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Full Length Research Paper

Improvement of mungbean varieties through induced mutations

Samiullah Khan and Sonu Goyal*

Mutation Breeding Laboratory, Department of Botany, Aligarh Muslim University, Aligarh-202 002 (U.P.) India.

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Mutations were induced in two mungbean varieties, K-851 and PS-16 using EMS and gamma rays as mutagens. Selection studies were conducted to improve the yield and to generate genetic variability in different quantitative traits viz., fertile branches per plant, pods per plant and seed yield per plant. Mean values in traits increase significantly over the controls and genetic parameters were recorded higher for the mutants isolated in M_5 generation. High values of heritability and genetic advance for the mutants indicate that further improvement could be made in next generations.

Key words: Mungbean, EMS, gamma rays, genetic variability, selection, yield.

INTRODUCTION

The possibility of improving yield and yield related traits through genetic manipulations have been clearly shown in recent years by high yielding varieties of cereal and pulses. Physical and chemical mutagenic agents cause genes to mutate at rates above the spontaneous base line, thus producing a range of novel traits and broadening of the genetic diversity of plants (Lagoda, 2007). Among the various pulses grown in India, mungbean (*Vigna radiata* (L.) Wilczek), a self-fertilized crop, occupies unique position in Indian agriculture and has been grown under various agro-ecological conditions. Despite this, progress in production and productivity in mungbean has remained far from satisfactory. This is because the breeding methodology applied to mungbean in the past has been purely conventional. Since, the conventional techniques employed in the improvement of mungbean have not keep pace with the demands of expanding population, the importance of development of high yielding mutant varieties has great relevance.

MATERIALS AND METHODS

Varieties used

Two varieties of mungbean namely K-851 and PS-16 were used in the present investigation. Both the varieties are well adapted to agro-climatic conditions of Uttar Pradesh. A brief description of the varieties is given in Table 1:

*Corresponding author. Email: goyalsoni@yahoo.com

Experimental procedure

The present study was carried out during summer seasons of 2003-2007 at the Agricultural Farm, Aligarh Muslim University, Aligarh. Uniform and healthy seeds of two varieties of mungbean, pre-soaked in distilled water for 9 h, were treated with 0.1 and 0.2% EMS (ethylmethane sulphonate) for 6 h. Solution of EMS was prepared in Phosphate buffer of pH 7. For gamma rays treatment, seed samples (having 12% moisture content) were packed in polythene cover and irradiated with 20 and 40 kR doses of gamma rays from ^{60}Co source. For each treatment three hundred seeds were used.

Three replications of 100 seeds each, were sown for every treatment in each variety in the field. The distance between seeds in a row and between the rows was kept 30×60 cms, respectively. Recommended agronomic practices were employed for preparation of field, sowing and subsequent management of the population.

Observations recorded in the M_1 generation

Seed germination

After recording germination counts, the percentage of seed germination was calculated on the basis of total number of seeds sown.

Pollen fertility

Pollen fertility was determined by staining the pollen grain with 1% acetocarmine solution. For this purpose, 15 plants at random were selected from each treatment including their controls for both the varieties and finally 5 young flower buds from each plant were used for microscopic analysis. Pollen grains which took stain and had a regular outline were considered as fertile, while shrunken, empty and unstained ones as sterile.

For raising M_2 generation, 30 healthy seeds of both the varieties from each normal-looking M_1 plant of all different treatments with

Table 1. A brief description of the varieties.

Name of variety	Pedigree	Distinguishing characters
K-851 (developed at Indian Institute of Pulses Research, Kanpur)	4453-3xType 1	Uniform maturity in 60-70 days, plant erect and semi tall, blackish brown pods, shining green medium size seeds, average yield 10+12 Q/ha.
PS-16 (developed at Indian Agricultural Research Institute, New Delhi)	Selection from germ plasm of Iran	Matures in 60-65 days, plant erect with medium height, seeds shining green, average yield 10-12 Q/ha.

Table 2. Analysis of variance.

Source	d.f.	SS	MSS	Expectations
Replication	r-1	SSr	SSr/(r-1)	MSSr/MSSe
Treatment	g-1	SSg	SSg(g-1)	MSSg/MSSe
Error(e)	(r-1)(g-1)	SSe	SSe/(g-1)(r-1)	

Where r = number of replications and t = number of treatments

their respective controls were planted in the plant progeny rows. The different treatments and controls comprised 30-progenies. Three replications were maintained in each treatment. M_2 plants, which differed from the control, were selected and further evaluated. Plants with 25-30% higher single plant seed yield in each treatment were selected and grown as M_3 generation. Based on plant progeny rows test, selected 15-20% M_3 plants with high seed yield were retained. They were grown in M_4 and M_5 generations in the plots with the spacing of 30 cm (plant to plant in row) and 60 cm (between the rows). Seed sowing and evaluation of progenies in M_3 and subsequent years were carried out according to the method suggested by Sakin and Yildirim (2004).

Data were collected on individual plants in M_4 and M_5 generations and analysed statistically to assess the extent of induced genetic variability for three quantitative characters viz., fertile branches per plant, pods per plant and total plant yield (g) of the mutants.

Analysis of Variance

Data were subjected to analysis of variance (ANOVA) (Table 2) using the methods suggested by Singh and Chaudhary (1985).

Analysis of genetic variability: From analysis of variance, the components of coefficient of variation viz., genotypic and phenotypic coefficient of variations were calculated by dividing the surface root of the genotypic and phenotypic variance by population mean and multiplying the resultant by hundred

$$Cvp\ (\%) = [(\sigma^2 p)^{1/2} / \overline{X}] \times 100$$

$$Cvg\ (\%) = [(\sigma^2 g)^{1/2} / \overline{X}] \times 100$$

Where; σp is the phenotypic standard deviation of the trait and σg is the genotypic standard deviation of the trait.

Components of variance:

$$\sigma^2 e = MSSe$$

$$\sigma^2 g = (MSSg - MSSe) / r$$

$$\sigma^2 p = \sigma^2 g + \sigma^2 e$$

Where; $\sigma^2 p$, $\sigma^2 g$ and $\sigma^2 e$ are phenotypic, genotypic and environmental variances, respectively and r represents the number of replicates, MSSg and MSSe are genotypic and environmental error mean sum of squares respectively.

$$h^2\ (\%) = (\sigma^2 g / \sigma^2 p) \times 100$$

The estimate of the expected genetic advance (GA, expressed as percentage of the mean value) was computed using the formula given by Allard (1960).

$$GA = k.\sigma p.h^2$$

Where;
h^2 = broad – sense heritability
σp = phenotypic standard deviation of the mean performance of the treated population.
k = 2.64, constant for 1% selection intensity

(C.D) between two means were calculated as follows:

Standard error (S.E.) = $(MSSe/r)^{1/2}$
Standard error of difference (Sed) = $(2.MSSe/r)^{1/2}$
Critical differences (C.D.) = $(2.MSSe/r)^{1/2} \times$'t'

't' is the tabulated value of 't' at 1% probability level of significance for the degrees of freedom of error mean square. If the mean difference between any two species is greater than the calculated C.D. value, then the difference is considered as significant.

RESULTS

Results of the present study are elaborated below.

Biological damage in M_1 generation

Data recorded on seed germination and pollen fertility are presented in Table 3. A gradual decrease in seed germination and pollen fertility was observed with increasing concentrations/doses of mutagens in both the varieties.

Table 3. Effects on mutagens on seed germination and pollen fertility.

Treatment	M_1 generation		M_2 generation	
	Seed germination (%age inhibition)	Pollen fertility (%age reduction)	Seed germination (%age inhibition)	Pollen fertility (%age reduction)
		Variety K-851		
Control	-	-	-	-
0.1% EMS	11.36	24.27	4.94	22.95
0.2% EMS	14.38	27.55	7.69	24.48
20 kR gamma rays	20.45	21.93	16.48	21.17
40 kR gamma rays	24.26	24.25	23.07	22.14
		Variety PS-16		
Control	-	-	-	-
0.1% EMS	10.94	22.50	4.49	21.44
0.2% EMS	14.08	24.93	6.17	22.16
20 kR gamma rays	12.50	22.26	11.23	20.61
40 kR gamma rays	15.63	24.47	14.04	21.03

Table 4. Brief description of the mutants isolated in M_4 generation.

Strain Number	Origin/Treatment	Duration of treatment	Remarks
K-851	K-851 (Control)	-	-
1. K-851-A	0.1% EMS	6 h	High yield
2. K-851-B	0.2% EMS	6 h	High yield
3. K-851-C	40 kR gamma rays	-	High yield
4. PS-16	PS-16 (Control)	-	-
5. PS-16-A	0.1% EMS	6 h	High yield
6. PS-16-B	20kR gamma rays	-	High yield

Both the varieties responded differently to various mutagenic treatments. Gamma rays treatments caused maximum inhibition in seed germination while EMS showing a more severe effect on pollen fertility in both the varieties. The var. K-851 showed a greater sensitivity to mutagenic treatments.

Although the inhibition percentage increased with the increasing concentrations/doses of the mutagens in both the varieties in M_2 also, the increase was not higher than in M_1 generation, showing that the effect of mutagens ceased to some extent in M_2 (Table 3).

Screening of high yielding mutants

The details of mutants isolated in M_4 generation and of their parents (controls) are given in Tables 4-8.

Since yield per plant is the most desirable character, certain mutants which were distinctly much superior to the others with regard to the seed yield per plant were selected in M_3 generation and grown in progeny rows in M_4 and M_5 generations and were evaluated for the number of fertile branches, number of pods and seed yield. The frequency of occurrence of mutant plants was rather low, considering the large size of M_3 population raised.

The mean values shifted in positive direction for all the three quantitative traits (Tables 5 and 4). The mutants K-851-B (0.2% EMS) and PS-16-B (20kR gamma rays) have given the highest seed yield of 17.30 and 20.16 g, respectively in M_4 generation against their respective controls which gave the mean yield 8.85 and 12.85 g. All these mutants isolated for a higher seed yield, have also shown higher values for the number of fertile branches and pods per plant as compared to the parental varieties. The mean values for the traits viz., fertile branches per plant, pods per plant and total plant yield of the mutants, isolated in M_5 generation, were recorded higher in comparison to the mean values for these traits of the M_4 mutants (Tables 7 and 8). The mutant PS-16-B (20kR gamma rays) showed decline in mean values for fertile branches per plant and pods per plant in M_5 generation (Table 8).

Coefficient of variation (phenotypic and genotypic), heritability and the genetic advance for the number of fertile branches, number of pods and the total plant yield were also recorded to be higher in all these mutants. Heritability was higher in M_5 than M_4 generation.

Table 5. Estimates of mean values and genetic parameters for various quantitative traits of the mutants isolated in M_4 generation.

Strain Number	Treatment	Mean±S.E.	Shift in $\overline{X}$	Cv_p (%)	Cv_g (%)	h^2 (%)	GA (% of $\overline{X}$)
Fertile branches / plant							
K-851	K-851 (Control)	5.60±0.02	-	22.15	10.20	21.19	12.38
1. K-851-A	0.1% EMS	11.22±0.11	+5.62	25.30	21.26	70.62	47.16
2. K-851-B	0.2% EMS	20.40±0.09	+14.80	24.20	23.16	91.59	58.51
3. K-851-C	40kR gamma rays	15.50±0.07	+9.90	30.15	26.00	74.36	59.18
C.D. (1%)		4.35					
Pods / plant							
K-851	K-851 (Control)	48.27±0.16	-	5.36	3.25	36.77	5.20
1. K-851-A	0.1% EMS	82.55±0.19	+34.28	24.00	23.21	96.28	61.00
2. K-851-B	0.2% EMS	80.64±0.08	+32.37	25.56	24.01	88.23	59.53
3. K-851-C	40kR gamma rays	76.00±0.12	+27.73	20.29	16.40	65.33	34.99
C.D. (1%)		22.24					
Total plant yield (g)							
K-851	K-851 (Control)	8.85±0.17	-	3.20	1.50	21.97	1.85
1. K-851-A	0.1% EMS	17.25±0.10	+8.40	9.95	8.20	67.93	17.84
2. K-851-B	0.2% EMS	17.30±0.14	+8.45	10.00	8.55	73.12	19.30
3. K-851-C	40kR gamma rays	16.28±0.18	+7.43	7.50	4.80	40.98	8.11
C.D. (1%)		5.47					

± S.E. = Standard error, Cv_p = Phenotypic coefficient of variation, Cv_g = Genotypic coefficient of variation.

Table 6. Estimates of mean values and genetic parameters for various quantitative traits of the mutants isolated in M_4 generation.

Strain Number	Treatment	Mean±S.E.	Shift in $\overline{X}$	Cv_p (%)	Cv_g (%)	h^2 (%)	GA (% of $\overline{X}$)
Fertile branches / plant							
PS-16	PS-16 (Control)	6.15±0.18	-	20.36	15.21	55.83	30.00
4. PS-16-A	0.1% EMS	12.50±0.16	+6.35	45.80	40.10	76.65	92.67
5. PS-16-B	20kR gamma rays	14.16±0.19	+8.01	42.28	38.19	75.28	87.47
C.D. (1%)		5.21					
Pods / plant							
PS-16	PS-16 (Control)	50.42±0.11	-	10.29	5.40	27.54	7.48
4. PS-16-A	0.1% EMS	92.56±0.12	+42.14	41.25	34.20	68.73	74.84
5. PS-16-B	20kR gamma rays	89.64±0.16	+39.22	37.11	30.00	65.35	64.02
C.D. (1%)		28.37					
Total plant yield (g)							
PS-16	PS-16 (Control)	12.85±0.17	-	4.23	1.95	21.28	2.37
4. PS-16-A	0.1% EMS	20.01±0.12	+7.16	10.24	6.15	36.07	9.75
5. PS-16-B	20kR gamma rays	20.16±0.10	+7.31	10.28	6.40	38.75	10.51
C.D. (1%)		4.96					

± S.E. = Standard error, Cv_p = Phenotypic coefficient of variation, Cv_g = Genotypic coefficient of variation

DISCUSSION

Mutation breeding is an efficient tool to amend and/or rectify certain character(s) without altering the other traits of the crop plants, in relatively short span, as compared to the conventional methods, especially when the traits under study show simple Mendelian inheritance. Basic information on the frequency and spectrum of mutations, treatment procedures and methods of handling the treated population, would be highly desirable for an effective

Table 7. Estimates of mean values and genetic parameters for various quantitative traits of the mutants isolated in M_5 generation.

Strain Number	Treatment	Mean±S.E.	Shift in $\overline{X}$	Cv_p (%)	Cv_g (%)	h^2 (%)	GA (% of $\overline{X}$)
Fertile branches / plant							
K-851	K-851 (Control)	5.60±0.02	-	22.15	10.20	21.19	12.38
1. K-851-A	0.1% EMS	11.41±0.43	+5.81	47.53	41.52	76.34	95.79
2. K-851-B	0.2% EMS	20.60±0.45	+15.00	25.84	24.56	90.33	61.61
3. K-851-C	40kR gamma rays	15.65±0.31	+10.05	28.92	25.63	78.54	59.97
C.D. (1%)		4.83					
Pods / plant							
K-851	K-851 (Control)	48.27±0.16	-	5.36	3.25	36.77	5.20
1. K-851-A	0.1% EMS	83.41±2.84	+35.14	22.00	21.24	93.21	54.13
2. K-851-B	0.2% EMS	80.76±0.85	+32.49	26.10	25.00	91.75	63.22
3. K-851-C	40kR gamma rays	77.41±1.07	+29.14	21.47	18.50	74.24	42.08
C.D. (1%)		25.16					
Total plant yield (g)							
K-851	K-851 (Control)	8.85±0.17	-	3.20	1.50	21.97	1.85
1. K-851-A	0.1% EMS	17.50±0.14	+8.65	12.51	10.24	67.06	22.15
2. K-851-B	0.2% EMS	17.65±0.06	+8.80	10.77	8.77	72.99	19.78
3. K-851-C	40kR gamma rays	16.55±0.10	+7.70	7.28	5.96	66.98	12.87
C.D. (1%)		8.29					

± S.E. = Standard error, Cv_p = Phenotypic coefficient of variation, Cv_g = Genotypic coefficient of variation

Table 8. Estimates of mean values and genetic parameters for various quantitative traits of the mutants isolated in M_5 generation.

Strain Number	Treatment	Mean±S.E.	Shift in $\overline{X}$	Cv_p (%)	Cv_g (%)	h^2 (%)	GA (% of $\overline{X}$)
Fertile branches / plant							
PS-16	PS-16 (Control)	6.15±0.18	-	20.36	15.21	55.83	30.00
4. PS-16-A	0.1% EMS	12.80±0.36	+6.65	31.75	28.16	78.63	6591
5. PS-16-B	20kR gamma rays	13.25±0.25	+7.10	41.21	34.81	71.35	77.62
C.D. (1%)		7.45					
Pods / plant							
PS-16	PS-16 (Control)	50.42±0.11	-	10.29	5.40	27.54	7.48
4. PS-16-A	0.1% EMS	96.00±0.84	+45.58	24.89	21.47	74.40	48.49
5. PS-16-B	20kR gamma rays	88.83±0.91	+38.41	21.93	17.84	66.17	38.31
C.D. (1%)		31.04					
Total plant yield (g)							
PS-16	PS-16 (Control)	12.85±0.17	-	4.23	1.95	21.28	2.37
4. PS-16-A	0.1% EMS	20.25±0.07	+7.40	9.04	5.88	42.38	10.11
5. PS-16-B	20kR gamma rays	20.50±0.06	+7.65	10.33	6.47	39.23	10.70
C.D. (1%)		7.05					

± S.E. = Standard error, Cv_p = Phenotypic coefficient of variation, Cv_g = Genotypic coefficient of variation.

use of this technique in the improvement of mungbean

Biological damage in M_1 generation

The relative sensitivity of mungbean varieties to various mutagenic treatments was assessed by studying the biological damage induced in M_1, in terms of seed germination and pollen fertility. In the present study, reduction in seed germination and pollen fertility was concentration/ dose dependent and linear. Promoting effects of low doses of gamma rays and EMS on biological parameters have been reported earlier in *Cicer arietinum* (Mujeeb, 1974 and Mahto et al., 1989) and *Vicia faba* (Vandana and Dubey, 1988). Gamma rays treatments caused maxi-

mum inhibition in seed germination than the EMS treatments in both the varieties. Reduction in germination in mutagenic treatments has been explained due to delay or inhibition of physiological and biological processes necessary for seed germination which include enzyme activity (Kurobane et al., 1979), hormonal imbalance (Chrispeels and Varner, 1967) and inhibition of mitotic process (Ananthaswamy et al., 1971). Yusuf and Nair (1974) inferred that gamma irradiation interfered with the synthesis of enzymes and at the same time accelerated the degradation existing enzymes involved in the formation of auxins and thus reduces the germination of seeds. Reduced seed germination due to mutagenic treatments may be the result of damage of cell constituents at medicular level or altered enzyme activity.

Maximum reduction in fertility was observed in EMS treatments as compared to gamma rays in both the varieties. In most cases, meiotic abnormalities are responsible for pollen sterility (Gaul, 1970; Sinha and Godward, 1972; Ramanna, 1974; Larik, 1975; Patil, 1992; Rehman, 2000; Mathusamy and Jayabalan, 2002 and Khan and Wani, 2005) because the meiosis is more prone to any conceivable type of disturbances. Contrary to this, Sato and Gaul (1967) in barley reported high pollen sterility coupled with low frequency of meiotic abnormalities after EMS treatments. This was attributed to small undetectable deletions or gene mutations. In addition to chromosomal aberrations, some genic and physiological changes might have caused pollen sterility. Based on seed germination and pollen fertility, var. K-851 was found to be more sensitive than the var. PS-16. Varietal differences were also reported earlier with respect to mutagen sensitivity in *Lathyrus sativus* (Nerker, 1976), *Lens culinaris* (Sharma and Sharma, 1981) and *A. hypogyea* (Venkatachalam and Jayabalan, 1995; Adu and Sanwan, 2004; Mensah and Odadoni, 2007). The sensitivity of an organism depends upon the mutagen employed, genetic makeup (Kaul, 1988), amount of DNA and its replication time in the initial stages (Varughese and Swaminathan, 1968) beside physical factors such as pH, moisture, oxygen and temperature (Konzak et al., 1965). Comparative mutagenicity of different mutagens in the two varieties viz., K-851 and PS-16 reflects the difference in their genome architecture. Genetic differences even though very small (as single gene difference) can induce significant changes in the mutagen sensitivity which, influence various plant characters.

Genetic variability

Estimates of genotypic coefficient of variation, heritability and genetic advance expected by selection for yield and its component traits are useful in designing an effective breeding programme. Means and estimates of different genetic parameters for three quantitative traits of mungbean provided ample evidence that mutagenic treatments could alter mean values and create additional genetic variability. A glance at the data for the number of fertile branches, number of pods and seed yield increased significantly in mutant lines K-851-A (0.1% EMS), K-851-B (0.2% EMS), K-851-C (40kR gamma rays), PS-16-A (0.1% EMS) and PS-16-B (20kR gamma rays) in comparison to the parental varieties (controls). Delayed selection is preferred as the deleterious mutations are generally eliminated in early generation. Estimation of heritability in broad sense gives the indication of heritable component of variability. It favours effective selection on single plant basis, if the heritability of that particular trait is high but if the trait concerned has lower heritability estimate, than breeder has to rely on progeny mean rather than on single plant. The results obtained on heritability showed high heritability estimates for fertile branches per plant and pods per plant whereas, total plant yield had moderate values of heritability. But, high heritability for yield has been reported in garden pea (Surejon and Sharma, 2000). The disparity in results could be because heritability is a property not only of a character but also of the population, environment and the circumstances to which the genotypes are subjected to. The heritability in M_5 generation was found higher than M_4 generation. High heritability indicates that the induce variability in mutant population was fixed by selection. These findings are in agreement with those of Sarkar (1986) and Borojevic (1991). For more efficient selection h^2 in conjunction with genetic advance are more reliable than h^2 alone (Johnson et al., 1955). Heritability along with genetic advance would be helpful in assessing the nature of gene action. In the present study, high genetic advance, as percenttage of mean, was noticed for fertile branches per plant and pods per plant. Both these traits also had high heritability which indicates that expression of these traits is governed by attentive gene action and as a result there is scope of improving these traits through selection procedure. Low genetic advance with moderate heritability was observed for total plant yield. It shows that this trait is most probably governed by non additive gene action.

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