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Comparative effectiveness and efficiency of gamma rays, ethyl methane sulfonate and maleic hydrazide in induction of chlorophyll mutations in black gram (*Vigna mungo* (L.) Hepper)

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Abstract

The present investigation was taken up to study the effectiveness and efficiency of gamma rays, ethyl methane sulfonate and maleic hydrazide in induction of chlorophyll mutations in two blackgram varieties viz., LBG 752 and TBG 104. The seeds were treated with different doses/concentrations of gamma rays (200 Gy, 300 Gy, 400 Gy, 500 Gy and 600 Gy), EMS (Ethyl methane sulfonate) (0.2%, 0.3%, 0.4%, 0.5% and 0.6%) and MH (Maleic Hydrazide) (0.01%, 0.02% and 0.03%). A wide spectrum of chlorophyll mutations viz., albina, xantha and chlorina was observed in M₂ generation and chlorina was the most frequent chlorophyll mutant in all the mutagen treated populations of both the varieties. There was a dose dependent increase in the frequency of chlorophyll mutations. This study showed that the effectiveness and efficiency of chlorophyll mutations declined considerably with the increase in dose of the mutagens. MH treatments were more effective followed by EMS and gamma rays treatments in both the varieties. With respect to efficiency, the gamma rays were more efficient followed by EMS and MH treatments in both the varieties for inducing chlorophyll mutations on the basis of injury, lethality and sterility except based on lethality in TBG 104, where MH treatments showed highest value followed by gamma rays and EMS treatments.

Keywords: blackgram, effectiveness, efficiency, gamma rays, EMS, MH, frequency and spectrum

Introduction

Blackgram is an important pulse crop of Indian agriculture. Among pulses, it ranks fourth in production and acreage. However, keeping in view the future demands for maintaining nutritional security, there is an immediate need to increase the production and productivity of blackgram. As genetic variability is essential for any crop improvement programme, induced mutations provide an important source for variability. In order to exploit induced mutagenesis for crop improvement, the basic studies on effectiveness and efficiency of a mutagen in a crop is necessary to recover high frequency of desirable mutations (Kousar and Suresh, 2010)^[12]. Mutagenic effectiveness is a measure of the frequency of mutations induced with application of unit dose of a mutagen, whereas mutagenic efficiency indicates the proportion of mutations in the M₂ generation in relation to the biological damage such as gross chromosomal aberrations, lethality, sterility caused in M₁ (Konzak *et al.*, 1965)^[11].

Though chlorophyll mutations are considered to be less important from crop improvement point of view, still they act as indicators of effectiveness of mutagenic treatments in inducing other mutations observed in M₂ generation. Chlorophyll mutations are most dependable indices for the evaluation of genetic effects of mutagenic treatments, since they are more conspicuous and are easily detectable. They have been extensively used to find out sensitivity of crop plants to mutagens and to elucidate effectiveness and efficiency of a mutagen (Gustafsson, 1940)^[9].

Therefore, the present investigation was undertaken to compare mutagenic effectiveness and efficiency of gamma rays, EMS and MH based on the frequency of chlorophyll mutations in M₂ generations of LBG 752 and TBG 104 blackgram varieties.

Material and methods

In the present investigation, two promising blackgram varieties of Andhra Pradesh viz., LBG 752 and TBG 104 were used. Well filled, undamaged and uniform sized seeds were handpicked from the seed lot and equilibrated to the moisture content of 12 per cent. For each dose/concentration 750 seeds were treated in each variety. For gamma irradiation studies, the seeds were irradiated with different doses of gamma rays viz., 200 Gy, 300 Gy, 400 Gy, 500 Gy and 600 Gy at BARC, Trombay. Similarly, for chemical mutagen treatment, the seeds were treated with five different concentrations viz., 0.2%, 0.3%, 0.4%, 0.5% and 0.6% of EMS and three different concentrations of MH viz., 0.01%, 0.02% and 0.03% in the Department of Genetics and Plant Breeding, Sri Venkateswara Agricultural College, Tirupati during *rabi*, 2017. The seeds of both varieties were soaked for six hours in distilled water before chemical mutagen treatment. The pre-soaked seeds after removal from the water were placed between folds of blotting paper to remove the water adhered on the surface. Then the seeds were immersed for six hours in the requisite concentration of mutagen with intermittent shaking. To ensure uniform absorption of the mutagen, the volume of the mutagen solution was maintained at a proportion of ten times to that of seed volume. The whole treatment was carried out at room temperature of $23 \pm 1^\circ\text{C}$. Immediately after the completion of treatment duration, treated seeds were thoroughly washed in running water for half-an-hour. Treated seeds of gamma rays, EMS and MH along with equal number of controls (untreated) were sown immediately in the field in a randomized block design with three replications. Data on biological abnormalities such as injury, lethality and sterility were recorded in M_1 generation. Normal looking plants of M_1 generation with sufficient seed in different treatments were selected to raise M_2 generation in compact family block design with two replications. Observations on chlorophyll mutations were recorded from 5th to 15th day of sowing and were classified in accordance with the system of Gustafsson (1940) [9]. The frequency of chlorophyll mutations was calculated on M_2 plant population basis as follows.

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

The effectiveness and efficiency of the mutagens were worked out by using the formula as suggested by Konzak *et al.* (1965) [11].

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

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

$$\text{Mutagenic efficiency (\%)} = \frac{M_p \times 100 / L}{\frac{M_p \times 100 / I}{M_p \times 100 / S}}$$

Where,

M_p - Chlorophyll or viable mutation frequency on M_2 plant basis

L - Percentage of lethality *i.e.*, percentage of reduction in survival of seedlings on 30th day after sowing

I - Percentage of injury *i.e.*, percentage of height reduction of seedlings on 30th day after sowing

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

Results and discussion

Frequency and spectrum of chlorophyll mutations

Chlorophyll mutations provide one of the most dependable indices for the evaluation of genetic effects of mutagenic treatments and have also been reported earlier in blackgram (Souframanien *et al.*, 2016; Dhasarathan *et al.*, 2017 and Tamilzharasi *et al.*, 2019) [16, 5, 18]. Gaul (1964) [6] reported that chlorophyll mutations are employed as markers for the evaluation of gene action of mutagenic factors in inducing mutation. Mutation in chlorophyll genes may induce chlorophyll mutation. The origin of chlorophyll deficiencies is mainly due to mutation in gene, which are responsible for synthesis of photosynthetic pigments. In the present investigation, the frequency and spectrum of chlorophyll mutants were observed in M_2 generation and are presented in Tables 1 and 2 for LBG 752 and TBG 104, respectively. In gamma rays treatments, the frequency of chlorophyll mutants in M_2 population of LBG 752 ranged between 1.846 (200 Gy) to 3.476 per cent (600 Gy) and in TBG 104, it was between 1.648 (200 Gy) and 2.887 per cent (600 Gy). In EMS treatments, the frequency of chlorophyll mutants ranged from 1.060 (0.2%) to 2.106 per cent (0.6%) in LBG 752 and from 2.515 (0.2%) to 4.195 per cent (0.6%) in TBG 104 mutated populations. In MH treatments, the chlorophyll mutant frequency varied from 0.889 (0.01%) to 1.147 per cent (0.03%) in LBG 752 mutated population and 3.506 (0.01%) to 7.082 per cent (0.03%) in TBG 104 mutated population. There was a dose dependent increase in the frequency of chlorophyll mutation in gamma rays, EMS and MH treated M_2 populations in both the varieties. Similar trend was also observed by Souframanien *et al.* (2016) [16], Goyal *et al.* (2019) [8] and Tamilzharasi *et al.* (2019) [18]. The increased chlorophyll mutation frequency at higher doses may be attributed to the chromosomal aberrations or saturation in the mutational events which may result in the elimination of mutant cells during growth (Brock, 1965) [3].

The two blackgram varieties viz., LBG 752 and TBG 104 responded differently to different mutagens with respect to frequency of chlorophyll mutations. In LBG 752, the average frequency of chlorophyll mutations was highest in gamma rays treated population (2.413 per cent) followed by EMS (1.609 per cent) and MH (1.036 per cent) treated populations. Whereas, in TBG 104, the frequency of chlorophyll mutations was highest in MH treated population (5.238 per cent) followed by EMS (3.221 per cent) and gamma rays (2.365 per cent) treated populations. Gamma rays treatments showed highest average chlorophyll mutation frequency in LBG 752, while MH treatments showed the highest average mutation frequency in TBG 104.

In this study, a wide spectrum of chlorophyll mutations were observed in mutagen treated populations of LBG 752 and TBG 104 in M_2 generation, which included albina, xantha and chlorina types (Plate 1). No chlorophyll mutations were observed in untreated (control) population. Among different types of chlorophyll mutants, chlorina was the most frequent in gamma rays, EMS and MH treated population of LBG 752 and TBG 104 followed by xantha and albina. The higher proportion of chlorina was in agreement with findings of

Dhasarathan *et al.* (2017)^[5] and Tamilzharasi *et al.* (2019)^[18] in blackgram. The albina type of chlorophyll mutants were not found in MH treated population of both the varieties.

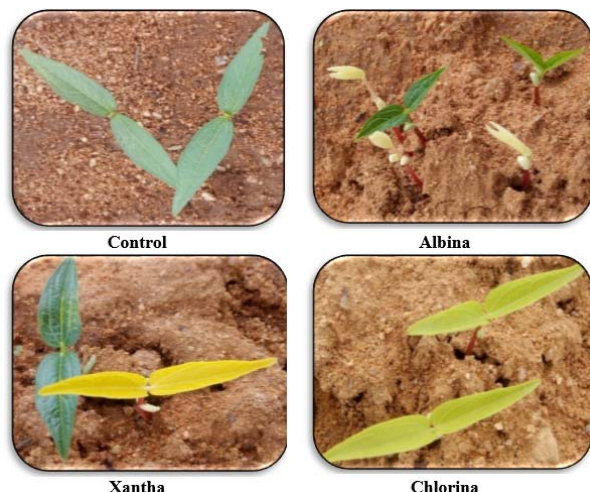


Plate 1: Chlorophyll mutants observed in M₂ generation

Mutagenic effectiveness and efficiency based on chlorophyll mutations

The Mutagenic effectiveness and efficiency based on chlorophyll mutations in LBG 752 and TBG 104 are presented in Tables 3 and 4, respectively. Konzak *et al.* (1965)^[11] had presented a detailed account of the mutagenic effectiveness and efficiency. The term effectiveness is a measure of gene mutation in relation to dose and efficiency is an estimate of biological effects induced, such as lethality, sterility and injury. To obtain high efficiency, the mutagenic effect must greatly surpass the damage in the cells and chromosomal aberrations as proposed by Gaul *et al.* (1972)^[7]. The effectiveness of gamma rays in inducing chlorophyll mutations ranged from 0.005 to 0.009 per cent with the highest effectiveness at 200 Gy of gamma rays in LBG 752, whereas in TBG 104 it varied from 0.005 to 0.008 per cent with highest effectiveness at 200 Gy of gamma rays. The effectiveness of EMS in inducing chlorophyll mutations ranged from 0.585 to 0.883 per cent in LBG 752 and in TBG 104, it ranged from 1.165 to 2.096 per cent with the highest mutagenic effectiveness in both the varieties at 0.2% of EMS. With respect to MH treatments in LBG 752, it ranged from 6.374 to 14.822 per cent and in TBG 104, it varied from 39.345 to 58.431 per cent and the highest effectiveness was noticed at 0.01% of MH in both the varieties.

In LBG 752, the highest average mutagenic effectiveness of chlorophyll mutants was recorded in MH treatments (10.040 per cent) followed by EMS treatments (0.704 per cent) and gamma rays treatments (0.007 per cent). Similarly, in TBG 104, the highest average mutagenic effectiveness of chlorophyll mutants was recorded in MH treatments (46.837 per cent) followed by EMS treatments (1.453 per cent) and gamma rays treatments (0.006 per cent). In the present study, the effectiveness of chlorophyll mutants showed a trend, which was inversely proportional to the increasing dose of mutagens. Among all mutagenic treatments studied, gamma rays at 200 Gy of gamma rays, 0.2% of EMS and 0.01% of MH were the most effective treatments in both the varieties. Among the mutagens, MH treatments were more effective followed by EMS and gamma rays treatments in both the varieties. Such a dose dependent findings agreed to the earlier response of Bhosale *et al.* (2013)^[1, 2], Bhosale and Hallale

(2013)^[1, 2] and Dhasarathan *et al.* (2017)^[5]. In contrast to this, Ramchander *et al.* (2018)^[13] reported high mutagenic effectiveness at higher doses and low mutagenic effectiveness at lower doses. Khan and Hashim (1979)^[10] and Reddi and Sunnetha (1992)^[14] reported that the effectiveness did not necessarily increase linearly with the increasing doses, rather every dose has its own effectiveness, independent of the other lower and higher doses.

The mutagenic efficiency varied depending on the criteria selected. The efficiency estimated on the basis of injury was generally higher compared with lethality and sterility. This observation was consistent with the findings of Souframanien *et al.* (2016)^[16]. The lethality, injury and sterility were the highest when higher doses of mutagenic treatments were used. In gamma rays treatments, the highest efficiency was observed at 200 Gy of gamma rays on the basis of lethality (0.119 per cent and 0.167 per cent), injury (0.265 per cent and 0.183 per cent) and sterility (0.300 per cent and 0.496 per cent) in LBG 752 and TBG 104. In LBG 752, the average mutagenic efficiency of gamma rays in inducing chlorophyll mutations based on lethality, injury and sterility was 0.061 per cent, 0.204 per cent and 0.120 per cent, respectively. In TBG 104, the average mutagenic efficiency of gamma rays in inducing chlorophyll mutations based on lethality, injury and sterility was 0.089 per cent, 0.142 per cent and 0.275 per cent, respectively.

In EMS treatments, the highest efficiency was observed at 0.2% of EMS on the basis of lethality (0.038 per cent and 0.423 per cent), injury (0.083 per cent and 0.664 per cent) and sterility (0.032 per cent and 0.932 per cent) in LBG 752 and TBG 104. In LBG 752, the average mutagenic efficiency of EMS in inducing viable mutations based on lethality, injury and sterility was 0.031 per cent, 0.062 per cent and 0.031 per cent, respectively. In TBG 104, the average mutagenic efficiency of EMS in inducing chlorophyll mutations based on lethality, injury and sterility was 0.149 per cent, 0.218 per cent and 0.348 per cent, respectively.

With respect to MH treatments, the highest efficiency was observed at 0.02% and 0.03% of MH on the basis of lethality (0.013 per cent), 0.02% of MH on the basis of injury (0.033 per cent) and 0.01% of MH on the basis of sterility (0.070 per cent) in LBG 752 and in TBG 104, the highest efficiency was observed at 0.03% of MH on the basis of lethality (0.081 per cent), 0.03% of MH on the basis of injury (0.149 per cent) and 0.01% of MH on the basis of sterility (0.280 per cent). In LBG 752, the average mutagenic efficiency of MH treatments in inducing chlorophyll mutations based on lethality, injury and sterility was 0.012 per cent, 0.030 per cent and 0.039 per cent, respectively. In TBG 104, the average mutagenic efficiency of MH treatments in inducing chlorophyll mutations based on lethality, injury and sterility was 0.603 per cent, 0.137 per cent and 0.164 per cent, respectively.

Based on lethality and injury, the average mutagenic efficiency in inducing chlorophyll mutations was highest in gamma rays followed by EMS and MH treatments, whereas based on sterility, it was highest in gamma rays followed by MH and EMS treatments. The efficiency of gamma rays, EMS and MH treatments declined considerably with the increase in dose of the mutagens with few exceptions. The decrease in efficiency at higher dose might be attributed to the failure in recovery of viable mutations proportion to the dose of the mutagen. This result was consistent with findings of Usharani and Kumar (2015)^[19], Souframanien *et al.* (2016)^[16] and Dhasarathan *et al.* (2017)^[5] in blackgram. The reason for the greater efficiency at lower concentrations of mutagen

would be due to the fact that the lethality, injury and sterility increased with the mutagen level at faster rate. So, the lower concentration caused relatively less damage which enabled the organisms to manifest the induced mutations more frequently (Shadakshari *et al.*, 2001). The high mutagenic efficiency, mostly at lower and intermediate doses of viable mutants than higher doses were previously reported by Surendar *et al.* (2014) in blackgram. Bhosale and Hallale (2013)^[1, 2] reported gradual increase in mutagenic efficiency with increasing concentration or doses of the mutagens. Based on the present results, gamma rays were more efficient

followed by EMS and MH treatments in both the varieties for inducing chlorophyll mutations on the basis of injury, lethality and sterility except based on lethality in TBG 104, where MH treatments showed higher value followed by gamma rays and EMS treatments. Deepalakshmi and Kumar (2003) also reported high efficiency of gamma rays in inducing chlorophyll mutations in blackgram. However, Bhosale and Hallale (2013)^[1, 2] and Usharani and Kumar (2015)^[19] reported EMS as the most efficient mutagen in blackgram.

Table 1: Frequency and spectrum of chlorophyll mutants in M₂ generation of LBG 752

Treatments	Total number of seedlings examined	Types of chlorophyll mutants			Total number of chlorophyll mutants	Relative percentage (Frequency) of chlorophyll mutants			Chlorophyll mutation frequency (%)
		Albina	Xantha	Chlorina		Albina	Xantha	Chlorina	
Control	2200	-	-	-	-	-	-	-	-
Gamma rays	200 Gy	1029	1	9	9	0.097	0.875	0.875	1.846
	300 Gy	455	0	2	8	0.000	0.440	1.758	2.198
	400 Gy	1072	0	9	15	0.000	0.840	1.399	2.239
	500 Gy	1215	0	2	26	0.000	0.165	2.140	2.305
	600 Gy	863	4	6	20	0.463	0.695	2.317	3.476
Mean	-	-	-	-	-	-	-	-	2.413
EMS	0.2%	1038	0	3	8	0.000	0.289	0.771	1.060
	0.3%	915	0	2	10	0.000	0.219	1.093	1.311
	0.4%	1488	1	6	17	0.067	0.403	1.142	1.613
	0.5%	1023	0	6	14	0.000	0.587	1.369	1.955
	0.6%	1187	2	10	13	0.168	0.842	1.095	2.106
Mean	-	-	-	-	-	-	-	-	1.609
MH	0.01%	1012	0	2	7	0.000	0.198	0.692	0.889
	0.02%	1214	0	1	12	0.000	0.082	0.988	1.071
	0.03%	1133	0	2	11	0.000	0.177	0.971	1.147
Mean	-	-	-	-	-	-	-	-	1.036

Table 2: Frequency and spectrum of chlorophyll mutants in M₂ generation of TBG 104

Treatments	Total number of seedlings examined	Types of chlorophyll mutants			Total number of Chlorophyll mutants	Relative percentage (Frequency) of chlorophyll mutants			Chlorophyll mutation frequency (%)
		Albina	Xantha	Chlorina		Albina	Xantha	Chlorina	
Control	2746	-	-	-	-	-	-	-	-
Gamma rays	200 Gy	789	0	2	11	0.000	0.253	1.394	1.648
	300 Gy	1385	2	3	26	0.144	0.217	1.877	2.238
	400 Gy	903	0	0	22	0.000	0.000	2.436	2.436
	500 Gy	1375	2	7	27	0.145	0.509	1.964	2.618
	600 Gy	1455	3	1	38	0.206	0.069	2.612	2.887
Mean	-	-	-	-	-	-	-	-	2.365
EMS	0.2%	1630	0	9	32	0.000	0.552	1.963	2.515
	0.3%	1410	2	2	36	0.142	0.142	2.553	2.837
	0.4%	1567	2	3	41	0.128	0.191	2.616	2.936
	0.5%	1519	12	7	36	0.790	0.461	2.370	3.621
	0.6%	1192	4	3	43	0.336	0.252	3.607	4.195
Mean	-	-	-	-	-	-	-	-	3.221
MH	0.01%	599	0	1	20	0.000	0.167	3.339	3.506
	0.02%	585	0	0	30	0.000	0.000	5.128	5.128
	0.03%	353	0	1	24	0.000	0.283	6.799	7.082
Mean	-	-	-	-	-	-	-	-	5.238

Table 3: Mutagenic effectiveness and efficiency based on chlorophyll mutants in LBG 752

Treatments	% Survival reduction at 30 th day (L)	% Height reduction at 30 th day (I)	% Pollen fertility reduction (S)	Chlorophyll mutation frequency (%) (Mc)	Mutagenic Effectiveness (%) Mc CxT (or) Gy	Mutagenic efficiency (%)			
						Mc L	Mc I	Mc S	
Control	-	-	-	-	-	-	-	-	
Gamma rays	200 Gy	15.55	6.97	6.16	1.846	0.009	0.119	0.265	0.300
	300 Gy	39.83	8.33	25.75	2.198	0.007	0.055	0.264	0.085
	400 Gy	49.24	12.29	28.54	2.239	0.006	0.045	0.182	0.078
	500 Gy	59.53	14.92	37.60	2.305	0.005	0.039	0.154	0.061
	600 Gy	71.77	22.13	45.45	3.476	0.006	0.048	0.157	0.076
Mean	-	-	-	-	0.007	0.061	0.204	0.120	

EMS	0.2%	28.01	12.81	32.87	1.060	0.883	0.038	0.083	0.032
	0.3%	38.37	22.25	42.82	1.311	0.729	0.034	0.059	0.031
	0.4%	51.85	29.90	54.27	1.613	0.672	0.031	0.054	0.030
	0.5%	68.97	34.89	60.40	1.955	0.652	0.028	0.056	0.032
	0.6%	81.74	37.76	73.54	2.106	0.585	0.026	0.056	0.029
Mean		-	-	-	-	0.704	0.031	0.062	0.031
MH	0.01%	84.90	32.24	12.67	0.889	14.822	0.010	0.028	0.070
	0.02%	85.51	32.87	44.21	1.071	8.924	0.013	0.033	0.024
	0.03%	88.26	40.68	49.87	1.147	6.374	0.013	0.028	0.023
Mean		-	-	-	-	10.040	0.012	0.030	0.039

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 TBG 104

Treatments	% Survival reduction at 30 th day (L)	% Height reduction at 30 th day (I)	% Pollen fertility reduction (S)	Chlorophyll mutation frequency (%) (Mc)	Mutagenic Effectiveness (%) $\frac{Mc}{C \times T}$ (or) Gy	Mutagenic efficiency (%)			
						$\frac{Mc}{L}$	$\frac{Mc}{I}$	$\frac{Mc}{S}$	
Control		-	-	-	-	-	-	-	
Gamma rays	200 Gy	9.88	9.00	3.32	1.648	0.008	0.167	0.183	0.496
	300 Gy	18.41	13.19	4.79	2.238	0.007	0.122	0.170	0.467
	400 Gy	43.73	14.86	16.27	2.436	0.006	0.056	0.164	0.150
	500 Gy	51.42	23.55	20.77	2.618	0.005	0.051	0.111	0.126
	600 Gy	58.78	35.05	20.97	2.887	0.005	0.049	0.082	0.138
Mean		-	-	-	-	0.006	0.089	0.142	0.275
EMS	0.2%	5.94	3.79	2.70	2.515	2.096	0.423	0.664	0.932
	0.3%	23.77	18.78	6.67	2.837	1.576	0.119	0.151	0.425
	0.4%	41.39	31.03	12.96	2.936	1.223	0.071	0.095	0.227
	0.5%	52.31	35.34	47.81	3.621	1.207	0.069	0.102	0.076
	0.6%	64.89	53.60	51.01	4.195	1.165	0.065	0.078	0.082
Mean		-	-	-	-	1.453	0.149	0.218	0.348
MH	0.01 %	86.46	23.95	12.50	3.506	58.431	0.041	0.146	0.280
	0.02 %	86.76	44.10	50.75	5.128	42.735	0.059	0.116	0.101
	0.03 %	87.51	47.46	63.91	7.082	39.345	0.081	0.149	0.111
Mean		-	-	-	-	46.837	0.603	0.137	0.164

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

Conclusion

The study on comparative effectiveness and efficiency of gamma rays, ethyl methane sulfonate and maleic hydrazide in induction of chlorophyll mutations on two black gram varieties viz., LBG 752 and TBG 104 revealed that the effectiveness and efficiency of chlorophyll mutations declined considerably with the increase in dose of the mutagens. It was also inferred from the present study that among the mutagens, MH treatments were more effective followed by EMS and gamma rays treatments in both the varieties. With respect to efficiency, the gamma rays were more efficient followed by EMS and MH treatments in both the varieties for inducing chlorophyll on the basis of injury, lethality and sterility except mutagenic efficiency based on lethality in TBG 104, where MH treatments showed higher value followed by gamma rays and EMS treatments. Hence, gamma rays could be considered as promising mutagen to get more frequency of desirable mutants than EMS and MH in blackgram as it was more efficient in both the blackgram varieties.

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