	46.85	9.77
CD (p=0.05)		0.83				
0.01% HZ	81.39±0.78	+0.27	9.66	5.15	44.12	9.15
0.02% HZ	79.20±0.64	-1.92	11.33	6.22	56.65	9.86
0.03% HZ	78.71±0.66	-2.41	12.89	7.83	57.78	10.32
0.04% HZ	79.78±0.78	-1.34	9.93	5.93	43.63	8.92
CD (p=0.05)		1.30				
0.01% SA	81.74±0.73	+0.62	9.16	5.02	43.52	8.33
0.02% SA	79.55±0.88	-1.57	10.92	6.05	52.26	9.21
0.03% SA	79.07±0.81	-2.05	11.83	6.72	54.80	9.56
0.04% SA	80.01±0.76	-1.11	8.99	5.37	42.71	7.99
CD (p=0.05)		0.98				
			M_3 generation			
Control	81.36±0.21	-	5.08	4.10	17.35	5.03
0.1% EMS	80.11±0.83	-1.25	7.63	5.91	32.02	7.35
0.2% EMS	77.26±0.88	-4.10	9.70	6.10	35.13	8.11

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Treatment	Mean±S.E.	Shift in $\bar{X}$	PCV (%)	GCV (%)	h^2(%)	GA (% of $\bar{X}$)
0.3% EMS	76.16±0.91	-5.20	11.03	7.53	37.17	8.37
0.4% EMS	79.29±0.85	-2.07	7.12	5.71	31.09	7.13
CD (p=0.05)		0.79				
0.01% HZ	80.54±0.78	-0.82	6.53	5.11	30.27	6.65
0.02% HZ	78.11±0.76	-3.25	8.37	5.96	31.16	7.56
0.03% HZ	77.19±0.85	-4.17	9.66	6.13	32.73	7.73
0.04% HZ	79.64±0.67	-1.72	6.73	5.56	28.26	6.79
CD (p=0.05)		0.76				
0.01% SA	80.94±0.62	-0.42	6.14	4.78	27.34	6.02
0.02% SA	78.25±0.57	-3.11	7.79	5.17	29.42	6.92
0.03% SA	77.58±0.52	-3.78	8.54	5.74	31.56	7.05
0.04% SA	80.08±0.48	-1.28	5.93	5.02	25.71	5.93
CD (p=0.05)		0.41				

**SE= Standard error; PCV=Phenotypic coefficient of variation; GCV=Genotypic coefficient of variation; h^2 =Heritability; GA=Genetic advance; CD=Critical difference*

Induced variability for days to maturity in M_2 and M_3 generations

Days to maturity showed a pronounced reduction under most mutagenic treatments in both generations, with EMS treatments being particularly effective (Table 2). With decreases of 3.87 days in M_2 and 5.60 days in M_3, the largest decrease was observed with 0.3% EMS. Induced genetic variability was higher and more selectable in the earlier generation, as evidenced by the fact that enhanced PCV, GCV, heritability, and genetic advance were more prominent in M_2 than in M_3 generation. The gradual fixing of features by selection is reflected in the fall in genetic parameters in M_3. Since early-maturing mutants are useful for surviving terminal heat and drought stress and are ideal for intercropping systems, inducing early maturity by mutagenesis has long been a significant breeding goal (Micke, 1979). According to Bolbhat *et al.* (2012), mutagens can effectively promote early maturity by causing physiological, biochemical, and hormonal changes that affect crop phenology.

Table 2: Estimates of mean values ($\bar{X}$), shift in $\bar{X}$ and genetic parameters for days to maturity in M_2 and M_3 generations of lentil

Treatment	Mean±S.E.	Shift in $\bar{X}$	PCV (%)	GCV (%)	h^2(%)	GA (% of $\bar{X}$)
			M_2 generation			
Control	137.35±0.33	-	5.33	4.20	16.73	5.13
0.1% EMS	137.47±0.93	+0.12	7.94	6.13	41.17	8.03
0.2% EMS	134.70±0.79	-2.65	8.53	6.35	43.74	8.65
0.3% EMS	133.48±0.99	-3.87	8.74	6.73	45.15	8.91
0.4% EMS	135.62±0.98	-1.73	7.34	5.84	38.67	7.54
CD (p=0.05)		1.13				
0.01% HZ	137.57±0.91	+0.22	7.28	5.83	39.30	7.78
0.02% HZ	135.30±0.94	-2.05	7.69	6.18	41.13	8.12
0.03% HZ	134.44±0.99	-2.91	8.10	6.43	43.38	8.28
0.04% HZ	135.85±0.95	-1.50	7.16	5.54	36.25	7.02
CD (p=0.05)		1.44				
0.01% SA	137.08±0.92	-0.27	6.64	5.68	37.68	7.31
0.02% SA	135.48±0.98	-1.87	7.14	6.03	38.59	7.83
0.03% SA	134.88±0.96	-2.47	7.57	6.25	41.13	6.92
0.04% SA	137.58±0.91	+0.23	6.30	5.13	34.75	6.68
CD (p=0.05)		0.94				
			M_3 generation			

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Treatment	Mean±S.E.	Shift in $\bar{X}$	PCV (%)	GCV (%)	h^2(%)	GA (% of $\bar{X}$)
Control	137.51±0.38	-	5.16	4.08	15.43	4.74
0.1% EMS	136.11±0.93	-1.40	7.62	5.88	32.13	7.93
0.2% EMS	132.99±0.87	-4.52	8.28	6.17	34.54	8.13
0.3% EMS	131.91±0.98	-5.60	8.36	6.38	36.11	8.68
0.4% EMS	134.81±0.96	-2.70	7.13	5.51	29.61	7.21
CD (p=0.05)		1.24				
0.01% HZ	136.29±0.90	-1.22	7.15	5.57	30.33	7.61
0.02% HZ	133.50±0.84	-4.01	7.31	5.98	31.88	7.91
0.03% HZ	132.89±0.86	-4.62	7.93	6.03	34.13	8.06
0.04% HZ	135.09±0.99	-2.42	6.89	5.13	27.15	6.96
CD (p=0.05)		1.09				
0.01% SA	136.49±0.88	-1.02	6.28	5.37	27.68	7.16
0.02% SA	134.58±0.85	-2.93	6.91	5.73	29.28	7.51
0.03% SA	133.46±0.97	-4.05	7.26	5.99	32.15	6.73
0.04% SA	135.61±0.99	-1.90	6.10	4.94	25.13	6.51
CD (p=0.05)		0.87				

Induced variability for plant height in M_2 and M_3 generations

A general reduction in plant height was observed across most treatments, with the exception of higher EMS and lower HZ concentrations in M_2. On the other hand, every treatment in M_3 generation showed a steady decline (Table 3). The most successful mutagen for causing dwarfism was sodium azide, with 0.03% SA resulting in the greatest decrease in plant height in M_3 (–5.12 cm). PCV, GCV, heritability, and genetic advance were among the genetic parameters that were consistently greater in M_2 generation. Heritability and genetic advance were particularly high under 0.03% SA. Because SA is a respiratory inhibitor that interferes with cellular energy metabolism and mitotic activity, it may have a larger dwarfing effect. Shortened internodal length, a feature commonly observed in induced dwarf mutants of grasses and grain legumes, was the main cause of reduced plant height (Talukdar & Biswas, 2006; Lather, 2000; Gaur *et al.*, 2008). Such reductions in stature are agronomically desirable due to improved lodging resistance.

Table 3: Estimates of mean values ($\bar{X}$), shift in $\bar{X}$ and genetic parameters for plant height (cm) in M_2 and M_3 generations of lentil

Treatment	Mean±S.E.	Shift in $\bar{X}$	PCV (%)	GCV (%)	h^2(%)	GA (% of $\bar{X}$)
			M_2 generation			
Control	42.22±0.37	-	3.25	2.12	23.24	4.11
0.1% EMS	42.01±0.61	-0.21	7.33	5.43	66.78	18.55
0.2% EMS	40.33±0.72	-1.89	8.11	5.97	71.20	19.43
0.3% EMS	40.43±0.50	-1.79	9.05	6.11	72.93	20.45
0.4% EMS	43.24±0.43	+1.02	7.43	4.22	74.24	15.03
CD (p=0.05)		0.77				
0.01% HZ	42.88±0.52	+0.66	8.22	5.66	77.71	21.17
0.02% HZ	39.67±0.47	-2.55	9.26	6.48	78.22	22.92
0.03% HZ	39.92±0.69	-2.30	9.67	6.98	80.12	25.34
0.04% HZ	41.10±0.44	-1.12	8.77	5.71	70.68	18.61
CD (p=0.05)		0.91				
0.01% SA	39.91±0.51	-2.31	9.26	6.11	80.12	25.12

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Treatment	Mean±S.E.	Shift in $\bar{X}$	PCV (%)	GCV (%)	h^2(%)	GA (% of $\bar{X}$)
0.02% SA	38.55±0.48	-3.67	10.13	7.66	81.30	27.10
0.03% SA	39.34±0.54	-2.88	11.59	8.14	87.55	28.53
0.04% SA	41.85±0.60	-0.37	9.26	6.73	73.07	21.21
CD (p=0.05)		0.70				
			M_3 generation			
Control	43.22±0.51	-	2.95	2.07	18.16	3.15
0.1% EMS	40.86±0.46	-2.36	6.37	4.11	44.73	11.01
0.2% EMS	39.32±0.55	-3.90	6.96	4.69	48.15	12.05
0.3% EMS	39.50±0.49	-3.72	7.91	4.51	50.03	12.86
0.4% EMS	41.35±0.50	-1.87	5.94	3.29	40.11	10.29
CD (p=0.05)		0.86				
0.01% HZ	40.70±0.65	-2.52	6.85	4.60	50.16	12.12
0.02% HZ	38.73±0.63	-4.49	7.02	5.03	51.33	12.25
0.03% HZ	38.51±0.44	-4.71	8.05	5.31	52.01	13.30
0.04% HZ	41.01±0.41	-2.21	6.70	3.96	45.21	11.25
CD (p=0.05)		0.80				
0.01% SA	38.99±0.72	-4.23	7.35	5.19	55.17	13.22
0.02% SA	38.37±0.53	-4.85	7.67	6.12	57.41	15.05
0.03% SA	38.10±0.57	-5.12	8.63	6.63	59.12	16.53
0.04% SA	39.57±0.68	-3.65	7.44	4.15	48.27	14.25
CD (p=0.05)		0.66				

Characterization of promising early-maturing and dwarf lentil mutants

A distinct and promising mutant was isolated from 0.03% HZ treatment, which exhibited significant reductions in days to maturity and plant height as compared to the control. Since dwarfing genes have traditionally been essential for enhancing lodging resistance, yield stability, fertility, and adaptability particularly during the Green Revolution, the co-expression of these traits is especially beneficial. Induced dwarf and early-maturing mutants have been shown to improve agronomic performance and cropping efficiency in rice, grass pea, cotton, and wheat (Talukdar, 2009; Andrew-Peter-Leon *et al.*, 2021; Wang *et al.*, 2024; Jin *et al.*, 2013). The identified mutant is an excellent option for breeding efforts aimed at creating lentil cultivar appropriate for a variety of agroclimatic conditions and intensive cropping systems due to its lower plant height, compact growth habit, and early maturity.

CONCLUSION

The current study showed that EMS (0.1–0.4%), HZ (0.01–0.04%), and SA (0.01–0.04%) chemical mutagenesis successfully increased the genetic variability for days to flowering, days to maturity, and plant height in lentils. Among these, intermediate HZ doses (especially 0.03%) induced the promising combination phenotypes, while 0.3% EMS was more successful in triggering earliness and 0.03% SA was better at lowering the plant height. There was more room for successful selection in earlier generations, as seen by the M_2 generation's continuously higher phenotypic and genotypic variability, heritability, and genetic advance than the M_3 generation. Therefore, early maturity and reduced plant stature are best achieved through selection in M_2 generation. However, subsequent generations are anticipated to consolidate these favourable features for use in lentil improvement initiatives.

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Authors' Contribution

Rafiul Amin Laskar conceptualized the study, designed the experiments, conducted field and laboratory work, analysed the data, and prepared the original manuscript. Mohammad Rafiq Wani provided technical guidance on mutagenesis, contributed to data interpretation, and critically reviewed and refined the manuscript. Roshan Jahan assisted in field experimentation, data collection, and preliminary analysis. Samiullah Khan contributed to the supervision of overall experimentation. All authors read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Ethics Approval Statement

This study did not involve human participants or animals.

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