



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

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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 M₁ generation and both chlorophyll and morphological mutations observed in the M₂ 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-N-nitrosourea, 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.) Merrill* 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 Atomic 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 thoroughly in running tap water to terminate the residual effect of the mutagenic chemicals.

For raising M₁ 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 M₁ 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 M₁ plants were harvested separately and sown as plant to row progeny basis in M₂ generation. 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 M₂ plant basis. Data on biological abnormalities such as seedling injury and lethality in M₁ generation and chlorophyll and morphological mutation frequency in M₂ 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 M₂ 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 M₁ 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.) Merrill*. 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 M₂ generation

Treatments	Lethality (%)	Seedling growth red (%)	On Chlorophyll mutant basis				On morphological mutant basis			
			Mutation frequency (MF)	Mutagenic efficiency MF/L	Mutagenic efficiency MF/I	Mutagenic effectiveness	Mutation frequency (MF)	Mutagenic efficiency MF/L	Mutagenic efficiency MF/I	Mutagenic effectiveness
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.) Merrill*. 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.) Merrill*.

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9. References

- Ambli K, Mullainathan L. Mutagenic effectiveness and efficiency of Gamma rays and Ethyl Methane Sulphonate in Pearl Millet (*Pennisetum typhoides* (Burn.F.) Stapf. And C.E.Hubb.) Var.CO (cu)-9. Academia Journal of Agricultural Research. 2016; 4(2):041-044.
- Ashraf M, Cheema AA, Rashid M, Zia-ul- Qamar. Effect of gamma rays on M1 generation in basmati rice. Pak. J Bot. 2003; 35(5):791-795.
- Bashir S, Wani AA, Nawchoo IA. Studies on mutagenic effectiveness and efficiency in Fenugreek (*Trigonella foenum-graecum* L.). African journal of Biotechnology. 2013; 12(18):2437-2440.
- Bhosale UP, Hallale BV. Mutagenic effectiveness and efficiency of Gamma rays and Ethyl Methane Sulphonate in Black gram (*Vigna mungo* (L.) Hepper). Bionanofrontier. 2013; 6(2):271-273.
- Bhosle SS, Kothekar VS. Mutagenic Efficiency and Effectiveness in Clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.). Journal of Phytology. 2010; 2(6):21-27.
- Blixt S, Gottschalk W. Mutation in the leguminosae. Agric. Hort. Genet. 1970; 33:33-85.
- Blixt S. Studies of induced mutations in peas XXVI. Genetically controlled differences in radiation sensitivity. Agri. Hort. Genet. 1970; 28:55-116.
- Botticella E, Sestili F, Hernandez-Lopez A, Phillips A, Lafiandra D. High resolution melting analysis for the detection of EMS induced mutations in wheat SbeIIa genes. BMC Plant Biol, 2011; 11:156.
- Burghate SK, Mishra MN, Chikhale NJ, Mahalle AM, Dhole VJ. Impact of mutagens its efficiency and effectiveness in groundnut (*Arachis hypogaea* L.). Scholarly Journal of Agricultural Science. 2013; 3(7):284-288.
- Chahal GS, Gosal SS. Principles and Procedures of Plant Breeding. Oxford: Alpha Science International Ltd, 2002, 399-412.
- Flibotte S, Edgley ML, Chaudhry I, Taylor J, Neil SE. Whole-genome sequencing profiling of mutagenesis in *Caenorhabditis elegans*. Genetics. 2010; 185:431-441.
- Girija M, Dhanvel D. Mutagenic Effectiveness and Efficiency of Gamma rays, EMS and their combined

- treatments in Cowpea (*Vigna unguiculata* L. Walp.). Global J Mol. Sci, 2009; 4:68-75.
13. Greene EA, Codomo CA, Taylor NE, Henikoff JG, Till BJ. Spectrum of chemically induced mutations from a large-scale reverse genetic screen in Arabidopsis. Genetics. 2003; 164:731-740.
 14. Gustafsson A. The mutation system of the chlorophyll apparatus. Lunds Univ. Arrks. N.F. Adv. 1940; 36:1-40.
 15. Johnson HW, Bornard HL. Soybean Genetics and Breeding. The soybean (Ed.). Norman, A.G. Pub. Head Press, 1976, 1-70.
 16. Khan IA. Comparative account of mutagenic efficiency of physical and chemical mutagens in mungbean. Mysore J. Agric. Sci. 1981; 15:231-233.
 17. Khan MH, Tyagi SD. Studies on effectiveness and efficiency of gamma rays, EMS and their combination in soybean [*Glycine max* (L.) Merrill.]. Journal of Plant Breeding and Crop Science. 2010; 2(3):055-058.
 18. Konzak CF, Nilan RA, Wagner J, Foster RJ. Efficient chemical mutagenesis. The use of induced mutations in plant breeding. (Rep. FAO/IAEA Tech. Meeting, Rome, 1964), Pergamon Press, 1965, 49-70.
 19. Kovacs E, Keresztes A. Effect of gamma and UV-B/C radiation on plant cell. Micron. 2002; 33:199-210.
 20. Kulthe MP, Mogle UP. Study of mutagenic efficiency of Ethyl methane sulphonate in Winged Bean. Science Research Reporter. 2014; 4(1):106-108.
 21. Kundi RS, Gill MS, Singh TP, Phul PS. Radiation- induced variability for quantitative traits in soybean (*Glycine max* Merill.) Crop Improv. 1997; 24(2):115-119.
 22. Laskar RA, Khan S. Enhancement of genetic variability through chemical mutagenesis in broad bean. Agrotechnol (2nd International conference on Agricultural and Horticultural Sciences. 2014; 2:4.
 23. Lawley PD, Martin CN. Molecular mechanisms in alkylation mutagenesis. Induced reversion of bacteriophage T4rII AP72 by ethyl methane sulphonate in relation to extent and mode of ethylation of purines in bacteriophage deoxyribonucleic acid. Biochem. J. 1975; 145:85-91.
 24. Mangaiyarkarasi R, Girija M, Gnanamurthy S. Mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate in *Catharanthus roseus*. Int. J Curr. Microbiol. App. Sci. 2014; 3(5):881-889.
 25. Minoia S, Petrozza A, D'Onofrio O, Piron F, Mosca G. A new mutant genetic resource for tomato crop improvement by TILLING technology. BMC Res. Notes, 2010; 3:69.
 26. Mishra D, Singh B. Studies on Effectiveness and Efficiency of Gamma rays in Green gram (*Vigna radiata* (L.) wilczek). SABRAO Journal of Breeding and Genetics. 2014; 46(1):34-43.
 27. Perry J, Brachmann A, Welham T, Binder A, Charpentier M. TILLING in *Lotus japonicus* identified large allelic series for symbiosis genes and revealed a bias in functionally defective ethyl methanesulfonate alleles toward glycine replacements. Plant Physiol. 2009; 151:1281-1291.
 28. Raja Ramesh Kumar P, Venkat Ratnam S. Mutagenic effectiveness and efficiency in varieties of Sunflower (*Helianthus annuus* L.) by separate and combined treatment with gamma rays and sodium azide. African J Biotech. 2010; 9:6517-6521.
 29. Ramya B, Nallathambi G, Ganesh Ram S. Mutagenic effectiveness and efficiency of gamma rays and EMS in Black gram (*V. mungo* L.). International Journal of Scientific Research. 2013; 2(11):6-9.
 30. Sangle SM, Kothekar VS. Mutagenic effectiveness and efficiency in pigeonpea International Journal of Advanced Scientific and Technical Research. 2013; 3(4):40-45.
 31. Satpute RA, Fultambkar RV. Mutagenic effectiveness and efficiency of gamma rays and EMS in Soya bean (*Glycine max* (L.) Merrill). Current Botany. 2012; 3(2):18-20
 32. Serrat X, Esteban R, Guibourt N, Moysset L, Nogués S. EMS mutagenesis in mature seed-derived rice calli as a new method for rapidly obtaining TILLING mutant populations. Plant Methods, 2014, 10:5.
 33. Sharma SK, Ritu Sood, Pandey DP. Studies on mutagen sensitivity, effectiveness and efficiency in Urdbean (*Vigna mungo* (L.) Hepper). Indian J Genet. 2005; 65:20-22.
 34. Shiwa Y, Fukushima-Tanaka S, Kasahara K, Horiuchi T, Yoshikawa H. Whole-genome profiling of a novel mutagenesis technique using proofreading-deficient DNA polymerase. Int. J Evol. Biol, 2012, 860797
 35. Sikder S, Biswas P, Hazra P, Akhtar S, Chattopadhyay A, Badigannavar AM, et al. Induction of mutation in tomato (*Solanum lycopersicum* L.) by gamma irradiation and EMS. Indian J Genet. 2013; 73(4):392-399.
 36. Sikora P, Chawade A, Larsson M, Olsson J, Olsson O. Mutagenesis as a tool in plant genetics, functional genomics and breeding. Int. J Plant Genom, 2011, 314-829.
 37. Thilagavathi C, Mullainathan L. Isolation of Macro Mutants and Mutagenic Effectiveness, Efficiency in Black gram (*Vigna mungo* (L.) Hepper). Global journal of Molecular Sciences. 2009; 4(2):76-79.
 38. Thompson O, Edgley M, Strasbourger P, Flibotte S, Ewing B. The million mutation project: a new approach to genetics in *Caenorhabditis elegans*. Genome Res. 2013; 23:1749-1762.
 39. Till BJ, Reynolds SH, Greene EA, Codomo CA, Enns LC. Large scale discovery of induced point mutations with high throughput TILLING. Genome Res. 2011; 13:524-530.
 40. Tiwari SP. Improvement of yield and yield potential in soybean: An analysis and synthesis. J Oilseed Res, 2003, 1-8.
 41. Watson WH, Cai J, Jones DP. Diet and apoptosis. Annu. Rev. Nur, 2000; 20:485-505.