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Effect of Different Doses of γ-rays (50 Gy, 100 Gy, 150 Gy, 200 Gy& 400 Gy) in M_3 Generation of Soybean (*Glycine max* (L.) Merril) Genotypes

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A B S T R A C T

Soybean genetic variation improvement is important for the development of superior cultivars. One of the greatest challenges in mutation breeding is random (uncontrolled) nature of induced mutagenesis. Large population requirement for desired mutant selection brings intensive labor. Choice of appropriate mutagen is one of the deciding factors on succession of the mutation breeding program. Physical, chemical, or biological agents are viable alternatives. Among physical mutagens ionizing radiation sources, particle (electrons, protons, neutrons, alpha and beta particles) or electromagnetic (X-rays, gamma rays), are widely used. Ionizing radiation interacts with genetic material and cause mutations on DNA sequences. Magnitude of mutagenic effect is proportional to the radiation dose Physical mutagen are still improving and mutation breeding proves its value to be fast, flexible, and viable in crop sciences. It is crucial to determine and optimize the effective radiation dose based on experimental plant variety, plant part, and radiation source. 80% of mutation breeding studies prefer physical mutagens and of 60% of this use gamma radiation. In both the soybean varieties, BSS-2 & RKS-18 the spectrum of viable mutation was high at lower dose of Gamma rays. In variety, BSS-2 twining stem, tricotyledon, tetracotyledon were observed while in M_3 generation. In RKS-18 stem mutants were not observed. Whereas in the variety, BSS-2, the spectrum of viable mutations was high at dose 100 Gy (14) while in RKS-18, the spectrum of viable mutations was high at lower dose 50 Gy (16).

Introduction

Soybean being predominantly self fertilized, inherent variability in this crop is much limited. Besides vulnerability of their flowers to artificial manipulation renders production of large scale variability through hybridization. Due to lack of sufficient natural variability for yield and its component traits in soybean, conventional methods of breeding have limited scope. In this regards, mutation breeding have been used to generate genetic variability and have been successfully utilized to improve yield and yield components of various crops Lagoda (2009). Yield and related characters are controlled by polygenic systems. Brock (1965, 1970) reported that these polygenes can be altered positively or negatively by mutations. Since the development of a variety is based on magnitude of genetic variability in base material and extent of variability for desired characters, the locked genetic variability in respect of linked genes is not released even by close or distant hybridization.

Under such situations mutation breeding is now playing an important role in developing new genetic resources and breakage of unwanted linkages (Manjaya, 2007, 2008). Induced mutations have generated a vast amount of genetic variability and are now widely used for the development of genes controlling important traits and understanding the functions and mechanisms of actions of these genes in plants.

Induced mutagenesis has been recognized as the most efficient method for induction of morphological and genetical variabilities in plants especially in those where genetic variability is limited (Bado *et al.,* 2015). Using mutation breeding, genetic improvement of any yield attributes either qualitative or quantitative trait, has been successfully achieved in soybean and also in other oil crops (Kharkwal and Shu, 2009).

Soybean flowers represent cleistogamous characteristics. Cleistogamy, which is described as the production of both open (chasmogamous, CH) and closed (cleistogamous, CL) floral forms by one species, is very common among angiosperms.

Soybean is pseudocleistogamous cleistogamy in which no morphological differences between CL and CH flowers occur other than a lack of expansion of petals and anthesis in CL flowers. It may also be induced by environmental stress factors, occasionally. Cleistogamy is observed both in cultivated soybean [*G. max* (L.) Merr.] and its wild relative [*G. soja* Sieb. & Zucc.]. Soybean usually produces both CH and CL flowers on the same plant. In these plants, fertilization occurs within closed petals of CL flowers (Valliyodan *et al*., 2016, 2021; Takahashi *et al*., 2021). The rates of natural cross-pollination have been observed between ranges of 0.03–1.14% in natural conditions for self-pollinating soybean plant (Malarkodi, 2008). Thus, cleistogamy may have influenced the genomic homogeneity and reduced genomic variation further in soybean along with domestication practices.

Materials and Methods

In *kharif* 2020 representative M_3 Gamma rays irradiated seeds (300 each) of two varieties of soybean, BSS-2 and RKS-18 (table no. 1) procured from the Department of Genetics and Plant Breeding, BAU, Ranchi were sown in western section. Seeds of both the varieties viz. BSS-2 and RKS-18 were exposed to five different doses of gamma rays (50 Gy, 100 Gy, 150 Gy, 200 Gy and 400 Gy) in the year 2014 and 2015 using Cobalt 60 sources in Gamma chamber at Bhabha Atomic and Research Centre, Mumbai (table 2) and their M_3 progenies were used as experimental materials. The frequency and spectrum of qualitative mutations was studied in the M_3 generation. In *Kharif* 2016, seeds of two varieties of Soybean BSS-2 & RKS-18 of five (5) different doses of γ-rays (50 Gy, 100 Gy, 150 Gy, 200 Gy & 400 Gy) were sown as progeny to row method in four row of 3.5 m length having 45 cm row to row spacing and 10cm plant to plant spacing in two replication in RBD design, along with control of both the varieties, BSS-2 and RKS-18. The assessments of qualitative characters were made only from selected families. The spectrums of different types of viable mutants were scored at various developmental stages in putative mutants population of M_3 generation. The mutants were classified taking into consideration the most conspicuous characters namely stature, leaf shape, seeds per pods and seed variants etc. The frequency, effectiveness, mutation rate, injury percentage, lethality percentage, injury percentage and spectrum of viable mutants were calculated and important characters of each mutant were recorded.

Results and Discussion

The spectrums of different types of viable mutants were scored at various developmental stages of M_3 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, mutation rate efficiency recorded (table 3) and spectrum of viable mutants were calculated and important characters of each mutant were recorded. The viable 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 soybean varieties, BSS-2 & RKS-18 the spectrum of viable mutation was high at lower dose of Gamma rays (Table 4). In the variety, BSS-2 maximum number of viable mutations (14) was recorded in 100 Gy of Gamma rays followed by 100 Gy dose (28).

Table.1 Salient feature of two experimental materials

Variety	Birsa Safed Soybean-2 (BSS-2)	Pratap Soya-2 (RKS-18)
Source	Birsa Agricultural University, Kanke, Ranchi	Borkhera farm, Agricultural University, Kota, Rajasthan
Parentage	JS 335 X MACS 58	MACS 450 X Monetta
Growth Type	Semi determinate	Determinate
Maturity	Medium	Medium
Flower Colour	White	Purple
Pod	Pubescent	Glabrous
Seed	Whitish yellow with light brown hilum	Yellowish seed with gray to black hilum
Specific features	Tolerant to bacterial pustules, Frogeye leaf spot, moderately resistant to Cercospora leaf spot & immune to Target leaf spot diseases. Tolerant to Blue beetle and Defoliator.	Tolerant to bacterial pustules, Girdle beetle and Leaf miner

Table.2 Treatment Details of M_3 and M_4 generation of soybean (*Glycine Max* (L.) Merril) genotypes

M_3		M_4	
T1	BSS2[50 Gy]	T11	BSS2[50 Gy]
T2	BSS2[100 Gy]	T12	BSS2[100 Gy]
T3	BSS2[150 Gy]	T13	BSS2[150 Gy]
T4	BSS2[200 Gy]	T14	BSS2[200 Gy]
T5	BSS2[400 Gy]	T15	BSS2[400 Gy]
T6	RKS-18[50 Gy]	T16	RKS-18[50 Gy]
T7	RKS-18[100 Gy]	T17	RKS-18[100 Gy]
T8	RKS-18[150 Gy]	T18	RKS-18[150 Gy]
T9	RKS-18[200 Gy]	T19	RKS-18[200 Gy]
T10	RKS-18[400 Gy]	T20	RKS-18[400 Gy]

Table.3 Frequency, mutation rate, efficiency and effectiveness of macro mutation induced by gamma rays in putative mutant population of two varieties (BSS-2 and RKS-18) of soybean M_3 generation.

Variety	Dose	Toal seeds sown		Total plants studied in M_3	Total plants segregated in M_3	Mutation Rate	Mutation Frequency(m)	Effectiveness	Efficiency m X100 /L	Efficiency m X100 /I
BSS – 2	Control	300		30		0.086				
	50 Gy	300		50	5		10	0.2	5154.63	40.45
	100 Gy	300		50	7		14	0.14	532.31	56.84
	150 Gy	300		50	3		6	0.04	78.94	20.44
	200 Gy	300		50	4		8	0.04	266.66	16.26
	400 Gy	300		50	2		4	0.01	93.24	8.01
RKS – 18	Control	300		30		0.106				
	50 Gy	300		50	8		16	0.32	1649.48	89.08
	100 Gy	300		50	5		10	0.1	215.05	35.54
	150 Gy	300		50	6		12	0.08	-1463.41	40.65
	200 Gy	300		50	3		6	0.03	301.50	14.01
	400 Gy	300		50	1		2	0.005	64.30	4.06

Table.4 Spectrum of viable mutants induced by gamma rays in putative mutant population of BSS-2 variety of soybean in M_3 generation (2017-18)

S. No	Mutant Character/Dose	50 Gy	100 Gy	150 Gy	200 Gy	400 Gy
PLANT						
1	Short height plant	1	1		2	-
2	Short height plant and early flowering	1	1	-		1
LEAF						
1	Bifoliate	1	1	2	-	-
2	Bifoliate and sessile				2	1
3	Trifoliate with one leaf sessile	1	1			
4	Quadrifoliate	1	1	2		1
5	Sesile quadrifoliate leaf					
6	Pentafoliate	1	1		1	
7	Hexafoliate					
8	Yellow leaf					
9	Small leaf		1			
10	small rounded ovate leaf					
11	Micro leaf					
12	Heart shaped leaf		2			
13	Obovate leaf shape					
STEM						
1	Twining stem	1	-	-	-	-
2	Tricotyledon		1	1		
3	Tetracotyledon	1			1	
4	Branched cotyledon					
5	Brown hypocotyl (Pigmentation present)					
SEED						
1	Light Brown Hilum		1			
2	Black Hilum					
3	Dark brown Hilum	1	1		1	1
4	Medium Brown seed coat					
5	Dark brown seed coat		1	1		
6	Light brown seed coat					
7	Small seed		1		1	
8	Large seed					
9	Elongated seed	1				
10	Brown patch on seed					
Total		**10**	**14**	**6**	**8**	**4**

Table.5 Spectrum of viable mutants induced by gamma rays in putative mutant population of RKS-18 variety of soybean in M_3 generation (2017-18)

S. No	Mutant Character/Dose	50 Gy	100 Gy	150 Gy	200 Gy	400 Gy
	Plant Height					
1	Short height plant	2	1	1	1	1
	Leaf					
1	Bifoliate	2	1	2	1	
2	Bifoliate and sessile	1	1	-	-	
3	Brown venation	-	-	1-		
	Flower Colour					
1	Light violet flower colour	2	1	-	1	
	Seed					
1	Small seeds	1	-	1		1
2	Elongated seeds	2	2	2		
3	Yellow seed coat					
4	Dark brown seed coat	3	2	2		
5	Black seed coat					
6	Broad Hilum	3	1	2	1	
7	Small Hilum		1	1	2	
8	Black Hilum					
9	Light Brown Hilum					
Total		**16**	**10**	**12**	**6**	2

In the variety, RKS-18 maximum number of viable mutants (29) was observed in 50 Gy dose (10). The possible cause of these macro mutations may be chromosomal aberrations, small deficiencies or duplications and most probably gene mutations. Maximum number of mutants was obtained for seed related traits such as (changed seed coat colour, shape, hilum colour) followed by leaf related traits. In both the variety BSS-2 and RKS-18 maximum number of Leaf mutant observed in M_3 generation included changes in leaflet number, leaf shape, leaf size, sessile leaves, lanceolate leaves, bifoliate leaves, tetrafoliate leaves, heart shape leaves. The variation in leaflet number did not give any variation in seed yield as compared to control.In variety BSS-2 mutant for leaf shape like like Bifoliate, Bifoliate and sessile, Trifoliate with one leaf sessile, Quadrifoliate, Pentafoliate, Small leaf, Heart shaped leaf were observed while in RKS-18 bifoliate, bifoliate and sessile, brown venation leaves were observed in M_3 generation. In variety, BSS-2 light brown hilum, dark brown hilum, dark brown seed coat, small seed, elongated seed were observed while in RKS-18 Small seeds, elongated seeds, dark brown seed coat, broad hilum, small hilum were observed in M_3 generation.

In variety, BSS-2 twining stem, tricotyledon, tetracotyledon were observed while in M_3 generation. In RKS-18 stem mutants were not observed. in the variety, BSS-2, the spectrum of viable mutations was high at dose 100 Gy (14) while in RKS-18, the spectrum (table- 5.) of viable mutations was high at lower dose 50 Gy (16). Mutation breeding passed important cross-roads successfully during recent advances mutagenesis will retain its place in crop science in next decades especially for the plants as soybean for which cross breeding is limited or not applicable.

Author Contributions

Priyanka Kumari: Investigation, formal analysis, writing—original draft. Manigopa Chakraborty: Validation, methodology, writing—reviewing. Milan Kumar Chakravarty:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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