Induced Genetic Variability through Physical and Chemical Mutagens in M_3 and M_4 Populations of Frenchbean (*Phaseolus vulgaris* L.)

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Rajmash (*Phaseolus vulgaris* L.), also known as rajma, French bean, kidney bean, common bean etc. is a popular pulse crop at hills and hill valleys but not so popular in the plains. Out of the two cultivars, 'Udai' and 'Malviya Rajmash 137' released for cultivation in plains, the cultivar 'Udai' is not widely accepted because of its shattering of pods at maturity, variegated seed colour and low yield as compared to Malviya Rajmash 137. The pure and healthy seeds of the variety 'Udai' were irradiated with gamma rays (5, 10, 15, 20 and 40 kR doses), ethyl methane sulphonate (10, 20, 40, 60 and 80 mM concentrations) and their combinations (5 kR + 10 mM, 10 kR + 20 mM, 15 kR + 40 mM, 20 kR + 60 mM and 40 kR + 80 mM doses/concentrations). In the present investigation, the dose of 15 kR was found to be most efficient among the different doses of gamma rays with few exceptions. In case of the EMS, the concentration of 20 mM was found to be the best. The most efficient treatment among the combination was found to be 10 kR gamma rays + 20 mM EMS.

Key Words: French bean, Genetic variability, Mutagens

Introduction

Pulses, also known as grain legumes, are an important group of crops, next to cereals. Legumes include the species of the family leguminoceae which are consumed directly by human beings, mostly as mature dry seeds, but occasionally as immature green seeds or green pods with immature seeds enclosed. Traditionally pulses have been cultivated in rainfed conditions due to some wrong notion that they are meant for poor and marginal lands and can be grown without much care and input. Thus, these energy rich crops are being cultivated largely under energy starvation conditions. With the development of high yielding varieties of cereals and oilseeds, pulses are being pushed more and more towards marginal areas. The non-availability of high yielding varieties and lack of protection against biotic and abiotic stresses compounding the problem (Asthana, 1998; The Times of India, 2001). The 146 legume species available worldwide, about 80 species are consumed by human and only 14 of them are of major economic importance. Among the different pulse crops grown in India, the most important are chickpea and pigeon pea followed by urdbean, mungbean, lentil, pea, cowpea, lathyrus, horsegram and rajmash. Rajmash (*Phaseolus vulgaris* L.) also known as rajma, frenchbean, kidney bean, haricut bean, navy bean, common bean etc. is grown as legume crop, mainly at hilly tracts of India. The crop is not so popular in plains and grown during Rabi season as a minor crop.

Materials and Methods

Present investigation was carried out at the Crop Research Centre, Institute of Agricultural Sciences, Banaras Hindu University during 2001-03. Seeds of Frenchbean (Rajmash) cultivar 'Udai' was treated with two mutagens, *viz*., gamma rays and ethyl methane sulphonate (EMS) singly as well as in combination. The different doses/ concentrations of gamma rays and ethyl methane sulphonate used for seed treatment are as five doses of gamma rays *viz.*, 5 kR, 10 kR, 15 kR, 20 kR and 40kR, five concentrations of ethyl methane sulphonate *viz.* 10, 20, 40, 60 and 80 mM and also combination treatments of both the mutagens were used. The total sixteen treatments including control *viz.* T_1-5 kR γ rays, T_2-10 kR γ rays, T_3-15 kR γ rays, T_4-20 kR γ rays, T_5-40 kR γ rays, T_6-10 mM EMS, T_7-20 mM EMS, T_8-40 mM EMS, T_9-60 mM EMS, T_{10}-80 mM EMS, T_{11}-5 kR γ rays + 10 mM EMS, T_{12}10 kR γ rays + 20 mM EMS,

T_{13}-15 kR γ rays+40 mM EMS, T_{14}-20 kR γ rays + 60 mM EMS, T_{15}-40 kR γ rays + 80 mM EMS and T_{16}–Control. Seeds harvested in M_2 generation from the above mentioned treatments and also seeds of different macro-mutants selected in M_2 generation were used as a experimental materials for study in M_3 generation. The following viable macro-mutants were detected in M_2 generation are dwarf mutant, early maturing mutant, late maturing mutant, non-shattering mutant, high yielding mutant, light seed coat colour

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mutant and dark seed coat colour mutant. The M_2 seeds were space planted in three replications to raise M_3 generation. Seeds of viable macro-mutations were also space planted in three replications individually. The population was screened for macro-mutations in M_3 generation.

Fifty competitive normal looking plants from each replication of each treatment were taken randomly to record the observation. The observations were recorded on selected plants for the following parameters, v*iz.* plant height, number of primary branches per plant, days to flowering, days to maturity, number of pods per plant, length of pod, number of seeds per plant, number of seeds per pod, yield per plant and 100-seed weight.

The M_3 seeds were space planted in three replications to raise M_4 generation and observations were recorded on selected plants for the same parameters as given in M_3 generation.

The statistical analysis for M_3 and M_4 generations was done for analysis of variance and other genetical parameter, *viz.*, Genotypic co-efficient of variation (GCV), Phenotypic co-efficient of variation (PCV), Heritability (h^2) and Genetic Advance (GA).

Results and Discussion

Analysis of Variance (ANOVA)

The analysis of variance for the ten quantitative characters under study in M_3 and M_4 generation revealed that the treatments differed significantly for all the characters studied in both the generations. The analysis of variances has been presented for both the generations for micro mutations and macro (viable) mutants in Table 1 and 2, respectively.

It is obvious from the ANOVA that the treatments were found significant for all the quantitative traits under study in micro mutations in both the generation, *i.e.*, M_3 and M_4 generations. It is also obvious from the ANOVA that the treatments were found significant for most of the quantitative traits under study in M_3 generation except average length of pod and in M_4 generation except average length of pod and 100-grain weight for macro mutants.

Performance of Viable Macro-mutants (Selected from M_2 Generation) in M_3 and M_4 Generations

A number of viable mutations for different morphological and yield traits were evaluated in M_3 and M_4 generations. These included mutations for plant height, earliness, non-shattering, high-yielding, lateness and seed coat colour. The mutations can be categorized into phenological and morphological mutants. Mutation affecting the time taken to flowering and maturity are grouped in to phenological while; those affecting the various plant parts constituted the morphological types (Table 3, 4).

Dwarf Mutant

The value of mean for plant height in control was 41.43 cm in M_3 generation. The plants with the height between 20-30 cm were grouped in to dwarf mutant category. The performance of dwarf mutant in M_3 generation for plant height was recorded with mean value of 27.73 cm which decreased significantly from the control and about same performance was found in M_4 generation whereas, mean value for plant height in control was recorded 43.17 cm and for dwarf mutant progeny it was recorded 27.60 cm. In M_3 this mutant showed a

Table 1. Analysis of variance for ten quantitative traits in Frenchbean cv. 'Udai' for micro-mutations in M_3 and M_4 generations

1	2	3	4								
Generation	Source	DF	Mean Sum of Squares (MSS)								
			Characters								
			Plant height	No. of primary branches per plant	No. of pods per plant	Average length of pods	No. of seeds per plant	No. of seeds per pod	Days to flowering	Days to maturity	100-grain weight	Grain yield per plant
M_3	Replication	2	0.0722	0.0764	0.2238	0.0087	1.251	0.0018	0.1171	0.3750	1.173	0.0505
	Treatments	15	16.81**	0.3141**	2.359**	0.8396**	27.42**	0.0428**	9.337**	10.36**	0.8289**	1.924**
	Error	30	0.2588	0.0191	0.0824	0.0344	0.6843	0.0071	0.2236	0.4138	0.1809	0.0484
M_4	Replication	2	0.0156	0.0308	0.0205	0.0173	0.4238	0.0012	0.8203	1.093	0.1230	0.0190
	Treatments	15	26.65**	0.3208**	2.842**	0.1314**	15.76**	0.241**	9.211**	10.95**	1.173**	1.20**
	Error	30	0.4789	0.0361	0.0575	0.0175	0.3853	0.0038	0.2554	0.2798	0.2164	0.0175

F Table Value at 1% = 4.31; ** Significant at 1%, level of significance

Table 2. Analysis of variance for 10 quantitative traits in Frenchbean cv. 'Udai' for macro-mutations in M_3 and M_4 generations

1	2	3	4									
Generation	Source	DF	Mean sum of squares (MSS)									
			Characters									
			Plant height	No. of primary branches per plant	No. of pods per plant	Average length of pods	No. of seeds per plant	No. of seeds per pod	Days to flowering	Days to maturity	100-grain weight	Grain yield per plant
M_3	Replication	2	2.794	0.0162	0.1588	0.0920	2.026	0.0087	0.4609	1.984	0.4326	0.0649
	Treatments	7	50.92**	0.7561**	8.52**	0.1455	110.23**	0.073**	20.23**	21.82**	1.564**	7.157**
	Error	14	0.6493	0.0343	0.1501	0.0775	2.234	0.0065	0.7446	1.110	0.2639	0.1574
M_4	Replication	2	1.444	0.0650	0.0112	0.1771	1.471	0.0133	0.8554	0.0312	0.2617	0.0426
	Treatment	7	60.04**	0.6580**	10.93**	0.0983	122.33**	0.0279*	17.82**	20.848**	1.446	8.834
	Error	14	0.4001	0.0407	0.1433	0.0815	2.383	0.0095	0.5522	0.3750	0.6820	0.0928

F Table value: At 1% level of significant=4.31; ** Significant at 1% level of significance; At 5% level of Significant =2.07; * Significant at 5% level of significance

Table 3. Mean performance of macro-mutants of Frenchbean cv. 'Udai' in M_3 generation

	Characters									
Control/macro-mutants	Plant height (cm)	No. of primary branches per plant	No. of pods per plant	Average length of pod (cm)	No. of seeds per plant	No. of seeds per pod	Days to flowering	Days to maturity	100-grain weight (g)	Grain yield per plant(g)
Control	41.43	4.20	1060	10.07	28.73	2.73	63.20	124.97	28.45	8.16
Dwarf mutants	27.73**	3.97	9.93*	9.63	25.27**	2.54**	63.93	126.43	28.76	7.27*
Early mutants	36.40**	4.00	9.93*	9.40**	25.57*	2.57*	57.53**	120.50**	26.98**	6.90**
Late mutants	38.80**	4.27	11.37*	9.73	28.73	2.45**	66.20**	130.27**	27.96	8.02
Non-shattering mutants	34.53**	3.60**	8.47**	9.47*	20.40**	2.41**	64.08	125.87	28.82	5.88**
High-yielding mutants	38.97**	5.23**	14.30**	9.43**	41.50**	2.90*	65.10**	125.57	26.99**	11.20**
Seed colour mutants (light)	37.47**	3.77**	10.23	9.57*	26.53	2.59*	64.20	125.10	28.14	7.47*
Seed colour mutants (dark)	37.77**	3.87**	10.37	9.50*	27.23	2.62	64.33	124.70	27.67	7.53
SE (d)	0.6569	0.1513	0.3163	0.2273	1.221	0.066	0.704	0.860	0.4194	0.324
CD (1%)	1.809	0.416	0.8698	0.625	3.355	0.1815	1.936	2.365	1.153	0.891
CD (5%)	1.343	0.3089	0.6458	0.4641	2.491	0.1347	1.437	1.756	0.8564	0661

** Significant at 1% * Significant at 5%

Table 4. Mean performance of macro mutants of Frenchbean co-efficient 'Udai' in M_4 generation

	Characters									
Control/macro-mutants	Plant height (cm)	No. of primary branches per plant	No. of pods per plant	Average length of pod (cm)	No. of seeds per plant	No. of seeds per pod	Days to flowering	Days to maturity	100-grain weight (g)	Grain yield per plant(g)
Control	43.17	4.40	10.83	9.85	28.60	2.63	62.40	123.73	27.22	7.78
Dwarof mutants	27.60**	4.03*	9.80*	9.53	25.70*	2.62	63.97*	125.70*	28.70*	7.37
Early mutants	36.87**	4.23	10.07*	9.57	26.57	2.63	58.32**	120.30**	28.27	7.50
Late mutants	38.60**	4.57	11.50*	9.57	30.57	2.66	66.70**	129.10**	26.75	8.18
Non-shattering mutants	34.80**	3.77**	8.87**	9.32	23.13**	2.61	64.50**	127.00**	28.63**	6.62**
High-yielding mutants	39.43**	5.27**	15.17**	9.73	44.10**	2.90**	64.67**	127.03**	27.68	12.21**
Seed colour mutants (light)	36.57**	4.03*	10.07*	9.57	27.37	2.71	64.27	126.20**	27.48	7.51
Seed colour mutants (dark)	37.63**	4.00*	10.40	9.53	27.83	2.67	63.93*	124.87	27.45	7.64
SE (d)	0.5164	0.1647	0.3155	0.2331	1.26	0.0797	0.606	0.50	0.674	0.248
CD (1%)	1.420	0.4529	0.8676	0.6410	3.465	0.2191	1.666	1.3751	1.853	0.6842
CD (5%)	1.054	0.3363	0.6442	0.4759	2.5729	0.1627	1.237	1.021	1.376	0.508

** Significant at 1%; * Significant at 5%

significant decrease in yield per plant but in M_4 generation it was observed non significant from the control.

In present investigation, the dwarf mutants (detected in M_2 generation) raised in M_3 and M_4 generation to confirm their performance and it is evident from experimental findings that mean performance for plant height recorded in M_3 and M_4 generation in agreement with the hypothesis. Egiazaryan (1981) and Fadl (1988) also observed mutant for plant height after treatment of seeds of Frenchbean with gamma rays and ethyl methane sulphonate.

Early Maturing Mutant

The mean value for days to maturity was recorded 120.5.days in M_3 which significantly decreased from the mean value of control (124.97). In M_4 generation it was recorded (120.3 days) for the mutant progeny which also significantly low from control (123.73 days). But this mutant was significantly low for grain yield per plant in M_3 generation whereas, in M_4 it was non significant for the same.

Late Maturing Mutant

The mean performance for days to maturity were recorded with 130.27 days and 129.10 days in M_3 and M_4 generations for mutant progeny which were significantly higher than the mean of control 124.97 days and 123.73 days in M_3 and M_4 generations, respectively. There was an increase in mean values for grain yield per plant (8.18 g) from the control (7.78 g) in M_4 generation but increase was non-significant. Increase in yield was recorded due to significant increase in number of pods per plant (11.50) from the control (10.83) in M_4 generation. In M_3 generation mean value for grain yield per plant (8.02 g) was recorded which was no significantly low from the control (8.16 g). A number of workers have also reported about early maturing mutants in Frenchbean after treatment with mutagens (Kimani, 1988; Manrique *et al*., 1988; Zagorcheva and Paryazov, 1983; Navale *et al*., 1998; Tulmann, 1994).

In present study, it was obvious from the result that early maturing mutant progenies were found earlier but significantly low yielder than the control. Hence, these mutants were not advantageous whereas, late maturing mutant progenies were found late and non-significantly high yielder due to increase in number of pods per plant.

Non-Shattering Mutant

Non-shattering mutants raised in M_3 and M_4 generation in replicated trials in the field which did not shatter even after fifteen to twenty days of maturity. Non-shattering mutants were found significantly low yielder (5.88 g and 6.62 g) as compared to be control (8.16 g and 7.78 g) in both the generations due to less number of branches and pods per plant. All plants of mutant progenies were found non-shattering in both the generations.

High Yielding Mutant

Mutants for higher yield raised in M_3 and M_4 generation in replicated trials and mean performance recorded for all quantitative traits in both the generations. These mutant progenies where found significantly high yielder in both the generations. The mean values for yield were recorded 11.20 g and 12.21 g in successive generations respectively which were found significantly in positive direction from the control. This increase in yield was due to significant increase in more number of primary branches per plant and number pods per plant from the control. Similar findings have been reported by Kandelaki (1981) and Nandi *et al*. (1995).

Light Seed Coat Colour Mutant

Mutants that were detected in M_2 generation for light seed coat colour raised in M_3 and M_4 generations in replicated trials in the field. The seed coat colour of this mutant (lighter colour) inherited to the M_3 and M_4 generations as in M_2 generation and for other quantitative traits were found either at par or decrease with the control.

Dark Seed Coat Colour Mutant

Mutant with dark seed coat colour raised in replicated trials in the field to see the performance for the seed coat colour in M_3 and M_4 generations. In case of dark mutant progenies white patches decreased leading to more dark red colour. None of the plants were obtained with change in colour in seeds obtained from all the pods and these mutants also showed same for dark seed coat colour in both the generations.This mutant also found either at par or decrease with the control for other quantitative traits.

Estimates of GCV, PCV, Heritability and Genetic Advance in M_3 and M_4 Generations for Micro Mutations

The estimates of phenotypic and genotypic co-efficient of variation (PCV and GCV), heritability in broad sense and genetic advance have been presented in Table 5.

Invariably, phenotypic co-efficient of variation values were higher than the corresponding genotypic co-efficient of variation in both the generations. Among all the parameters under study, maximum values for GCV and PCV were obtained for number of seeds per plant (11.65 and 12.09) followed by yield per plant (10.53 and 10.93) in M_3 generation and number of pods per plant (9.18 and 9.46) followed by number of seeds per plant (8.22 and 8.52) in M_4 generation. Lower values of GCV and PCV were obtained for days to maturity, 100-grain weight, and days to flowering in M_3 generation and in M_4 generation, it was obtained for days to maturity, 100-grain weight and average length of pod. Almost a similar finding was reported by Arya and Rana (1999) in French bean. Almost all the characters exhibited high values for heritability in both the generations. The highest heritability was recorded for plant height (0.955) followed by days to flowering (0.931), number of seeds per plant (0.929) and grain yield per plant (0.928) in M_3 generation and for grain yield per plant (0.957) followed by plant height (0.948), number of pods per plant (0.942) and number of seeds per plant (0.930) in M_4 generation. The 100-grain weight exhibited the minimum value of heritability (0.544 and 0.596) in both the generations, respectively. The number of seeds per pod also exhibited low values of 0.624 and 0.638 of heritability in M_3 and M_4 generations, respectively. The highest value of genetic advance was scored for number of seeds per plant (5.93) followed by plant height (4.73) days to maturity (3.54) and days to flowering (3.47) in M_3 generation and in M_4 generation it was scored for plant height (5.92) followed by number of seeds per plant (4.5), days to maturity (3.74) and days to flowering (3.42). The minimum values (0.18 and 0.14) of genetic advance was recorded for the number of seeds per pod.

Several reports regarding heritability and genetic advance has been reported in Frenchbean. Singh *et al.* (1994) recorded high values of heritability and genetic advance for yield per plant. Somal *et al.* (1995) found maximum heritability for days to fifty per cent flowering, followed by 100-seed weight and plant height. Nandi *et al.* (1995) found that yield per plant had moderately

Table 5. Estimates of genotypic, phenotypic co-efficient of variation, heritability and genetic advance for micro-mutations of Frenchbean Cv. 'Udai' in M_3 and M_4 generations

Characters	M_3 generations					M_4 generation				
	GCV	PCV	h^2	GA	GA as percent of mean	GCV	PCV	h^2	GA	GA as percent of mean
Days to flowering	2.71	2.81	0.931	3.47	5.40	2.71	2.82	0.924	3.42	5.36
Days of maturity	1.45	1.54	0.889	3.54	2.83	1.51	1.57	0.927	3.74	2.99
Plant height	6.43	6.58	0.955	4.73	12.95	7.81	8.02	0.948	5.92	15.65
No. of primary branches	8.15	8.91	0.837	0.590	15.37	7.07	8.31	0.724	0.54	12.41
No. of pods per plant	9.03	9.50	0.902	1.70	17.62	9.18	9.46	0.942	1.93	18.40
Average lengthof pods	5.57	5.92	0.886	1.00	10.77	2.04	2.47	0.684	0.33	3.46
No. of seeds per plant	11.65	12.09	0.929	5.93	23.14	8.22	8.52	0.930	4.50	16.34
No. of seeds per pod	4.10	5.19	0.624	0.18	6.80	3.11	3.89	0.638	0.140	5.30
100-grain weight	1.58	2.14	0.544	0.71	2.41	1.99	2.58	0.596	0.90	3.17
Grain yield per plant	10.53	10.93	0.928	1.57	20.90	8.06	8.24	0.957	1.27	16.32

Table 6. Estimates of genotypic, phenotypic co-efficient of variation, heritability and genetic advance for macro-mutants of Frenchbean Cv. 'Udai' in M3 and M_4 generations

Characters	M_3 generations					M_4 generation				
	GCV	PCV	h^2	GA	GA as percent of mean	GCV	PCV	h^2	GA	GA as percent of Mean
Days to flowering	4.01	4.23	0.897	4.97	7.82	3.77	3.95	0.912	4.72	7.42
Days to maturity	2.09	2.26	0.861	5.02	4.00	2.08	2.14	0.948	5.24	4.17
Plant height	11.17	11.39	0.963	8.27	22.57	12.11	12.23	0.980	9.09	24.68
No. of primary branches per plant	11.93	12.75	0.875	00.95	23.11	10.58	11.58	0.835	00.85	19.85
No. of pods per plant	15.69	16.10	0.949	03.35	31.45	17.50	17.86	0.960	03.83	35.36
Average length of pods	01.57	03.30	0.226	00.15	01.56	00.79	03.11	0.064	00.04	0.421
No. of seeds per plant	21.43	22.09	0.942	11.99	42.83	21.63	22.27	0.944	12.65	43.27
No. of seeds per pod	05.74	06.53	0.773	00.27	10.38	02.93	04.68	0.392	00.10	3.74
100-grain weight	02.35	02.99	0.622	01.07	03.82	01.82	03.49	0.272	00.54	1.94
Grain yield per plant	19.57	20.22	0.937	03.05	39.10	21.07	21.41	0.969	03.46	42.71

high heritability and genetic advance. High heritability values for days to flowering has also been reported by Somal *et al.* (1997). Jai Dev and Gupta (1997) found high heritability for most of the traits but genetic advance was moderate for different traits in M_2 generation. Similar finding has been reported by Nandi *et al.* (1988) and Dikshit *et al.* (1999). The highest values of heritability and genetic advance for days to flowering followed by days to maturity, yield per plant and 100-grain weight have been reported by Roy (2001). Singh *et al*. (2001) recorded highest estimates of heritability and genetic advance for number of pods per plant, number of seeds per pod and 100-seed weight in Mungbean.

Estimates of GCV, PCV, Heritability and Genetic Advance in M_3 and M_4 Generations for Macro-Mutants

The estimates of phenotypic and genotypic co-efficient of variation, heritability in broad sense and genetic advance have been presented in the Table 6.

Invariably, phenotypic co-efficient of variation values were higher than the corresponding genotypic co-efficient of variation. Among all the parameters under study, maximum values for GCV and PCV were obtained for number of seeds per plant (21.43 and 22.09) followed by grain yield per plant (19.57 and 20.22) in M_3 generation and in M_4 generation, it was found for the same characters but values were (21.63 and 22.27) followed by (21.07 and 21.41). Low values of GCV and PCV were obtained for average length of pods, 100-grain weight and days to maturity in both the generations. Almost all the characters exhibited high values for heritability in both the generations. The highest heritability was recorded for plant height (0.963) followed by number of pods per plant (0.949), number of seeds per plant (0.942) and grain yield per plant (0.937) in M_3 generation whereas, in M_4 generation, maximum heritability was exhibited by plant height (0.980) followed by grain yield per plant (0.969), number of pods per plant (0.960) and days to maturity (0.948). The average length of pods exhibited the minimum value of heritability (0.226 and 0.064) in M_3 and M_4 generations, respectively. The 100 grain weight also exhibited low values of 0.622 and 0.272 in M_3 and M_4 generations, respectively. The highest values of genetic advance were recorded for number of seeds per plant (11.99 and 12.65) and followed by plant height (8.27 and 9.09) in M_3 and M_4 generations, respectively. The minimum values (0.15 and 0.04) of genetic advance were recorded for the average length of pods in M_3 and M_4 generation, respectively.

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