Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2014; 2(12B): 943-947 **ISSN 2347-9515 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Research Article

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

Shahnawaz Khursheed[1] and Samiullah khan[2]
Mutation Breeding Lab, Aligarh Muslim University, Aligarh-202 002, India

***Corresponding author**
Shahnawaz Khursheed
Email: shahnawazkhursheed95@gmail.com

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 M_1 generation. Later on the M_1 generation seeds were collected and sown in next season to raise the M_2 generation. Different parameters related to growth like seed germination, plant survival and percentage reduction in pollen fertility were studied in M_1 generation. Quantitative characters were studied in M_2 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 M_1 generation while as in M_2 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 $B_1$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 M_1 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 M_1 generation. Seeds of M_1 plants were collected separately and were sown to raise M_2 generation in 2011-2012. The M_2 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.

Percentage inhibition
or
Percentage injury
or
Percentage reduction

$$= \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$$

Statistical analysis

Data collected for quantitative traits in M_2 generation were subjected to statistical analysis in order to assess the extent of induced variation, as indicated below:

Mean ($\overline{X}$)

The mean was computed by taking the sum of a number of values (X_1, X_2, …….. X_n) and dividing by the total number of values (N) involved, thus;

$$\overline{X} = \frac{X_1 + X_2 + \ldots\ldots\ldots X_n}{N}$$

Where, $X_1, X_2, \ldots\ldots X_n$ = 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.

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

Where, ($\overline{X}$) = Mean of the observations involved
$X_1, X_2 \ldots\ldots\ldots\ldots\ldots Xn$ = observation
N = Total number of observations

Standard error (S.E.)

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

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

RESULTS

Biological study

Data recorded in M_1 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.

A B C D

Plate-I

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

A B C D E F

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 M_1 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 M_2 generation.

Treatment	Branches⁄plant $\bar{X}$±S.E.	Fertile branches⁄plant $\bar{X}$±S.E.	Pods⁄plant $\bar{X}$±S.E.	100 Seeds weight(g) $\bar{X}$±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 M_2 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	-	-

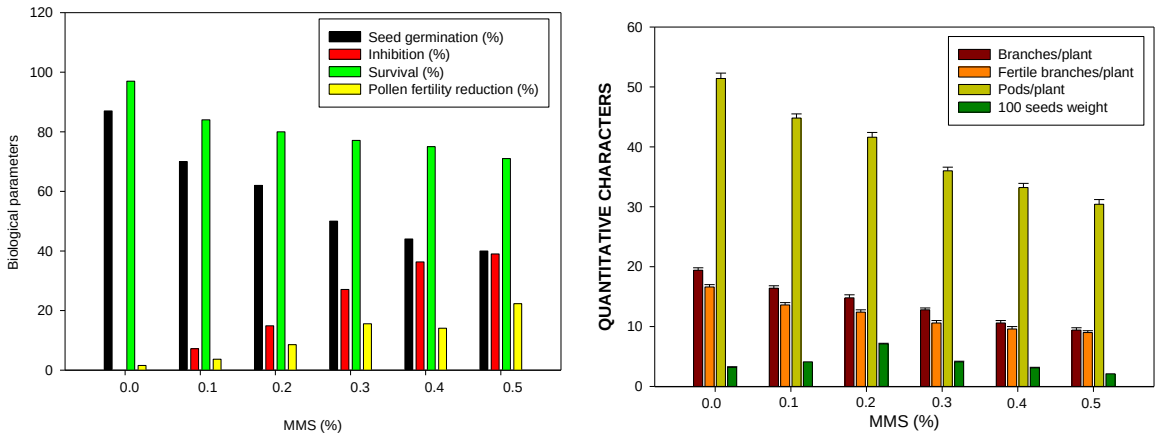

Fig-1: Bilogical parameters and quantitative characters

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 pliotropic 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 pliotropic 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 pliotropic 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.

ACKNOWLEDGEMENT

The authors are thankful to the Chairman, Department of Botany, Aligarh Muslim University, Aligarh, India for providing necessary infrastructural support for carrying out research experiment.

REFERENCES

1. Muehlbauer FJ, Cubero JI, Summerfield RJ; Lentil (Lens culinaris Medik.). p. 266-311. In: R.J. Summerfield and E.H. Roberts (eds.), Grain Legume Crops. Collins, 8 Grafton Street, London, UK. 1985.
2. FAO; The State of Food Insecurity in the World 2013. Climate change excerpts. 2013.
3. Selvaraj R, Jayakumar S; Effect of gamma rays and ethylmethane sulphonate on qualitative and quantitative traits in sunflower (Helianthus annus L.) Cv. Morden. Madras Agric J., 2004; 91:206-210.
4. Singh SP, Singh RP, Prasad JP, Agrawal RK, Shahi JP; Induced genetic variability for protein content, yield and yield components in microsperma lentil (Lens culinaris Medik.). Madras. Agric. J., 2006; 94:7-13.
5. United States Department of Agriculture, USDA Nutrient database, 2011.
6. Bhat TA, Khan AH, Parveen S, Wani NA; MMS induced closed flower mutant in Broad bean (Vicia faba L.) Indian J.Genet, 2006; 66:249-250.
7. Khan S, Rehman MU, Siddique BA, Azad SA; Mutagen induced biological damage and chlorophyll mutations in Vigna radiata L. Wilczek. J. Indian Bot. Soc., 1998; 77:143
8. Sato M, Gaul H; Effects of ethylmethane sulphonate on the fertility of barley. Rad. Bot. 7, 7-15.
9. Jayabalan N, Rao GR; Gamma radiation induced cytological abnormalities in Lycopersicon esculentum Mill var. Pusa Ruby. Cytologia, 1987; 52:1-4.
10. Sharma SK, Sharma B; Induced chlorophyll mutations in Lentil. Indian Journal of Genetics and Plant Breeding, 1981; 41:328-333.
11. Shah TM, Mirza JI, Haq MA, Atta BM; Induced genetic variability in chickpea (Cicer arietinum L.). Frequency and spectrum of chlorophyll mutations. Pak. J. Bot., 2006; 38(4): 1217-1226.
12. Khan S, Siddiqui BA; Genetic variability and correlation studies in yield and yield components in mungbean (Vigna radiata (L.) Wilczek). J. Indian Bot. Soc, 1997; 71:69-71.
13. Kharkwal MC; Induced mutations in chickpea (Cicer arietinum L.). VI. Significance of induced altered correlations. Indian J. Genet, 2003; 63:219-224.
14. Scossiroli RE; 2nd Int. Wheat Genetics Symposium, 1964;85-101.
15. Wani MR, Lone MA, Sheikh SA, Dar MS, Tak MA, Ahmad P, Khan S; Induction and assessment of genetic variability for yield and yield contributing traits of Chickpea [Cicer arietinum L.]. Journal of plant genomics, 2012; 2:28-33.
16. Potdukhe NR, Wanjari SS, Raut SK; Gamma rays induced variability in durum wheat. Agric. Sci. Digest, 1994;14:121-125.
17. Ignacimuthu S, Babu CR; Induced variation in protein quantity in the wild and cultivated Urdbeans. India J. Genet., 1989; 49:173-181.
18. Tickoo JL, Chandra N; Mutagen induced variability in mungbean (Vigna radiata wilczek L.). The Indian Journal of Genetics and Plant Breeding, 1999; 59:193-201.