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Mutagenic effectiveness and efficiency of gamma rays and EMS in *Glycine max (L.) Merill*

Sapna S Rajderkar[1*], SB Sakhare[2]
[1] Junior Research Assistant, Department of Agricultural Botany, Dr. PDKV, Akola, Maharashtra, India
[2] Associate Professor, Department of Agricultural Botany, Dr. PDKV, Akola, Maharashtra, India

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Abstract
Mutation has been successfully employed in breeding of several food crop varieties. The aim of this study was undertaken to assess the effectiveness and efficiency of EMS and gamma rays in two varieties of soybean. Genetically pure, uniform and dry seeds were treated with different doses/ concentrations of gamma rays (10KR, 20KR, 30KR and 40KR) and EMS (0.1%, 0.2%0.3% and 0.4%). Mutagenic effectiveness and efficiency of gamma rays and EMS was calculated on the biological damage (lethality and seedling injury) in the M_1 generation and both chlorophyll and morphological mutations observed in the M_2 generation. The results indicated that, mutagenic effectiveness and efficiency decreased with increase in mutagenic treatments.

Keywords: EMS, gamma rays, variation, effectiveness, efficiency

1. Introduction
Soybean is one of the most economic and valuable agricultural commodities because of its unique chemical composition and multiple uses as food, feed and industrial material. Furthermore, soybean also contain many biologically active components including iso-flavins, lecithin, saponine, oligosaccharides, phytosterols, trypsin inhibitor, lectins etc [41].

The productivity of soybean in India 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 [40]. Narrowing down of the genetic base is due to the repeated use of few parents for breeding programme. The breeding efforts in soybean would be enhance if the range of genetic variability could be broadened.

In soybean, creation of variation through hybridization is a tedious process due to small i.e 5 to 8 mm fragile flowers that makes it difficult to carry out the process of emasculation injuring the parts of the flower and are prone to heavy flower shedding (over 75%) even under favorable conditions [15]. These coupled with complete self-fertility impose limitation on the success of hybridization programme. Hence the classical breeding methods have got limited application in soybean. Mutagenesis provides a powerful technique to improve plant breeding and assist functional and genomic analysis of crop plants.

Agricultural production has witnessed a sharp rise at the global level due to application of various tools of improvement including induced mutagenesis. Sweden has greatly advanced in mutation breeding since 1929 due to efforts of scientists like Gustaffsson at Svaloff Research Station. Mutagenesis provides a powerful technique to improve plant breeding and assist functional and genomic analyses of crop plants. This technique was first introduced with the use of x-ray and radium radiations followed by fast neutron and gamma radiation [32]. Because such application of physical mutagens required specialized equipment, chemical mutagens were introduced later. Chemical mutagens are used widely because they are easier to handle and increase mutation frequency [36]. It is a coherent tool used in mutation breeding program for creating new alleles (Laskar and Khan, 2014). Various chemical mutagens have been prepared, such as sodium azide, ethyl methanesulphonate (EMS) and N-ethyl-N-nitrosourea, which produce different side effects on the genetic structure of treated populations.These chemicals can cause point mutations, insertions, and/or deletions in the genomic strands, leading to phenotypic changes, which could be desirable traits for important crops [11, 13]. EMS, an alkylating agent, commonly is used as a chemical mutagen for DNA lesions. Unlike N-ethyl-Nnitrosourea, EMS induces a biased spectrum of G/C-to-A/T transitions. These transitions most likely occur due to the alkylation at the O6 or N7 position of guanine, which leads to the replacement of cytosine with thymine base pairing [23] known as EMS

Gamma rays are the most energetic form of electromagnetic radiation; their energy level is from ten to several hundred kilo electron volts and they are considered as the most penetrating compared to other radiations [19]. It is known to be the most popular mutagens for their simple application, good penetration, reproducibility, high mutation frequency and less disposal problems [10].

Mutagenic effectiveness is a measure of the frequency of mutations induced by a unit dose of mutagen while mutagenic efficiency is an estimate of biological effects induced such as lethality, injury and sterility. The observation of non-random pattern of variation in mutagenic effectiveness and efficiency demonstrates that the genotypic response to different mutagens is of genetic origin and depends upon the physical and chemical properties of the mutagen [17]. Therefore, this study was undertaken to gather information on effectiveness and efficiency of different doses/ concentrations of gamma rays and EMS in Soybean.

2. Materials and methods

Uniform and dry seeds of *Glycine max (L.) Merill* was taken for the induction of mutation using gamma rays and EMS. The gamma radiation was given to the dry seeds with different doses of gamma rays (10KR, 20KR, 30KR and 40KR) at Bhabha Automic Research Center, Mumbai. For EMS treatment, the seeds were presoaked in distilled water for 6 hours, were subjected to chemical mutagens, Ethyl Methane sulphonate (EMS) for 2 hours. The concentrations used for EMS were 0.1%, 0.2%, 0.3% and 0.4%. After the EMS treatment, the treated seeds were washed throughly in running tap water to terminate the residual effect of the mutagenic chemicals.

For raising M_1 generation, the treated seeds were sown along with control at the Department of Agricultural Botany, Dr. PDKV, Akola in a split plot design with three replication. The spacing was maintained at 15 cm (Plant to plant in a row) and 30 cm (between the rows) in the field. In the M_1 generation seedling height and plant survival was studied. The data on biological damage (Injury, and Lethality) was computed as the reduction in plant height and reduction in plant survival. All the surviving M_1 plants were harvested separately and sown as plant to row progeny basis in M_2 gneration. Screening was done for chlorophyll and viable mutation. Chlorophyll mutations were classified in accordance with the system of Gustaffson (1940) and Blixt and Gottschalk (1975) [6]. Frequency of viable mutations was calculated M_2 plant basis. Data on biological abnormalities such as seedling injury and lethality in M_1 generation and chlorophyll and morphological mutation frequency in M_2 generation were used to determine the mutagenic efficiency and effectiveness of mutagen.

2.1 Mutagenic effectiveness and efficiency

Mutagenic effectiveness means mutations induced by a unit dose of mutagen (KR (or) Concentration x Time) while mutagenic efficiency gives an idea of the damage such as lethality, injury and pollen sterility. The formulae proposed by Konzak *et al.*, 1965 were followed for the calculations of mutagenic effectiveness and efficiency by incorporating the mutation frequency values recorded for each mutagenic treatment.

$$\text{Mutagenic effectiveness (Physical mutagen)} = \frac{\text{Mutation Frequency (MF)}}{\text{Dose of Physical mutagen (KR)}}$$

$$\text{Mutagenic effectiveness (Chemical mutagen)} = \frac{\text{Mutation Frequency (MF)}}{\text{Conc. of chemical mutagen x duration of treatment}}$$

$$\text{Mutation efficiency} = \frac{\text{Mutation Frequency (MF)}}{\text{Biological damage in M1 generation}}$$

Where,

MF - Chlorophyll or Viable or total mutation per on M_2 plants basis

KR - Dose of mutagenic radiation in Kilo rad

I - Percentage of injury or reduction in seedling height.

L - Percentage of lethality or reduction in plant survival

3. Results & Discussion

The effect of different doses or concentrations of gamma rays and EMS on the biological damage caused by the mutagens in M_1 generation [plant survival reduction (lethality) and seedling height reduction (Injury), mutation frequency, mutagenic effectiveness and efficiency are depicted in Table 1.

3.1 Mutagenic effectiveness and efficiency

In mutation breeding it is necessary to determine the effectiveness and efficiency of mutagen. Frequency of mutations induced by mutagenic treatment is an index of the effectiveness of mutagen. By observation of the values, the major trends pertaining to this parameter influenced by different doses/concentrations of mutagen can be understood. In general, the effectiveness decreased with increasing doses/concentrations of mutagens. The maximum mutagenic effectiveness was observed in 0.2% concentration of EMS in both the varieties i.e JS-335 (2.50) and JS-9560 (1.59) respectively and the minimum mutagenic effectiveness was recorded in 30KR in JS-335(0.026) and JS-9560(0.016) on chlorophyll mutant basis and mutagenic effectiveness found maximum in 0.2% EMS treatment in both varieties on morphological mutant basis. This was in confirmation with the findings of Thilagavathi and Mullainathan (2009) [1] in Black gram, Khan and Tyagi (2010) [17] in Soya bean, Satpute *et al.* (2012) [31] in Soya bean, Bhosale and Kothekar (2010) [5] in Cluster bean, Sikder *et al* (2013) [35] in Tomato, Burghate *et al* (2013) [9] in Ground nut, Mangaiyarkarasi *et al* (2014) [24] in *Catharanthus roseus*, Kulthe and Mongle (2014) in Winged bean, Ambli and Mullainathan (2016) [1] in Pearl Millet.

Mutagenic efficiency gives an idea of the proportion of mutations in relation to other associated undesirable biological effects such as injury and lethality induced by the mutagen. Efficient mutagens and their treatments are indispensable for the cost effective use of the mutagen as a tool for the induction of mutations and their direct and indirect utilization in successful breeding programme.

On the basis of lethality the maximum mutagenic efficiency was recorded at 0.2% EMS i.e 1.08 in var. JS-335 and 0.69 in var. JS-9560 on chlorophyll mutant basis and 25.87 in var. JS-335 and 16.45 in JS-9560 on morphological mutant basis; whereas minimum mutagenic efficiency was recorded in 10kR gamma rays treatment in both the varieties on both chlorophyll mutants and morphological mutants.

On the basis of injury the maximum mutagenic efficiency was recorded at 0.2% EMS concentration i.e 1.65 in var. JS-335 and 1.20 in JS-9560, whereas the minimum mutagenic efficiency was observed in higher doses of mutagens i.e 40Kr in JS-335 (0.22) and 0.4% EMS in JS-9560 (0.29) on chlorophyll mutant basis. However maximum mutagenic efficiency were recorded at 0.2% EMS concentration i.e 39.22 in JS-335 and 28.59 in JS-9560 as well as minimum in 40kR in JS-335 (1.91) and in 30kR in JS-9560 (3.06) on morphological mutants basis.

Mutagenic efficiency decreased with an increase in the dose/ concentration of mutagens. Similar results were reported earlier in Sunflower (Raja Ramesh Kumar and Venkat Ratnam, 2010) [28], Pigeon pea (Sangle and Kothekar, 2013) [5] Black gram (Bhosale and Hallale, 2013) [4] and Green gram (Mishra and Singh, 2014). When the mutation rates based on efficiency were compared, EMS was found to be more efficient than gamma rays in *Glycine max (L.) Merill*. Similar observation has been recorded by Girija and Dhanvel (2009) [12], Sharma *et al.*, (2005) [33], Ramya *et al.* (2013) [29] and Bashir *et al.*, (2013) [3.]. Such difference in effects of mutagen on different materials might be due to seed metabolism and onset of DNA synthesis. Kundi *et al.*

(1997) [21] reported differential sensitivity within crop and even genotype. It was opined that the sensitivity depends upon the genetic architecture and mutagens employed besides the amount of DNA, its replication time in initial stages and degree of heterochromatin (Blixt, 1970) [6].

Table 1: Frequency and Spectrum of chlorophyll mutation, mutagenic effectiveness and efficiency in M_2 generation

Treatments	Lethality (%)	Seedling growth red (%)	On Chlorophyll mutant basis				On morphological mutant basis			
			Mutation frequency (MF)	Mutagenic efficiency		Mutagenic effectiveness	Mutation frequency (MF)	Mutagenic efficiency		Mutagenic effectiveness
				MF/L	MF/I			MF/L	MF/I	
JS-335										
10 kR	23.88	14.58	0.64	0.16	0.26	0.06	0.38	1.61	2.63	0.38
20 kR	27.22	23.67	1.70	0.39	0.45	0.08	1.91	7.03	8.09	0.96
30 kR	37.50	31.56	0.78	0.23	0.27	0.02	0.64	1.71	2.03	0.21
40 kR	41.28	43.60	2.38	0.23	0.22	0.06	0.83	2.01	1.91	0.18
Mean	32.47	28.35	1.38	0.25	0.30	0.05	0.94	3.09	3.66	0.43
0.1%	23.10	12.04	0.77	0.47	0.89	0.96	2.92	12.65	24.27	3.65
0.2%	31.48	20.76	4.00	1.09	1.65	2.50	8.14	25.86	39.22	5.08
0.3%	46.7	29.48	2.83	0.65	1.03	1.17	5.65	12.10	19.17	2.35
0.4%	52.69	34.05	2.94	0.56	0.86	0.91	4.70	8.93	13.82	1.46
Mean	30.79	19.26	2.63	0.69	1.11	1.39	5.35	14.88	24.12	3.13
Control	0	0	0.00	0.000	0.00	0.00	0.38	1.61	2.63	0.38
JS-9560										
10 kR	21.76	10.64	0.59	0.27	0.55	0.05	0.39	1.80	3.68	0.39
20 kR	30.70	21.29	0.83	0.59	0.84	0.04	2.62	8.56	12.34	1.30
30 kR	41.76	34.25	0.48	0.31	0.37	0.01	1.04	2.51	3.06	0.85
40 kR	47.70	39.35	1.15	0.26	0.31	0.02	1.81	5.89	7.14	0.70
Mean	35.48	26.38	0.76	0.38	0.52	0.03	1.72	4.69	6.56	0.81
0.10%	29.71	11.57	0.75	0.27	0.69	0.94	1.07	3.61	9.29	1.34
0.20%	33.76	19.44	2.56	0.69	1.20	1.59	5.55	16.45	28.57	3.47
0.30%	49.28	29.16	2.06	0.29	0.49	0.86	1.74	3.543	5.98	0.72
0.40%	57.80	35.18	2.38	0.18	0.29	0.74	1.42	2.47	4.06	0.44
Mean	34.11	19.07	1.94	0.36	0.67	1.03	2.45	6.52	11.98	1.49
Control	0	0	0.00	0.000	0.00	0.00	0.39	1.80	3.68	0.39

7. Conclusions

The present investigation was carried out to study the mutagenic effectiveness and efficiency of Gamma rays and EMS in *Glycine max (L.) Merill*. The mutagenic effectiveness and efficiency decreased with increased in doses/ concentrations of mutagens. The results indicate that lower doses/ concentrations of mutagens are effective in induction of mutations for crop improvement in *Glycine max (L.) Merill.*

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