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GAMMA-RAYS AND ETHYL METHANE SULPHONATE INDUCED MUTATION IN *MICROSPERMA* LENTIL (*LENS CULINARIS* L. MEDIKUS)

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ABSTRACT

Mutagenic sensitivity was studies in the lentil variety HUL-57 (Malavia Vishwanath) by treating the seeds with 10kR, 20kR and 30kR of gamma-rays and chemical 0.3% concentration of ethyl methane sulphonate (EMS) mutagens singly and in combinations of both. Mutagenic treatment showed highly significance differences for all the morphological and yield traits in both of the generations (M_1 and M_2). The highest reduction in germination (42 %), plant survival (23.85 %), root length (76.4 %) and shoot length (90.22 %) were observed at combine treatment of gamma rays (30 kR) and EMS (0.3%). Root length showed more sensitivity as compared to shoot length. The frequency of chlorophyll mutants was quite narrow as only three types of mutant, *viz*., *albino*, *xantha* and *viridis* were observed. The induction of *Albino* (2) mutants was obtained only at 0.3 % EMS, whereas *xantha* and *viridis* types of mutants were recorded at all the doses, maximum being at 0.3 % of EMS mutagens. For induction of chlorophyll mutation 0.3 % EMS alone mutagens proved to be the best option. Thus, it may be concluded that the mild doses of mutagens may be more useful to induce desired type of chlorophyll mutants.

INTRODUCTION

Lentil is a self pollinated diploid (2n = 14) annual winter crop, belonging to the family leguminosae (Singh, 2012). Lentil is one of the most valuable and oldest crops grown an area of 1.48 million hectares and production 1.03 million tonnes with an average yield of 697 kg/ha in India (Anonymous, 2012). It has relatively higher in protein content (ranging from 22-34.6 per cent), carbohydrate and calories than other legumes but also a rich source of minerals, vitamins (thiamine, riboflavin, niacin etc.), crude fibers and excellent quality forage for animals. It also contains some anti-nutritional factors, such as, trypsin inhibitors, hemagglutinins and oligosaccharides that cause flatulence (Kay, 1979; Adsule *et al.*, 1989; Singh, 2012).

Being an important pulse crop, its production and productivity is very low as compare to other countries. It may be due to its cultivation under rainfed/ dryland conditions on residual moisture in marginal environment (Tyagi and Khan, 2011). Heat stress at flowering and maturity in lentil has been recognized as one of the key factors affecting the yield of the crop. None of the genotypes of lentil evolved till date has resistance/ tolerance against heat stress. Thus, there is need to induce desirable variability in lentil to isolate a mutant(s) which may show some degree of tolerance/ resistance against heat stress.

In several breeding tools, mutation breeding has been proved to be one of the potent tools to increase the genetic variability and yield potentiality of lentil crop (Singh *et al.*, 2000, Shah *et al.*, 2011). Whereas, the induced mutants through physical (Gama-rays) and chemical (EMS) mutagens can increase yield as well as improve several other quantitative and qualitative traits in lentil, as reported by several workers Cheema (2006), Gaikwad and Kothekar (2004), Sinha and Lal (2007) and Singh *et al.*, 2007. The choice of mutagen, their dose and procedure for mutation breeding in lentil is an important step for creating new genetic variability. In this experiment, an attempt was made by using physical and chemical mutagen to inducing variability in lentil cultivar HUL 57. These mutant lines may be use for creating new genetic variability and will be utilized in future breeding programme to improve the yield and quality traits in lentil.

MATERIALS AND METHODS

Two year field and laboratory experiment were carried out to study the mutagen in 2010-11 and 2011-12 with the help of 2560 healthy and dry seeds of lentil variety HUL-57 (Malvia vishwanath) at Dryland Agricultural Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (UP). Three doses of physical mutagen - gamma-rays (Co^{60} source), namely, 10 kR, 20 kR and 30 kR and a chemical mutagen *i.e.*, ethyl methane sulphonate (EMS) with its concentration of 0.3 % were used in single and in combinations with gamma rays. Three hundred twenty pure, uniform, healthy and dry (12% moisture) seeds for each treatment of *micro-sperma* lentil cultivar HUL 57 (Malvia vishwanath) was irradiated with

gamma rays at three doses *viz*, 10 kR, 20 kR and 30 kR at National Botanical Research Institute (NBRI) Lucknow, U.P. Three hundred seeds were sown in the field in *Rabi* season 2010-11 and remaining twenty seeds were kept for laboratory observations. EMS solution with 0.3 % concentration was prepared by mixing appropriate volume of ethyl methane sulphonate and phosphate buffer (pH 7.0). Eight lots of (two untreated + 2 lots irradiated with 10kR + 2 separate lots of each treated with 20 kR and 30 kR of gamma rays, respectively) three hundred twenty pure, uniform, healthy and dry seeds (12% moisture) were subjected to presoaking in distilled water for 6 hours at room temperature. The soaked seeds were properly dried with blotting paper and then transferred to EMS solution (0.3%) singly and in combination at Research laboratory of the Department of Genetics and Plant Breeding. The seeds were kept in EMS solution atleast 12 hours. During this period the seeds were given intermittent shaking throughout the period of treatment to maintain uniformity and then the mutagen solution were drained out. The treated seeds will be then washed in running tap water for 6 hours to remove residual chemical from the seeds.

However, in combination treatment of gamma rays and EMS were also done as described earlier. For this, three lots of the gamma ray treated 320 lentil seeds were soaked in distilled water for six hours and then treated with EMS solution of 0.3% concentration in each treatment, separately and kept it for 12 hours at room temperature followed by washing in running tap water for six hours. All treated and untreated (used as control) 320 seeds soaked in distilled water for six hours. Immediately after the treatment seeds were sown in the field to raise the M_1 generation during *Rabi season* on 13th November, 2010 and remaining 20 seeds were used in laboratory experiment.

The field experiment was conducted in three replications followed by Randomized Block Design with maintaining a distance of 25 cm between the rows and 5 cm between the plants. Recommended cultural practices and plant protection measures were followed during the crop period to establish the healthy crop. The M_1 plants were studied critically and carefully. Any deviations in the characters of treated plants from the control (parent) plants were screened and marked. The morphological variations were noticed and recorded. Single plant seeds were harvested separately in separate seed paper bag from each suspected mutant plants during M_1 generation and to raise M_2 generation. The data on different quantitative traits *viz*., days to 50 % flowering, plant height, number of primary branches/ plant, number of secondary branches/ plant, number of pods/ plant and grain yield/ plant from 20 randomly selected plants from each replication in M_1 and M_2 generation were recorded. However, the remaining 20 seeds of M_1 generation out of 320 seeds from each treatment of gamma rays, EMS alone/ or in combination along with respective controls of HUL 57, were grown in sterilized Petri dishes in the laboratory conditions, for recording observations on germination percentage, root and shoot length reduction on seven days and fourteen days interval after sowing in Petri-dishes. Physiological damage due to exposure of mutagenic treatments was assessed by measuring the root and shoot length reduction with respect to control after 7 days and 14 days of germination.

The harvesting of plants at maturity was done in two steps: For macro mutational study: the 50 plants from each treatment were randomly selected and one main primary branch per plant bearing secondary branch with pods was harvested separately. For micro mutational study: all the remaining plants were bulked and harvested treatment wise for micro mutational study. The same numbers of plants were also harvested from the control. For raising M_2 generation, the seeds of the separate progeny lines of the selected M_1 panicle for macro-mutational and the bulk seeds for micro-mutational studies of all the treatment of both the varieties were sown on 18th November, 2011 in field under three replications along with the control following plant-to-progeny method. There was likelihood of getting huge number of qualitative and quantitative mutants. The distance between and within the rows were kept at 25 cm and 5 cm, respectively. Timely cultural practices were performed to maintain a healthy crop. The spectrum and frequency of chlorophyll mutations were analyzed with help of chlorophyll meter (SPAD meter) on the basis of the M_1 main primary branch pods sown in M_2 generation plants as progeny to row method of cultivar HUL 57 according to Nilan (1967) The mutagenic effects were determined by comparing the mean of M_1 and M_2 generation. The standard statistical procedure was used for analysis of data by using Singh and Chaudhary (1997).

RESULTS AND DISCUSSION

The analysis of variance for the quantitative traits *viz*., days to 50 % flowering, plant height, number of primary branches/ plant, number of secondary branches/ plant, number of pods/ plant and grain yield/ plant were studied in M_1 and M_2 generation of lentil cv. HUL 57, presented in Table 1. It revealed significant differences between the treatments for all quantitative traits in both the generations, however significant differences between the replication for plant height in M_2 generations, only. It indicates all the treatments differed from each other with respect to various concentrations of mutagens. These finding were inconformity of the results of Ali and Shaikh

Table 1: ANOVA for different morphological traits in M_1 and M_2 generations in lentil after mutagenic treatments

Source of variation	Degree of freedom		Days to 50% flowering		Plant height (cm)		Primary branches/ plant (no.)		Secondary /plant (no.)		branches pods/ plant (no.)		Number of Grain yield/ plant (g)
Generation	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	
Replication	2	0.07	25.07	2.41	4.39*	0.04	0.05	0.14	0.32	33.93	21.09	0.02	0.02
Treatment	7	256.62*	0.94*	29.45*	18.00*	0.36*	0.07*	5.34*	1.82*	4360.83*	2413.92*	3.89*	0.17*
Error	14	14.72	1.38	2.22	1.03	0.02	0.02	0.45	0.32	22.10	20.60	0.12	0.02

* Significant at 5% level of probability

Table2: Effect of mutagenic treatment on germination, plant survival and reduction of root and shoot length in lentil in M_1 generation

Treatment/ Dose of mutagen	Germination In per cent	Survival In Per cent	0n 7 days of sowing		0n 14 days of sowing					
			Mean root length (cm)	Root length reduction as per cent of control	Mean shoot length (cm)	Shoot length reduction as per cent of control	Mean root length (cm)	Root length reduction as per cent of control	Mean shoot length (cm)	Shoot length reduction as per cent of control
Control	97.60	99.38	1.15	100	2.85	100	2.50	100	6.75	100
Gamma-rays										
10 kR	83.90	88.98	0.59	48.7	0.69	75.8	1.18	52.8	2.46	63.56
20 kR	71.4	84.67	0.51	55.6	0.66	76.9	0.94	62.4	1.71	74.67
30 kR	66.50	80.97	0.67	41.7	0.78	72.6	0.62	75.2	1.14	83.11
Ethyl methane sulphonate (EMS)										
0.3 %	72.25	78.58	0.44	61.7	2.20	22.8	0.62	75.2	1.15	82.96
Gamma-rays + EMS										
10 kR+0.3 % EMS	65.20	91.41	0.72	37.3	1.49	47.7	0.85	66	1.96	70.96
20 kR+0.3 % EMS	62.90	82.09	0.70	39.1	0.97	65.9	0.72	71.2	1.3	80.74
30 kR+0.3 % EMS	55.60	75.53	0.42	63.4	0.71	75.0	0.59	76.4	0.66	90.22

(2007), Shah *et al.* (2011), Singh *et al.* (2012) and Singh (2012).

In general, there was decrease in length of root and shoot, germination percentage and plant survival (%) with the increase in doses of gamma-rays or combined treatment of gamma-rays + EMS as compared with the control (Table 2). Similar trends were also observed by Kumari and Singh (1996), Ali and Shaikh (2007) and Singh *et al.* (2007). Germination percentage was almost showed linearly in the mutagenic population, the effect was more apparent in the combined treatment of gamma rays + EMS. The behavior of plant was observed to be changed for survival of plants scored at maturity where combined treatment of gamma rays (30 kR) + EMS (0.3 %) had drastic reduction in survival as compared to control hence M_1 generation was more affected as compared to M_2 generation.

The response of mutagens were compared for root and shoot length on 7th days and 14th days interval after sowing in petri-plates. Root length showed more sensitivity to combined treatment (30 kR + 0.3 % EMS) as compared to shoot length which was affected more with gamma-rays at 20 kR dose in both 7th days and 14th days, interval. In general, combined treatments at higher doses of gamma-rays (30 kR) showed more reduction in both root and shoot lengths. However, the response of combined treatments at lower dose of gamma-rays (10 kR) + 0.3 % EMS was similar in both root and shoot length. Suggesting root and shoot injury was positively correlated with the increasing doses of mutagens. EMS and their combine treatments had greater damaging effect as compared to gamma-rays for both root and shoot lengths. These results support the finding of Sinha and Lal (2007).

There were significant differences between the mean values of treated and untreated (control) population in both (M_1 and M_2) generations represented in Table 3. Also, there was negative shift towards earliness and was maximum at 10kR dose of gamma rays (69 days and 71 days in M_1 and M_2 generations, respectively) although maximum range was recorded at 30 kR dose and minimum at 10 k R in M_1 generation whereas maximum range at 20 kR and minimum at 10 kR in M_2 generation, respectively. The flowering was delayed significantly at 30 kR of gamma rays with 0.3 % of EMS mutagens in both the generations; whereas, the earliness was associated with 10 kR dose of gamma rays in both the generations. Invariably, the variability increased at all the doses/ concentration of the mutagen in both the generations (M_1 and M_2). The significance reduction of plant height (except 20 kR in the M_2) and increased coefficients of variation was recorded in all the treatment for both the generations. Maximum coefficient of variation (18.38 in the M_1 and 12.66 in the M_2 generations) was observed in the combined treatment of 20 kR gamma – rays + 0.3 % EMS in both the generations. Mean of number of primary branches also decreased where as variability increased in all the doses of mutagens in both the generations. The significance reduction in mean values of primary branches/ plant was recorded at higher dose/ concentration of the physical (30 kR gamma rays) and chemical (0.3 % EMS) mutagen individually and combined treatment of mutagens (30 kR gamma rays + 0.3 % EMS) in both the generations. While, the maximum reduction in mean values were observed in secondary branches/ plant at higher dose of combined treatment (30kR+0.3% EMS) of mutagens in both the generations. In case of number of pods/ plant and grain yield/ plant, mean performance was decreased and variability increased in majority of the treatments in both M_1 and M_2 generation. The present study was in conformity with the finding of Tripathi and Dubey (1992), Singh *et al.* (2006), Ali and Shaikh (2007), Sinha and Lal (2007) and Meshram *et al.*, 2013. Thus, combined treatments at higher dose of both mutagens such as gamma-rays and EMS showed more damaging/ deleterious effects.

The spectrum and frequency of chlorophyll mutants were computed on the basis of the M_2 plants of lentil cv. HUL 57, and presented in Table 4. The chlorophyll mutants, such as, *albino* – white leaves without chlorophyll (lethal), *xantha* - complete yellow colour of leaves (lethal) and *viridis* - uniform light yellow green colour of leaves, (viable) were scored in M_2 generation at the seedling stage. The *albino* and *xantha* mutants did not survive (lethal mutants) whereas *viridis* was observed as a viable mutant. The frequency of chlorophyll mutants in M_2 generation was induced by different doses of gamma rays

Table 3: Mean and Coefficient of variation for different quantitative traits in M_1 and M_2 generations

Treatment/ Dose of mutagen	Days to 50% flowering (days)				Plant height (cm.)				Primary branches/ plant (no.)			
	M_1 Mean	CV	M_2 Mean	CV	M_1 Mean	CV	M_2 Mean	CV	M_1 Mean	CV	M_2 Mean	CV
Control	80	2.16	81	5.06	35.48	11.62	48.59	9.88	2.92	22.64	2.4	23.93
Gamma-rays												
10 kR	69*	2.72	71*	6.7	34.44	12.84	47.16	9	2.87	23.39	2.33	27.68
20 kR	71*	3.47	76*	8.23	36.5	10.68	46.53*	11.43	2.61*	25.57	2.2	28.16
30 kR	71*	3.58	76*	8.79	34.43	10.27	46.53*	12.26	2.04*	31.39	2.14*	32.69
Ethyl methane sulphonate (EMS)												
0.3 % EMS	73*	2.76	75*	9.22	34.83	13.21	41.38*	12.83	2.41*	31.08	2.13*	32.45
Gamma-rays + EMS												
10Kr + 0.3%EMS	72*	2.34	74*	9.25	34.5	12.11	43.26*	12.01	2.17*	33.04	2.02*	33.43
20kR + 0.3%EMS	74*	3.03	76*	9.84	28.82*	18.38	43.00*	12.66	2.08*	33.34	2.08*	36.53
30kR + 0.3%EMS	74*	3.18	77*	10.17	27.87*	17.93	41.82*	12.2	1.99*	35.75	1.98*	40.41
SEm±	0.55		0.68		0.86		0.59		0.09		0.08	
CD at 5 %	1.68		2.06		2.61		1.78		0.28		0.25	

Treatment/ Dose of mutagen	Secondary branches/ plant (no.)				Number of pods/ plant (no.)				Grain yield/ plant (g.)			
	M_1 Mean	CV	M_2 Mean	CV	M_1 Mean	CV	M_2 Mean	CV	M_1 Mean	CV	M_2 Mean	CV
Control	14.16	21.7	14.72	18.8	122.99	25.4	177.2	18.6	3.85	39	4.47	18.1
Gamma-rays												
10 kR	13.22	31.5	13.11*	19.5	111.12*	34.5	156.61*	19.8	3.62	50.6	3.88*	20.9
20 kR	12.86*	35.7	13.13*	18.7	110.29*	45.8	143.7*	22	3.61	58.9	3.99*	21.8
30 kR	9.85*	35.1	12.96*	23.9	90.76*	45.4	140.2*	20.1	2.34*	53.3	3.89*	26.4
Ethyl methane sulphonate (EMS)												
0.3 % EMS	10.76*	33.1	13.72*	23.8	31.73*	48	126.57*	22.8	1.56*	55	3.84*	29.7
Gamma-rays + EMS												
10Kr + 0.3%EMS	11.19*	33.9	13.55*	20.1	35.56*	45.3	120.75*	23.9	1.11*	55.2	4.06*	25.6
20kR + 0.3%EMS	11.12*	37.5	13.60*	18.4	30.15*	43.3	93.43*	20.9	0.963*	56.5	3.60*	25.7
30kR + 0.3%EMS	10.60*	32.9	11.86*	24	28.84*	52.2	89.59*	20.3	0.939*	56.9	3.83*	27.8
SEm±	0.39		0.33		2.71		2.62			0.2		0.08
CD at 5 %	1.18		0.99		8.23		7.95			0.62		0.23

*Significant at 5% level of significance, CD = Critical difference, SEm = Standard error of mean

Table 4: Spectrum and frequency of chlorophyll mutants in M_2 generation

Treatment/ Dose of mutagen	Number of plants scored	Chlorophyll mutations			Chlorophyll mutation frequency
		Albino	*Xantha*	*Viridis*	
Control	1074	-	-	-	-
Gamma-rays					
10 kR	837	-	5	6	1.31
20 kR	970	-	4	5	0.92
30 kR	687	-	7	7	2.09
Ethyl methane sulphonate (EMS)					
0.3 % EMS	599	2	15	17	5.67
Gamma-rays + EMS					
10 kR + 0.3 % EMS	499	-	4	6	2.00
20kR + 0.3 % EMS	483	-	6	8	2.89
30 kR + 0.3 % EMS	770	-	5	6	1.44

and EMS alone and/or in combination treatment at fair frequency. However, the spectrum of chlorophyll mutations was quite narrow as only three types of mutant, viz., *albino*, *xantha* and *viridis* were observed. The induction of *albino* mutants was obtained only at 0.3 % EMS. Whereas *xantha* and *viridis* types of mutants were recorded at all the doses of mutagens, maximum being at 0.3 % of EMS treatment alone. Among the chlorophyll mutants, viridis occurred in highest frequency followed by xantha and albino mutants. Thus, for induction of chlorophyll mutation 0.3 % EMS alone proved to be the best option in lentil cv HUL 57. Similar type of chlorophyll mutants were also reported by Wani and Khan (2003), Solanki *et al.* (2004) and Ali and Sheikh (2007). The differential response of treatments to induce chlorophyll mutants is possibly due to difference in the genetic makeup of the variety used for mutagenesis.

Chlorophyll mutations provide one of the most dependable indices for the evaluation of genetic effects of mutagenic treatments and have been reported in lentil and other several crops by several workers (Sharma and Sharma, 1986, Singh and Singh, 2003, Singh *et al.*, 2006, Singh *et al.*, 2007 and Kumari *et al.*, 2013). In the present study, differential response

of the treatment to induce the chlorophyll mutations was observed and it demonstrated that the total frequency of chlorophyll mutations was higher in *viridis* type. Similar reports for treatment differences in lentil and other crops were reported by many workers (Reddi and Suneetha, 1992, Singh *et al.*, 1998, Singh *et al.*, 2000, Gaikwad and Kothekar, 2004, and Shah *et al.*, 2011). However, the frequency of chlorophyll mutants was found independent of mutagenic doses of gamma rays as reported by Solanki and Sharma (1999), Wani and Khan (2003) and Singh *et al.* (2007). Since, the experiment was started with a view to induce and isolate heat stress tolerant mutants in M_2 generation. Although mean of the treated population has decreased in most of treatments including their yields. However a wide range of coefficient of variability for days to 50% flowering and other yield and yield traits shows the possibilities of heat stress mutants in the M_3 and onward generations, hence single M_2 plant progenies will be advanced and selection for desired trait is made.

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Only original research papers are considered for publication. The authors may be asked to declare that the manuscript has not been submitted to any other journal for consideration at the same time. Two hard copies of manuscript and one soft copy, complete in all respects should be submitted. The soft copy can also be sent by e-mail as an attachment file for quick processing of the paper.

FORMAT OF MANUSCRIPT

All manuscripts must be written in English and should be typed double-spaced with wide margins on all sides of good quality A4 paper.

First page of the paper should be headed with the title page, (in capital, font size 16), the names of the authors (in capitals, font size 12) and full address of the institution where the work was carried out including e-mail address. A short running title should be given at the end of the title page and 3-5 key words or phrases for indexing.

The main portion of the paper should be divided into Abstract, Introduction, Materials and Methods, Results, Discussion (or result and discussion together), Acknowledgements (if any) References and legends.

Abstract should be limited to 200 words and convey the main points of the paper-outline, results and conclusion or the significance of the results.

Introduction should give the reasons for doing the work. Detailed review of the literature is not necessary. The introduction should preferably conclude with a final paragraph stating concisely and clearly the aims and objectives of your investigation.

Materials and Methods should include a brief technical description of the methodology adopted while a detailed description is required if the methods are new.

Results should contain observations on experiment done illustrated by tables and figures. Use well known statistical tests in preference to obscure ones.

Discussion must not recapitulate results but should relate the author's experiments to other work on the subject and give their conclusions.

All tables and figures must be cited sequentially in the text. Figures should be abbreviated to Fig., except in the beginning of a sentence when the word Figure should be written out in full.

The figures should be drawn on a good quality tracing/ white paper with black ink with the legends provided on a separate sheet. Photographs should be black and white on a glossy sheet with sufficient contrast.

References should be kept to a minimum and listed in alphabetical order. Personal communication and unpublished data should not be included in the reference list. Unpublished papers accepted for publication may be included in the list by designating the journal followed by "in press" in parentheses in the reference list. The list of reference at the end of the text should be in the following format.

1. **Witkamp, M. and Olson, J. S. 1963.** Breakdown of confined and non-confined Oak Litter.*Oikos*. **14**:138-147.
2. **Odum, E.P. 1971.** *Fundamentals of Ecology*. W. B. Sauder Co. Publ. Philadelphia.p.28.
3. **Macfadyen, A.1963.** The contribution of microfauna to total soil metabolism. In:*Soil organism*, J. Doeksen and J. Van Der Drift (Eds). North Holland Publ. Comp., pp 3-16.

References in the text should be quoted by the **author's name and year** in parenthesis and presented in year order. When there are more than two authors the reference should be quoted as: first author followed by *et al.,* throughout the text. Where more than one paper with the same senior author has appeared in on year the references should

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