



## Ethyl methane sulphonate induced variability and meiotic aberrations in two economically important mutants of faba bean (*Vicia faba*)

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### ABSTRACT

Effect of mutagenesis was studied in faba bean (*Vicia faba* L.) varieties 05/249 local and 05/233 HBP. Seeds of *Vicia faba* varieties 05/249 local and 05/233 HBP were subjected to 0.02%, 0.04%, 0.06%, and 0.08% of ethyl methane sulphonate (EMS). The mutagenic treatments of EMS in M<sub>1</sub> and induced variation in mean values for various quantitative traits such as, plant height, days to flowering, days to maturity, pod length, number of fertile branches/plant, number of pods/plant, number of seeds/pod, 100 seed weight (g) and total plant yield (g) in M<sub>2</sub> generation. The present study also provides a comparative account of cytological effects of EMS on meiotic aberrations in two varieties. Different types of meiotic abnormalities as laggards, bridges, chromosome stickiness, stray chromosomes and precocious movement of chromosomes, disturbed polarity and multinucleate condition were recorded. The mean value of plant height, fertile branches/ plants and pods/plants were found to be significant in both the varieties while, seeds/pod and 100 seeds weight did not show any significant difference from the control. Yield/plant increased significant by in the var. 05/249 local but was not significant in var. 05/233 HBP.

**Key words:** Ethyl methane sulphonate, Meiotic aberrations, Quantitative traits, *Vicia faba*

Faba bean (*Vicia faba* L.) is used as vegetables in developing countries. It is a common breakfast food in the Middle East, Mediterranean region, China and Ethiopia. Mutations can be beneficially utilized for tailoring better varieties of crop plant (Kozgar *et al.* 2012). Induced mutagenesis may be of special use of broadening the genetic variability of quantitatively inherited characters. With efficient selection procedure, it is possible to get a desirable stuff in the mean values of characters under consideration. Against this background, the aim of the present study was to study the biological damage in M<sub>1</sub> and induced variability in mean values for quantitative traits in M<sub>2</sub> generation in faba beans, a self pollinated crop. Cytological studies constitute an important component in breeding program involving the development of new original forms with aid of experimental mutagenesis (Srivastava and Kapoor 2008). The increased frequency of meiotic anomalies with increasing concentrations of mutagens was reported in several crops by many workers (Goyal and Khan 2009).

### MATERIALS AND METHODS

Experiment conducted at the agricultural fields of the Aligarh Muslim University, Aligarh in *rabi* growing season of October 2008 the normal looking M<sub>1</sub> plants were harvested and their seeds were sown in October 2009 to raise M<sub>2</sub> generation. The soil texture of the field was loamy sandy. The experimental design was used as factorial arrangement in randomized complete block with three replications. Each plot consisted of 4 rows, 5m long with 50 cm spaced between rows and 5 cm distance between plants in the row. Two varieties of faba bean namely, 05/249 local and 05/233 HBP were used in the present study. Seeds of both the varieties were obtained from the India Agricultural Research Institute, New Delhi. Healthy seeds of uniform size of each variety were used. Seeds were soaked in distilled water for 9 hours and then treated with four different concentrations with ethyl methane sulphonate, prepared in phosphate buffer at 7.0 pH, for 6 hours with constant intermittent shaking. The treated seeds were washed in running tap water for 2 to 3 times to remove the residual effect of the mutagen sticking to the seed coat. One set of seeds was soaked in distilled water for 15 hours to act as control for comparison. Data were collected on mean values for days to flowering, days to maturity, plant height and on certain yield and yield components in M<sub>2</sub> generation.

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For meiotic analysis, young flower buds from each treatment and control in both the varieties were fixed in Carnoy's fluid (1 part glacial acetic acid: 3 parts chloroform: 6 parts of ethyl alcohol) for 30 minutes. The material was transferred to propionic alcohol saturated with ferric acetate for 24 hours. The flower buds were washed with and preserved in 70% alcohol. Anthers were smeared in 1% acetocarmine solution and pollen mother cells were examined for their behavior at various stages of microsporogenesis. Photographs were taken from temporary preparation.

RESULTS AND DISCUSSION

The chemical mutagen used in the present study produced adverse effect on seed of germination, seedling height, pollen fertility, and plant survival at maturity (Table 1). Data recorded on seed germination, biological damage in  $M_1$ , after 10 days of sowing of faba bean differed in their response to mutagenic treatments (Kozar *et al.* 2012). The percentage of seed germination gradually decreases with increasing concentrations of mutagens. Similar result was also reported by Fatma (2007) in *Vicia faba* and Khan *et al.* (2000) in *Vigna radiata*. Treatment with 0.08% EMS had more toxic effect on both the varieties of faba bean (var.05/249 local and

05/233 HBP). The maximum seed germination was observed to be 100% in the control population (\*\*).Pollen character is one the important stable and genetically controlled character. The highest percentage of pollen sterility was 8.39 and 7.54 at 0.08% EMS treatment in the varieties 05/249 local and 05/233HBP, respectively. The dose dependent increase in pollen sterility was observed. Two varieties of faba bean differed in response of chemical mutagen, var. 05/249 local was found to be more sensitive (\*\*\*\*). It may be concluded that lower concentrations of EMS for increasing mutation rate and obtaining desirable spectrum of mutations in faba bean.

Studies on quantitative characters in  $M_2$

A significant decrease in days to maturity by about 2 days was recorded in the var. 05/233HBP. High mutagenic treatments reduced plant height significantly in both the varieties. Most of treatments of EMS brought about a significant increase in yield components expect seed/pod and 100-seed weight in both the varieties (Table 3 and 4). Enhancement of the frequency and spectrum of mutation in a predictable manner and consequent achievement of desirable plant characteristics is an important goal of mutation research. Although high seed yield is the ultimate

Table 1 Effect of EMS on seeds germination, plant survival, pollen fertility and seedling height in the two varieties in *Vicia faba*

Concentration	Seed germination		Plant survival at maturity (%)	Pollen fertility		Seedling height (% age injury)
	Actual (%)	% age inhibition		Actual (%)	% age inhibition	
<i>Var.05/249local</i>						
Control	100.00		79.00	99.18		
0.02%	93.33	-6.67	65.00	97.77	-1.42	-7.20
0.04%	80.00	-20.00	67.00	96.39	-2.81	-15.14
0.06%	66.97	-33.33	59.00	94.99	-5.03	-17.62
0.08%	46.67	-53.33	59.00	90.86	-8.39	-41.56
<i>Var.05/233HBP</i>						
Control	100.00		72.00	98.76		
0.02%	93.33	-6.67	58.00	97.80	-0.97	-4.25
0.04%	86.67	-13.33	59.00	96.44	-2.32	-12.89
0.06%	73.33	26.67	52.00	94.22	-4.40	-31.29
0.08%	60.00	-40.00	48.00	91.31	-7.54	-50.37

Table 2 Frequency of meiotic aberrations induced by EMS in *Vicia faba*

Concentrations of EMS	Total no. of PMCs observed		Metaphase-I/II		Anaphase-I/II		Telophase-I/II		Total no. of abnormal PMCs		% of total abnormal PMCs in all stages	
	05/249 local	05/233 HBP	05/249 local	05/233 HBP	05/249 local	05/233 HBP	05/249 local	05/233 HBP	05/249 local	05/233 HBP	05/249 local	05/233 HBP
	Control	136	130									
0.02 %	131	134	2.12	1.44	1.43	1.44	1.43	0.72	7	5	4.98	3.60
0.04 %	129	137	2.84	1.98	2.04	1.45	1.59	1.42	11	9	6.47	4.85
0.06 %	125	122	3.12	2.48	2.59	2.02	2.43	1.67	14	12	8.14	6.17
0.08 %	128	127	4.50	3.22	3.56	3.01	3.34	2.33	18	15	11.40	8.56

Table 3 Mean (X), shift in mean, SD and CV of various quantitative characters in *Vicia faba* var 05/249 local

Concentrations	Days to flowering		Days to maturity		Plant height		Fertile branches/plant		Pods/plant		Number of seeds/pod		100 Seeds weight (g)		Total plant yield (g)		Pod length	
	Mean±SD	CV, Shift in mean	Mean±SD	CV, Shift in mean	Mean±SD	CV, Shift in mean	Mean±SD	CV, Shift in mean	Mean±SD	CV, Shift in mean	Mean±SD	CV, Shift in mean	Mean±SD	CV, Shift in mean	Mean±SD	CV, Shift in mean	Mean±SD	CV, Shift in mean
<i>Var:05/local</i>																		
Control	49.67±0.18	1.45, -	109±1.08	3.81, -	71.33±3.23	17.66, -	9.40±3.35	124.47, -	17.87±2.77	59.99, -	3.13±0.13	16.48, -	33.31±0.24	2.79, -	15.60±2.30	57.08, -	5.32±0.16	12.04, -
0.0.2%EMS	50.93±0.23	1.73, + 1.26	109.00±1.00	3.55, + 0.33	49.53±5.18	40.54, +21.8	10.27±3.02	113.98, + 0.87	20.33±3.59	67.78, + 2.46	2.33±0.21	34.99, +0.80	33.34±0.24	2.79, + 0.03	15.18±2.45	62.63, +0.42	5.11±0.17	12.91, +0.21
0.04% EMS	51.06±0.21	1.56, + 1.39	110.00±1.19	4.21, + 0.67	63.47±3.02	18.48, +7.86	7.40±0.99	51.53, +2.00	24.33±3.54	56.39, +6.46	2.60±0.19	28.33, +0.53	33.65±0.22	2.79, +0.34	20.98±2.60	47.83, +5.32	4.70±0.24	20.14, +0.60
0.06% EMS	52.00±0.22	1.62, +2.33	111.33±1.14	3.97, +2.00	63.93±2.55	15.48, -7.40	7.20±1.19	63.90, -2.20	34.73±9.04	100.74, +16.86	2.53±0.16	25.26, -0.60	33.51±0.25	2.53, 0.20	29.48±7.62	89.44, +13.88	4.72±0.23	18.98, +0.60
0.08% EMS	52.87±0.27	2.00, +3.20	110.67±1.08	3.99, +1.34	56.60±2.41	16.53, -14.73	9.60±0.87	35.17, +0.20	43.00±10.68	96.14, +25.13	2.20±0.22	39.17, -0.93	33.71±0.22	2.56, 0.40	37.56±8.33	85.87, +21.96	4.60±0.18	15.28, -0.72
LSD (P= 0.05) (*)	0.62		3.12		9.68		6.09		19.12		0.53		0.66		14.62		0.59	
LSD (P= 0.01) (**)	0.82		4.15		12.88		8.11		25.43		0.7		0.88		19.46		0.57	

Table 4 Mean (X), shift in mean, SD and CV of various quantitative characters in *Vicia faba* var 05/233 HBHP

Concentrations	Days to flowering		Days to maturity		Plant height		Fertile branches/plant		Pods/plant		Number of seeds/pod		100 Seeds weight (g)		Total plant yield (g)		Pod length	
	Mean±SD	CV, Shift in mean	Mean±SD	CV, Shift in mean	Mean±SD	CV, Shift in mean	Mean±SD	CV, Shift in mean	Mean±SD	CV, Shift in mean	Mean±SD	CV, Shift in mean	Mean±SD	CV, Shift in mean	Mean±SD	CV, Shift in mean	Mean±SD	CV, Shift in mean
<i>Var:05/233HBHP</i>																		
Control	45.08±0.20	1.68, -	102.27±0.23	0.86, -	70.46±3.95	21.71, -	10.47±1.58	58.44, -	18.67±3.65	75.79, -	3.13±0.01	16.48, -	34.21±0.26	2.93, -	17.08±3.3	75.63, -	5.23±1.35	14.68, -
0.0.2% EMS	45.00±0.22	1.87, -0.80	104.07±0.23	0.85, +1.80	62.93±5.70	35.07, -7.53	12.26±2.65	83.61, +1.79	25.40±4.37	66.73, +6.73	2.53±0.19	29.33, -0.60	34.38±0.21	2.33, +0.17	22.68±5.86	71.17, +5.60	5.14±1.32	12.92, -0.09
0.04% EMS	45.73±0.23	1.92, -0.07	103.87±0.19	0.72, +1.60	48.02±8.67	69.58, -22.26	7.40±1.15	60.16, -3.07	24.80±3.62	56.45, +6.13	2.47±0.17	25.94, -0.66	34.25±0.30	3.37, +0.04	23.13±3.36	56.16, +6.05	4.68±0.25	19.88, -0.55
0.06% EMS	45.00±0.22	1.87, -0.08	103.80±0.20	0.75, +1.53	42.20±5.08	46.67, -28.26	8.20±1.36	64.39, -2.27	27.67±5.47	76.64, +9.00	2.00±0.24	46.29, -1.13	34.09±0.22	3.08, -0.21	28.58±6.93	93.78, +11.50	4.76±0.25	19.10, -0.47
0.08% EMS	44.80±0.22	1.92, -	103.03±0.16	0.62, +0.76	36.20±3.13	33.46, -34.26	14.27±2.79	75.79, +3.80	32.73±3.29	38.98, +14.06	2.07±0.21	38.65, -1.06	34.53±0.22	2.74, +0.32	27.95±3.06	42.40, +10.87	4.47±0.20	17.71, -0.76
LSD (P= 0.05) (*)	0.62		0.58		15.94		5.72		11.76		0.54		0.72		12.46		0.86	
LSD (P= 0.01) (**)	0.82		0.77		21.2		7.61		15.64		0.72		0.96		16.57		1.18	

aim for legumes breeders, yield is a composite character and, therefore, can be manipulated through the various components characteristics. Thus manipulation of plant structural component to induce desirable alternations in the yield components provides valuable materials for the breeders. A range of genetic variability was induced in the present study, several of which are useful from the breeder's point of view.

#### Studies on quantitative characters in $M_2$

Mean and coefficient of variation for nine quantitative traits of faba bean provide ample evidence that mutagenic treatments could alter mean values and create additional genetic variability for polygenic traits. Wani and Khan (2006) reported variable response of quantitative characters to various mutagenic treatments in *Vigna radiata*. The extent of variation in mean values and CV was not same in two varieties showing the varietal differences. Growth and yield parameters were affected by EMS in various ways. Higher concentration of mutagen produced adverse effects on all the traits on the other hand; lower concentrations of mutagen had significant effects on them. Beneficial effects of mutagens when applied at lower doses have earlier been recorded in a number of crops (Khan and Wani 2006, Wani and Khan 2007). Pleiotrophic effect as has been reported by Fatma (2007) also succeeded in inducing the leaf mutation in faba bean similar to the present finding. Bushy plants characterized by increase in branching have better yield potential because of their greater number of nodes and consequently increase in number of fruits and seeds.

#### Cytological studies

Meiosis was normal in the control plants. However, a number of meiotic aberrations were recorded in plants raised from seeds treated with EMS which increased with mutagen concentrations. Most of the abnormalities were present in all the treatments, precocious movement of chromosomes were dominant at metaphase (Fig 1 A and B). Among the abnormalities at anaphase, laggards, bridges and stickiness (Fig 1 C, D, E and F) were dominant, whereas dominant telophasic abnormalities were disturbed polarity and multinucleate condition (Fig 1 G and H). By EMS treatments, the percentage of abnormal PMCs ranged from 4.98-11.40% in var 05/249 local and 3.60-8.56% in var. 05/233 HBP (Table 2). Precocious movement of chromosomes might have occurred due to disturbed homology for chromosome pairing, disturbed spindle mechanism or inactivation of spindle mechanism (Agarwal and Ansari 2001). Laggards were present in almost all treatments and occurrence of laggards in the present study has also been reported earlier by many workers such as Iqbal and Dutta (2007) in *Withania somnifera* (L.) Dun, Khan *et al.* (2009). Delayed terminalisation and/or failure of chromosomal movement, following spindle fiber discrepancies have lead to lagging

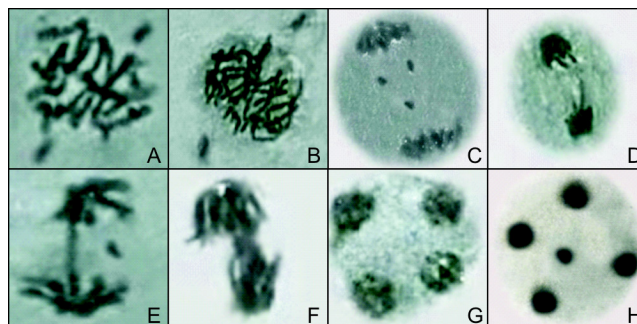


Fig 1 (A-H) Meiotic Aberrations Induced by EMS in two varieties of *Vicia faba*, A: Metaphase-I: Precocious separation of chromosomes in var. 05/249 local. (0.02% EMS), B: Metaphase-I: Precocious separation of chromosomes in var. 05/233 HBP. (0.06% EMS), C: Anaphase-I: Stickiness and laggards in var. 05/249 local. (0.08% EMS), D: Anaphase-I: Stickiness and broken chromatin bridge in var. 05/233 HBP. (0.04% EMS), E: Anaphase-I: Chromatin bridge and laggard in var. 05/249 local. (0.06% EMS), F: Anaphase-I: Stickiness in var. 05/233 HBP. (0.02% EMS), G: Telophase-II: Disturbed polarity in var. 05/249 local. (0.08 % EMS), H: Telophase-II: Multinucleate condition in var.05/233 HBP. (0.04% EMS).

chromosomes. Stickiness among the chromosomes occurred at metaphase and anaphase I and II stages. Stickiness was a common meiotic abnormality reported by various workers such as Chidambaram *et al.* (2008). Rao and Lakshmi (1980) hold the view that stickiness could be due to disturbances of cytochemically balanced reactions by the effects of alkylating agents. Bridges occurred both at anaphase I and II and telophase I & II stages. Bridges might have occurred as a result of delayed terminalisation, stickiness of chromosome ends, failure of chromosome movement, late terminalisation, unequal separation of chromosomes (Iqbal and Datta 2007). The laggards and non-oriented chromosomes when fail to reach the poles in time to be included in the main telophase nucleus, form micronuclei leading to multinucleate condition. Disturbed polarity has been observed by Sharma *et al.* (2009) in *Vicia faba*. The disturbed polarity at anaphase and telophase stages could be due to spindle disturbances.

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