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Ethyl methane sulphonate induced genetic variability and heritability in *macrosperma* and *microsperma* lentils

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Abstract

Dry and healthy seeds of two lentil cultivars, LH90-54 (*macrosperma*) and LH89-48 (*microsperma*) were treated with three doses of ethyl methane sulphonate (0.1, 0.2 and 0.4 %). In both the cultivars, all the M_1 plants with sufficient seed from each treatment and control were taken to raise independent M_2 plant progenies. Wider range of means in both positive and negative directions along with overall positive shift in mean for all the polygenic traits, except pod-initiation height and 100-seed weight, were observed in different treatments in M_2 generation. In both the cultivars, medium dose induced highest amount of variation. The estimates of variance, GCV and PCV for different polygenic traits increased significantly over control values in all the treatments of both the cultivars. Higher estimates of heritability and genetic advance in M_2 population indicated tremendous scope for the improvement of seed yield and its component traits through selection in the mutagenized material.

Key words

Ethyl methane sulphonate, Genetic advance, Heritability, Lentil

Introduction

In India, lentil is one of the most important *Rabi* pulse crops, second only to chickpea. The cultivated lentils belong to two broad groups: small seeded (*Lens culinaris* Medik. var. *microsperma* Zhukovsky) and large seeded (*Lens culinaris* Medik. var. *macrosperma* Zhukovsky). The large seeded cultivars are more mutable than the small seeded in chickpea (Khan *et al*., 2005; Pathania and Sood, 2006) and mungbean (Singh *et al*., 2005). Studies on mutagenesis using ethyl methane sulphonate (EMS) as a mutagen in lentil are limited and have been conducted in both the groups i.e., *microsperma* (Tripathi *et al*., 1994; Wani and Khan, 2003) and *macrosperma* (Solanki *et al*., 2004; Solanki and Phogat, 2005). These studies have shown that lentil is a highly sensitive and mutable crop. In majority of the studies on mutations in crop plants, EMS has been reported to be more efficient and effective to induce mutations (Barshile and Boddu, 2012; Goyal *et al.,* 2012; Kozgar *et al.*, 2011; Roychowdhury and Jagatpati, 2011), however, Shirsat *et al.* (2010) observed that EMS was less effective than sodium azide (SA) which showed the highest mutagenic rate. Mutation breeding is more advisable for this crop because exploitation of exotic variability through recombination breeding is tedious since pollination is difficult due to tiny size of its flowers.

Differential responses of cultivars to EMS treatments for induction of mutations have been reported in basmati rice (Wattoo *et al.,* 2012), chickpea (Khan *et al*., 2005; Pathania and Sood, 2006), green gram (Singh *et al*., 2005), opium poppy (Chatterjee *et al.,* 2012) and soybean (Kartika and Subba Lakshmi, 2006; Khan and Tyagi, 2009). The main limitation for success of mutation breeding is lack of suitable mutagens and screening technique that helps in creation, identification and isolation of useful genetic variability. Mutagenesis, especially artificial mutagenesis has provided abundant useful variations for crop breeding and improvement and shortened the breeding process as compared to other traditional breeding programmes (Zou *et al*., 2011). Therefore, in the present investigation using EMS as mutagen, an attempt was made to create and assess the polygenic variability for various traits and to identify mutagenic treatments that possess highest amount of genetic variability for its further exploitation.

Materials and Methods

Healthy seeds of uniform size of both lentil cultivars, *macrosperma* (LH90-54) and *microsperma* (LH89-48) were treated with three doses (0.1, 0.2 and 0.4 %) of ethyl methane sulphonate (EMS). The treated seeds, along with control, were sown immediately in field to raise M_1 generation. In both the cultivars all M_1 plants with sufficient seed from each treatment and control were taken to raise individual progenies in M_2 generation. Spacing between different rows and plants was kept at 30.0 cm and 5.0 cm, respectively. Observations were recorded on five randomly chosen normal looking plants from each M_2 progeny for eight quantitative traits of economic importance viz., plant height (cm), pod-initiation height (cm), number of fruiting branches/plant, number of fruiting pods/plant, 100-seed weight (g), seed yield/plant (g), biomass/plant (g) and harvest index (%).

The mean, variance (interfamily and intrafamily) and genotypic and phenotypic coefficients of variation (GCV and PCV, %) were calculated using standard statistical procedures (Snedecor and Cochran, 1967). Heritability in broad sense (h^2) and genetic advance as per cent of mean (GA) were calculated following the procedures suggested by Hanson *et al.* (1956) and Johnson *et al.* (1955), respectively.

Results and Discussion

A large number of progenies and plants in different treatments of EMS were used for the study of micromutations in both the cultivars in M_2 generation (Table 1). The range of means obtained in the mutagenic treatments in both the cultivars in M_2 generation was wider in both directions, i.e., positive and negative than control for economic traits (Tables 2,3).

A negative shift in mean values for all the traits was observed at highest dose of mutagen (0.4 %), whereas in case of 100-seed weight, the lowest dose, followed by the highest and the medium doses, were observed to be the most effective for increasing the mean values. Talebi *et al*. (2012) showed negative correlation between doses of EMS applied and character means in rice, *i.e*., with increase in dose, character means decreased, whereas Kozgar *et al.* (2011) observed a linear correlation between the doses and plant yield in mungbean and urdbean, *i.e*., with increase in dose, character means also increased. With regard to positive shift in the mean of seed yield and its contributing traits; medium dose, followed by the lowest and the highest dose, was observed to be most effective. In case of plant height and pod-initiation height with increase in dose the mean values decreased. Since yield is a complex trait increase or decrease in mean seed yield in M_2 generation is a consequence of corresponding changes in other yield contributing characters like, plant height, pod-initiation height, branches and pods per plant, 100-seed weight, etc. Thus, increase in mean seed yield may be explained on the basis of mutations induced with positive effects for one or more components. A positive shift in mean of different traits over control is attributed to the induction of comparatively a higher proportion of positive mutations than negative mutations (Waghmare and Mehra, 2000; Solanki and Sharma, 2001; Kartika and Subba Lakshmi, 2006; Barshile and Boddu, 2012).

Table 1: Population size of EMS induced M_2 generation for study of micromutations in LH90-54 and LH89-48 lentil cultivars

Variety / Treatment	Population size	
	Progenies	Plants
LH90-54		
Control	96	480
0.1%	55	275
0.2%	71	355
0.4%	77	395
LH89-48		
Control	98	490
0.1%	59	295
0.2%	77	385
0.4%	86	430

Variability in different traits estimated through variance (interfamily and intrafamily), GCV and PCV increased in different treatments over control in both the cultivars (Tables 2, 3). The analysis of variance revealed significant interfamily variances for different traits in all the EMS treatments and non-significant in control. In all the treatments of both the cultivars, the interfamily variances for all the traits were higher than the intrafamily variances, therefore, selection within family would not be useful. In both cultivars, medium dose induced highest amount of variation. GCV and PCV of different traits increased in the treated material over control values (PCV > GCV). Higher values of PCV than GCV for all the traits indicated the influence of environment in expression of these traits. The highest estimates of GCV and PCV in both the cultivars were observed for biomass/plant, followed by harvest index, seed yield/plant, fruiting pods and branches/plant, whereas lowest estimates were observed for plant height. Both GCV and PCV for all the traits were observed to be highest at medium dose (0.2 %), followed by highest (0.4 %) and lowest (0.1 %) doses, except in case of plant height in LH89-48 where PCV increased with increase in dose of mutagen. Induction of polygenic variation has been reported earlier in different pulse crops (Waghmare and Mehra, 2000; Solanki and Sharma, 2001; Kartika and Subba Lakshmi, 2006; Barshile and Boddu, 2012).

High heritability coupled with high genetic advance indicates the expected effectiveness of selection for the character(s) under consideration. Very little information is available in pulse crops, particularly lentil, regarding heritability and genetic advance under mutagenic treatment. Higher heritability with high genetic advance was observed for quantitative traits like, number of pods/plant and number of seeds/plant in chickpea (Barshile and Boddu, 2012). Almost

Table 2: Range, mean, variance and genetic variability for different traits in EMS induced M_2 generation of *macrosperma* lentil (LH90-54)

Trait/Treatment	Range	Mean	Variance		GCV (%)	PCV (%)	h^2 (%)	GA (% of mean)
			Inter-family	Intra-family				
Plant height (cm)								
Control	26.7 -34.6	30.1	7.7	4.2	2.8	7.4	14.3	2.2
0.1%	17.5 -37.0	31.9	27.3**	11.4	5.6	12.0	21.8	5.4
0.2%	13.6 -43.8	30.8	44.9**	14.6	8.0	14.8	29.3	8.9
0.4%	14.1 -38.4	30.0	38.5**	13.5	7.4	14.0	27.0	8.0
Pooled	13.6 -43.8	30. 9	36.9**	13.2	7.0	13.6	26.0	7.4
Pod -initiation height (cm)								
Control	8.3 -11.5	9.8	4.3	2.1	6.8	16.3	17.3	5.8
0.1%	6.9 -14.3	10.1	16.2**	5.4	14.6	27.2	28.6	16.0
0.2%	5.3 -16.5	9.3	27.8**	8.2	21.3	37.4	32.3	24.9
0.4%	4.4 -13.2	8.7	23.4**	7.7	20.4	37.8	29.0	22.6
Pooled	4.4 -16.5	9.4	22.5**	7.1	18.8	34.1	30.0	21.2
Fruiting branches per plant								
Control	10.0 -18.3	14.5	16.3	9.5	8.0	22.7	12.5	5.8
0.1%	6.5 -35.3	15.6	67.8**	30.2	17.6	39.4	19.9	16.2
0.2%	5.1 -43.7	17.5	103.4**	37.4	20.8	40.6	26.1	21.8
0.4%	4.8 -29.9	13.8	58.1**	22.5	19.3	39.4	24.0	19.5
Pooled	4.8 -43.7	15.6	76.4**	30.0	19.2	39.8	23.3	19.2
Fruiting pods per plant								
Control	35.5 -98.3	73.5	910.0	553.8	11.5	34.0	11.4	8.0
0.1%	14.8 -176.3	75.8	2235.1**	988.6	20.8	46.4	20.1	19.3
0.2%	19.6 -203.7	80.6	2958.3**	1099.2	23.9	47.6	25.3	24.8
0.4%	12.5 -165.4	71.3	2176.2**	846.3	22.9	46.8	23.9	23.0
Pooled	12.5 -203.7	75.9	2456.5**	978.0	22.5	46.9	23.1	22.4
100 -seed weight (g)								
Control	3.3 -3.8	3.5	0.44	0.31	4.6	16.6	7.7	2.6
0.1%	3.2 -4.3	3.5	1.00**	0.42	9.7	20.9	21.6	9.3
0.2%	2.9 -4.7	3.3	1.85**	0.71	14.5	29.3	24.3	14.7
0.4%	3.0 -4.4	3.4	1.23**	0.51	11.2	23.8	22.0	10.8
Pooled	2.9 -4.7	3.4	1.36**	0.55	11.8	24.7	22.6	11.6
Seed yield per plant (g)								
Control	2.5 -4.6	3.4	3.6	2.3	15. 0	47.1	10.2	9.8
0.1%	2.1 -6.7	3.7	7.6**	4.0	22.9	58.7	15.3	18.5
0.2%	1.2 -10.5	4.1	12.1**	5.3	28.4	62.9	20.4	26.5
0.4%	0.9 -3.4	3.0	5.7**	2.5	26.7	59.1	20.4	24.8
Pooled	0.9 -10.5	3.6	8.5**	3.9	26.0	60.2	18.7	23.3
Biomass per plant (g)								
Control	5.9 -13.8	9.2	37.4	21.1	19.6	53.6	13.4	14.8
0.1%	4.9 -23.3	9.7	66.1**	31.4	27.2	63.8	18.1	23.8
0.2%	4.1 -30.9	10.5	96.4**	36.9	32.9	66.5	24.4	33.4
0.4%	3.5 -18.6	8.9	64.2**	24.8	31.5	64.2	24.1	31.9
Pooled	3.5 -30.9	9.7	75.6**	31.0	30.5	64.8	22.2	29.7
Harvest index (%)								
Control	31.8 -42.7	37.0	489.9	319.2	15.8	50.8	9.7	10.1
0.1%	26.4 -55.1	38.1	957.5**	459.1	26.2	62.0	17.8	22.8
0.2%	21.9 -56.8	39.0	1258.2**	483.2	31.9	64.8	24.3	32.4
0.4%	17.2 -43.5	36.0	967.8**	413.6	29.2	63.6	21.1	27.7
Pooled	17.2 -56.8	37.7	1061.2**	452.0	29.1	63.5	21.1	27.6

Table 3: Range, mean, variance and genetic variability for different traits in EMS induced M_2 generation of *microsperma* lentil (LH89-48)

Trait/Treatment	Range	Mean	Variance		GCV (%)	PCV (%)	h^2 (%)	GA (% of mean)
			Inter-family	Intra-family				
Plant height (cm)								
Control	27.1-32.6	29.2	6.8	3.7	2.7	7.1	14.4	2.1
0.1%	18.6-41.2	30.8	26.9**	10.9	5.8	12.2	22.7	5.7
0.2%	14.0-38.1	31.7	42.6**	13.5	7.6	13.9	30.1	8.6
0.4%	13.8-37.3	28.5	34.5**	13.0	7.3	14.6	24.8	7.4
Pooled	13.8-41.2	30.3	34.7**	12.5	6.9	13.6	25.9	7.2
Pod-initiation height (cm)								
Control	7.9-11.2	9.6	4.5	1.9	7.5	16.2	21.5	7.2
0.1%	6.6-15.5	9.8	15.1**	5.2	14.4	27.3	27.6	15.5
0.2%	5.1-17.2	9.2	25.8**	7.9	20.6	36.8	31.2	23.7
0.4%	4.8-14.9	8.8	20.8**	7.6	18.5	36.4	25.8	19.3
Pooled	4.8-17.2	9.3	20.6**	6.9	17.8	33.5	28.2	19.5
Fruiting branches per plant								
Control	9.4-17.1	13.8	14.7	7.7	8.6	21.9	15.4	6.9
0.1%	6.1-30.2	14.8	53.8**	24.9	16.2	37.4	18.8	14.5
0.2%	5.8-39.4	16.7	84.9**	33.1	19.3	39.5	23.8	19.4
0.4%	4.9-26.3	13.6	51.4**	20.4	18.3	37.9	23.3	18.2
Pooled	4.9-39.4	15.0	63.4**	26.1	17.9	38.3	22.0	17.4
Fruiting pods per plant								
Control	43.9-81.4	62.5	579.6	342.1	11.0	31.6	12.2	7.9
0.1%	18.7-159.6	64.5	1519.4**	679.4	20.1	45.1	19.8	18.4
0.2%	16.4-201.3	68.5	2037.5**	789.4	23.1	47.1	24.0	23.3
0.4%	22.5-153.8	61.6	1599.7**	623.1	22.7	46.4	23.9	22.8
Pooled	16.4-201.3	64.9	1718.9**	697.3	22.0	46.4	22.6	21.5
100-seed weight (g)								
Control	1.6-2.0	1.8	0.12	0.08	5.0	16.5	9.1	3.1
0.1%	1.7-2.2	2.0	0.31**	0.13	9.5	20.4	21.7	9.1
0.2%	1.4-2.2	1.6	0.42**	0.16	14.3	28.8	24.5	14.5
0.4%	1.4-2.0	1.7	0.27**	0.11	10.5	22.2	22.5	10.3
Pooled	1.4-2.2	1.8	0.33**	0.13	11.4	23.8	22.9	11.3
Seed yield per plant (g)								
Control	2.2-3.4	2.7	2.0	1.3	13.9	44.4	9.7	8.9
0.1%	1.8-4.3	3.1	5.3**	2.8	22.8	58.6	15.2	18.3
0.2%	1.5-4.9	3.4	8.2**	3.6	28.2	62.5	20.4	26.2
0.4%	1.3-3.1	2.2	3.1**	1.4	26.5	60.0	19.5	24.1
Pooled	1.3-4.9	2.9	5.5**	2.6	25.8	60.4	18.4	22.9
Biomass per plant (g)								
Control	6.9-11.7	7.7	22.7	15.3	15.8	53.2	8.8	9.7
0.1%	5.6-14.5	8.2	44.3**	21.5	26.0	62.3	17.5	22.4
0.2%	4.9-16.8	8.9	64.2**	24.8	31.5	64.2	24.1	31.9
0.4%	4.2-10.7	7.0	36.9**	14.9	30.0	62.8	22.8	29.5
Pooled	4.2-16.8	8.0	48.5**	20.4	29.2	63.1	21.5	27.9
Harvest index (%)								
Control	30.5-39.7	35.0	392.7	261.1	14.7	48.4	9.2	9.1
0.1%	27.8-56.7	37.8	879.9**	429.2	25.1	60.3	17.4	21.6
0.2%	22.5-56.1	38.2	1146.4**	452.7	30.8	63.7	23.5	30.8
0.4%	19.3-35.6	31.4	659.3**	287.5	27.5	60.6	20.5	25.6
Pooled	19.3-56.7	35.8	895.2**	389.8	27.8	61.5	20.5	26.0

invariably, the medium dose resulted in the highest values of heritability for different traits over control in both the cultivars, followed by highest and lowest doses. In both the cultivars, pod-initiation height, followed by plant height showed highest heritability estimates, whereas lowest estimates were observed for seed yield/plant and harvest index. Similar to heritability estimates, medium dose, followed by highest and lowest doses resulted in highest estimated values of GA for different traits over

control in both the cultivars. Highest GA was obtained for biomass/plant, followed by harvest index and seed yield/plant, whereas lowest estimates were observed for plant height and 100-seed weight. Therefore, the results suggest that medium dose of EMS was most effective for increasing the character means and variation, resulting in higher estimates of heritability and genetic advance. These findings are congruent to earlier results reported by various workers in different pulse crops (Barshile and Boddu, 2012; Kartika and Subba Lakshmi, 2006; Solanki and Sharma, 2001).

The perusal of results obtained in the present investigation clearly depicts that substantial amount of genetic variability by different treatments was generated for all the polygenic traits of economic importance, resulting in higher estimates of heritability and GA than control in both the cultivars. Higher estimates of variance, h^2 and GA were observed in the *macrosperma* cv., LH90-54 than the *microsperma* cv., LH89-48 making it quite clear that *macrosperma* cultivars are more mutable than the *microsperma* cultivars. Findings of the present investigation in both the cultivars suggest tremendous scope for improving seed yield and its component traits by exercising selection in M_2 generation.

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References

Barshile, J. and A. Boddu: Genetic improvement of chickpea (*Cicer arietinum* L.) cultivar 'Vijay' (Phule G 81-1-1) through induced mutagenesis. *Biorem. Biodiv. Bioavail.*, **6**, 103-106 (2012).

Chatterjee, A., S. Shukla, A. Rastogi, B.K. Mishra and S.P. Singh: Induction of variability and selection of elite lines for specific alkaloids from a population of induced mutagenesis in opium poppy (*Papaver somniferum* L.). *Biorem. Biodiv. Bioavail.*, **6**, 94-102 (2012).

Goyal, S., M.I. Kozgar and S. Khan: Effectiveness and efficiency of gamma rays, ethyl methane sulphonate and their combinations in *Vigna mungo* (L.) Hepper. *Biorem. Biodiv. Bioavail.*, **6**, 113-115 (2012).

Hanson, C.H., H.F. Robinson and R.E. Comstock: Biometrical studies on yield in segregating population of Korean lespedeza. *Agron. J.*, **48**, 268-272 (1956).

Johnson, H.W., H.F. Robinson and R.E. Comstock: Estimation of genetic and environmental variability in soybeans. *Agron. J.*, **47**, 314-318 (1955).

Karthika, R. and B. Subba Lakshmi: Effect of gamma rays and EMS on two varieties of soybean. *Asian J. Plant Sci.*, **5** , 721-724 (2006).

Khan, M.H. and S.D. Tyagi: Studies on induction of chlorophyll mutations in soybean (*Glycine max* L. Merrill). *Front. Agric. China*, **3**, 253-258 (2009).

Khan, S., M. R. Wani, M. Bhat and P. Kouser: Induced chlorophyll mutations in chickpea (*Cicer arietinum* L.). *IJAB*, **7**, 764-767 (2005).

Kozgar, M.I., S. Goyal and S. Khan: EMS induced mutational variability in *Vigna radiata* and *Vigna mungo*. *Res. J. Bot.*, **6**, 31-37 (2011).

Pathania, A. and B.C. Sood: Spectrum and frequency of mutations induced by gamma rays and EMS in chickpea (*Cicer arietinum* L.). *Res. on Crops*, **7**, 453- 457 (2006).

Roychowdhury, R. and T. Jagatpati: Assessment of chemical mutagenic effects in mutation breeding programme for M_1 generation of carnation *(Dianthus caryophyllus)*. *Res. Plant Biol.*, **1**, 23-32 (2011).

Shirsat, R.K., M.N. Mohrir, M.A. Kare and A.S. Bhuktar: Induced mutations in horse gram: mutagenic efficiency and effectiveness. *Rec .Res. Sci. Tech.*, **2**, 20-23 (2010).

Singh, V.P., K. Srivastava and S. Singh: Gamma ray and EMS induced mutations in mungbean [*Vigna radiata* L. Wilczek]. *Crop Res.*, **29**, 480-485 (2005).

Snedecor, G.W. and W.G. Cochran: Statistical Methods. 6th Edn., Iowa State University Press, Ames, Iowa, USA (1967).

Solanki, I.S. and B. Sharma: N-nitroso-N-ethyl urea-induced genetic variability for quantitative characters in lentil (*Lens culinaris* Medik.). *Nat. J. Pl. Improv.*, **3,** 102-106 (2001).

Solanki, I.S. and D.S. Phogat: Chlorophyll mutation induction and mutagenic effectiveness and efficiency in *macrosperma* lentil (*Lens culinaris* Medik.). *Nat. J. Plant Improv.*, **7**, 81-84 (2005).

Solanki, I.S., D.S. Phogat and R.S. Waldia: Frequency and spectrum of morphological mutations and effectiveness and efficiency of chemical mutagens in *Macrosperma* lentil. *Nat. J. Plant Improv.*, **6**, 22-25 (2004).

Talebi, A.B., A.B. Talebi and S. Behzad: Ethyl methane sulphonate (EMS) induced mutagenesis in Malaysian rice (cv. MR219) for lethal dose determination. *American J. Plant Sci.*, **3**, 1661-1665 (2012).

Vandana, A. Tripathi and D.K. Dubey: Frequency and spectrum of mutations induced by ethyl methane sulfonate (EMS) and diethyl sulfate (DES) in lentil var. K–85. *Lens Newsl.*, **21**, 16-19 (1994).

Waghmare, V.N. and R.B. Mehra: Induced genetic variability for quantitative characters in grasspea (*Lathyrus sativus*). *Indian J. Genet.,* **60**, 81-87 (2000).

Wani, M.R. and S. Khan: Chlorophyll mutations in lentil. *Tropical Agriculturist*, **154**, 21-26 (2003).

Wattoo, J.I., K. Aslam, S.M. Shah, G. Shabir, M. Sabar, S.A. Naveed, R. Waheed, Samiullah, Q.H. Muqaddasi and M. Arif: Ethyle methane sulphonate (EMS) induced mutagenic attempts to create genetic variability in Basmati rice. *J.Plant Breed. Crop Sci.*, **4**, 101-105 (2012).

Zou, J., N. Wang and J. Meng: Mutagenesis. In: Genetics, Genomics and Breeding of Oilseed Brassicas. Science Publishers, Inc., Lebanon, USA, pp. 158-173 (2011).