

Mudasir Hafiz KHAN, Sunil Dutt TYAGI

# Induced morphological mutants in soybean [*Glycine max* (L.) Merrill]

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**Abstract** The seeds of two soybean cultivars viz., Pusa-16 and PK-1042 were treated with 15, 30, and 45 kR of gamma rays, three concentrations of ethyl methane sulphonate (EMS) (0.1%, 0.2%, and 0.3%), and their combinations. Various morphological mutants were recorded in  $M_2$  and  $M_3$  generations. These morphological mutants were named on the basis of the part of the plant body affected. Eight different morphological mutants, i.e., tall, dwarf, gigas, black pod, smooth pod, black spotted seed, bold seeded, and early maturing were isolated. Among them, early maturing and bold seeded mutants had the potential to be incorporated into breeding programs.

**Keywords** morphological mutants, gamma rays, ethyl methane sulphonate, soybean

## 1 Introduction

Soybean, *Glycine max* (L.) Merrill belonging to the family Leguminosae, subfamily Papilionoideae, and genus *Glycine* L., has become an increasingly important agricultural commodity with a steady increase of annual production worldwide. In 1999, the global production of soybean was 155.1 million metric tonnes, with the major producers being the United States, Brazil, Argentina, China, and India (USDA, 2000). In 1954, China emerged as a leading country in soybean production and export. However, since then, the United States has become the world leader (Liu, 1997), producing about half of the total world soybean crop. The worldwide increasing demand of soybean is due to its unique composition, excellent nutritional value, health benefits, and adaptability to varied

climatic conditions. It is widely believed that soybean originated in China 4000 to 5000 years ago, and from there, its cultivation spread to Japan, Korea, and Southeast Asia (Morse, 1950; Anonymous, 1993).

Mutations are the ultimate source of variability in organisms. Mutations can be used for plant breeding in many different ways. The direct use of mutations is valuable supplementary approach to plant breeding, particularly when it is desired to improve 1 or 2 easily identifiable characters in an otherwise well adapted variety. Induced mutation is thus the ultimate source of all the genetic variability in crop plants that may be difficult to bring through cross breeding and other breeding procedures, since mutations give rise to nonexisting variations. In the present investigation, mutations were induced in the Pusa-16 and PK-1042 varieties of soybean by treating the seeds with different doses/concentrations of gamma rays, EMS, and their combinations. The plants of the  $M_2$  generation were screened for morphological mutations. Most of these were found to breed true in the  $M_3$  generation. The present investigation deals with the characterisation of the different morphological mutations isolated in the  $M_2$  and  $M_3$  generations of Pusa-16 and PK-1042 cultivars of soybean.

## 2 Materials and methods

### 2.1 Experimental material

Seeds of two soybean varieties, viz., Pusa-16 and PK-1042, were obtained from the Division of Genetics, Indian Agricultural Research Institute, New Delhi, India.

### 2.2 Methods

#### 2.2.1 Physical and chemical treatment of seeds

The experimental materials were divided into three treatment groups:

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Mudasir Hafiz KHAN (✉), Sunil Dutt TYAGI  
Department of Plant Breeding and Genetics, Kisan (P. G.), College,  
Simbhaoli, Ghaziabad (U. P.) 190009, India  
E-mail: kmudasirhafiz@yahoo.com

1) Treatment with physical mutagen, i.e., 15, 30, and 45 kR of gamma rays.

2) Treatment with chemical mutagen, i.e., 0.1%, 0.2%, and 0.3% concentration of EMS.

3) Treatment with both physical and chemical mutagens, i.e., 15 kR + 0.2% EMS, 30 kR + 0.2% EMS, and 45 kR + 0.2% EMS.

There were 100 irradiated seeds of 15, 30, and 45 kR subjected to 0.2% EMS treatment for eight hours. The seeds treated with chemical mutagen were washed in running water before sowing.

The treated materials along with two controls (untreated) were immediately sown in single unreplicated plot with four rows at spacing of 30 cm × 10 cm at Research Farm of Kisan (P. G.), College, Simbhaoli.

### 2.2.2 Recording of data

The data were recorded from 20 randomly selected plants from each treatment. The seeds of  $M_1$  plants were collected and again sown in the field in the next season to raise  $M_2$  generation on the selected plant bulk progeny method, and similarly, those of  $M_2$  generation were raised to grow as  $M_3$  generation. The  $M_2$  population was screened for the presence of morphological mutations. Most of the  $M_2$  mutants were found to breed true in the  $M_3$  generation. The morphological mutants were characterized on the basis of growth, development, and reproductive performance.

## 3 Results

### 3.1 Morphological mutants and their characteristics

Five and four types of visible morphological mutants were isolated in cv. PK-1042 and Pusa-16, respectively. The various types of morphological mutants including gigas, tall, dwarf, black pod, smooth pod, black spotted seed, bold seeded, and early maturing were isolated in  $M_2$

generation (Fig. 1). The characteristics of each of the above mutants are presented below:

#### 3.1.1 Gigas

This group of mutants was characterized by crumpled or crinkled appearance of leaves, glossy or waxy surface, and leathery texture. The stems were fully hairy. These mutants (Fig. 1(a)) were more vigorous than the parents Pusa-16 and PK-1042 but carried lesser flowers and pods than parents.

#### 3.1.2 Dwarf

The plants with height ranging from 28–39 cm and possessing small leaves were grouped under this category. The mean height of the dwarf mutants of Pusa-16 was 31.25 cm (Fig. 1(b)) as against 69.40 cm in control.

#### 3.1.3 Tall

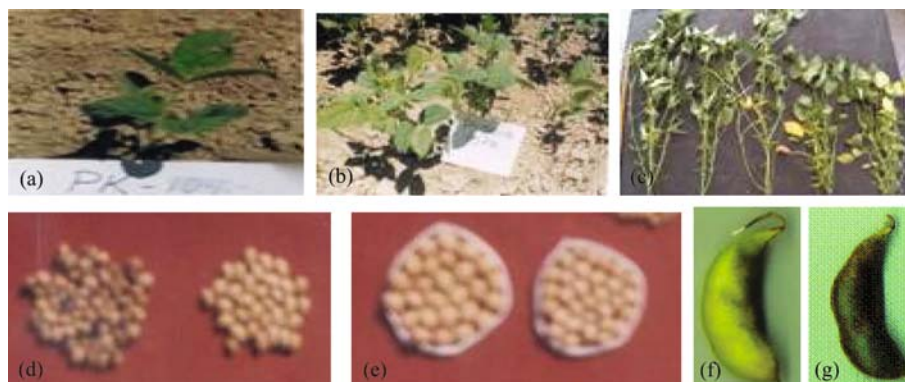
The plants with height ranging from 103 to 106 cm were grouped under this category. The mean height of these tall mutants of Pusa-16 (Fig. 1(c)) was 113.25 cm as against 69.40 cm in control.

#### 3.1.4 Black spotted seed

These mutants were characterized by black spotted seeds (Fig. 1(d)). The 100-seed weight was found to be 11.05 g in mutant as compared to 12.40 g in parent cultivar PK-1042. This mutant recorded 59.25 pods per plant as compared to 45.50 pods per plant in the control.

#### 3.1.5 Bold seed

The mutants (Fig. 1(e)) had considerably larger seeds than those in the control (Pusa-16). The plants of these mutants



**Fig. 1** Morphological mutants in mutagen treated soybean

Note: (a)–(g) represent gigas, dwarf, tall, bold seed, black spotted seed, smooth pod, and black pod mutants, respectively.

were almost similar to control plants except for 100-seed weight. The 100-seed weight for Pusa-16 mutant was 13.32 g as compared to 12.24 g in control. Plant height and other yield contributing characters of this mutant were almost similar with that of the control plants.

### 3.1.6 Smooth pod

In this category, mutants with nonhairy and glabrous pods as against hairy pods in the parent cultivar PK-1042 (Fig. 1 (f)) were isolated and named as smooth pod mutants.

### 3.1.7 Black pods

In this category, mutants with black pod color but similar in viability and structure to the parent cultivar PK-1042 (Fig. 1(g)) were selected.

### 3.1.8 Early maturing

These mutants demonstrated a feature of early pod maturity. The pod maturity was attained within 92.75 days in PK-1042 as compared to 111.75 days in control. The height of the early maturing mutants was on the increasing side coupled with a good yielding potential.

A summary of the data on some of the quantitative characters of morphological mutants selected in Pusa-16 and PK-1042 are presented in Tables 1 and 2.

## 3.2 Scoring of morphological mutants

The frequencies of morphological mutations and mutants in Pusa-16 and PK-1042 are presented in Table 3.

The data revealed that in Pusa-16, the frequencies of the mutants decreased with the increase in the dose of gamma rays, whereas in EMS and combined treatments, the decrease was noticed upto the intermediate dose levels followed by increase at the higher doses. In PK-1042, combined treatments exhibited the highest frequency of mutants followed by gamma rays and EMS treatments, and no definite increasing or decreasing trend was noticed. In this cultivar, the intermediate dose of combined treatment showed the highest frequency of mutants, while in Pusa-16, the highest frequency was exhibited by 0.1% EMS followed by 15 kR of gamma rays.

## 3.3 Frequency and spectrum of morphological mutations

The frequencies and spectrum of different morphological mutants in  $M_2$  generation in two cultivars (Pusa-16 and PK-1042) of soybean are presented in Tables 4 and 5, respectively.

The data revealed that in Pusa-16, the highest frequencies of morphological mutants were obtained from gamma rays followed by EMS and combined treatments, whereas in PK-1042, the higher frequency of mutants were recorded from combined treatments followed by gamma rays and EMS treatment. On the basis of spectrum of mutants, the data revealed that four types of mutants were isolated in Pusa-16, whereas five types of mutants were recovered from PK-1042. It may be noted that gigas mutant was recovered from both the cultivars with highest frequency in Pusa-16. The early maturity, tall, bold seed, black pod, and smooth pod mutants were recovered from lower or intermediate doses, while dwarf, gigas, and black spotted seed mutants were mostly isolated from high dose levels. In Pusa-16, among all the mutagens, 0.1% EMS

**Table 1** Performance for various characters of morphological mutants in  $M_2$  generation in Pusa-16 cv. of soybean

item		day to maturity	plant height/cm	Pods per plant	seed yield/g	biological yield/g	100-seed weight/g	harvest index/%	protein content/%
control	A	106–108	65–75	34–55	21–29	44–52	11.72–12.86	44.17–62.85	33.73–37.70
	B	107±0.86	69.40±0.12	41.10±15.41	24.7±0.97	48.30±0.23	12.14±0.01	51.60±0.40	36.01±0.82
	C	1.47	4.20	18.22	12.38	9.86	2.48	14.08	5.42
dwarf	A	106–110	28–39	16–29	7–19	40–56	11.60–12.82	12.50–47.50	32.50–37.10
	B	108.5±0.12	31.25±0.32	21.0±0.35	15.0±0.34	51.25±0.47	12.11±0.33	30.59±0.89	35.40±0.03
	C	1.76	16.81	26.66	36.51	17.42	4.34	46.95	5.99
tall	A	105–110	103–126	42.85	22–39	52–83	11.65–12.87	31.94–54.38	33.0–37.65
	B	107.75±0.11	113.25±0.45	57±0.91	28.75±0.38	66±0.67	12.14±0.03	43.90±0.45	35.94±0.09
	C	2.06	8.51	33.56	27.59	21.46	4.55	21.41	5.73
gigas	A	107–111	66–79	18–29	10–18	43–72	11.03–12.00	44.15–76.20	32.82–35.20
	B	109±0.16	72.40±0.35	22.10±0.72	14.26±0.72	59.20±0.56	11.72±0.23	66.72±0.39	34.40±0.19
	C	1.63	13.20	21.40	21.45	13.85	3.83	13.15	4.86
bold seed	A	107.111	63.78	42.69	23–37	46–62	13.15–13.42	50–64	36.51–38.32
	B	108.75±0.11	70.25±0.39	49.25±0.82	29.25±0.40	51.25±0.46	13.32±0.01	56.72±0.39	37.09±0.05
	C	1.57	9.03	26.75	22.05	14.37	0.89	11.12	2.24

Note: A, B, and C indicates the range, mean±SE, and coefficient of variability, respectively.

**Table 2** Performance for various characters of morphological mutants in M<sub>2</sub> generation in PK-1042 cv. of soybean

item		day to maturity	plant height/cm	Pods per plant	seed yield/g	biological yield/g	seed yield/g	harvest index/%	protein content/%
control	A	111–113	45–67	39–56	22–33	44–58	12.01–13.01	39.29–68.18	36.89–38.53
	B	111.75±0.30	57.40±0.26	45.50±0.27	27.40±0.14	53.90±0.16	12.40±0.01	51.30±0.37	37.15±0.03
	C	0.86	14.46	11.07	12.46	7.44	2.26	17.28	2.43
smooth pod	A	111–113	42–65	39–69	21–38	42–72	11.49–12.84	36.20–54.76	35.62–37.10
	B	111.75±0.07	56.50±0.74	47.25±1.04	26.75±0.55	59.25±0.92	12.29±0.04	45.55±0.68	36.45±0.04
	C	0.86	18.42	30.83	28.69	21.67	4.68	21.01	1.68
back spotted seed	A	1110.115	40–72	32–72	20–34	36.74	10.42–11.46	39.70–55.55	36.10–37.95
	B	112.25±0.13	59.25±0.81	52.25±0.96	26.75±0.37	59.25±0.98	11.05±0.03	48.86±0.44	37.15±0.05
	C	1.98	23.20	31.50	22.08	28.15	4.04	15.23	2.09
early maturing	A	89–96	44–78	34–62	24–38	45–66	11.96–13.10	48.33–57.57	36.10–37.65
	B	92.75±0.33	60.25±1.50	48.25±1.28	30.25±0.64	56.25±0.99	12.60±0.05	53.70±0.44	36.95±0.08
	C	3.22	23.09	28.83	19.16	15.93	3.95	7.40	1.92
gigas	A	109–112	36–59	9–23	4–19	28–44	11.75–12.92	14.28–47.50	33.10–37.40
	B	110±0.11	49.50±0.77	13.0±0.53	11.25±0.50	36.25±0.56	12.35±0.004	30.41±1.23	36.05±0.16
	C	1.29	19.69	51.79	56.29	19.68	4.46	51.36	5.60
black pod	A	110–113	39–62	36–72	21–35	36–66	11.90–12.96	39.06–53.84	35.49–38.69
	B	111.17±0.09	55.25±0.84	49±124	26±0.47	56.25±0.94	12.32±0.04	46.75±0.59	37.32±0.11
	C	1.13	19.68	32.91	23.92	21.84	3.79	16.62	3.71

Note: A, B, and C indicates the range, mean±SE, and coefficient of variability, respectively.

**Table 3** Frequencies of morphological mutations recorded as % M<sub>2</sub> seedlings in M<sub>2</sub> generation in two cultivars of soybean

serial no.	treatment	number of M <sub>2</sub> seedlings		frequency of mutants (% M <sub>2</sub> seedlings)	
		Pusa-16	PK-1042	Pusa-16	PK-1042
1	control	0.00	0.00	0.00	0.00
2	15 kR	535	740	2.24	1.08
3	30 kR	595	560	1.68	1.07
4	45 kR	480	400	1.46	1.75
5	0.1% EMS	520	600	2.88	1.50
6	0.2% EMS	565	674	0.70	1.19
7	0.3% EMS	685	525	0.87	0.76
8	15 kR + 0.2% EMS	435	485	1.61	1.03
9	30 kR + 0.2% EMS	440	420	0.68	2.38
10	45 kR + 0.2% EMS	385	375	1.30	1.60

treatment was found to be more effective as compared to other mutagens, while in PK-1042, 30 kR + 0.2% EMS dose of combined treatment was more effective in inducing the morphological mutation. The lowest frequency of mutants in Pusa-16 was exhibited by combined treatments at 30 kR + 0.2% EMS, and in PK-1042, it showed by 0.3% EMS treatment.

Thus, the effectiveness of different mutagens in inducing morphological mutation in Pusa-16 was found in the following order:

0.1% EMS > 15 kR > 30 kR > 45 kR = 15 kR + 0.2% EMS > 0.3% EMS > 45 kR + 0.2% EMS + 0.2% EMS > 0.2% EMS > 30 kR + 0.2 EMS.

However, in PK-1042, the effectiveness of different mutagens showed a different order.

30 kR + 0.2% EMS > 0.1% EMS > 15 kR = 0.1% EMS > 45 kR > 30 kR = 45 kR + 0.2% EMS > 15 kR + 0.2% EMS > 0.3% EMS.

## 4 Discussion

In the present investigation, it was observed that all mutants showed several distinct characters. Prominent among them are mutants like tall, bold seed, early maturing, and black spotted seed mutant, where the

**Table 4** Frequencies and spectrum of different types of morphological mutants recorded in M<sub>2</sub> generation in Pusa-16 cultivar of soybean

serial no.	treatment	morphological mutations/%			
		dwarf	tall	gigas	bold seed
1	control	0.00	0.00	0.00	0.00
2	15 kR	0.00	1.68	0.00	0.56
3	30 kR	0.00	0.67	1.00	0.00
4	45 kR	1.46	0.00	0.00	0.00
5	0.1% EMS	0.00	0.77	0.00	2.11
6	0.2% EMS	0.00	0.71	0.0	0.00
7	0.3% EMS	0.58	0.00	0.29	0.00
8	15 kR + 0.2% EMS	0.00	0.00	1.61	0.00
9	30 kR + 0.2% EMS	0.00	0.00	0.23	0.45
10	45 kR + 0.2% EMS	1.29	0.00	0.00	0.00

**Table 5** Frequencies and spectrum of different types of morphological mutants recorded in M<sub>2</sub> generation in PK-1042 cultivar of soybean

serial no.	treatment	morphological mutations/%				
		gigas	black pod	smooth pod	black spotted seed	early maturing
1	control	0.00	0.00	0.00	0.00	0.00
2	15 kR	0.00	0.81	0.27	0.00	0.00
3	30 kR	0.00	0.00	0.71	0.00	0.36
4	45 kR	0.75	0.00	0.00	1.00	0.00
5	0.1% EMS	0.00	0.83	0.00	0.00	0.67
6	0.2% EMS	0.00	0.00	0.74	0.00	0.45
7	0.3% EMS	0.76	0.00	0.00	0.00	0.00
8	15 kR + 0.2% EMS	0.00	0.41	0.62	0.00	0.00
9	30 kR + 0.2% EMS	0.24	0.00	0.00	2.14	0.00
10	45 kR + 0.2% EMS	0.53	0.00	0.00	1.07	0.00

morphology of the vegetative parts as well as yield were affected toward the positive side against the control populations. In earlier studies, workers have reported induction of viable mutations by the mutagens (Kharkwal, 2000, in chickpea and Singh et al., 2000, in mungbean). The tallness as observed in the present study is due to an initial increase in the internode length, sometimes accompanied by an increase in internode number (Jana, 1963). He further opined that increased length of the cells and their number per unit area can also contribute to tallness. Earlier, tall mutants were reported by Begum et al. (1995) and Solanki et al. (2004) in lentil. Plants with reduced height (dwarf) were identified in Pusa-16. The mean plant height of dwarf mutants was 31.25 cm as against 69.40 cm of the parent cultivar. The mutant plants possessed small leaves, few flowers, and small pods, and the grain yield was low. The dwarfness could be due to the distinction of auxins (Smith and Kerstein, 1942), interference with the synthesis of new DNA (Pelc and Howard, 1955), and reduction in the internodal length (Kumar et al., 1967). Dwarf mutants have earlier been reported by Satpute (1994) in safflower and in lentil by Ramesh and Dhananjay (1996) and Solanki and Sharma (2002).

A few mutants for smooth pods were also found in PK-1042 (Table 2). These mutants were inferior in seed yield and other yield contributing characters against the control population. Dwivedi and Singh (1985) reported smooth pod mutants in black gram. Early maturing mutant obtained in the present study showed normal growth and rapid productivity. The yield, pods per plant, and 100-seed weight of these mutants were also high as compared to the parent population. These results are in conformity with the findings of Ramesh and Tyagi (1999), Thakur and Sethi (1993), and Solanki and Sharma (2002). Jana (1962) explained that early maturity may be due to physiological changes caused by irradiation and increased production of flowering hormone. Variations in seed coat color (black spotted seed), pod color (black pod), and seed size (bold seed) were also observed in the present investigation. The yield and other yield contributing characters of bold seed and black spotted seed mutants were higher as compared to the control populations. Variation in the seed coat color may be attributed to the genetic factors like pigmentation factor, pigment complementary factor, and modifying factors. Variations in seed coat color were earlier reported by Sharma (1989) and Reddy (1991) while variations in

seed size were reported by Singh and Raghuvanshi (1991) in black gram. One mutant, viz., gigas, was also recorded in the present study. The yield and other contributing characters of this mutant were lower as compared to the control population.

In the present study, some of the abovementioned mutants differed for more than one character from the parent cultivars. For example, some of the early maturing mutants were taller and also possessed higher number of pods per plant. In this regard, two possibilities may be considered: the first possibility is that this may be result due to the pleiotropic gene effects of the mutated gene, and a second possibility assumes that they are mutations for several genes (each controlling a separate trait) that was very closely linked but mutated simultaneously during the mutation treatment. Because of their close linkage, the single genes of the group were not separated from each other by crossover events. Therefore, the complex of diverging characters was transferred as a unit from generation to generation, showing a monogenic inheritance in crosses.

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