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Studies on Effectiveness and Efficiency of Gamma Rays, Ethyl Methane Sulphonate and Sodium Azide in Mungbean (*Vigna radiata* (L.) Wilczek)

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ABSTRACT: In the present study, dry seeds of two mungbean varieties *viz.*, WGG-42 and LGG-460 were treated with different doses/ concentrations of gamma rays (200 Gy, 300 Gy, 400 Gy, 500 Gy and 600 Gy), ethyl methane sulphonate (0.2%, 0.3%, 0.4%, 0.5% and 0.6%) and sodium azide (1 mM, 2 mM and 3 mM) to study the mutagenic effectiveness and efficiency of gamma rays, ethyl methane sulphonate and sodium azide in induction of chlorophyll mutations in M₂ generation. Three types of chlorophyll mutants *viz.*, albina, xantha and chlorina were observed in M₂ generation and occurrence of chlorina was found to be the most frequent in all the mutagen treated populations of both the varieties. The frequency of chlorophyll mutations increased with the increase in dose/concentration of mutagens in both the genotypes, but EMS treatments showed the highest frequency of chlorophyll mutations than gamma rays and SA treatments. The results indicated that the values of mutagenic effectiveness and efficiency gradually decreased with increases in dose/concentration of mutagens. The EMS treatments were found to be more effective and efficient in inducing chlorophyll mutants than gamma rays and sodium azide in both the varieties. Thus, it is concluded that the lower doses/concentrations of mutagens are more useful to induce different type of chlorophyll mutants in mungben.

Keywords: Gamma rays, EMS, SA, chlorophyll mutants, effectiveness and efficiency, M₂, mungbean.

INTRODUCTION

Mungbean [Vigna radiata (L.) Wilczek] (2n=22) is one of the important pulse crop grown extensively in both tropical and sub-tropical regions of the world. Although numbers of varieties have been recommended for cultivation, the productivity is still low which may be attributed to narrow genetic base of the varieties resulting in low yield potential and susceptibility to biotic and abiotic stresses. Hence, there is an immediate need to improve the productivity of mungbean through breeding high yielding varieties coupled with tolerant to biotic and abiotic stresses. Existence of adequate genetic variability is a prerequisite for the genetic improvement of any crop. Induced mutations have been used to generate genetic variability and have been successfully utilized to improve yield and yield components of various crops. Many physical and chemical mutagens have been used for induction of useful mutants in a number of crops. Induction of chlorophyll mutations in general is considered as a measure to assess the effectiveness of various mutagens (Awnindra and Singh 2007; Lavanya et al., 2022). The usefulness of any mutagen in plant breeding depends not only on its effectiveness but also upon its efficiency. Mutagenic effectiveness is a measure of the frequency of mutations induced by unit mutagen dose, whereas mutagenic efficiency is the measure of proportion of mutations in relation to undesirable

changes like lethality, sterility and meiotic aberrations *etc.* The frequency of induced chlorophyll mutations has been considered a reliable index for estimating the potency of mutagens due to the ease of their detection and greater accuracy in scoring (Gustafsson, 1940) and chlorophyll mutations although not useful for plant breeding purpose, may be used to assess the efficiency and effectiveness of mutagens in order to select suitable mutagen at appropriate concentration so as to use them in applied mutagenesis programme (Navdeep *et al.*, 2021). Hence, the present investigation was undertaken to study the frequency and spectrum of chlorophyll mutations along with the mutagenic effectiveness and efficiency of different doses of gamma rays, EMS and SA treatments in mungbean.

MATERIAL AND METHODS

Dry seeds of two mungbean genotypes *viz.*, WGG-42 and LGG-460 administered with mutagenic treatments of gamma rays (200 Gy, 300 Gy, 400 Gy, 500 Gy and 600 Gy), ethyl methane sulphonate (0.2%, 0.3%, 0.4%, 0.5% and 0.6%) and sodium azide (1 mM, 2 mM and 3 mM). Dry seeds were irradiated with gamma rays at Bhaba Atomic Research Centre (BARC), Trombay For chemical treatment, seeds were pre-soaked for 6 h in water initially. Then, the seeds were immersed for 6 h in the requisite concentration of mutagens ethyl methane sulphonate and sodium azide with intermittent shaking. The whole treatment was carried out at a room

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temperature of 23±1°C for 6 h. Treated seeds were thoroughly washed with running water to bleach out the residual chemicals and then dried on blotting paper after treatment. The treated seeds along with controls were sown in the field in a Randomized Block Design (RBD) with three replications to raise the M₁ generation. Data on biological abnormalities such as injury, lethality and sterility were recorded in M_1 generation. The M_2 generation was raised from individual M1 plants following plant to progeny method in Compact Family Block Design (CFBD) with two replications. Standard agronomic package of practices were followed to raise the crop. Observation on different types of chlorophyll mutations in each treatment were recorded daily from emergence of seedlings to 15th days after sowing and were classified in accordance with the system of Gustafsson (1940).

The frequency of chlorophyll mutations was estimated as follows.

Mutation frequency (%) =
$$\frac{\text{Number of mutants}}{\text{Total number of } M_2 \text{ plants}} \times 100$$

The effectiveness and efficiency of the mutagens were worked out by using the formulae as suggested by Konzak *et al.* (1965).

Mutagenic effectiveness (%) =
$$\frac{M_p \times 100}{Gy \text{ or } T \times C}$$

Where,

 M_p - Chlorophyll mutation frequency on M_2 plant basis Gy - Dose of gamma radiation

T - Duration of mutagenic treatment C - Concentration of mutagen Mutagenic (0) - Mp/L (1)

 $Mp/S \times 100$

Where,

 $\begin{array}{l} Mp \mbox{ - Chlorophyll mutation frequency on } M_2 \mbox{ plant basis } L \mbox{ - Percentage of lethality } i.e., \mbox{ percentage of reduction in survival of seedlings on } 30^{th} \mbox{ day } \end{array}$

I - Percentage of injury *i.e.*, percentage of height reduction of seedlings on 30^{th} day

S - Percentage of sterility *i.e.*, percentage of reduction in pollen fertility

RESULTS AND DISCUSSION

The frequency of induced chlorophyll mutations in M_2 generation has been considered a reliable index for estimating the potency of mutagens due to the ease of their detection and greater accuracy in scoring (Gustafsson, 1940) and the chlorophyll mutation serves not only as a measure for evaluating effectiveness and efficiency of mutagens, but also as indicators to predict the size of vital factor mutations.

In the present study, the frequency and spectrum of chlorophyll mutants were observed in M_2 generation and are presented in Table 1 and 2 for WGG-42 and LGG-460, respectively. In WGG-42 variety (Table 1), the chlorophyll mutation frequency in gamma rays treatments varied from 1.030% (200 Gy) to 1.806% (600 Gy). Likewise, the mutation frequency of EMS treatments varied from 1.142% (0.2%) to 2.926% (0.6%). Similarly, the mutation frequency of SA

treatments varied from 0.765% (1 mM) to 1.496% (2 mM). In LGG-460 variety (Table 2), the highest frequency of chlorophyll mutations was observed at 600 Gy (1.640%) and lowest frequency was observed at 200 Gy (1.209%) of gamma rays. As far as EMS treatments concerned, the highest frequency was observed at 0.6% (2.824%) and lowest frequency was observed at 0.2% (1.333%). Similarly, in SA treatments the highest frequency was observed at 3 mM (1.311%) and lowest frequency was observed at 1 mM (0.732%). While such mutations were not observed in control populations.

In the present investigation, the frequency of chlorophyll mutations increased with parallel increase in dose/concentration of gamma rays, EMS and SA treatments in both the genotypes (Tamilzharasi et al., 2019; Navdeep et al., 2021). Wani et al. (2011); Rukesh et al. (2017); Mahto et al. (2018) were also observed similar types of chlorophyll mutations in mungbean. The frequency of chlorophyll mutations were higher in EMS treatments followed by gamma rays treatments and SA treatments in both the genotypes. The variety WGG-42 appeared to produce a higher number of chlorophyll mutations than LGG-460 variety. In the present study, both the genotypes of mungbean were found to respond to the mutagenic treatments differently. Arulselvi et al. (2016); Rukesh et al. (2017); Lavanya et al. (2022) were also found varietal differences in the expression of chlorophyll mutation frequency similar to the observations made in the present investigation. The observed differential response to mutagenic treatments specifically with regard to chlorophyll mutation frequency might be due to alteration of genes concerned with the development and expression of chlorophyll.

A wide spectrum of chlorophyll mutations were observed in mutagen treated populations of both the varieties in M₂ generation and is presented in Table 1 and 2. Such chlorophyll mutations were not observed in the controls of both the varieties. The spectrum of chlorophyll mutations was determined as the relative proportion of different types of mutations to the total number of chlorophyll mutations. In this study, three types of chlorophyll mutations were observed in both the genotypes (Fig. 1 and 2). They are namely, albina, xantha and chlorina with variable frequency. In WGG-42 variety (Table 1), chlorina was the most frequent mutation isolated in all the treatments, followed by xantha. Whereas, albina was isolated in all the treatments except 400 Gy of gamma rays, 0.2% of EMS and 2 mM of SA. In LGG-460 variety (Table 2), chlorina type of mutants had the highest frequency among chlorophyll mutations in all the treatments followed by xantha and albina with some exceptions. These findings were in accordance with Sanjai et al. (2014); Rukesh et al. (2017); Navdeep et al., 2021) who also reported three types of chlorophyll mutations and among which chlorina was the highest proportion in their studies using gamma rays. Similarly, Wani et al. (2011); Vairam et al. (2014); Digbijaya et al. (2019) also observed chlorina and xantha in all the treatments of gamma rays and EMS at higher proportions.

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The effectiveness and efficiency of different treatments of the three mutagens (Gamma rays, EMS and SA) in both the mungbean genotypes viz., WGG-42 and LGG-460 were calculated following the methods suggested by Konzak et al. (1965). In the present study, effectiveness of mutagenic treatments in both the varieties differed considerably. The mutagenic effectiveness showed a trend, which was inversely proportional to the increasing dose/concentrations of mutagens in both the genotypes and the same pattern was also found in mutagenic efficiency. The effectiveness and efficiency of different treatments of the three mutagens in WGG-42 was presented in the Table 3. In WGG-42, the highest effectiveness was observed at 200 Gy (0.005) and the lowest effectiveness was observed at 600 Gy (0.002) of gamma rays. Similarly, in EMS treatments the highest effectiveness was observed at 0.2% (0.952) and the lowest effectiveness was observed at 0.6% (0.813). Whereas, in SA treatments the highest effectiveness was observed at 1 mM (0.127) and the lowest effectiveness was observed at 3 mM (0.068). In LGG-460 variety (Table 4), the highest effectiveness was observed at 200 Gy (0.006) and the lowest effectiveness was observed at 500 Gy and 600 Gy (0.003) of gamma rays. Similarly, in EMS treatments the highest effectiveness was observed at 0.2% (1.111) and the lowest effectiveness was observed at 0.6% (0.784). Whereas, the SA treatments concerned, the highest effectiveness was observed at 1 mM (0.122) and the lowest effectiveness was observed at 3 mM (0.073). EMS was found to be most effective mutagen than gamma rays and SA.

Mutagenic efficiency varies depending upon the criteria selected. The lethality, injury and sterility were the highest when higher doses of mutagenic treatments were used. Mutagenic efficiency analyzed based on lethality, in WGG-42 variety (Table 3), the highest efficiency was observed at 200 Gy (0.037) of gamma rays, 0.5% (0.057) of EMS and 2 mM (0.020) of SA. In LGG-460 variety (Table 4), the highest efficiency was observed at 200 Gy (0.072) of gamma rays, 0.2% (0.068) of EMS and 3 mM (0.016) of SA. When efficiency was analyzed based on injury, in WGG-42 variety (Table 3), the highest efficiency was found at 200 Gy (0.214) of gamma rays, 0.2% (0.160) of EMS and 1 mM (0.092) of SA. In LGG-460 variety (Table 4), the highest efficiency was found at 200 Gy (0.298) of gamma rays, 0.2% (0.180) of EMS and 1 mM (0.056) of SA. When analysis was based on sterility, in WGG-42 variety (Table 3), the highest efficiency was found at 200 Gy (0.128) of gamma rays, 0.2% (0.165) of EMS and 1 mM (0.105) of SA. In LGG-460 variety (Table 4), the highest efficiency was observed at 200 Gy (0.180) of gamma rays, 0.2% (0.218) of EMS and 1 mM (0.176) of SA.

In the present investigation, mutagenic efficiency decreased with the increasing dose/concentration of the mutagens with respect to injury, lethality, sterility in both the varieties. This was also reported by Sweta (2014); Rukesh et al. (2017); Mahto et al. (2018); Das and Arjun (2020); Vasudevan et al. (2023) in mungbean. in mungbean. The higher efficiency obtained at lower and intermediate doses of mutagens might be due to the fact that the lethality, injury, sterility etc. increases with mutagen concentration at a rate faster than the frequency of mutations (Blixt, 1964). In the present study, EMS was found to be most effective and efficient mutagen than gamma rays and SA. Effectiveness and efficiency of mutagens had also been worked out by Kuldeep and Singh (2013); Mishra and Singh (2014); Goyal et al. (2019) and reported that EMS was found to be more effective and efficient than gamma rays in mungbean.

Treatments		Total number of seedlings	Types of chlorophyll mutants			Total number of chlorophyll	Relative percentage (Frequency) of chlorophyll mutants			Chlorophyll mutation frequency
		examined	Albina	Xantha	Chlorina	mutants	Albina	Xantha	Chlorina	(%)
Control										
	200 Gy	1650	3	5	9	17	0.182	0.303	0.545	1.030
Gamma rays	300 Gy	1768	6	3	14	23	0.339	0.170	0.792	1.301
	400 Gy	1700	0	8	17	25	0.000	0.471	1.000	1.471
	500 Gy	1668	5	4	18	27	0.300	0.240	1.079	1.619
	600 Gy	1550	9	7	12	28	0.581	0.452	0.774	1.806
	0.2%	1664	0	8	11	19	0.000	0.480	0.661	1.142
	0.3%	1602	4	7	16	27	0.250	0.437	0.999	1.685
EMS	0.4%	1350	5	6	19	30	0.370	0.444	1.407	2.222
	0.5%	1050	3	8	17	28	0.286	0.762	1.619	2.667
	0.6%	1196	9	11	15	35	0.753	0.920	1.254	2.926
SA	1 mM	1700	4	3	6	13	0.235	0.176	0.353	0.765
	2 mM	936	0	6	8	14	0.000	0.641	0.855	1.496
	3 mM	1300	3	6	7	16	0.231	0.462	0.538	1.231

Table 1: Frequency and spectrum of chlorophyll mutations in M₂ generation of WGG-42.

Treatments		Total number of	Types of chlorophyll mutants			Total number of	Relative percentage (Frequency) of chlorophyll mutants			Chlorophyll mutation	
		seedlings examined	Albina	Xantha	Chlorina	chlorophyll mutants	Albina	Xantha	Chlorina	frequency (%)	
Control											
	200 Gy	1820	5	7	10	22	0.275	0.385	0.549	1.209	
	300 Gy	1650	2	6	15	23	0.121	0.364	0.909	1.394	
Gamma rays	400 Gy	1716	4	8	14	26	0.233	0.466	0.816	1.515	
	500 Gy	1750	8	0	20	28	0.457	0.000	1.143	1.600	
	600 Gy	1768	5	7	17	29	0.283	0.396	0.962	1.640	
	0.2%	1500	4	7	9	20	0.267	0.467	0.600	1.333	
	0.3%	1136	3	6	12	21	0.264	0.528	1.056	1.849	
EMS	0.4%	1610	9	0	22	31	0.559	0.000	1.366	1.925	
	0.5%	1398	6	8	19	33	0.429	0.572	1.359	2.361	
	0.6%	1204	3	11	20	34	0.249	0.914	1.661	2.824	
	1 mM	1502	0	6	5	11	0.000	0.400	0.333	0.732	
SA	2 mM	1464	4	7	6	13	0.273	0.205	0.410	0.888	
	3 mM	1144	3	5	7	15	0.262	0.437	0.612	1.311	

Table 2: Frequency and spectrum of chlorophyll mutations in M₂ generation of LGG-460.

Table 3: Mutagenic effectiveness and efficiency based on chlorophyll mutants in M₂ generation of WGG-42.

Treatments		% Survival			Chlorophyll	Mutagenic	Mutagenic efficiency (%)		
		reduction at 30 th day (L)	% Height reduction at 30 th day (I)	% Pollen fertility reduction (S)	mutation frequency (%) (Mc)	effectiveness (%) <u>Mc</u> Gy (or) T x C	Mc L	Mc I	Mc S
Con	Control		-	-	-	-	-	-	-
	200 Gy	27.87	4.82	8.05	1.030	0.005	0.037	0.214	0.128
Commo	300 Gy	46.15	10.73	13.08	1.301	0.004	0.028	0.121	0.099
Gamma	400 Gy	57.62	13.23	16.19	1.471	0.003	0.026	0.111	0.091
rays	500 Gy	77.48	18.12	19.85	1.619	0.003	0.021	0.089	0.082
	600 Gy	89.86	24.60	23.57	1.806	0.002	0.020	0.073	0.077
	0.2%	29.02	7.12	6.92	1.142	0.952	0.039	0.160	0.165
	0.3%	33.71	13.73	13.51	1.685	0.936	0.050	0.123	0.125
EMS	0.4%	43.64	18.72	19.92	2.222	0.926	0.051	0.119	0.106
	0.5%	47.13	29.76	25.57	2.667	0.889	0.057	0.090	0.104
	0.6%	79.02	36.27	30.26	2.926	0.813	0.037	0.081	0.100
	1 mM	41.71	8.33	7.27	0.765	0.127	0.018	0.092	0.105
SA	2 mM	73.08	21.93	18.62	1.496	0.125	0.020	0.068	0.080
	3 mM	86.15	33.70	25.97	1.231	0.068	0.014	0.037	0.047

L: Lethality; I: Injury; S: Sterility; Gy: Dose of gamma radiation; T: Duration of mutagenic treatment; C: Concentration of mutagen

Table 4: Mutagenic effectiveness and efficiency based on chlorophyll mutants in M2 generation of LGG-460.

Treatments		%				Mutagenic	Mutagenic efficiency (%)			
		Survival reduction at 30 th day (L)	% Height reduction at 30 th day (I)	% Pollen fertility reduction (S)	Chlorophyll mutation frequency (%) (Mc)	effectivene ss (%) <u>Mc</u> Gy (or) T x C	Mc L	Mc I	Mc S	
Cont	Control		-	-	-	-	-	-	-	
	200 Gy	16.74	4.05	6.73	1.209	0.006	0.072	0.298	0.180	
Gamma	300 Gy	22.94	10.51	13.66	1.394	0.005	0.061	0.133	0.102	
	400 Gy	37.18	12.17	19.94	1.515	0.004	0.041	0.125	0.076	
rays	500 Gy	55.42	14.08	23.23	1.600	0.003	0.029	0.114	0.069	
	600 Gy	69.32	20.60	28.85	1.640	0.003	0.024	0.080	0.057	
	0.2%	19.66	7.42	6.13	1.333	1.111	0.068	0.180	0.218	
	0.3%	30.05	13.57	10.88	1.849	1.027	0.062	0.136	0.170	
EMS	0.4%	47.15	19.89	15.39	1.925	0.802	0.041	0.097	0.125	
	0.5%	60.27	28.43	22.40	2.361	0.787	0.039	0.083	0.105	
	0.6%	76.51	36.08	28.03	2.824	0.784	0.037	0.078	0.101	
	1 mM	56.82	13.04	4.17	0.732	0.122	0.013	0.056	0.176	
6 A	2 mM	77.35	20.01	14.75	0.888	0.074	0.011	0.044	0.060	
SA	3 mM	81.91	33.23	23.17	1.311	0.073	0.016	0.039	0.057	

L: Lethality; I: Injury; S: Sterility; Gy: Dose of gamma radiation; T: Duration of mutagenic treatment; C: Concentration of mutagen

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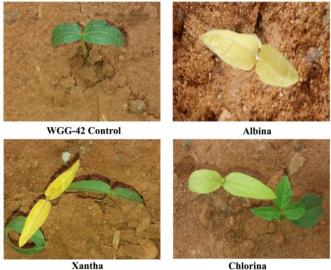


Fig. 1. Chlorophyll mutants observed in M₂ generation of WGG-42.

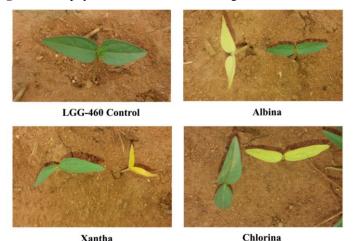


Fig. 2. Chlorophyll mutants observed in M2 generation of LGG-460.

CONCLUSIONS

From the results of the present study, it can be inferred that the frequency of chlorophyll mutations increased with the increase in dose/concentration of mutagens in both the genotypes, but EMS treatments showed the highest frequency of chlorophyll mutations than gamma rays and SA treatments. Mutagenic effectiveness and efficiency gradually decreased with increases in dose/concentration of mutagens. It was also inferred from the present study, EMS treatments were found to be more effective and efficient in inducing chlorophyll mutants than gamma rays and SA treatments in both the varieties *viz.*, WGG-42 and LGG-460. These results suggest that high mutation rates could be obtained with lower/moderate dose/concentration of mutagens in mungbean.

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