Int J Pharm Bio Sci 2012 Oct; 3(4): (B) 750 - 756

Research Article **Botany**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ALKYLATING AGENT ETHYL METHANE SULPHONATE (EMS) INDUCED VARIABILITY IN TWO ECONOMICALLY IMPORTANT MUTANTS OF *VICIA FABA* L.

RUBINA PERVEEN*, ALKA AND SAMIULLAH KHAN

Mutation Breeding Laboratory,Department of Botany, Aligarh Muslim University, Aligarh- 202002 (India)

ABSTRACT

Seeds of *Vicia faba* L. varieties 05/249 local and 05/233 HBP were subjected to induce variability in faba bean an economically important crop 0.02%, 0.04%, 0.06%, and 0.08% of ethyl methane sulphonate (EMS). The mutagenic treatments of ethyl methane sulphonate caused biological damage, which was measured by M_1 parameters. Data on various quantitative traits such as, plant height, days to flowering, and days to maturity, pods length, number of fertile branches/plant, number of pods / plant, number of seeds / pod, 100 seeds weight (g) and total plant yield (g) were recorded in both the varieties of faba bean. The variants were morphologically quite distinct, as compared to the control and to each other. The increase in mean value of plant height, fertile branches/ plant and pods/plant were found to be significant in both the varieties, though seeds/ pod differed from the control but the values were insignificant . Number of seeds/pod and 100 seeds weight did not show any significant difference. The total plant yield did not show significant in var. 05/233 HBP but it is found significant in var. 05/249 local.

KEY WORDS: ***Vicia faba,*** **EMS, quantitative traits**

RUBINA PERVEEN
Mutation breeding Laboratory,Department of Botany, Aligarh Muslim University, Aligarh- 202002 (India)

Corresponding author

INTRODUCTION

The pulse crops can improve soil fertility by fixing atmospheric nitrogen and increase inorganic matter of the soil by adding leaves and other plant parts. In order to achieve higher production of pulse crops, Cultivated faba bean is used as human food in developing countries. It can be used as vegetables either green or dried, fresh or canned. It is a common breakfast food in the Middle East Mediterranean region, China and Ethiopia (Bond et al., 1985). It is one of the most important winter crops for human consumption in the Middle East. Wide variation of protein content (20-41%) has been reported (Chaven et al., 1989). Protein concentration is influenced by both genetic and environmental factors and it has been reported that inheritance of this trait is additive with some partial dominance (Bond et al., 1985). Amino acid content as mg/g of nitrogen varies from 37-69 mg for methionine, 44-49mg for cystine and 333-400mg for lysine (Chevan et al., 1989). Legumin is the predominant globulin and has a larger proportion of arginine, threonine and tryptophan (Hulse, 1994). The whole dried seeds contain (per 100g) 344 calories 10.1% moisture, 1.3g fat, 59.4g total carbohydrates, 6.8g fibre, 3.0g ash, 104mg Ca, 301mg P, 6.7mg Fe, 8mg Na, 1123mg K, 130µg β carotene equivalent, 0.38 mg thiamine, 0.24 mg riboflavin, 2.1mg niacin, and 162mg tryptophan. Flour contain: 340 calories, 12.4% moisture, 25.5g protein, 1.5g fat, 58.8g total carbohydrates, 1.5g fibre, 1.8g ash, 66mg Ca, 354mg P, 6.3mg Fe, 0.42mg thiamine, 0.28mg riboflavin, and 2.7mg niacin, The amino acid content except for methionine is reasonable and well balanced (Bond et al., 1985).

Induced mutagenesi may be of special use for broadening the genetic variability of quantitatively inherited characters. The mutagenic treatments induced mutation affecting plant height, growth habit, branching and stem structure, leaf morphology, inflorescence, calyx, flower, pod, fertility and seed colour have been reported in faba bean (Sinha et al., 1987: Tyagi and Gupta, 1991; Ashutosh and Dubey, 1992; Vanadana et al., 1994; Ramesh and Dhananjay, 1996; Tyagi and Ramesh 1998; Solanki and Sharma, 1999; Jeena and Singh 2000). Therefore the planned mutagenesis can create more variability with efficient selection procedure;It is possible to get a desirable stuft in the mean values of characters under consideration. Against this background, the aims of present study are to study the biological damage, spectrum of induced variability and the shift in means for quantitative traits as compared to the control in M1 generation.

MATERIALS AND METHODS

Two varieties of faba bean (*Vicia faba* L.) namely, 05/249 local and 05/233 HBP were used in the present study. Seeds of both varieties were obtained from Genetic Section of India Agricultural Research Institute of New Delhi. Certified 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 sodium phosphate buffer at 7.0 pH for 6h 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 kept untreated to act as control for comparison. Seeds were sown in the earthen pots of 30 cm diameter. Seedling survival was calculated on the 30^{th} day after sowing in pots while, the plant survival was calculated after transplantation of seedling in field. As soon as, the 5^{th} leaf stage emerged out, the seedling were space transplanted in the field at a distance of 90cm apart in complete Randomized Block Design (CRBD) with 2 replicates in each dose and control to observe number of fertile branches per plants, number of pods per plants, length of

pods, number of seeds per pod, height of plant and total plant yield and plant survival in M_1

RESULT

Data recorded on seed germination of faba bean differed in their response to mutagenic treatments with regard to germination recorded after 10 days of sowing. Treatments 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 control population of both varieties. It ranged 93.33-46.67% in EMS treated population of the var. 05/249 local. It showed that the percentage of seed germination gradually decreased with increasing concentration of mutagens.

Plant survival at maturity and lethality percentage (Table 1)

Mutagenic treatments of EMS showed differing effects on the plant survival in var.05/249 local and var. 05/233 HBP, lowest percentage of plant survival at maturity was found at 0.08% of EMS treatment. The highest percentage of plant survival was found in control in both varieties. At highest concentration of mutagen (0.08% EMS), the percentage of plant survival at maturity was 59.00% in var.05/259 local and 48.00% in var. 05/233 HBP. The lethality was found highest in both varieties at 0.08% 0f EMS treatments.

Pollen fertility and sterility (Table 1)

Pollen character is one the important stable and genetically controlled character. The pollen fertility was highest in 0.02% EMS treatment in both the varieties, however, the decreased with an increase in concentration of mutagen and the lowest percentage of pollen fertility was found in 0.08% of EMS treatment in both the varieties. A progressive increase in EMS concentrations was found with an increase in pollen sterility in both the varieties. The highest sterility was -8.39 and -7.54at 0.08% EMS treatment in varieties 05/249 local and 05/233HBP respectively.

Table 1

Effect of mutagens on Seed germination, Plant survival, Pollen fertility and Seedling height in two varieties in Vicia faba L.

Concentration	Seed germination		Plant survival at maturity (%)	Pollen fertility		Seedling height (% age injury)
	Actual (%)	% age inhibition		Actual (%)	% age inhibition	
Var.05/249local						
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
Var.05/233HBP						
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

Mean (X), shift in mean, S.D. and C.V. of various quantitative characters in Vicia faba var. 05/249 local and 05/233 HBP

Concentrations	Days to Flowering Mean± SD CV, Shift in mean	Days to maturity Mean± SD CV, Shift in mean	Plant height Mean± SD CV, Shift in mean	Fertile branches/plat Mean± SD CV, Shift in mean	Pods/plant Mean± SD CV, Shift in mean	Number of Seeds/pod Mean± SD CV, Shift in mean	100 Seeds Weight (g) Mean± SD CV, Shift in mean	Total plant Yield (g) Mean± SD CV, Shift in mean	Pod length Mean± SD CV, Shift in mean
Var.05/local									
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±00.21 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
L_{SD} at 5 % (*)	**0.62**	**3.12**	**9.68**	**6.09**	**19.12**	**0.53**	**0.66**	**14.62**	**0.59**
L_{SD} at 1 % (**)	**0.82**	**4.15**	**12.88**	**8.11**	**25.43**	**0.70**	**0.88**	**19.46**	**0.57**
Var.05/233HBP									
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
L_{SD} at 5 % (*)	**0.62**	**0.58**	**15.94**	**5.72**	**11.76**	**0.54**	**0.72**	**12.46**	**0.86**
L_{SD} at 1% (**)	**0.82**	**0.77**	**21.20**	**7.61**	**15.64**	**0.72**	**0.96**	**16.57**	**1.18**

Days to flowering (Table 2)
Most of the mutagenic treatments were found enhancing with regard to days to flowering in both the varieties.

Days to maturity (Table)
Most of the treatments of EMS brought about a significant decrease in days to maturity in both the varieties of faba bean.

Plant height (Table 2)
Significant difference in plant height was recorded in var. 05/249 local for all mutagenic treatments. But significant difference was found in plant height for 05/233HBP of faba bean.

Fertile branches/plant (Table 2)
Most of treatment of EMS brought about a significant increase in fertile branches/plant in both the varieties.

Pods/plant (Table)
Significant difference in pods/ plant was recorded in all mutagenic treatments of EMS in both the varieties.

No. of seeds/pod and 100 seeds weight (g) (Table2)
No significant difference in number of seeds/pod and 100-seeds weight (g) was recorded in both the varieties of faba bean.

Total plant yield (g) (Table)
No significant difference in total plant yield was recorded in var. 05/233 HBP. But all treatments of EMS brought about significant difference in total plant yield in var. 05/249 local.

DISCUSSION

These two varieties of faba bean differed in response of chemical mutagens are known to produce adverse effects on germination seedling growth and plant growth in M_1 generation. Delayed maturity, varying degrees of sterility, and reduced survival are other features recorded in M_1 generation after mutagen treatments (Blixit, 1960; Sjodin, 1962; Nerkar, 1970; Goud, 1972; Sinha and Godward, 1972; Dixit and Dubey, 1981; Parveen, 2004; Fatma, 2007). The chemical mutagen applied during present study produces adverse effects of germination, seedling height, pollen fertility, and plant survival at maturity.

The dose dependent pollen sterility with increase in mutagenic concentration was observed in present study. Similar results were also reported by Vandana and Dubey (1988), Fatma (2007) in *Vicia faba* and Khan et al., (2000) in *Vigna radiate*. The lower concentration of mutagens showed less pollen sterility compared to the higher concentrations. It may be concluded that such mutagenic treatments could be used favourably for increased mutation rate and obtaining desirable spectrum of mutation in faba bean.

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 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 wide range of morphological variations was induced in the present study several of which are useful from the breeder's point of view. The differences in the frequencies of leaf mutations may be due to the number of genes with pleiotrophic effect as has been reported by Sjodin (1974), Rao Jana (1976), Fillppetti an De pace (1986) and Fatma (2007) also succeeded in inducing the leaf mutation in faba bean similar to the present finding. Bushy plants characterized by increased branching have better yield potential because of their greater number of nodes and consequently increased number of fruits and seeds.

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. Khan (1990) and Wani and Khan (2006) reported variable response of quantitative characters to various mutagenic treatments in *Vigna radiate*. The extent of variation in mean values and CV was not same in two varieties showing the varietals differences. Variety 05/233HBP was found to be more sensitive than 05/259 local. Growth and yield parameters were effected by EMS in various ways. Higher concentration of mutagens produced adverse affects on all the traits. On the other hand, lower concentration of mutagens had no significant adverse effects on them. Growth promoting effects of mutagens when applied at low doses have earlier been recorded in a number of crops (Sax, 1963; Singh et al., 1978, Venkteshwarlu et al., 1978; Trivedi and Dubey, 1998; Khan and Wani 2004; Khan and Wani 2006; Wani and Khan, 2007).

REFRENCES

1. Ashutosh, T and D.K. Dubey. (1992). Effects of Separate and Simuitaneous Application of Gamma Rays and N-nitroso-N-methyl urea on Germination, Growth, Fertlility and Yeild of Two Lentil Varieties. LENS Newsletter 19(1): 9-13.
2. Blixit, S.; Ehrenberb, L. and Gelin, O. (1960). Quatitative studies of induced mutation in peas, III. Mutagenic effects of ethylene imine. Agric. Hort. Genet. 18: 109-123.
3. Bond, D.A.; Lawes; G.C. Hawtin. M.C. Saxena, and J.s. stephens (1985). Faba bean (Vicia faba L.). pp.199-265. In: R.J. Summerfield and E.H. Robert (etds.) Grain Legumes Crops. William Collins sones Co. Ltd. 8 Grafton street, London WIX 3LA, UK.
4. Chavan, J.K., L.S. Kute and S.S. Kadam (1989). In: CRC Hand book ofworld Legumes.pp. 223-245. D.D. Salinkhe and S.S. kadam (eds.), Boca Rataon, Florida, USA: CRC Press.
5. Dixit, P. and Dubey, D.K.(1981). Studies on the effect of separate and simultaneous application of gamma rays and NMU on lentil (*Lens culinaris* Medic). 1. Germanation, growth, fertility and yield. Bot. Progress 4: 10-15.
6. Fatma, S. (2007). Studies induced variability in faba bean. M.sc. dissertation, Aligarh Muslim University, Aligrh
7. Fillipetti, A. and C. De Pace (1986). Improvement of seed yield in *Vicia faba* L. by using experimental mutagenesis. II. Comparison of gamma radiation and ethyl methane sulphonate (EMS) in production of morphological mutant. Euphytica 35: 49-59.
8. Hulse, J.H. (1994). Nature, Composition and utilization of food legumes. 77-79. In: F.J. Muehlbauer and W.J. Kaiser (eds.), Expanding the production and use of cool season Food Legumes. Kluwer Acedemic Publisher, Dordrecth. The Netherlands.
9. Jeena, A.S. and I.S. Singh. (2000). Field evalution of of wild relatives of lentil. Indian J.Puelses Res. 13(1): 50-51.
10. Khan, S. (1990). Studies on chemical mutagenesis in mutagens [*Vigna radiate* (L.) Wilczek]. Ph.D. thesis, Aligarh Muslim University, Aligarh
11. Khan, S. and M.R. Wani (2004). Studies on the effect of EMS and MMS on biological damage and quantitative characters of mungbean. VEGETOS 17: 15-20.
12. Khan, S. and Wani, M.R. (2006). MMS and SA induced genetic variability for quantitative traits in mungbean. Indian J. Pulses Res. 19 (1): 50-52.
13. Khan, S.; M.R. Wani and K. Perveen. (2007). Sodium azide induced high yielding early mutant in lentil. Agric. Sci. Digest. 26: 65-66.
14. Khan, S: Rehman, M.; M. bhat and B.A. Siddiqui (2000). MMS induced biological damage and polygenic variability in green gram [*Vigna radiate* (L.) Wilczek]. 23 (2): 126-129.

15. Nerker , Y. S. (1970). Study on the induction of mutation in *Lathyrus sativus* with special reference to the elimination of neurotoxic principle. Ph.D. Thesis, IARI, New Delhi.
16. Parveen, K. (2004). Studies on the induction of polygenic variability in chick pea (*Cicer arietinum* L.). M.phil dissertation, Aligarh. Muslim. University, Aligarh
17. Ramesh, B. and S. Dhananjay. (1996). Developmental morphology of morphology of induced semidwarf and stunted mutants in lentil. Indian Journal of Genetics and Plant Breeding. 56 (3): 335-340.
18. Rao, S.A. and Jana (1976). Leaf mutayions induced in black gram by X rays and EMS. Environmental and Experimental Botany 16: 151-154.
19. Sax, K. (1963). The stimulation of plant growth by ionizing radiations. Radioation Botany. 3: 179-186.
20. Singh, R.B.; B.D. Singh; R.M. Singh and Vijay Laxmi (1978). Seedlong injury, pollen sterility and morphological mutations induced by gamma rays and EMS in pearl millet. Indian Journal of genetic and Plant Breeding 38 (3): 380-389.
21. Sinha, S.S. and V.K. Singh (1987). Radiation studies in *Lens culinaris.* Effect of acute gamma radiation on germination, growth and survival. Proc Natl Symposium or Cytogenetic Research in India- An Appraisal. pp 72, Patna.
22. Sinha, S.S.N. and M.B.E. Godward, (1972). Radiation studies in *lens culinaris*. Indian Journal of Genetics and Plant Breeding 32: 331-339.
23. Sjodin, J. (1962). Some observation in X_1 and X_2 of *Vicia faba* L. after treatments with the different mutagens. Hereditas 48: 565-586.
24. Solanki, I.S. and B. Sharma. (1999). Induction and isolation of morphological mutations in different mutagenic damage groups in lentil (*Lens culinaris* Medik). Indian J. Genet. 59(4): 479-485.
25. Trivedi, S.C. and D.K. Dubey (1988). Effect of gamma rays on seed germination and seedling growth in *Triticale* var. Rahun. Abstract in proceeding of the 69th Indian science congress part III-24.
26. Tyagi, B.S. and P.K. Gupta. (1991). Induced macromutation in Lentil. LENS. 18: 3-7.
27. Tyagi, N.K. and B. Ramesh. (1998). Characteristic and Development Morphology of Reduced Plant Height Mutants in Lentil. LENS Newsletter. 25 (1&2): 6-10.
28. Vanadana and D.K. Dubey (1988). Effect of ethyl methane sulphonate (EMS) and diethyl sulphate (DES) on germination, growth fertility and yield of *Vicia faba* L. FABIS NEWSletter 20: 25-29.
29. Vandana ; A. Tripathi and D.K. Dubey. (1994). Frequency and spectrum of mutations induced by ethylmethane sulphonate (EMS) and diethyl sulfate (DES) in lentil var. K-85. LENS, 21: 16-19.
30. Venkateshwarlu, S.; R.M. Singh abd B.D. Singh (1978). Radiosensitivity and frequency of chlorophyll mutations in pigeon pea. Indian Journal of Genetics and plant Breeding 38: 90-94.

Wani, M.R. and s. Khan (2006). Estimates of genetic variability in mutated population and the scope of selection yield attributes in *Vigna radiata* (L.). wilezzek. Egyptian Journal of Biology. 8: 1-6.