Madras Agric. J., 102 (4-6): 140-143, June 2015
https://doi.org/10.29321/MAJ.10.001086

Mutagenic Effectiveness and Efficiency in Soybean [*Glycine max* (L.) Merrill]

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Comparative mutagenic effectiveness and efficiency of EMS and gamma rays were studied in two varieties of soybean (Co(Soy)3 and JS-335) in terms of M_2 progenies ; lethality and injury on the basis of chlorophyll and viable mutation frequency. Both mutagens produced high frequency as well as wide spectrum of mutations. The frequency of mutation was high at higher concentration/ dose of mutagen. EMS was the most effective and efficient mutagen compared to gamma rays. Higher the dose, higher the mutagenic effectiveness and lower the efficiency. JS 335 was the most sensitive variety to both mutagens.

Key words: Effectiveness, Efficiency, Mutation frequency, Soybean, EMS and Gamma Rays

Soybean or golden bean is an attractive dual purpose crop because of its richness in seed protein and oil. The crop has fetched higher importance in international markets because of its widespread use in most of the food and cosmetic industries. Demand for soybean is increasing steadily, which is not possible to meet out with the current productivity level. Soybean is a self pollinated crop with narrow genetic base, because of which the breeders are unable to exploit the yield potential of the crop. Since the soybean flowers are tiny and fragile, it impedes the process of genetic improvements through hybridization and other conventional breeding approaches. Thus, the best way to improve soybean lines is mutation breeding. Mutation breeding of plants is useful to improve the character if such character is not located in the germplasm of a species. It is also used for generating variability in the existing varieties (Van Harten, 1998; Yaqoob and Rashid, 2001; Khan and Goyal, 2009).

The effectiveness of a mutagen is expressed as the magnitude of effects produced after a particular dose of the mutagen, while, mutagenic efficiency gives an idea of the proportion of useful mutations in relation to other associated undesirable biological effects, such as gross chromosomal aberrations, lethality and sterility induced by the mutagen (Konzak *et al.*, 1965).

Selection of an effective and efficient mutagen is very essential to recover high frequency of desirable mutations in any mutation breeding studies (Solanki and Sharma, 1994; Mahabatra, 1983). Hence, previous knowledge of effectiveness and efficiency of different mutagens in a number of genotypes is indispensable to decide upon the doses/ concentrations of mutagens. The present investigation was therefore undertaken to explicit the response of two soybean genotypes to gamma radiation and EMS.

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Materials and Methods

Dry seeds of the soybean varieties Co(Soy)3 and JS 335 were treated with gamma rays (200 Gy, 250 Gy and 300 Gy) and EMS (35 *mM*, 40 *mM* and 45 *mM*) as per the procedure given below.

Gamma irradiation

Well filled, uniform sized, dry seeds were packed in butter paper covers and placed in the Gamma chamber installed at Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore and exposed to gamma irradiation from the cobalt 60 gamma source for appropriate time for each dose based on the half life of the source.

Treatment with EMS

Dry seeds of each variety were presoaked in distilled water for 10 hours prior to the mutagen treatment. After draining the water, presoaked seeds were placed between folds of blotting paper to remove water adhering to the surface. Then, the seeds were immersed for four hours in the requisite concentration of mutagen. Intermittent shaking was given once in half an hour for uniform absorption of the solution. Immediately after the completion of treatment duration, the treated seeds were thoroughly washed in running tap water for half an hour to eliminate the residual effect of the chemical. The excess moisture in the seed coat was removed by using folds of blotting paper.

The gamma rays and EMS treated seeds along with untreated seeds of both the varieties were sown in a randomized block design (RBD) with three replications each consisting of 600 seeds for raising M_1 generation. The seeds were sown with spacing of 30 cm between rows and 10 cm between plants during Summer, 2012. Selfed seeds of individual M_1 plants were harvested separately and grown as single plant progeny in M_2 generation during Kharif, 2013.

Recording observations

The M_2 population at seedling stage were screened for both frequency and spectrum of different types of chlorophyll mutations from the day of germination upto fifteenth day after sowing. The classification and characterization of various chlorophyll mutants was done according to Gustafsson (1940) and Bilxt (1970). Plant survival was recorded from emergence till the age of three weeks after germination and expressed as percentage of control. The damage (lethality / injury) was computed as the reduction in plant height (I) and survival (L) for each treatment. The frequency and spectrum of different types of viable mutants were scored at various developmental stages of M_2 plants. These mutants were classified for deviation from the normal and taking into consideration the most conspicuous characters namely, stature, duration, leaf shape, pod size *etc*. Mutation frequency was calculated as the percentage of mutated progenies and plants. Both mutagenic effectiveness and efficiency were determined as per the formulae suggested by Konzak *et al*. (1965).

Results and Discussion

Chlorophyll and viable mutations were calculated on M_2 plant basis. Frequency of mutations increased with increase in dose/concentration of the mutagen (Tables 1 and 2) except for EMS treatments in JS 335. The frequency of chlorophyll and viable mutations induced by EMS was comparatively higher than that of gamma ray treatments. The highest frequency of chlorophyll mutations (3.91%) was observed in Co(Soy)3 following EMS treatment at 45 *mM* (Table 1a and 1b) while, for viable mutations, the highest frequency (9.24%) was observed in 300 Gy gamma ray treatments (Table 2a and 2b).

Table 1a. Mutagenic effectiveness and efficiency based on chlorophyll mutations in M_2 generation of Co(Soy)3

Treatments	Lethality (%)(L)	Injury (%)(I)	Mutation frequency(Mc)	Effectiveness (%)	Efficiency (%) $\frac{Mc \times 100}{L}$	Efficiency (%) $\frac{Mc \times 100}{I}$
Gamma rays						
200 Gy	58.93	17.71	0.20	1.00	0.34	1.13
250 Gy	62.13	29.17	0.36	1.44	0.58	1.23
300 Gy	68.07	36.46	1.68	5.60	2.47	4.61
EMS						
35 mM	21.26	20.83	1.74	1.24	8.18	8.35
40 mM	52.08	30.21	1.46	0.91	2.80	4.83
45 mM	55.81	35.42	3.91	2.17	7.01	11.04

The spectrum of chlorophyll mutants were recorded and classified according to Gustafsson (1940) and Blixt and Gottschalk (1970) as Albino, Xantha, Chlorina and Viridis types. The viable mutants were recorded as tall, dwarf, early and late maturity, trailing and erect growth habit, striped seeds and male sterility.

Table 1b. Mutagenic effectiveness and efficiency based on chlorophyll mutations in M_2 generation of JS 335

Treatments	Lethality (%)(L)	Injury (%)(I)	Mutation frequency(Mc)	Effectiveness (%)	Efficiency (%) $\frac{Mc \times 100}{L}$	Efficiency (%) $\frac{Mc \times 100}{I}$
Gamma rays						
200 Gy	73.97	10.94	1.09	5.45	1.47	9.97
250 Gy	77.04	23.44	1.08	4.32	1.40	4.61
300 Gy	68.59	32.81	2.41	8.03	3.51	7.34
EMS						
35 mM	78.50	9.38	1.54	1.10	1.96	16.43
40 mM	58.07	17.19	2.13	1.33	3.67	12.39
45 mM	46.66	31.25	2.20	1.22	4.72	7.04

Mutagenic effectiveness

The mutagenic effectiveness was found to be the highest at higher concentration with all the mutagenic treatments. However, mutagenic effectiveness based on viable mutations in JS 335 was recorded high in lower doses irrespective of the mutagen. On the basis of chlorophyll mutation frequency, gamma ray treatments were found to be effective than EMS. The maximum effectiveness based on chlorophyll mutations was observed at

Table 2a. Mutagenic effectiveness and efficiency based on viable mutations in M_2 generation of Co(Soy)3

Treatments	Lethality (%)(L)	Injury (%)(I)	Mutation frequency(Mv)	Effectiveness (%)	Efficiency (%) Mc X 100 / L	Mc X 100 / I
Gamma rays						
200 Gy	58.93	17.71	3.31	16.55	5.62	18.69
250 Gy	62.13	29.17	3.04	12.17	4.90	10.43
300 Gy	68.07	36.46	9.24	30.80	13.57	25.34
EMS						
35 mM	21.26	20.83	2.49	1.78	11.72	11.96
40 mM	52.08	30.21	3.13	1.95	6.00	10.34
45 mM	55.81	35.42	5.15	2.86	9.22	14.53

300 Gy of gamma rays in JS 335 (8.03 %) followed by Co(Soy)3 (5.6 %) (Table 1a and 1b). The maximum effectiveness based on viable mutations was observed at 300 Gy of gamma treatments (30.80%) in Co(Soy)3 followed by 200 Gy of gamma rays (17.70%) in JS 335 (Table 2a and 2b). The present study showed that the effectiveness increased with increase in concentration of EMS and gamma rays in most of the cases. This was in confirmation with the findings of Packiaraj (1988) in cowpea and Sharma *et al*. (2005) in blackgram.

Mutagenic efficiency

Efficiency of chlorophyll and viable mutations was based on lethality (L) and injury (I) in M_1 plant and M_2 seedling basis. The lethality based maximum efficiency was achieved by 300 Gy of gamma rays (13.57%) in Co(Soy)3 followed by 40 *mM* of EMS (12.94%) in JS 335 for viable mutants (Tables 2a and 2b). The injury based maximum efficiency was observed at 35 *mM* of EMS (61.47%) followed by 40 *mM* of EMS (43.71%) (Tables 2a and 2b).

Likewise, lethality based maximum efficiency due to chlorophyll mutations was achieved by 35 *mM* of EMS in Co(Soy)3 (8.18%) followed by 45 *mM* of EMS (7.01%) in Co(Soy)3 and JS 335 (4.72%). Similarly, injury based maximum efficiency due to chlorophyll mutations was achieved by 35 *mM* of EMS (16.43 %) followed by 40 *mM* of EMS in JS 335 (12.39 %) and 45 *mM* EMS in Co(Soy)3 (11.04%) (Tables 1a and 1b).

Table 2b. Mutagenic effectiveness and efficiency based on viable mutations in M_2 generation of JS 335

Treatments	Lethality (%)(L)	Injury (%)(I)	Mutation frequency(Mv)	Effectiveness (%)	Efficiency (%) Mc X 100 / L	Mc X 100 / I
Gamma rays						
200 Gy	73.97	10.94	3.54	17.70	4.79	32.37
250 Gy	77.04	23.44	3.20	12.80	4.15	13.65
300 Gy	68.59	32.81	4.61	15.37	6.72	14.06
EMS						
35 mM	78.50	9.38	5.76	4.11	7.34	61.47
40 mM	58.07	17.19	7.51	4.69	12.94	43.71
45 mM	46.66	31.25	4.86	2.70	10.42	15.56

On the whole, mutation frequency was high at 300 Gy of gamma rays followed by 45 *mM* of EMS. EMS was found to be the most effective and efficient mutagen as reported previously by Shah *et al.,* 2008; Girija and Dhanavel, 2009 and Satpute *et al*., 2012. The order of effectiveness at different dose was 45 *mM*> 40 *mM* > 35 *mM*. While, the order of doses with respect to efficiency was reverse *ie*. 35 *mM* > 40 *mM* > 45 *mM*. This insisted that a highly effective mutagen need not necessarily to be most efficient one. This was in accordance with the results of Shah *et al.,* 2008. The higher efficiency of a mutagen indicates relatively less biological damage (seedling injury, sterility etc.,) in relation to mutations induced (Kharakwal, 1998; Sarker, 1985).

The present study indicated that the mutagenic efficiency decreased with the increasing dose of mutagens. The decrease in efficiency at higher doses was reported by Sharma *et al.,* 2005; Shah *et al.,* 2008; Kavithamani *et al.,* 2008; Mundhe, 2008; Pavadai *et al.,* 2009; Wani, 2009; Tambe *et al.,* 2010; Khan and Tyagi, 2010 and Satpute *et al*., 2012. Higher efficiency at the lower concentration of the mutagen appears mainly due to the fact that injury, lethality and sterility increases with an increase in the mutagen concentration than actual mutations (Kharkwal, 1998; Cheema *et al*., 2003).

Acknowledgement

The authors are thankful to the Programme Officer, BRNS (Board of Research in Nuclear Sciences) Mumbai for their financial assistance and BARC (Bhabha Atomic Research Centre), for their valuable support.

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Received after revision: February 22, 2015; Accepted: April 20, 2015