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GAMMA RAY INDUCED VIABLE MUTANTS IN SOYBEAN (*Glycine max.* (L.) Merrill)

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ABSTRACT
Two well recommended high yielding varieties of Soybean for Jharkhand namely, BSS-2 and RKS-18 were exposed to five different doses (50 Gy, 100 Gy, 150 Gy, 200Gy and 400 Gy) of gamma rays using cobalt 60 (Co^{60}) sources of Gamma chamber at Bhabha Atomic & Research Centre, Mumbai and their M2 progenies were screened thoroughly. Morphological (viable) mutants were observed and recorded in M_2 generation from early seedling stage till physiological maturity with different doses of Gamma rays. The frequency of viable mutants ranged from 3.12% to 53.12% in variety BSS-2, while it ranged from 3.25% to 100% in the variety RKS-18. The viable mutation frequency was found increasing with increase in dose of mutagen in both the varieties BSS-2 and RKS-18. In the variety,BSS-2 the maximum number of viable mutants was isolated in the lowest dose (50Gy) dose, while in the variety RKS-18 the highest number of viable mutants was found in 50Gy and 100Gy showing that lower doses are more effective in inducing mutants than higher doses (200Gy and 400Gy). The present study reveals a good scope for isolation of induced morphological mutants of Soybean which can be utilized in future breeding programme.

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) also known as golden bean is an important oilseed crop. Among the major oilseed crop, Soybean is one of the most important oilseed crop. It contains 40% good quality protein rich in lysine and 20% oil high in essential fatty acids (omega-6 and omega-3). In addition, it contains good amounts of minerals, salts, vitamins and isoflavones. Due to the versatile nature of this crop, its contribution to industrial, agricultural, and medicinal sectors is significantly increasing. There is a strong global demand of soybean for its oil and meal. In India the productivity of soybean is much low in comparison with the world average. The main attributes identified for low productivity are limited genetic diversity, narrow genetic base of Indian soybean varieties and stagnant genetic potential for yield (Tiwari, 2003). Narrowing down of the genetic base is due to the repeated use of few parents for breeding programmes (Satyavathi *et al.*, 2006). At this context, widening of genetic base is a major concern and challenge put forward to the Indian soybean breeders.

To meet the growing demand, more variability is required for development of new high yielding varieties of Soybean with desired characters. In soybean, creation of genetic variation through hybridization is a tedious process due to small and fragile flowers, which make it very difficult to carry out the process of emasculation and injuring the parts of the flower are prone to heavy flower shedding even under favorable conditions (Datta & Maiti, 1966; Lal & Haque ,1971). These coupled with complete self-fertility impose limitations on the success of hybridization programme. As a consequence, lack of required amount of variability, limits the scope for the selection of better genotypes. Frequency of natural mutations is very low and information on mutagenesis induced population is scanty (Singh 2009). Hence, artificially induced mutations are the best way to enlarge genetic variability, considerably within a short time (Patil *et at.,* 2003; Singh and Singh 2003). A wide range of characters which have been improved through induced mutation breeding include plant architecture, yield, flowering and maturity duration, quality and tolerance to biotic and abiotic stresses. About 90% of mutant varieties have been developed using physical mutagens such as X-rays, χ-rays, thermal and fast neurons where χ-rays alone account for the development of 65% of the mutant varieties (Kharkwal, 2009). In general mutation breeding has been playing a key role in self pollinated crop with limited variability. Under such situation induced mutagenesis has been recognized as the most efficient method for induction of morphological and genetical variability in soybean plants. Gamma rays have been proved economical and effective in inducing mutation. Mutation breeding has become increasingly popular in recent times as an effective tool for crop improvement (Acharya *et al.,* 2007). The present investigation was undertaken with the objective of inducing mutations in two well adapted varieties of Soybean employing ã- radiation to obtain putative mutants with desirable and economically important characters.

MATERIALS AND METHODS

The material for this study comprised of two varieties of Soybean, BSS-2(medium height, white flowers and whitish yellow seed with light brown hilum) and RKS-18(tall, purple flowers and yellowish seed with gray to black hilum). In Kharif 2014, two thousand (2000) seeds of both varieties, irradiated with five different doses of ã-rays (50Gy, 100Gy, 150Gy, 200Gy & 400Gy) using Co^{60} sources of Gamma chamber at Bhabha Atomic & Research Centre, Mumbai were sown along with control untreated seeds in the single plot design at research farm of Birsa Agricultural University, Kanke, Ranchi. M_2 seeds from individual plants were harvested separately. For qualitative characters assessment, representative M_2 seeds from 5649 families were sown in Kharif 2015 in non replicated trial as progeny to row method in single row of 1m length (20 seeds per row) having 45 cm row to row spacing and 10 cm plant to plant distance along with control untreated . The soil type of the experimental site was lateritic with the pH 5.8. In this region, Soybean cultivation is mainly dependent on monsoon rains. The climate of this place is subtropical type and the average annual rainfall of this area is approximately 1400 mm which is mostly erratic and punctured with occasional dry spells. Nearly 80% of the total rainfall comes during four monsoon months (mid June to mid October).The data recorded for different characters in all the five treatments of M2 generations and control were subjected to statistical analysis on the basis of t-test and z-test (R.A Fisher 1922)**.** The frequency and spectrum of different types of viable mutants were scored at various developmental stages of M_2 plants. The mutants were classified taking into consideration the most conspicuous characters namely stature, leaf shape, seeds per pods and seed variants *etc*. The frequency and spectrum of viable mutants were calculated and important characters of each mutant were recorded.

RESULTS AND DISCUSSION

Mutational event may be accompanied by a larger or small change in phenotype; such changes have highest significance in plant breeding (Brock, 1970, Sigurbjornsson, 1972). Gaul (1964) had classified viable mutations as macro and micromutation, while Swaminathan (1964) grouped them as macro mutations and systemic mutations. A number of new commercial varieties have been originated from induced macro mutants and proved to be important in attaining different breeding objectives. In general, macro mutants play an important role in plant breeding as it may lead to the evolution of new genotypes.

Table 1: Frequency of viable mutants induced by gamma rays

Variety	Dose	Total plants studied in M2	Total plants showing viable mutation	Mutation Frequency
BSS - 2	Control	72	-	-
	50 Gy	7845	245	3.12
	100 Gy	1358	139	10.24
	150 Gy	64	34	53.12
	200 Gy	13	5	38.46
	400 Gy	44	21	47.73
RKS - 18	Control	67	-	-
	50 Gy	2032	66	3.25
	100 Gy	1533	91	5.94
	150 Gy	581	41	7.06
	200 Gy	257	17	6.61
	400 Gy	4	4	100

In present investigation, some morphological (viable) mutants were observed in M_2 generation from early seedling stage to till the plant attained physiological maturity with different doses of Gamma rays and an increase in number of viable mutants were recorded. The M_2 progeny was screened thoroughly and various mutations were recorded throughout the crop duration. The frequency of viable mutation was recorded (Table 1).The mutations affecting different morphological features of the plants were recorded and grouped (Table 2 & 3).

The frequency of viable mutations in M_2 plants showed inconsistent relationship with mutagen dose.(Table 1). The frequency of viable mutants ranged from 3.12% to 53.12 % in variety BSS-2 while it ranged from 3.25% to 100% in the variety RKS-18.

In M_2 generation of the variety, BSS-2, mutation frequency increased with increase in dose of Gamma rays. Highest value of mutation frequency (53.12) was recorded in 150 Gy, while lowest was recorded in 50 Gy (3.12). In the variety, RKS-18 it increased parallel with increase in dose of Gamma rays with exceptionally slight decrease in 200 Gy. 400 Gy recorded highest mutation frequency (100 percent).

The spectrum of viable macro mutations observed in the present study consisted of dwarf stunted growth, leaf aberrant and seed character variation and flower colour variation as shown in (Table 2&3). Reduced plant height is an important trait in plant breeding, because short genotypes are resistant to lodging (Austin *et al.,* 1980). The mutants with mean height of the plant of more than 20 cm but less than 35cm were considered as dwarfs in this study. The mean height of control plants were 58.68 cm in BSS-2 and 51cm in RKS-18, whereas in dwarf mutant it was 10 cm in BSS-2 and 9 cm in RKS-18. Dwarf mutants were recorded at all doses of treatment (Table 2&3). Some dwarf mutants also showed early flowering but it does not showed any variation in grain yield as compared to control plants. Among the treatments maximum number of mutants was recorded at 50Gy in both the varieties. Similar mutants were observed by Sinha (1998) and Juliet Hepzib and Subramaniam (2002) and Mesharam *et al.* (2013) in Blackgram. Kumar and Dubey (1998) in *Lathyrus sativum*. Ramesh and Seetharami Reddy (2002) in Rice, Yadava *et al.* (2003) in Kodo-millet, Patil *et al.* (2003) and Pavadai (2006) in Soybean and Ahirwar *et al.* (2014) in Lentil.Leaf mutants observed in M_2 generation included change in leaflet number, leaf shape, leaf size, sessile leaves, leaf and leaf vein discoloration (Table 2&3). The variety BSS-2 showed more prominent and frequent leaf mutation as compared to the variety RKS-18. Maximum number of variations recorded was for change in leaflet number like (bifoliate, quadrifoliate, pentafoliate and hexafoliate) (Fig. 1, 2, 3 & 6). In the variety, RKS-18 bifoliate and bifoliate sessile leaves were observed (Fig. 5). Apart from the mutants with changed leaflet number many small leaf mutants were also recorded in the variety BSS-2 in 100 Gy and 150 Gy doses the change in leaflet number did not show any alteration in grain yield as compared to control. Similar mutants were also observed by Sengupta and Datta (2005) in Sesame. In the variety, BSS-2 mutants for leaf

Figure1 : Pentafoliate leaflets

Figure 2 : Quadrifoliate laeflets

Figure 3: Bifoliate leaflets

Figure 4: Dwarf and early flowering plant

Figure 5 : Bifoliate sessile leaflets

Figure 6: Hexafoliate leaflets

Figure7 : Bifoliate leaflet with bifurcated venetation

Figure 8 : Lanceolate shaped leaves

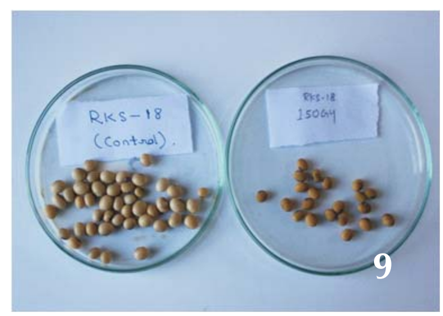

Figure 9 : Yellow seed coat

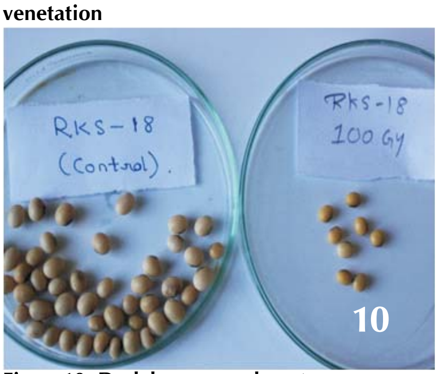

Figure10: Dark brown seed coat

Figure11 : Perfect black hilum

Figure 12 : Twinging stem

shape like small ovate shaped leaves, heart shaped bifurcated leaf, bifurcated venation (Fig. 7), lanceolate leaf shape(Fig. 8) were recorded. The variety RKS-18 did not showed any mutants for leaf shape. Many sessile leaves (without pedicels) were also observed in both the varieties. Mutant leaves with alteration in colour like yellow patches on leaves, yellow leaf margin observed in the BSS-2 while no such mutants were recorded in the variety RKS-18. 100 Gy dose of Gamma rays was found more prominent in causing this type of discolouration in the leaves. Leaf veins discolouration (brown) was observed in both the varieties.

Bold seeded mutants, which had bigger seeds than (control), were recorded only in the variety, BSS-2 at 100 Gy dose while the small seeded mutants which had small sized seeds than that of control were observed at all other doses. In the RKS-18 all five doses recorded small seeded mutant. In Green gram

Table 2: Spectrum of viable mutants induced by gamma rays in BSS-2 variety of soybean

MUTANT CHARACTER / DOSE	Score	50 GY	100 GY	150 GY	200 GY	400 GY
PLANT HEIGHT						
1.Short height plant	-	0	1	2	1	6
2.Short height plant and early flowering	-	10	0	0	0	0
LEAF						
1.Bifoliate	-	5	1	6	1	0
2.Bifoliate and sessile	-	0	1	2	1	4
3.Trifoliate with an small leaf	-	0	0	0	0	0
4.Trifoliate with one leaf sessile	-	2	0	0	0	0
5.Quadrifoliate	-	66	24	8	1	1
6.Sessile quadrifoliate leaf	-	0	0	0	0	2
7.Pentafoliate	-	14	4	0	0	0
8.Hexafoliate	-	0	0	0	0	0
9.Yellow leaf	-	1	1	0	0	0
10.Small leaf	-	0	2	1	0	1
11.Small rounded ovate leaf	4	0	2	0	0	0
12.Micro leaf	-	0	1	0	0	0
13.Heart shaped leaf	-	2	2	2	0	0
14.Obviate leaf shape	-	0	1	0	0	0
15.Irregular leaf shape	-	0	4	0	0	0
16.Yellow patch on leaf	-	0	1	0	0	0
17.Yellow leaf margin	-	1	1	0	0	0
18.Brown venation	-	4	0	0	0	0
STEM						
1.Twining stem	-	6	0	0	0	0
2.Tricotyledon	-	6	5	0	0	0
3.Tetracotyledon	-	3	1	0	0	0
4.Branched cotyledon	-	1	0	0	0	1
5.Brown hypocotyls (Pigmentation present)	9	6	4	0	0	0
SEED						
1.Light Brown hilum	3	32	32	1	0	1
2.Black Hilum	6	19	8	0	1	2
3.Dark brown hilum	4	17	7	2	0	1
4.Medium Brown seed coat	5	15	21	0	0	0
5.Dark brown seed coat	6	0	0	6	0	1
6.Light brown seed coat	4	5	1	0	0	0
7.Dark yellow seed coat	1	19	8	0	0	0
8.Small seed	3	7	3	2	0	1
9.Large seed	7	0	2	0	0	0
10.Elongated seed	3	1	1	2	0	0
11.Brown patch on seed	-	3	0	0	0	0
Total		245	139	34	5	21

Table 3: Spectrum of viable mutants induced by gamma rays in RKS-18 variety of soybean

MUTANT CHARACTER/DOSE	SCORE	50 GY	100 GY	150 GY	200 GY	400 GY
PLANT HEIGHT1.Short height plant	_	2	3	2	0	2
2.Short height plant and early flowering	4	2	1	0	1	0
LEAF						
3.Bifoliate	_	3	1	1	0	0
4.Bifoliate and sessile	_	2	1	1	0	0
5.Brown venation	_	0	0	0	1	0
FLOWER COLOUR						
1.Light violet flower colour	_	0	1	1	0	0
SEED						
1.Small seeds	3	18	16	6	4	1
2.Elongated seeds	3	1	0	0	0	0
3.Yellow seed coat	1	21	22	0	0	0
4.Dark brown seed coat	6	8	5	5	0	0
5.Black seed coat	7	0	1	0	0	0
6.Broad hilum	_	2	2	2	2	0
7.Small hilum	_	0	2	8	3	0
9.Light Brown hilum	3	0	0	3	4	1
Total		66	91	41	17	4

Ramya *et al.* (2014) isolated bold seeded mutants and Usharani and Kumar (2015) reported bold and small seeded mutants in Urdbean. In the variety BSS-2, light brown and medium brown seed coat was observed in lower doses (50 Gy and 100 Gy), while dark brown seed coat was observed at 150 Gy and 400 Gy. Besides dark yellow seeded mutants and brown pigmentation on seed coat were observed in 50 Gy dose, while no such kind of pigmentation was found in control plants .In the variety RKS-18, dark brown at 50,100 & 150 Gy , yellow at 50 & 100 Gy and black at 150 Gy seed coat mutants were recorded(Table 3, Fig. 9,10). Similar variation in seed colour was reported by Pavadai (2006) in Soybean and Ramya *et al.* (2014) in Blackgram, Black discolouration of hilum was noticed in both the varieties. In the variety BSS-2, dark brown to perfect black hilum discolouration was also observed (Fig. 11), while in RKS-18 only light brown discolouration of hilum was recorded. In RKS-18 an increased in hilum diameter (broad hilum) was noticed. Tak shi and Abe (1994) reported yellow hilum in Soybean. Cober *et al.* (1998) assessed that the colour of hilum ranges from yellow to black depending upon genetic background of the line and environmental conditions. Elongated seed shape mutants were also observed in both the varieties. In the varietyBSS-2, it was observed at 50 Gy, 100 Gy and 150 Gy while it was only at 50 Gy in the variety, RKS-18. All these types of seed mutations did not show any variation in grain yield except the bold seeded mutant which showed slight increase in grain yield.

From the early growth stage the M_2 plants showed mutations which were observed in form of increase in number of cotyledons (tricotyledanary/tetracotyledanary/branched cotyledons).This type of early stage mutants were observed in the variety, BSS-2 in lower doses (50 Gy and 100 Gy). Brown pigmentation in hypocotyls region was also observed in the variety, BSS-2 in lower doses (50 Gy and 100 Gy).

Twining mutants were also recorded at 50 Gy in the variety, BSS-2 (Fig. 12).The stem was twining upto 30 cm between there was no variation for traits like flowers, number of pods and grain yield as compared to control. Twining type mutants has been reported earlier by Malarkodi (2008) in Urdbean.

Flower colour mutants were observed only in the variety RKS-18 in 100 Gy and 150 Gy. The mutant plants had light purple colour flowers instead of the purple colour flowers of RKS-18.

The putative mutants isolated in the present study included mutants with agronomically desirable features which could possibly be utilized in future for breeding programmes. In both the varieties, BSS-2 & RKS-18 the spectrum of viable mutation was high at lower dose of Gamma rays. In the variety, BSS-2 maximum number of viable mutations (245) was recorded in 50 Gy of Gamma rays followed by 100 Gy dose (139). In the variety, RKS-18 maximum number of viable mutants (91) was observed in 100 Gy dose followed by 50 Gy (66). Maximum number of mutants were obtained for seed related traits such as (changed seed coat colour, shape, hilum colour) followed by leaf related traits. The possible cause of these macro mutations may be chromosomal aberrations, small deficiencies or duplications and most probably gene mutations. Hanafiah (2010) opinioned that low irradiation (micro dose) can produce variance of desired character. Sakin (2002) also observed mutation in quantitative characters of wheat at low doses of Gamma irradiation. Besides, low dose of irradiation which allows mutations to happen in the minor genes that can be observed in coming generation without harmful effect.

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