

# Study of the Cytogenetical Effects on Meiotic Chromosomal Abnormalities Induced by Mutagens in Soybean

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Received: 14 Dec 2017/Revised: 19 Jan 2018/Accepted: 28 Feb 2018

**ABSTRACT-** An attempt was made to study the cytogenetical effects of gamma rays and ethyl methane sulphonate on meiotic chromosomal abnormalities in two cultivars viz., PKV-1 and JS-335. The most frequently observed aberrations in meiosis were univalents, trivalent, multivalents chromosomal fragments, desynapsis of chromosome, laggards, and clumping of chromosomes etc. The physical mutagens were more effective than chemical mutagens. The effect of gamma-rays and ethyl methane sulphonate shows chlorophyll mutations such as Chlorina, Xantha, Albina, and Alboviridis in an M<sub>2</sub> generation in both the cultivars. Cultivar JS-335 showed more pronounced effect than cultivar PKV-1. Gamma-rays recorded maximum macro mutations as compared to chemical mutagens (EMS). The frequency and spectrum of morphological mutation indicated that variety JS-335 was more sensitive than PKV-1. Different response of the two varieties to various mutagens was noticed.

**Key-words-** Chromosomal aberrations, Chlorophyll mutation, EMS, Gamma radiation, Mutagens

## INTRODUCTION

Soybean, *Glycine max* (L.) Merrill is one of the world's most important sources of vegetable oil and protein. It is a highly self-pollinated crop, cultivated all over the world. Soybean being an autogamous crop, the naturally existing genetic variability may not be sufficient to achieve the desired improvement. Each kind of breeding method involves creation and utilization of genetic variability by means of hybridization, recombination and selection. Due to small size of flowers, emasculation and pollination in soybean is very tedious and costly. Alternatively artificially induced mutation is the best way to widen the genetic variability of a species considerably within a short time.

Mutation breeding is relatively a quicker method for improvement and creating genetic variability in crops. Attempts to induce mutations in soybean would be quite useful in creating genetic variability. Mutation is a sudden heritable change brought about in nucleotide base pairs either by addition, deletion or substitution <sup>[1]</sup>. It may be caused by the various factors which lead to a change in the coded information finally expressed in terms of a changed phenotype, through alteration in the chain of events like transcription and translation.

In other words, the biochemical pathway gets affected resulting in the modified manifestation of a gene.

Recently, generation of genetic variability by induced mutagenesis provided a base for strengthening crop improvement programme and represents a more efficient source of genetic variability than the gene pool conservers by nature <sup>[2]</sup>. Hence, mutation breeding can be applied to altering specific characters in otherwise good varieties, by incorporating some useful changes such as earliness, high oil and protein content, high yields, non-shattering and disease and insect resistant in a comparatively shorter time than conventional breeding methods.

The choice of mutagens holds great importance in changing the spectrum of mutations in a predictable manner. Considering the above facts the present study intended to assess the effect of gamma rays, Ethyl methane sulphonate (EMS) on induction of variability in two varieties of soybean.

## MATERIALS AND METHODS

Dry and healthy seeds of soybean variety JS-335 and PKV-1 were treated separately with chemical (EMS) and physical (gamma-rays) mutagens. 500 dry seeds of two cultivars were exposed to 15, 20, 25, 30 kR gamma-rays & also the same number were presoaked in distilled water for 6 hours and then flooded with freshly prepared 0.05%, 0.10%, and 0.15% aqueous solution of ethyl methane sulphonate for 6 hours. Then the treated seeds were thoroughly washed in running tap water.

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Quick Response Code	Website: www.ijlssr.com
	 DOI: 10.21276/ijlssr.2018.4.2.10

The treated seeds were immediately sown in the field with 45x10 cm spacing in two rows each treatment along with irradiated seeds and respective control to grow M1 generation. The sowing was done in Factorial randomized block design (FRBD) replicated thrice in the Field of Arts, Commerce and Science College in July 2012.

The seeds from each M1 plants were harvested separately and sown subsequently in progeny row basis in Rabi season (November 2012) to screen different chlorophyll and morphological mutations. Screening for chlorophyll mutations was done during the first 15 days of sowing of M2 generation. Mutation types were identified following the classification of Gustaffson [3]. Twenty-five plants from each treatment were randomly selected from the M2 population for raising M3 progeny in randomized block design with four replications during kharif season 2013. The observations were recorded on fifteen plants from each treatment per replication for almost all the characters studied in M2 generation. The statistical analysis was carried out as per the standard method of "Analysis of Variance" [4].

**RESULTS AND DISCUSSION**

Data on the frequency of morphological mutations observed in both soybean varieties viz. PKV-1 and JS-335 were presented in Table 1. The data revealed that the gamma rays doses recorded maximum macro mutations

as compared to EMS. However, the frequency and spectrum of morphological mutants indicated that cv. JS-335 was more sensitive than cv. PKV-1. As regards the mutation rate on the M1 family basis it was found to be higher than the M2 plant basis in both the varieties. Amongst mutagens, EMS in cv. PKV-1 and gamma-rays in JS-335 recorded highest mutation rate on M1 family and M2 plant basis.

The frequency of macro mutations expressed on M2 population basis as well as M1 progeny basis was found increased as the dose of gamma-rays increased. The observations recorded are in agreement with those of Raut *et al.* [5] in Soybean, Narsinghani and Kumar [6]; Venkateswarlu *et al.* [7]; Venkateswarlu *et al.* [8] and Nandarajan and Ramalingani [9] in pigeon pea. Whereas, lower concentration in EMS recorded that the highest frequency in both the varieties. Study of spectrum of viable cultivars showed that numbers of viable mutations were induced for growth habit mutants followed by leaf mutants and then economic mutations. The cultivar JS-335 produced relatively high number of macro mutations as compared to cv. PKV-1. In general, on an overall basis it can be said that, irrespective of varieties, the physical mutagen i.e. gamma-rays was more effective than the EMS (Table 1).

**Table 1:** Frequency spectrum of macro mutations induced by different mutagens

Treatment	Total M2 Plants	Mutation Frequency		Growth habit mutants	Mutants for duration	Pod characters			Leaf type	Hair characters			High yielding
		M1 Family basis	M2 Plant basis			Non Shattering	Large seeds	Bold seeded		Hairy	Non hairy	Red hairy	
<b>PKV-1</b>													
Control	2534	-	-	-	-	-	-	-	-	-	-	-	-
15 kR γ rays	2460	2.22	0.29	0.14	-	-	-	0.20	-	-	-	-	0.04
20 kR γ rays	2009	6.66	0.95	-	0.40	0.05	0.10	-	0.05	-	-	-	-
25 kR γ rays	1985	5.55	1.86	0.35	1.21	-	-	-	-	-	0.25	-	0.5
30 kR γ rays	1913	5.55	1.31	0.58	0.11	-	-	-	-	-	-	-	0.21
0.05 % EMS	2260	8.88	1.15	0.58	0.27	0.13	0.04	-	0.04	-	-	-	0.09
0.10% EMS	1860	5.55	1.94	1.34	-	-	-	0.10	-	-	-	-	-
0.15% EMS	1948	3.33	0.21	0.05	0.10	-	-	-	-	-	-	-	0.05
<b>JS- 335</b>													
Control	2753	-	-	-	-	-	-	-	-	-	-	-	-
15 kR γ rays	2640	8.88	1.48	0.15	0.46	-	0.07	0.07	0.07	0.07	-	-	-

<b>20 kR <math>\gamma</math> rays</b>	2430	6.66	2.06	1.69	0.78	-	-	-	0.12	0.12	-	-	0.29
<b>25 kR <math>\gamma</math> rays</b>	2289	5.55	0.92	-	-	0.09	-	-	0.48	0.21	-	-	0.13
<b>30 kR <math>\gamma</math> rays</b>	2123	11.49	2.83	1.13	0.52	-	-	-	-	-	-	0.14	0.14
<b>0.05 % EMS</b>	2439	6.66	1.35	0.16	0.12	-	0.16	0.04	0.77	0.77	-	-	0.04
<b>0.10% EMS</b>	2386	5.55	0.96	0.54	-	0.04	-	-	-	-	-	-	0.38
<b>0.15% EMS</b>	2067	2.22	0.19	0.19	-	-	-	-	-	-	-	-	-

**Meiotic abnormalities-** The various chromosomal aberrations recorded chromatin bridges, univalent, trivalent, quadrivalent, pentavalent, chromosomal fragments, desynapsis of chromosomes, laggards and clumping of chromosomes etc. The formation of trivalent and multivalent suggested that the exchange hypothesis may have operated in mutagen induced chromosome abnormalities as reported by Zeerak<sup>[10]</sup> in brinjal. The

high chromosome stickiness may be due to reduction of correctly polymerized nucleic acid on the chromosome producing characteristic errors in spiralization, which combined with superimposed excess of non polymerized nucleic acid causing surface stickiness<sup>[11]</sup>. High frequencies of chromosomal aberrations were recorded in gamma rays than by EMS indicating greater efficiency of gamma rays for inducing meiotic abnormalities (Table 2).

**Table 2:** Meiotic abnormalities as influenced by different mutagen

Mutagen	No of cells observed	Total cells with abnormalities	% abnormalities	Diakinesis				Metaphase I		Anaphase I		Telophase II
				Trivalent	Tetra valent	Multivalent	De-synapses	Trivalent	Tetravalent	Chromatin bridges	Laggards	Micronuclei
<b>PKV 1</b>												
<b>Control</b>	250	2	0.8	-	-	-	(0.8)	-	-	-	-	-
<b><math>\gamma</math> rays</b>	860	35	4.06	1.04	0.81	0.46	-	0.46	0.34	0.34	0.23	0.34
<b>EMS</b>	740	23	3.10	1.08	0.67	0.27	-	0.40	0.27	0.40	-	-
<b>JS 335</b>												
<b>Control</b>	305	3	0.98	-	-	-	-	-	-	-	-	-
<b><math>\gamma</math> rays</b>	915	48	5.24	1.31	0.98	0.54	-	0.43	0.32	0.54	0.65	0.43
<b>EMS</b>	872	32	3.66	0.80	1.03	0.22	-	0.34	0.34	0.45	0.22	0.22

**Chlorophyll mutations and viable mutation-** Four different kinds of chlorophyll mutant, viz. albina, xantha, chlorine, chlorine, and alboviridis were observed in both the cultivars. The data revealed that the different mutagens used differed significantly from each other for inducing chlorophyll mutations. However, gamma-rays in variety PKV-1 and ethyl-methane sulphonate in JS-335 was found most effective in inducing chlorophyll mutations so far as the different doses or concentration are concerned, all the doses were found effective in inducing chlorophyll mutations. Higher doses of gamma

rays showed the maximum number of chlorophyll mutations in both the varieties viz. PKV-1 and JS-335. Similarly, the higher concentration of EMS-induced maximum mutations in both the varieties PKV-1 & JS-335.

The frequency of chlorophyll mutations on M<sub>1</sub> family basis was found highest in 20 kR gamma-rays and 0.10% EMS concentration in both the varieties. Similarly, the mutations the frequency of the chlorophyll mutants on M<sub>2</sub> plant basis were highest in 25 kR gamma-rays dose in both the varieties while 0.10% EMS concentration in PKV-1 and 0.15% in JS-335 produced highest frequency of chlorophyll mutations.

The similar results were also observed by Badaya and Mehrotra<sup>[12]</sup>; Constanin<sup>[13]</sup>; and Wakode<sup>[14]</sup> in Soybean and Nandanwar and Khamankar<sup>[15]</sup> in Mung bean. However, gamma-rays in both the varieties were found most effective in inducing chlorophyll mutations than EMS. Gamma-rays and EMS both induced maximum chlorophyll mutations namely Albina, Xantha, Alboviridis, and Chlorina.

The similar spectrum of chlorophyll mutations was also reported by Rajput and Sarwar<sup>[16]</sup> in Soybean<sup>[17]</sup> in Pigeon pea<sup>[18]</sup> in gram pea,<sup>[19]</sup> in soybean. The order of frequency of chlorophyll mutations induced by various mutagens in PKV-1 can be represented as Xantha> Chlorina> Alboviridis> Albina and in JS-335 can be represented as Xantha> Chlorina> Alboviridis> Albina (Table 3).

**Table 3:** Frequency spectrum of chlorophyll mutations as influenced by different mutagens

Treatment	No. of M <sub>2</sub> plants	Chlorophyll mutants				Mutation frequency	
		Albina	Xantha	Alboviridis	Chlorina	M <sub>1</sub> family basis	M <sub>2</sub> family basis
<b>PKV-1</b>							
<b>Control</b>	2534						
<b>15 kR <math>\gamma</math> rays</b>	2460	0.12	0.49	-	0.29	5.56	0.89
<b>20 kR <math>\gamma</math> rays</b>	2009	0.20	0.78	0.20	-	13.33	1.20
<b>25 kR <math>\gamma</math> rays</b>	1985	0.20	0.81	-	0.46	4.44	1.20
<b>30 kR <math>\gamma</math> rays</b>	1913	0.31	0.42	0.47	0.16	11.11	1.36
<b>0.05 % EMS</b>	2260	0.13	0.21	-	0.31	13.33	0.66
<b>0.10% EMS</b>	1860	-	0.32	0.48	0.60	17.78	1.39
<b>0.15% EMS</b>	1948	0.05	0.21	0.46	0.46	7.77	1.19
<b>JS- 335</b>							
<b>Control</b>	2753						
<b>15 kR <math>\gamma</math> rays</b>	2640	0.19	0.30	0.11	0.03	6.67	0.64
<b>20 kR <math>\gamma</math> rays</b>	2430	-	0.49	0.04	0.33	11.11	0.86
<b>25 kR <math>\gamma</math> rays</b>	2289	0.18	0.83	0.13	0.26	5.56	1.40
<b>30 kR <math>\gamma</math> rays</b>	2123	0.09	0.57	0.38	0.28	10.34	1.32
<b>0.05 % EMS</b>	2439	0.04	0.57	0.29	0.21	13.33	1.11
<b>0.10% EMS</b>	2386	0.04	0.38	0.50	0.38	16.67	1.30
<b>0.15% EMS</b>	2067	0.19	0.53		0.58	6.67	1.31

## CONCLUSIONS

These mutants need to be studied in M4 and onwards generation for purification and these mutants can be used for the improvement of soybean. The economical mutants provide the base for selections for further generations which is the source of developing new lines also a large amount of the variability is created in the population which can be utilized as a source for developing new lines for different desirable characters.

## ACKNOWLEDGMENT

The authors are highly grateful to the North Maharashtra University, Jalgaon and Principal of Institution to carry out the present investigation.

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### How to cite this article:

Patil GP, Sharma CT. Study of the Cytogenetical Effects on Meiotic Chromosomal Abnormalities Induced by Mutagens in Soybean. *Int. J. Life Sci. Scienti. Res.*, 2018; 4(2): 1675-1679. DOI:10.21276/ijlssr.2018.4.2.10

Source of Financial Support: Nil, Conflict of interest: Nil