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Effectiveness and Efficiency of Mutagens in gamma irradiated and EMS treated population of blackgram (*Vigna mungo* (L) Hepper)

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ABSTRACT: *The present investigation was carried out to study the impact of mutagens on biological parameters such as seed germination, shoot and root length, plant survival, plant height, pollen fertility and seed fertility and effectiveness and efficiency of mutagens by subjecting the two promising varieties* viz., *MDU (Bg) 1 and VBN (Bg) 4 to gamma irradiation and EMS.* LD_{50} *was noticed at 300 Gy and 30 mM of gamma irradiation and EMS respectively against different doses (200, 250, 300 and 350 Gy) / concentration (20, 30 and 40 mM) of mutagens used. All growth parameters showed decreasing fashion to increasing doses / concentrations of mutagens. Seed germination, plant survival and pollen fertility were drastically reduced with increasing of mutagens. Mutagenic effectiveness and efficiency was increased with increasing doses / concentrations of mutagens. Even though, lower dose of both mutagens showed maximum effectiveness and efficiency.*

Keywords: *Blackgram, Mutagenesis, Chlorophyll mutants, effectiveness and efficiency*

INTRODUCTION

Blackgram is an important pulse crop, occupying distinctive position in Indian agriculture. Among the pulses, it stands fourth position in production and acreage. Blackgram is rich in protein and dietary amino acids for men and farm animals. The discovery that X-rays induced mutations in the fruitfly (*Drosophila melanogaster*) (Muller, 1927) and in barley (Stadler, 1928) paved a new field – induced mutagenesis, which later was to become the most important tool in locating genes on chromosomes, studying gene structure, expression and regulation, and for exploring genomes.

Investigational mutagenesis is a vital source which creates mutation in higher frequencies in cultivated crops. Excellent source of precious materials for breeding work can be offered by creating wide collection of mutations based on productive characters. Mutation breeding may be a successful tool for producing variability in the existing varieties (Khan & Goyal, 2009). A crop plant can be enhanced in productivity, resistance to biotic and abiotic stresses when the existing genetic variability for the specific trait is enormous in the considered population or species. Induced mutagenesis has been successfully used to generate variability, fractioning for isolating mutants with advantageous characters of economic importance such as superior dwarf plant types, synchronous maturity, high grain yield, larger seed size and seed colour *etc*., (Kharkwal *et al*., 2004). Biological damage caused by mutation for germination, seedling injury, pollen sterility and survival at maturity may be considered as an indication of mutagenic effect (Gaul, 1964).

The worth of a mutagens in mutation breeding depends not only on its mutagenic effectiveness (mutations per unit dose of mutagens), but also on its mutagenic efficiency (mutation in relation to undesirable changes like sterility, lethality, injury *etc*.). The choice of effective and efficient mutagens is very essential to recover a high frequency and spectrum of desirable mutations.

The present investigation was undertaken to study the impact of mutagen on biological materials in M_1 generation such as germination, shoot length, root length, plant height at 30 days after sowing,

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pollen fertility and survival on 30 days after sowing, plant height at maturity and seed fertility. The frequency of chlorophyll mutations was scored to study the effectiveness and efficiency of mutagen.

MATERIALS AND METHODS

Identical, Well filled, sun dried and genetically pure seeds of blackgram promising varieties *viz*., MDU (Bg) 1 and VBN (Bg) 4 were treated with Gamma radiation from ^{60}Co source fixed in the gamma chamber 1200 installed at Tamil Nadu Agricultural University, Coimbatore at 200, 250, 300 and 350 Gy doses and ethyl methane sulphonate (Sigma chemical Co. Ltd. USA) at 20, 30 and 40 mM concentrations. For each treatment, 500 seeds of each variety were pre soaked in double distilled water for 6 hrs initially (Malarkodi, 2008). Thereafter, pre-soaked seeds were soaked in requisite concentration of EMS prepared in phosphate buffer at the pH 7 for six hours (Sri Devi and Mullainathan, 2012) 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 28 ± 1°C.

Immediately after the completion of treatment duration, the treated seeds were thoroughly washed in running water for half-an-hour. After mutagenic treatment seeds were sown immediately in the field in a randomized block design along with control (The untreated seeds). All M_1 plants were harvested individually and forwarded to M_2 generation spacing adopted was 30 cm between rows and 10 cm between plants with single seed per hole. Recommended fertilizers, plant protection measures and the general cultural practices were uniformly followed for all the treatments. The M_2 plants at seedling stage were scored for chlorophyll mutations up to 15th day after sowing. The percentage of different types of chlorophyll mutants to the total number of chlorophyll mutants induced by various doses of each mutagen was worked. Chlorophyll mutation frequency per 100 M_2 seedlings was calculated. Mutation frequency was calculated as percentage of mutated M_2 progenies for both chlorophyll and morphological mutations in each treatment. The Mutagenic effectiveness and efficiency were calculated on the basis of formulae suggested by Konzak *et al*., 1965.

Mutagenic Effectiveness

$$Mutagenic\ effectiveness = \frac{M \times 100}{Gy(or)Tc}$$

Where,

- M - Mutation frequency for 100 M_2 plants.
- T - Duration of mutagenic treatment
- c - Concentration of mutagen in mM.
- Gy - Dose of mutagenic radiation in Grey for physical mutagen.

Mutagenic Efficiency

Mutagenic efficiency = M x 100 / L
M x 100 / I
M x 100 / S

Where,

- M - Mutation frequency for 100 M_2 plants.
- L - Percentage of lethality *i.e.,* reduction in survival on 30th day.
- I - Percentage of injury *i.e.*, reduction in plant height on 30th day.
- S - Percentage of sterility *i.e.*, reduction in seed fertility.

RESULT AND DISCUSSION

In this present study impact of different doses / concentrations of gamma rays and EMS on biological parameters such as germination, shoot length, root length, survival on 30 Days after sowing, plant height at 30 Days after sowing, pollen fertility, plant height at maturity and seed fertility were investigated. LD_{50} was noticed at 300 Gy of gamma rays and 30 mM of EMS for both cultivars. Germination percentage was found to decrease with increase of mutagens. The seed germination was decreased from 77.02% to 46.90% for gamma rays and 61.28% to 45.87% for EMS in MDU (Bg) 1 over control. In blackgram variety VBN (Bg) 4, Gamma irradiation has range of germination reduction from 74.15% to 48.29% and EMS varied from 73.33% to 46.07% over control. This shows significant influence of mutagen on germination.

A drastic and prominent reduction on germination per cent was noticed in EMS treatments than Gamma rays. Similar reports were noticed in blackgram by Sagade and Apparao (2011), Thilagavathi and Mullainathan (2011) and Ramya *et al*. (2014). In both cultivars all the treatments showed significant differences for shoot and root length reduction per cent over control for both mutagenic treatments (Table 1 & 2). The higher per cent reduction in shoot length (39.45% - MDU (Bg)1 and 35.65% – VBN (Bg) 4) and root length (37.50% - MDU(Bg)1 and 33.99% – VBN (Bg) 4) was recorded in higher dose of gammarays (350 Gy). In EMS treatments, higher concentration (40mM) showed increased shoot

Table 1
Impact of Gamma Ray and ethyl methane Sulphonate on Biological Parameters of MDU (Bg) 1 in M_1 Generation

Treatments	*Germination reduction over control (%)*	*Shoot length reduction over control (%)*	*Root length reduction over control (%)*	*Plant height on 30th DAS reduction over control (%)*	*Survival on 30th DAS reduction over control (%)*	*Pollen fertility over control (%)*	*Seed fertility reduction over control (%)*
Gamma rays							
200 Gy	77.02	83.56	82.99	93.83	67.82	83.09	90.06
250 Gy	69.65	70.55	64.93	88.79	65.20	66.17	86.28
300 Gy	47.26	56.58	56.60	84.52	48.41	53.44	78.24
350 Gy	46.90	39.45	37.50	81.29	46.73	49.08	73.48
EMS							
20 mM	61.28	86.71	71.18	94.95	73.88	72.25	89.03
30 mM	50.00	67.53	61.11	87.11	49.00	51.68	84.96
40 mM	45.87	44.38	44.44	80.42	45.41	47.88	81.58

Table 2
Impact of Gamma Ray and ethyl methane Sulphonate Treatments on Biological Parameters of VBN (Bg) 4 in M_1 Generation.

Treatments	*Germination reduction over control (%)*	*Shoot length reduction over control (%)*	*Root length reduction over control (%)*	*Plant height on 30th DAS reduction over control (%)*	*Survival on 30th DAS reduction over control (%)*	*Pollen fertility over control (%)*	*Seed fertility reduction over control (%)*
Gamma rays							
200 Gy	74.15	83.95	77.23	88.98	72.87	84.08	91.21
250 Gy	61.98	66.67	58.42	85.06	60.18	74.92	87.56
300 Gy	49.02	53.47	43.89	80.60	39.32	53.81	82.33
350 Gy	48.29	35.65	33.99	74.64	47.06	46.54	76.67
EMS							
20 mM	73.33	85.71	72.94	94.54	71.27	75.87	88.55
30 mM	49.02	66.53	63.04	88.54	48.63	51.57	84.18
40 mM	46.07	39.18	37.29	81.04	44.87	47.02	80.85

(44.38% - MDU (Bg) 1 and 39.18% – VBN (Bg) 4) and root length (44.44% - MDU (Bg) 1 and 37.29% – VBN (Bg) 4) reduction per cent over control.

Seedling injury is broadly used as an index of determining biological effects of various physical and chemical mutagens in M_1 generation (Swaminathan *et al.*, 1962). Plant height was also found to be significantly reduced in higher doses of physical and chemical mutagenic treatments. All the treatments resulted retardation in height of plants. The maximum plant height reduction on 30 DAS (81.29%) was observed in higher dose of gamma rays (350 Gy) than EMS (80.42%) in MDU (Bg) 1. But contrast to MDU (Bg) 1, the maximum plant height reduction was found in EMS (81.04%) than gamma irradiation (74.64%). The differential sensitivity of biological material to different mutagen may be due to the metabolic processes affected at embryonic level (Ashri and Herzog, 1972). In the present investigation, survival per cent reduction increased with increase of mutagens in both varieties.

A significant was noticed in all treatments. This might have been due to the effect of mutagens on merismatic tissues of seed (Ramya *et al.*, 2014). The maximum reduction (45.41% - MDU (Bg) 1 and 44.87% - VBN (BG) 4) was observed in higher concentration (40 mM) of EMS than gammarays. The decrease in survival of plants at maturity is due to hasty infusion of chemical mutagen and their capability to produce chromosomal aberrations (Swaminathan *et al.*, 1962). The survival percentage and mean value of M_1 generation decreased with increase in dose of treatments (Pavadai *et al.*, 2010) in soybean.

Pollen fertility in M_1 generation is the first indication of genetic effectiveness of the treatments. The pollen fertility and seed fertility decreased with increase of mutagens. In most cases, meiotic abnormalities are responsible for pollen fertility reduction (Muthu samy and Jayabalan, 2002) in cotton and chickpea (Khan and Wani, 2005).

MUTAGENIC EFFECTIVENESS AND EFFICIENCY

Mutagens induce differential genetic and cytogenetic changes (Fahmy and Fahmy, 1959). Thus the mutagenic effectiveness and efficiency will also depend upon the nature of induced mutations. The data revealed that, generally frequency of chlorophyll mutations increased with the increase in dose / concentration of gammarays amd EMS in both the cultivars *viz*. MDU (Bg) 1 and VBN (Bg) 4 (Table 3.). In MDU (Bg) 1 maximum frequency of chlorophyll mutations were observed in lower dose of gamma irradiation. But generally chlorophyll mutation frequency showed increase with increase of gamma irradiation. EMS treatments showed maximum (1.814%) at higher concentration (40 mm). A linear increase of chlorophyll mutation frequency was observed with increase of both mutagens in VBN (Bg) 4.

In the present study, the effectiveness of different varieties by gamma ray was assessed by their mutagen dose and the efficiency was assessed on three different biological parameters *viz.,* plant survival, plant height, and seed sterility. The data depicted that the mutagenic effectiveness ranged from 5.24 % (250 Gy) to 11.69 % (200 Gy) for gamma irradiation in MDU (Bg) 1. It is varied from 0.92 % (20 mM) to 1.57 % (30 mM) for EMS. Lower doses of gamma irradiation and higher concentration of EMS showed maximum effectiveness in the variety MDU (Bg) 1.

A gradual increase in mutagenic effectiveness was found with increase of gamma rays in VBN (Bg) 4. Maximum effectiveness (7.62 %) was niticed in 350 Gy of gamma irradiation. Lower concentration (20mM) EMS treatment had given maximum (1.38 %) effectiveness. Gamma irradiation recorded maximum effectiveness than EMS treatments in both cultivars. This results were associated with results of Deepalakshmi and Ananda Kumar (2003) in blackgram; Awnindra K. Singh, 2007) in greengram and Kale (2007) in cowpea.

The mutagenic efficiency was calculated out based on injury, lethality and sterility. The mutagenic efficiency gives an initiative of the proportion of mutations in relation to other associated undesirable biological effects such as injury, lethality and sterility induced by the mutagen (Konzak *et al.,* 1965). Higher dose / concentration of gamma rays and EMS was found to be more efficient based on lethality in MDU (Bg) 1. Based on injury and sterility, lower doses (200 Gy) of gamma irradiation and higher concentration

Table 3

Mutagenic Effectiveness and Efficiency in the M_2 Generation of MDU (Bg) 1 (Chlorophyll mutants).

Treatments Gammarays (Gray)	*Per cent survival Reduction on 30th day (Lethality) (L)*	*Per cent Height Reduction on 30th day (Injury) (I)*	*Seed fertility reduction (Sterility) (S)*	*Mutation frequency (%) (Mp)*	*Effectiveness (%)*		*Efficiency (%)*	
					$\frac{M_p X100}{Kr/mM}$	$\frac{M_p x100}{L}$	$\frac{M_p x100}{I}$	$\frac{M_p x100}{S}$
200 Gy	32.18	6.17	9.94	2.339	11.69	0.226	37.909	23.54
250 Gy	34.80	11.21	13.72	1.310	5.24	3.764	11.685	9.548
300 Gy	51.59	15.48	21.76	1.689	5.63	3.273	10.910	7.761
350 Gy	53.27	18.71	26.52	1.882	5.37	3.532	10.058	7.096
EMS (mM)								
20 mM	26.12	5.05	10.97	0.737	0.92	2.821	14.594	6.718
30 mM	51.00	6.73	15.04	1.878	1.57	3.078	27.904	12.486
40 mM	54.59	19.58	18.42	1.814	1.13	3.32	9.264	9.847

Table 4

Mutagenic Effectiveness and Efficiency in the M_2 Generation of VBN (Bg) 4 (Chlorophyll mutants)

Treatments Gammarays (Gray)	*Per cent survival Reduction on 30th day (Lethality) (L)*	*Per cent Height Reduction on 30th day (Injury) (I)*	*Seed fertility reduction (Sterility) (S)*	*Mutation frequency (%) (Mp)*	*Effectiveness (%)*		*Efficiency (%)*	
					$\frac{M_p X100}{Kr/mM}$	$\frac{M_p x100}{L}$	$\frac{M_p x100}{I}$	$\frac{M_p x100}{S}$
200 Gy	27.13	11.02	8.79	1.153	5.77	4.25	10.46	13.12
250 Gy	39.82	14.94	12.44	1.368	5.47	3.44	9.16	10.99
300 Gy	60.68	19.40	17.67	1.964	6.55	3.24	3.24	11.11
350 Gy	52.94	25.36	23.33	2.667	7.62	5.04	5.04	11.43
EMS (mM)								
20 mM	28.73	5.46	11.45	1.103	1.38	3.84	20.20	9.63
30 mM	51.37	11.46	15.82	0.912	0.76	1.48	7.96	5.76
40 mM	55.13	18.96	19.15	1.669	1.04	1.89	8.80	8.72

(30 mM) of EMS found to be more efficient among all gamma rays and EMS treatments. In VBN (Bg) 4, a gradual reduction in mutagenic efficiency was noticed with increasing doses / concentration of both mutagens based on injury and sterility. Gamma irradiation showed greatest effectiveness than EMS treatments in both cultivars. Similar reports were obtained by Sagade and Apparao (2011) in blackgram; Ashok Kumar *et al.* (2010) and Dube *et al.* (2011) in cowpea; Dayanidhi Mishra and Baburam Singh (2013) in greengram.

CONCLUSION

Efficient mutagens and their treatments are obligatory 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. In this study it is concluded that gamma irradiation at lower doses is more efficient than EMS. At lower doses gamma irradiatin can produces more variability in the biological materials used for breeding program.

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