

Original Research Article

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Studies on Sensitivity of Blackgram (*Vigna mungo* (L.) Hepper) Varieties to Physical and Chemical Mutagens under *in-vitro* Condition

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ABSTRACT

Keywords

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The present investigation was taken up to study the sensitivity of two blackgram varieties viz., LBG 752 and TBG 104 to three mutagens on biological parameters and to determine the lethal dose (LD₅₀) of mutagens. 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 sulphonate) (0.2%, 0.3%, 0.4%, 0.5% and 0.6%) and MH (Maleic Hydrazide) (0.01%, 0.02% and 0.03%). MH treatment resulted in drastic reduction in seed germination percentage, shoot length and root length followed by EMS treatment and gamma rays treatment in both the varieties. The reduction in these characters with increasing dose/concentration of mutagens was more prominent in LBG 752 than TBG 104 indicating more sensitive response of LBG 752 to mutagen treatment than TBG 104. LD₅₀ values were estimated based on seed germination percentage and the values were 500.16 Gy for gamma rays, 0.396% for EMS and 0.016% for MH in LBG 752 and 552.92 Gy for gamma rays, 0.554% for EMS and 0.028% for MH in TBG 104. These findings would greatly help for cost effective selection of variety and mutagens for successful generation of variation in mutation breeding programmes aimed at blackgram crop improvement.

Introduction

Blackgram (*Vigna mungo* (L.) Hepper) (2n=22) popularly known as urdbean, belongs to family Fabaceae. It is highly nutritious crop rich in seed protein content (24-28 per cent) and contributes 76 per cent carbohydrate, 3 to 5 per cent fibre, 1.74 per cent fat, vitamins like thiamine (B1), riboflavin (B2) and niacin

(B3) and also rich in minerals like phosphorus, calcium, magnesium and potassium etc. (Elangaimannan *et al.*, 2008). This crop has an important role in meeting the dietary protein requirement, in particular, in South India where its products are daily used in different forms like idli, dosa and vada etc. The perusal of the statistics of blackgram showed that India is the largest producer in

the world with an area of 5.28 million hectares with a production potential of 3.49 million tonnes and a productivity of 662 kg ha⁻¹ (Indiastat, 2017 - 18). Among the pulses, blackgram ranks fourth in area and production after chickpea, pigeonpea and mungbean and is one of the most highly prized pulses of India. However, the average yield of blackgram is very low in comparison to major grain legumes like chickpea and pigeonpea. Hence, in view of its growing importance and its suitability to various cropping systems and niches, the production potential of blackgram crop should be enhanced by developing high yielding genotypes. However, as the genetic variability is very low in this crop due to cleistogamous nature and narrow genetic base among the released cultivars, the progress of breeding programmes is not in pace with the growing demands. As the genetic variability is essential for any crop improvement programme, induced mutagenesis could be one of the viable options to bridge this gap. In order to induce variable mutations both physical and chemical mutagens are most commonly used in crop plants.

In mutation breeding, the success is highly proportionate to the choice of the genotype and the dose of the mutagen which are directly related to the production of desired mutants. The dose that leads to 50% lethality (LD₅₀) has often been chosen for induction of mutations as the lower dose cannot cause mutation in seeds and the higher dose leads to death of the mutated seeds and other deleterious effects. Hence, understanding the sensitivity of genotypes to the mutagens and also determining the LD₅₀ values of mutagens is highly useful for establishment of mutation breeding programme in a cost effective way.

Therefore, the present investigation was undertaken to study the sensitivity of two different blackgram varieties (LBG 752 and TBG 104) to gamma rays, EMS (Ethyl

Methane Sulphonate) and MH (Maleic Hydrazide) treatments in M₁ generation by considering the effects of mutagens on traits like germination, shoot length and root length and to determine the LD₅₀ value for different mutagens by probit analysis.

Materials and Methods

The plant material in the present investigation consisted of two promising blackgram varieties of Andhra Pradesh and Telangana viz., LBG 752 and TBG 104. The seeds of these varieties were irradiated with five 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 *kharif*, 2017-18. The seeds of both the varieties were soaked for six hours in distilled water before the 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±1^oC. Immediately after the completion of treatment duration, the treated seeds were thoroughly washed in running water for half-an-hour. A set of untreated seed served as control. The treated seeds with respective control were kept for germination under *in-vitro* condition following completely randomized design with three replications to fix LD₅₀ values for

different mutagens. Five days after sowing, the seed germination percentage, shoot length and root length were recorded in each and every seedling as follows. The percentage of number of seeds that gave rise to normal seedlings with root and shoot growth from the total number of seeds kept for germination was considered as seed germination percentage. The shoot length was measured from the collar region to the tip of the shoot and the root length was measured from the collar region to the tip of the primary root. The sensitivity of two blackgram varieties for different mutagens was assessed based on the rate of reduction in different characters like seed germination, shoot and root length. Probit analysis (Finney, 1971) based on seed germination was carried out to determine LD₅₀ value.

Results and Discussion

The data recorded on seed germination, shoot length and root length under *in-vitro* condition for LBG 752 and TBG 104 mutagen treated population were statistically analysed separately and the details of analysis of

variance are furnished in Table 1 and 2. The analysis of variance indicated significant treatment differences for all the three characters studied in gamma rays, EMS and MH treated population of both the varieties.

The results on effect of gamma rays, EMS and MH treatments on biological parameters like seed germination, shoot length and root length in LBG 752 and TBG 104 varieties of blackgram are presented and discussed below (Table 3).

Significant reduction in mean germination percentage than their respective control was observed with increase in dose/concentration of all the mutagens in both the varieties except 200 Gy of gamma rays and 0.2% of EMS treatments in TBG 104 (Table 3). In gamma rays treatment, the mean seed germination percentage ranged from 38.67 (600 Gy) to 86.67 per cent (200 Gy) in LBG 752 and 45.33 (600 Gy) to 97.67 per cent (200 Gy) in TBG 104 as against 98.33 per cent and 99.33 per cent in their respective control (LBG 752 and TBG 104).

Table.1 Analysis of variance for three characters in M₁ generation of LBG 752 under *in-vitro* condition

S. No.	Characters	Mean sum of squares	
		Treatments (df: 13)	Error (df: 28)
1.	Seed germination (%)	1721.19**	8.05
2.	Shoot length (cm)	75.04**	3.06
3.	Root length (cm)	4.98**	0.41

** Significant at 1% level

Table.2 Analysis of variance for three characters in M₁ generation of TBG 104 under *in-vitro* condition

S. No.	Characters	Mean sum of squares	
		Treatments (df: 13)	Error (df: 28)
1.	Seed germination (%)	1274.86**	10.19
2.	Shoot length (cm)	59.12**	1.64
3.	Root length (cm)	5.22**	0.68

** Significant at 1% level

Table.3 Effect of mutagens on biological parameters in M₁ generation of LBG 752 and TBG 104 under *in-vitro* condition

Treatments	LBG752			TBG104		
	Seed germination (%)	Shoot length (cm)	Root length (cm)	Seed germination (%)	Shoot length (cm)	Root length (cm)
Control	98.33	16.71	4.52	99.33	15.87	4.54
200 Gy	86.67*	13.57*	3.77	97.67	11.52*	3.77
300 Gy	74.67*	12.80*	3.53	89.00*	11.20*	3.58
400 Gy	63.00*	11.77*	3.47	74.33*	8.44*	3.35
500 Gy	52.33*	10.25*	2.85*	55.67*	8.06*	3.13*
600 Gy	38.67*	8.71*	1.98*	45.33*	7.74*	2.81*
Mean	68.94	12.30	3.35	76.88	10.47	3.53
Ethyl methane sulphonate (EMS)						
Control	98.33	16.71	4.52	99.33	15.87	4.54
0.2%	81.67*	11.67*	3.15*	96.67	8.35*	2.93*
0.3%	69.00*	6.50*	2.92*	89.33*	7.82*	2.55*
0.4%	50.67*	5.33*	1.73*	76.00*	6.23*	1.13*
0.5%	37.67*	3.69*	0.91*	57.33*	2.32*	0.88*
0.6%	23.33*	2.87*	0.29*	42.33*	1.58*	0.41*
Mean	60.11	7.80	2.25	76.83	7.03	2.07
Maleic Hydrazide (MH)						
Control	98.33	16.71	4.52	99.33	15.87	4.54
0.01%	72.67*	3.09*	1.24*	68.00*	2.67*	1.23*
0.02%	46.33*	2.17*	1.18*	59.33*	1.63*	1.09*
0.03%	18.00*	0.93*	1.10*	46.33*	1.11*	0.99*
Mean	58.83	5.73	2.01	68.24	5.32	1.96
	SE(m): 1.64 CD(0.05): 4.74	SE(m): 1.01 CD(0.05): 2.92	SE(m): 0.37 CD(0.05): 1.07	SE(m): 1.84 CD(0.05): 5.34	SE(m): 0.74 CD(0.05): 2.14	SE(m): 0.48 CD(0.05): 1.38

* Significant decrease than control at P=0.05

Table.4 Probit analysis for calculating LD₅₀ in gamma rays, EMS and MH treated populations of LBG 752

		Number of seeds evaluated	Number of plants killed	Observed mortality percentage	Corrected mortality percentage	Log ₁₀ of doses	Empirical probit unit
Control		75	2	2.67	-	-	-
Gamma rays	200 Gy	75	11	14.67	12.30	2.30	3.84
	300 Gy	75	21	28.00	26.00	2.48	4.36
	400 Gy	75	29	38.67	37.00	2.60	4.67
	500 Gy	75	37	49.33	47.90	2.70	4.95
	600 Gy	75	47	62.67	61.60	2.78	5.30
Ethyl Methane Sulphonate (EMS)	0.2%	75	14	18.67	16.40	-0.70	4.02
	0.3%	75	24	32.00	30.10	-0.52	4.48
	0.4%	75	38	50.67	49.30	-0.40	4.98
	0.5%	75	48	64.00	63.00	-0.30	5.33
	0.6%	75	58	77.33	76.70	-0.22	5.73
Maleic Hydrazide (MH)	0.01%	75	21	28.00	26.00	-2.00	4.36
	0.02%	75	41	54.67	53.40	-1.70	5.09
	0.03%	75	62	82.67	82.20	-1.52	5.92

LD₅₀ value for gamma rays = 500.16 Gy

LD₅₀ value for EMS = 0.396%

LD₅₀ value for MH = 0.016%

Table.5 Probit analysis for calculating LD₅₀ in gamma rays, EMS and MH treated populations of TBG 104

		Number of seeds evaluated	Number of plants killed	Observed mortality percentage	Corrected mortality percentage	Log ₁₀ of doses	Empirical probit unit
Control		75	3	4.00	-	-	-
Gamma rays	200 Gy	75	5	6.67	2.80	2.30	3.09
	300 Gy	75	9	12.00	8.30	2.48	3.62
	400 Gy	75	21	28.00	25.00	2.60	4.33
	500 Gy	75	35	46.67	44.40	2.70	4.86
	600 Gy	75	43	57.33	55.60	2.78	5.14
Ethyl Methane Sulphonate (EMS)	0.2%	75	7	9.33	5.60	-0.70	3.41
	0.3%	75	15	20.00	16.70	-0.52	4.03
	0.4%	75	22	29.33	26.40	-0.40	4.37
	0.5%	75	33	44.00	41.70	-0.30	4.79
	0.6%	75	45	60.00	58.30	-0.22	5.21
Maleic Hydrazide (MH)	0.01%	75	24	32.00	29.20	-2.00	4.45
	0.02%	75	32	42.67	40.30	-1.70	4.75
	0.03%	75	41	54.67	52.80	-1.52	5.07

LD₅₀ value for gamma rays = 552.92 Gy

LD₅₀ value for EMS = 0.554%

LD₅₀ value for MH = 0.028%

Fig.1 Plots of log doses versus probits for calculation of LD₅₀ of gamma rays in LBG 752

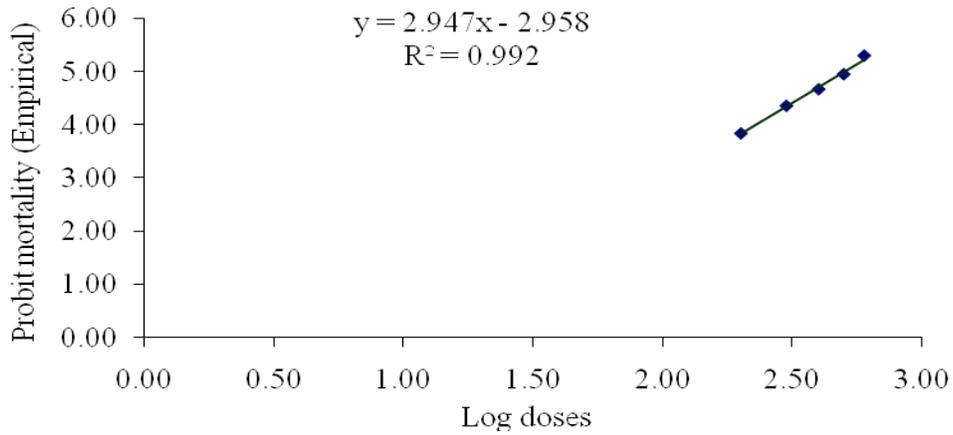


Fig.2 Plots of log concentrations versus probits for calculation of LD₅₀ of EMS in LBG 752

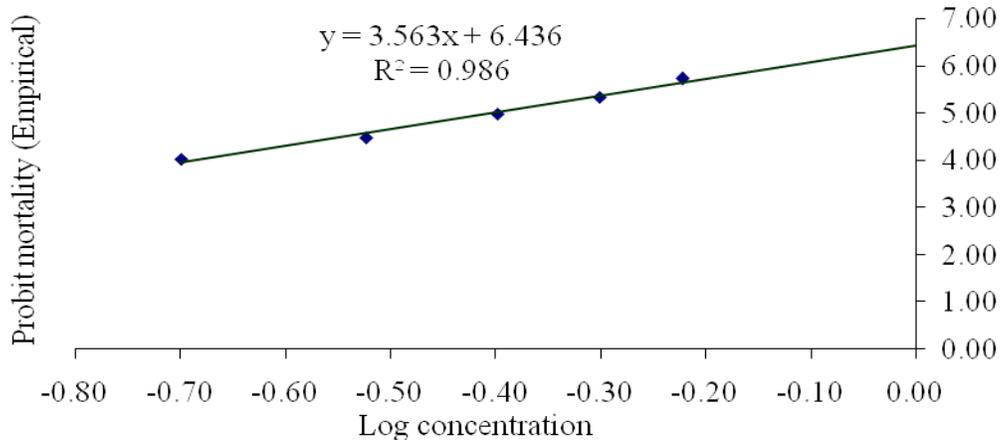


Fig.3 Plots of log concentrations versus probits for calculation of LD₅₀ of MH in LBG 752

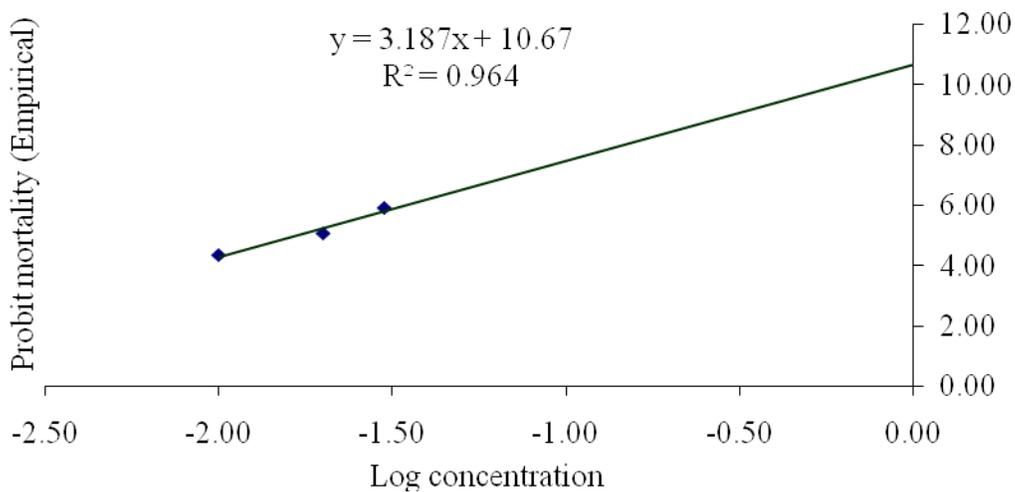


Fig.4 Plots of log doses versus probits for calculation of LD₅₀ of gamma rays in TBG 104

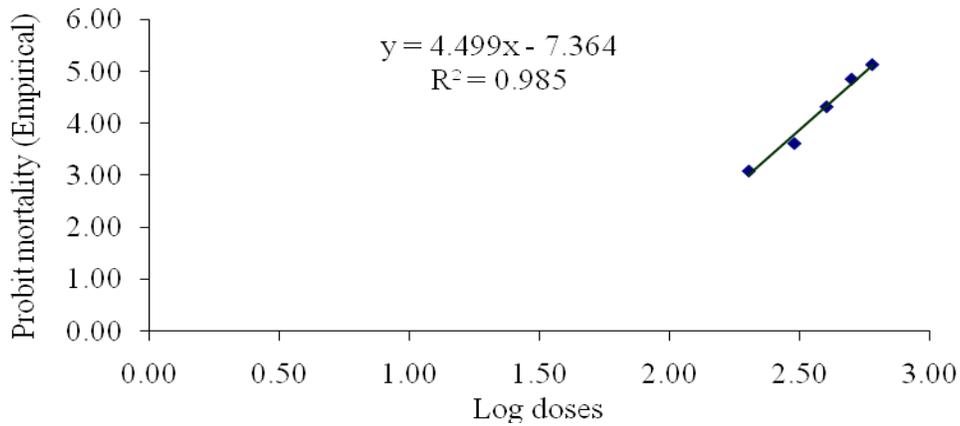


Fig.5 Plots of log concentrations versus probits for calculation of LD₅₀ of EMS in TBG 104

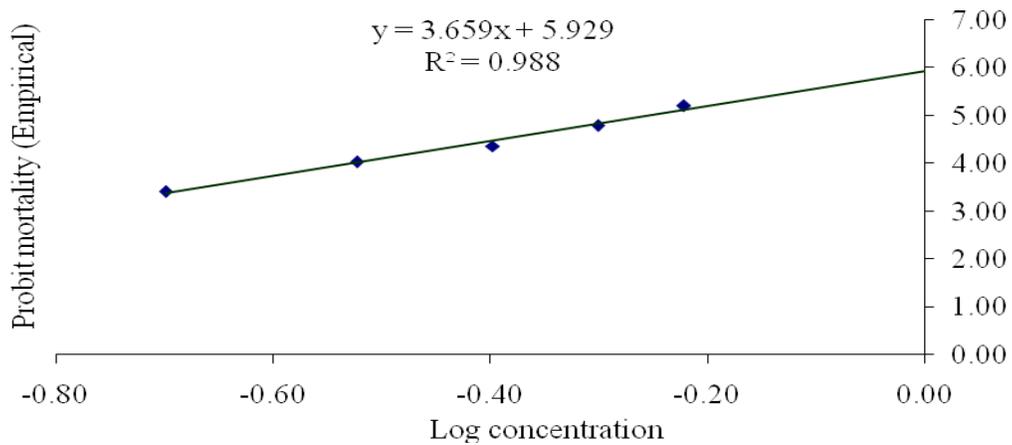


Fig.6 Plots of log concentrations versus probits for calculation of LD₅₀ of MH in TBG 104

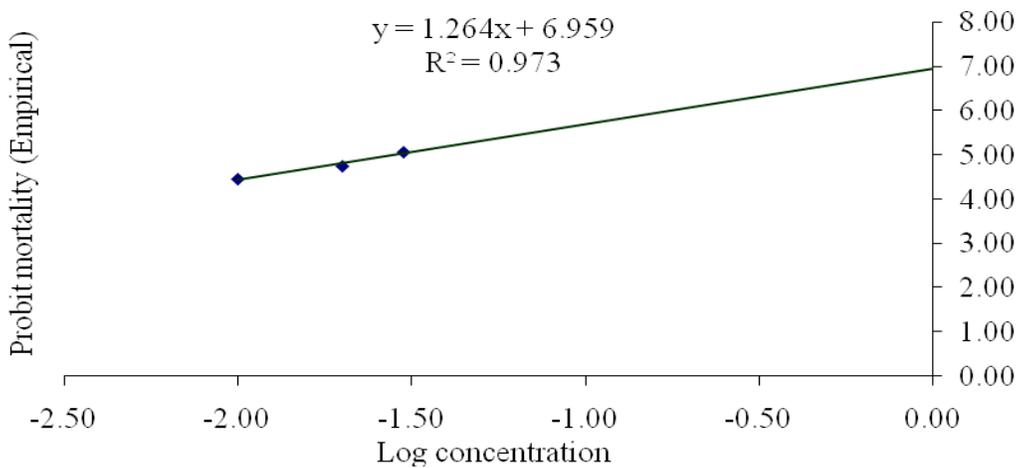


Plate.1 Effect of mutagens on germination, shoot length and root length in M1 generation of LBG 752



Plate.2 Effect of mutagens on germination, shoot length and root length in M1 generation of TBG 104



In EMS treatment, germination percentage ranged from 23.33 (0.6%) to 81.67 per cent (0.2%) in LBG 752 and 42.33 (0.6%) to 96.67 per cent (0.2%) in TBG 104. Whereas, in MH treatment seed germination percentage ranged from 18.00 (0.03%) to 72.67 per cent (0.01%)

in LBG 752 and 46.33 (0.03%) to 68.00 per cent (0.01%) in TBG 104. In LBG 752, the mean of seed germination in gamma rays, EMS and MH treated population was 68.94%, 60.11% and 58.83%, respectively. With respect to TBG 104, the mean of seed

germination in gamma rays, EMS and MH treated population was 76.88%, 76.83% and 68.2%, respectively.

Reduction in seed germination percentage due to mutagenic treatment was also reported by Ramya *et al.*, (2014), Dhasarathan *et al.*, (2014) and Usharani *et al.*, (2017) in blackgram. Maherchandani (1975) reported that reduction in germination percentage might be due to the disturbance of promoters and inhibitors balance, probably, in favour of inhibitory materials. In the present investigation, at higher dosage of mutagens, the seed germination got delayed and the seedlings were shorter which subsequently died in short period.

The shoot length in all the treated population of both the varieties was significantly reduced as compared to respective control (Table 3). The shoot length varied from 8.71 (600 Gy) to 13.57 cm (200 Gy) in LBG 752 and 7.74 (600 Gy) to 11.52 cm (200 Gy) in TBG 104 in gamma rays treatment; 2.87 (0.6%) to 11.67 cm (0.2%) in LBG 752 and 1.58 (0.6%) to 8.35 cm (0.2%) in TBG 104 in EMS treatments; and 0.93 (0.03%) to 3.09 cm (0.01%) in LBG 752 and 1.11 (0.03%) to 2.67 cm (0.01%) in TBG 104 in MH treatments as against the values of 16.71 cm (LBG 752) and 15.87 cm (TBG 104) of their respective control. In LBG 752, the mean of shoot length in gamma rays, EMS and MH treated population was 12.30 cm, 7.80 cm and 5.73 cm, respectively. With respect to TBG 104, the mean of shoot length in gamma rays, EMS and MH treated population was 10.47 cm, 7.03 cm and 5.32 cm, respectively.

Regarding the root length, the mean root length recorded was significantly lesser than their respective control in all the mutagen treatments in both the varieties except 200 Gy, 300 Gy and 400 Gy doses of gamma rays treatment (Table 3). The root length ranged

from 1.98 (600 Gy) to 3.77 cm (200 Gy) in LBG 752 and 2.81 (600 Gy) to 3.77 cm (200 Gy) in TBG 104 in gamma rays treatment while, in EMS treatments it varied from 0.29 (0.6%) to 3.15 cm (0.2%) in LBG 752 and 0.41 (0.6%) to 2.93 cm (0.2%) in TBG 104. In MH treatment, the root length ranged from 1.10 (0.03%) to 1.24 cm (0.01%) in LBG 752 and 0.99 (0.03%) to 1.23 cm (0.01%) in TBG 104 as against the values of 4.52 cm (LBG752) and 4.54 cm (TBG 104) of respective control. In LBG 752, the mean of shoot length in gamma rays, EMS and MH treated population was 3.35 cm, 2.25 cm and 2.01 cm, respectively. With respect to TBG 104, the mean of shoot length in gamma rays, EMS and MH treated population was 3.53 cm, 2.07 cm and 1.96 cm, respectively. Such reports of reduction in shoot and root length was also reported by Surendar and Vanniarajan (2014), Lavanya *et al.*, (2016), Usharani *et al.*, (2017) and Veni *et al.*, (2017) in blackgram.

In all three mutagen treatments *viz.*, gamma rays, EMS and MH, the seed germination percentage, shoot length and root length were lesser than their respective control and these characters decreased progressively with the increase in doses/concentration of all the mutagens in both the varieties *viz.*, LBG 752 and TBG 104. In the present investigation, the high proportion of seed lethality due to mutagen treatment might be associated with weakening of intra-chromosomal linkage or accumulation of deleterious mutations in different genomes. The reduction in germination might be due to the alkylation of sulphahydral (-SH) group of important proteins causing death of the seeds (Ehrenberg and Lundquist, 1961).

The reduction in shoot and root length could be attributed to the effects of mutagens on the physiological system (Gaul, 1977). According to Rupinder and Kole (2005) severe reduction

in plumule to radical length and physical injuries of radicles indicated that the root inhibition arises primarily from the effect on meristems by arresting the synthesis of growth stimulating auxins and consequent inhibition of cell division. The influence on shoot and root growth has been related to many factors which may be attributed to chromosomal abnormality with height reduction, reduction in auxin levels, inhibition of auxin synthesis, failure of assimilation mechanism and chromosomal damage cum mitotic inhibition (Riley, 1954).

Probit analysis for determination of LD₅₀ dose of mutagens

The LD₅₀ values were determined with the help of probit analysis for the physical and chemical mutagens based on seed germination percentage. LD₅₀ value is essential to fix the optimum dose that cause maximum of mutation with minimum of damage to the plant. The LD₅₀ value also helps in knowing the sensitivity of different genotypes to the mutagens. In the present study, probit analysis was carried out in mutagen treated populations of LBG 752 and TBG 104 blackgram varieties to determine LD₅₀ values for gamma rays, EMS and MH treatments under *in-vitro* condition. The results obtained on observed mortality percentage for gamma rays, EMS and MH treated population are presented in Tables 4 and 5 (Fig.1 to 6).

Mean observed mortality percentage increased with increase in the dose/concentration of all the mutagen treatments in both the varieties. In LBG 752 variety, the mean mortality percentage of gamma ray treatment increased from 14.67 per cent (200 Gy) to 62.67 per cent (600 Gy) and in TBG 104 variety, it increased from 6.67 (200 Gy) to 57.33 per cent (600 Gy). The mean mortality percentage of EMS treatment increased from 18.67 per cent (0.2%) to 77.33

per cent (0.6%) in LBG 752 variety and in TBG 104 variety, it increased from 9.33 per cent (0.2%) to 60.00 per cent (0.6%) of EMS. Whereas, mean observed mortality percentage of MH treatment increased from 28.00 per cent (0.01%) to 82.67 per cent (0.03%) in LBG 752 variety and in TBG 104 variety, it increased from 32.00 per cent (0.01%) to 54.67 per cent (0.03%). Further, the highest mortality percentage was observed at 600 Gy of gamma ray, 0.6% of EMS and 0.03% of MH treatments in both the varieties. However, between LBG 752 and TBG 104 varieties, highest mortality percentage was recorded in LBG 752 at all the doses.

The LD₅₀ values were 500.16 Gy for gamma rays, 0.396% for EMS and 0.016% for MH in LBG 752 and 552.92 Gy for gamma rays, 0.554% for EMS and 0.028% for MH in TBG 104 (Table 5). From the results, it was observed that TBG 104 found to be more tolerant to different mutagen treatments than LBG 752. The differences in the sensitivity of the varieties of blackgram based on LD₅₀ values indicated the differential response of specific genotypes to different mutagens. Such variations were also reported by Surendar and Vanniarajan (2014) in blackgram. However, minor differences were observed in LD₅₀ doses between genotypes by Dhasarathan (2014), Ramya *et al.*, (2014) and Veni *et al.*, (2017) in blackgram.

In conclusion the study on effect of various mutagens on two blackgram *viz.*, LBG 752 and TBG 104 in M₁ revealed that among all the treatments, MH treatment resulted in drastic reduction in seed germination percentage, shoot length and root length followed by EMS and gamma rays treatments in both the varieties. It was also clear that the reduction in all these characters with increasing dose/concentration of mutagens was more conspicuous in LBG 752 than TBG 104 indicating more sensitivity of LBG 752 to

mutagen treatment than TBG 104. These findings would greatly help for cost effective selection of variety and mutagens for successful generation of variation in mutation breeding programmes aimed at blackgram crop improvement.

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