

Research Article

Mutagenic effects of methyl methanesulphonate on the growth and yield characteristics in Lentil (*Lens culinaris* Medik.) var. DPL-15.

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Abstract: Induced variability in growth and yield characteristics in lentil (*Lens culinaris* Medik.) var. DPL-15 were studied by using chemical mutagen methyl methanesulphonate (MMS). Healthy seeds of lentil were treated with five different concentrations as 0.1%, 0.2%, 0.3%, 0.4% and 0.5% of mutagen. The seeds were sown in pots to raise the M₁ generation. Later on the M₁ generation seeds were collected and sown in next season to raise the M₂ generation. Different parameters related to growth like seed germination, plant survival and percentage reduction in pollen fertility were studied in M₁ generation. Quantitative characters were studied in M₂ generation like branches per plant, fertile branches per plant, pods per plant and 100 seeds weight. Dose dependent decrease in seed germination and plant survival were observed in M₁ generation while as in M₂ generation dose dependent decrease in different parameters like branches per plant, fertile branches per plant, pods per plant were observed but there was increase in 100 seeds weight at lower concentrations of MMS. The shift in mean values in positive direction for 100 seeds weight indicate that selection for this character would be effective in subsequent generations. The weight of 100 seeds is a dependable index of yielding ability of pulses. Although the mean 100 seeds weight showed a slight increase, the genetic variability induced for this character can play an important role in overcoming the yield barriers.

Keywords: *Lens culinaris* Medik. var. DPL-15, MMS, Induced variability, Methyl methanesulphonate, Quantitative characters, 100 seeds weight.

INTRODUCTION

India is the largest producer as well as consumer of pulses. The major pulses grown in India are lentil, pigeon pea, chickpea, mungbean and urdbean. The yield of lentil is low compared to other Pulses. Moreover lentil has relatively higher contents of protein, carbohydrates and calories compared to other legumes [1]. As per the reports of various International Organisations like FAO[2], around one in eight people in the world are likely to have suffered from chronic hunger, not having enough food for an active and healthy life. The rates of food insecurity and malnutrition poses a great threat to human civilization. Therefore, genetic variability should be induced to develop new pulse varieties with better quality of proteins. Mutation Breeding is an important tool to cope these problems. Mutagenesis has proved an important tool in enhancing the natural mutation rate hence increasing the genetic variability and increasing the chances for obtaining desired traits. The induction of mutations in polygenic system, controlling the quantitative characters is important for crop improvement. It has proven an important tool in bringing genetic variability in self-pollinated crops as already reported by several authors earlier[3-4]. Plant

used in the present study is an important pulse crop namely lentil (*Lens culinaris* Medik.) var. DPL-15, a self-pollinated crop. The seeds of lentil are important source of protein with approximately 25% protein content. Apart from higher level of proteins, lentils also contain dietary fiber, folate, Vitamin B₁ and minerals[5]. The mutagenic treatments induced mutations affecting plant height, branching and leaf morphology have been reported in lentil[6].

The main objective of the present study was to enhance the variability in lentil to increase the yield. The increase in yield can overcome the food insecurity problem. Moreover, the higher content of proteins in lentil makes it a suitable experimental plant.

MATERIALS AND METHODS

Healthy seeds of *Lens culinaris* Medik. var. DPL-15 were pre-soaked in double distilled water for 12 hours and then treated with different concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 percent) of MMS for 5 hours. The solution of MMS was prepared in phosphate buffer of pH 7. Another set of 100 seeds were soaked in distilled water to act as a control. The treated seeds were thoroughly washed in running tap water to remove

the residual effect of mutagen. The seeds were sown in 10"×15" pots to raise the M₁ generation in the crop season 2010-2011. Different biological parameters related to growth like seed germination, inhibition, plant survival and reduction in pollen fertility were studied in M₁ generation. Seeds of M₁ plants were collected separately and were sown to raise M₂ generation in 2011-2012. The M₂ generation was screened for quantitative characters like plant height, number of branches per plant, number of fertile branches per plant, number of pods per plant and 100 seeds weight. Different formulas were used to calculate the different parameters as:

Seed germination

After recording germination counts, the percentage of seed germination was calculated on the basis of total number of seeds sown in the pots.

$$\text{Germination(\%)} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds sown}} \times 100$$

Inhibition

The following formula was used to calculate the percentage of inhibition, injury or reduction.

$$\begin{aligned} &\text{Percentage inhibition} \\ &\text{or} \\ &\text{Percentage injury} \\ &\text{or} \\ &\text{Percentage reduction} \\ &= \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100 \end{aligned}$$

Statistical analysis

Data collected for quantitative traits in M₂ generation were subjected to statistical analysis in order to assess the extent of induced variation, as indicated below:

Mean (\bar{X})

The mean was computed by taking the sum of a number of values (X₁, X₂, X_n) and dividing by the total number of values (N) involved, thus;

$$\bar{X} = \frac{X_1 + X_2 + \dots + X_n}{N}$$

Where, X₁, X₂,X_n = Observations

N = Total number of observations involved

Standard deviation (S.D.)

The standard deviation was calculated by the following formula for each parameter of study.

$$\text{S.D.} = \sqrt{\frac{(\bar{X} - X_1)^2 + (\bar{X} - X_2)^2 + \dots + (\bar{X} - X_n)^2}{N}}$$

Where, (\bar{X}) = Mean of the observations involved
X₁, X₂,X_n = observation

N = Total number of observations

Standard error (S.E.)

$$\text{S.E.} = \frac{\text{S.D. of sample}}{\sqrt{N}}$$

Where, S.D. = Standard deviation
N = Number of observations

RESULTS

Biological study

Data recorded in M₁ generation on seed germination, plant survival at maturity and pollen fertility in control and mutagen treated population are presented in Table-1.

The control showed 96.66% seed germination, whereas the germination was decreased with increasing concentrations of MMS and the range was 78.33-95%. Higher concentrations of mutagen produced more inhibition in seed germination. In control population, plant survival was 95% at maturity. In MMS treated plants, survival was low and it ranged from 70.18-94.80%. Though about 2% pollen sterility was recorded in control plants but it increased with increase in the concentrations of mutagen. The maximum pollen sterility was 23.21% at 0.5% MMS treatment.

MORPHOLOGICAL MUTANTS

Data on morphological mutants namely dwarf, tall and bushy are given in Table-3. In general, mutation frequency increased with increase in concentration of mutagen. Dwarf and bushy mutants were not recorded at lower concentration of mutagen whereas at higher concentration of mutagen tall and bushy mutants were not observed. Moreover, different variations in leaflet size were observed in treated population also ranging from broad to small size leaflets.

QUANTITATIVE CHARACTERS

Data recorded on different quantitative characters namely, number of branches per plant, number of fertile branches per plant, number of pods per plant and 100 seeds weight are given in Table-2. Mean values of the quantitative characters, except 100 seeds weight shifted in negative direction. Weight of 100 seeds in control population was 3.2g where as in 0.1-0.3% mutagen treated population it ranged from 7.1-3.1g. The weight of 100 seeds decreased considerably at the highest concentrations (0.4 and 0.5% MMS). The shift in mean values in positive direction for 100 seed weight indicate that selection for this character would be effective in subsequent generations. The weight of 100 seeds is a dependable index of yielding ability of pulses. Although the mean 100 seeds weight showed a slight increase, the genetic variability induced for this character can play an important role in overcoming the yield barriers.

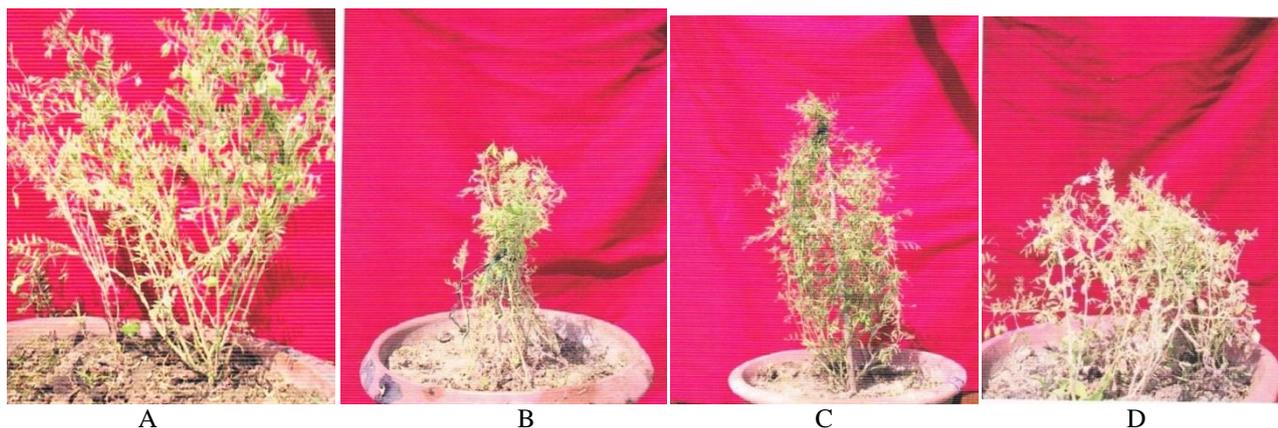


Plate-I

A. Control mutant , B. Dwarf mutant, C. Tall mutant, D. Bushy mutant

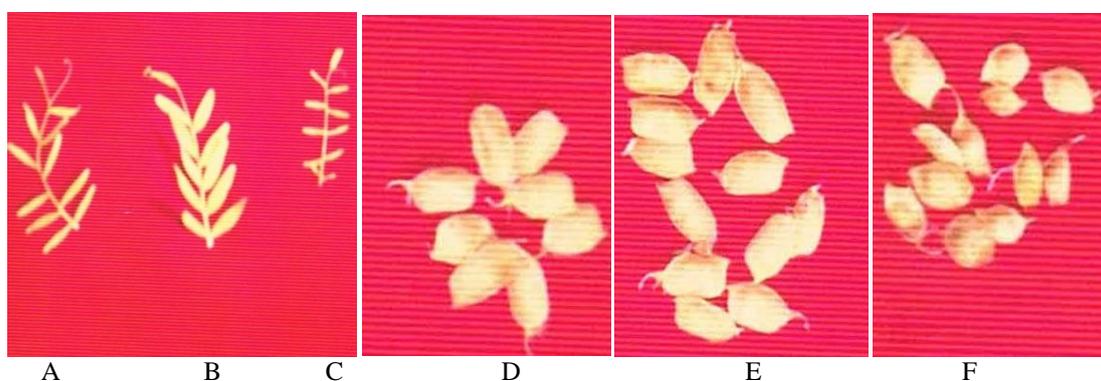


Plate-II

A. Leaflet of control plant, B. Broad leaflet of variant, C. Small leaflet of variant, D. Pods of control plant, E. Large pods of variant, F. Small pods of variant

Table 1: Effect of different concentrations of MMS on seed germination, plant survival at maturity and pollen sterility of *Lens culinaris* Medik. var. DPL-15 in M₁ generation.

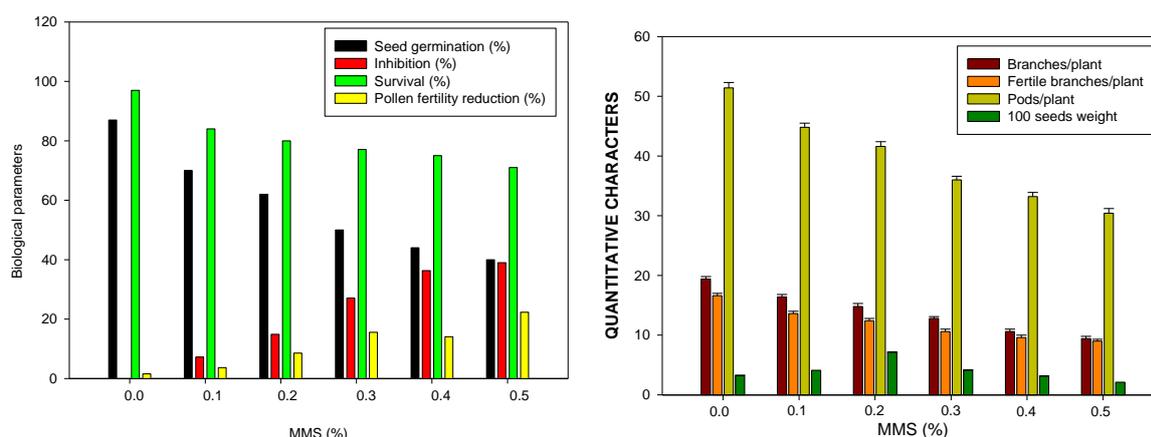
Treatment	Germination(%)	Plant survival(%)	Percentage reduction in Pollen fertility
Control	96.66	95	1.22
0.1% MMS	95	94.80	4.96
0.2% MMS	91.66	89.48	7.24
0.3% MMS	86.66	84.22	14.33
0.4% MMS	80	73.69	19
0.5% MMS	78.33	70.18	23.21

Table 2: Effect of different concentrations of MMS on the quantitative characters of *Lens culinaris* Medik. var. DPL-15 in M₂ generation.

Treatment	Branches/plant $\bar{X} \pm S.E.$	Fertile branches/plant $\bar{X} \pm S.E.$	Pods/plant $\bar{X} \pm S.E.$	100 Seeds weight(g) $\bar{X} \pm S.E.$
Control	19.4±0.4	16.6±0.4	51.4±0.9	3.2±0.1
0.1% MMS	16.4±0.4	13.6±0.4	44.8±0.7	4.1±0.0
0.2% MMS	14.8±0.5	12.4±0.4	41.6±0.8	7.10±0.1
0.3% MMS	12.8±0.3	10.6±0.4	36±0.6	4.11±0.1
0.4% MMS	10.6±0.4	9.6±0.4	33.2±0.7	3.1±0.1
0.5% MMS	9.4±0.4	9.0±0.3	30.4±0.8	2.1±0.0

Table 3: Table showing frequency of morphological mutants of *Lens culinaris* Medik. var DPL-15 in different concentrations of MMS in M₂ generation.

Treatment	No. of M ₂ plants	Mutant/Frequency (%)		
		Dwarf	Tall	Bushy
Control	50	-	-	-
0.1% MMS	50	-	(1)2	-
0.2% MMS	50	(1)2	(2)4	(2)4
0.3% MMS	50	(2)4	(4)8	(2)4
0.4% MMS	50	(3)6	-	(4)8
0.5% MMS	50	(4)8	-	-

**Fig-1: Biological parameters and quantitative characters**

DISCUSSION

Mutagenesis induction is an important tool to bring variability in plants in short period of time. Reduction in seed germination in mutagen treated population has been explained earlier by workers [7] due to the delay or inhibition in physiological and biological processes necessary for seed germination. High frequency of pollen sterility was observed at higher concentrations of mutagen. This may be the result of meiotic abnormalities caused by the mutagen. The percentage of seed survival was decreased with the increase in concentration of mutagen. According to Sato and Gaul [8], the reduction in seedling survival occurs due to cytogenetic damage and physiological disturbance. In the present experiment seed germination, plant survival at maturity and pollen fertility decreased with increase in concentrations of mutagen. These are in conformity of findings of Jayabalan and Rao [9] in *Lycopersicon esculentum*, Sharma and Sharma in *Lens culinaris* [10].

Data on morphological mutants namely dwarf, tall and bushy are given in Table-3. In general, mutation frequency increased with increase in concentration of mutagen. Morphological mutants, as recorded in the present study, might be the result of pleiotropic effects of mutated genes or chromosomal aberrations.

Different variations in leaflet size were also observed in treated population. These variations ranged from broad size to small size leaflets. Different morphological mutants have also been observed by Shah *et al* in Chickpea also [11].

Different quantitative characters are shown in Table 2, the mutation frequency increased linearly with increase in concentrations of mutagen. Higher concentrations of mutagens produced large number of mutants. This might be the result of pleiotropic effects of mutated genes or chromosomal aberrations or gene mutations. As is shown in the Table-2, 100 seed weight increased up to the 0.2% MMS concentration. The shift in mean values in positive direction indicates that more positive mutations have occurred for these traits up to the concentration 0.2%. Similar correlation was reported earlier by Khan and Siddiqui [12] in mungbean and Kharkwal [13] in Chickpea. These results are also in conformity with the earlier findings of Scossiroli [14] and Wani *et al.*, [15]. However, no significant increase in seed weight was reported by Portdukhe *et al* [16] in durum wheat after gamma rays treatment. The improvement of quantitative characters might be result of pleiotropic effects of mutated genes or chromosomal aberrations. In recent years, the role of mutation breeding in increasing the variability in quantitative characters has been proved beyond doubt [17-18].

CONCLUSION

As is clear from the experiment that with increasing concentrations MMS, mutation frequency increased resulting in negative shifting of characters from mean values. 100 seeds weight at lower concentrations of MMS showed positive results. This may overcome the yield barriers in future. Moreover, in future we can lower the concentrations of MMS from 0.1% to 0.01% likewise to get more positive results. This may overcome the food insecurity problem in future.

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