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Fixation of Lethal Dose$_{50}$ and Effect of Mutagens in M_1 Generation under Laboratory Condition

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A B S T R A C T

The current study was conducted to find out the LD_{50} dose, shoot length and root length induced by both gamma rays and EMS in blackgram variety VBN 4, under laboratory condition. The investigation was carried out by exposing the seeds with different doses of gamma rays (10, 20, 30, 40, 50, 60, 70, 80 and 90kR) and various concentrations of EMS (30, 40, 50, 60 and 70mM) along with the control. The effect of mutagens on germination and seedling traits like, shoot and root length was observed on 5th day after sowing. The germination percentage and seedling growth decreased with increase in doses/concentrations of the mutagens. The lethal dose 50 (LD_{50}) was found at 50kR of gamma rays and 60mM of EMS treatments.

Introduction

Pulses are also known as grain legumes are prominent source of vegetable protein. They are also an important source of energy and minerals. Pulse crops have the unique character of retaining and restoring soil fertility through biological nitrogen fixation and increasing soil physical properties by their well spread root system. They add organic matter into the soil in the form of leaf mould. Pulses are often attributed as "poor man's diet," which are really important in Indian diet as a source of protein. Though pulses contributed significant role in human consumption they have not yet reached a comfortable level of production. To alleviate protein-energy malnutrition, a minimum of 50g pulses/capita/day should be available. Blackgram or Urdbean is the important food grain legume and rich in protein. It contains about 20.8 to 30.5% protein, which is almost three times that of cereals. Its total carbohydrates range from 56.5 to 63.7%. It is adapted to different agro-climatic conditions, short duration crop and used for intercropping

and multicropping systems. In India, it covers in an area of 32.46 lakh hectares and produces 19.59 lakh tonnes with a productivity of only 604 kg per ha. The annual production of blackgram is 3.59 lakh tonnes from an area of 3.74 lakh hectares in Tamil Nadu and the productivity is 960 kg/ha, still the per capita availability is low (Ministry of Agriculture, 2015).

Crop improvement of blackgram through hybridization and recombination is very difficult, because of their autogamous nature (Deepalakshmi and Anandakumar, 2004). Due to autogamous nature, they lack genetic variability also. For any breeding programme, the genetic variability is important for further crop improvement. Mutation breeding is a suitable choice of creating variability in self pollinated pulse crop like blackgram, greengram and cowpea *etc*. Induced mutation increases the value of additional variability for all traits. Further mutation breeding is a relatively quick method of improvement of crops. Mutants can also be incorporated into crossing programme as conventional alleles to obtain the desired genotype.

Hence, the current study aims at creation of variation through induction of mutation through physical and chemical mutagens *viz.*, gamma rays (ionizing radiation) and Ethyl Methane Sulphonate (Mono functional alkylating agent) respectively. Few studies were made under laboratory as well as field conditions to decide the LD_{50} value of both gamma rays and EMS for blackgram. The effect of these mutagens on seed germination under laboratory condition in the M_1 generation has also been observed.

Materials and Methods

The promising blackgram variety namely VBN 4 (Vamban-4) was selected to induce mutation by gamma rays and EMS to determine the LD_{50} dose. Selfed seeds were obtained from the germplasm collection maintained by the National Pulses Research Centre, Vamban. This study was carried out in Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Madurai during the year 2009-2011.

a) Mutagenic treatment

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 of physical and chemical mutagens, a random sample of 75 seeds was treated, to fix the LD_{50} value in the laboratory.

b) Induction of mutation - fixing LD_{50} value under laboratory

(i) Physical mutagen - gamma rays

For fixing LD_{50} value of physical mutagen, nine sets containing 75 well filled seeds were treated with gamma rays (10kR to 90kR with an interval of 10kR) in the gamma chamber installed at the Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore where cobalt-60 serves as source of gamma rays. Non-irradiated dry seeds are also taken to utilize it as control.

(ii) Chemical mutagen - EMS

The chemical mutagen, Ethyl Methane Sulphonate ($CH_3SO_2OC_2H_5$, Molecular weight 124.16, Boiling point 80/100 mm Hg and density D_4^{25} = 1.203 g/ml) was stored in dry air at 0^oC to maintain its purity. Prior to use it was removed from refrigerator and placed in a desiccators over calcium chloride to reach the room temperature. Five different concentrations of EMS ranging from 30mM

to 70mM with 10mM interval were used to fix LD_{50} value. Five sets containing 75 well filled seeds were presoaked for 16 hours in distilled water initially (Malarkodi, 2008). The presoaked seeds after removal from the water were placed between folds of blotting paper to remove water adhering on the surface. Then the seeds were immersed for six hours in the requisite concentration of EMS with intermittent shaking. To ensure uniform absorption of the mutagen, the volume of mutagen solution was maintained at proportion of ten times of that of the seed volume.

The whole treatment was carried out at a room temperature of 28±1°C. A sample of 75 seeds was soaked in distilled water for the respective duration to utilize it as control. 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 EMS and the excess moisture in the seed coat was removed by using folds of blotting paper. The seeds were then subjected to germination test. Based on the effect of physical and chemical mutagen on germination, LD_{50} value was obtained.

c) Handling the mutated population

(i) Laboratory study of M_1 generation

A total of 75 seeds were sown in germination paper by roll towel method under a temperature of 28±1°C in three replications of 25 seeds each for all treatments. The treatments selected for fixing LD_{50} are as follows.

(a) Gamma rays : 10, 20, 30, 40, 50, 60, 70, 80 and 90kR
(b) Ethyl Methane Sulphonate : 30, 40, 50, 60 and 70mM

(ii) Laboratory observations

The seedling injury was recorded from the seedlings under controlled condition. Five days after sowing, the germination percentage, shoot length and root length were recorded in each and every seedling. The shoot length was measured from the collar region to the tip of the shoot, while that of root was measured from the collar region to the tip of the primary root.

Results and Discussion

LD_{50} dose is of very importance to understand the sensitivity of various genotypes to the critical dose of mutagens creating 50 per cent mortality. In mutation breeding experiments, evaluation of the effect of mutagens on the M_1 generation is relatively a common procedure. Statistical analysis was done by using the seed germination values in VBN 4 blackgram variety for both the mutagens to calculate the Lethal Dose (LD_{50}) value. The data on seed germination percentage under laboratory conditions are presented in table 1 (Fig. 1). Under laboratory conditions, the mean germination percentage ranged from 20.02 (90kR) to 85.33 (10kR) in gamma ray treatment and 42.67 (70mM) to 84.00 (30mM) in EMS treatment. In all the gamma ray and EMS treatments, the germination percentage recorded was lesser than their respective control. In gamma ray treatment, the reduction in germination percentage has been ranged from 8.25 (10kR) to 78.47 (90kR) and 50 per cent reduction was obtained at 50kR of gamma ray treatment. In EMS treatments, the per cent reduction was low at 30mM which was 8.91 and high at 70mM which recorded 53.73 per cent and 50 per cent reduction was obtained at 60mM. Thus the LD_{50} value for germination was fixed as 50kR for gamma rays treatments and 60mM for EMS treatments (Plate 1). A minor difference in LD_{50} dose between various

genotypes of same species is a common nature in mutation study as the resistance offered by the biological experimental material mainly depends on extent, maturity duration and moisture level etc., at the time of mutagen treatment. Such variations in LD_{50} were observed in mungbean (Krishnaswami *et al.,* 1977; Jain and Khandelwal, 2008) and urdbean (Vanniarajan and Vijendra Das, 1996; Anbu Selvam *et al.,* 2010). Decrease in seed germination percentage was directly proportional to the increase in dosage of mutagens. Reduce in germination percentage due to inhibitory action of the treated mutagen is observed in pulses (Hussein and Disouki, 1976; Kundu, 1980; Khan, 1988; Misra, 1992; Sharma *et al.,* 2005; Khan and Wani, 2006; Karthika and Subba lakshmi, 2007; Sagade and Apparao, 2011), cereals (Gnanamurthy *et al.,* 2012; Talebi *et al.,* 2012). Maherchandani (1975) observed that reduction in germination percentage may be due to the disturbance of promotors and inhibitors balance, probably, in favour of inhibitory materials.

Pronounced inhibitory effect of gamma irradiation and chemical mutagen on the growth of shoot length and root length in seedlings were observed in the present investigation under laboratory conditions. The shoot length ranged from 8.54 (90kR) to 24.29cm (10kR) in gamma ray treatment while 7.30 (70mM) to 22.05cm (40mM) in EMS treatments. In both physical and chemical mutagenic treatments, shoot length was low at 90kR and 70mM respectively. The per cent reduction has been ranged from 6.29 (10kR) to 67.06 (90kR) in gamma ray treatment and the trend observed was regular with continuous reduction for mean shoot length, when the dose level increased. In EMS treatment the per cent reduction was ranged from 14.27 (40mM) to 71.62 (70mM) and there was an irregular trend in mean shoot length (Table 2) (Fig. 2).

In all the gamma ray and EMS treatments, the mean root length recorded was lesser than their respective control. The root length ranged from 1.57 (90kR) to 5.67cm (10kR) in gamma ray treatment while 1.20 (70mM) to 5.94cm (30mM) in EMS treatments. In both physical and chemical mutagenic treatments, root length was low at 90kR and 70mM respectively. The per cent reduction ranged from 5.66 (10kR) to 73.88 (90kR) in gamma ray treatment and there was an irregular trend in mean root length. In EMS treatment the per cent reduction was has been ranged from 12.00 (30mM) to 82.22 (70mM) and mean root length showed declining trend with increasing doses of mutagen (Table 2) (Fig. 2).

EMS treatments resulted in somewhat drastic reduction in shoot length compared to gamma rays, which produced gradual reduction. But in case of root length gamma ray treatments resulted in drastic reduction but EMS treatments showed declining trend with increasing doses of mutagen. The reduction in shoot and root length enhanced with increase in doses of both gamma rays and EMS treatments. Such a report of reduction in root and shoot length was confirmed by Ramaswamy (1973), Bhaskaran (1978), Khan (1988) in blackgram and Jebaraj (1978); Kumar and Mishra (1999) in greengram. Mahna *et al.,* (1990) reported about reduction in shoot and root length when compared to control in blackgram. According to Rupinder and Kole (2005) severe reduction in plumule to radicle length and physiological injuries of radicles indicated effective mutagenesis, this clearly 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.

Table.1 Effect of mutagens on germination percentage of Blackgram variety VBN 4 in M_1 generation under laboratory condition

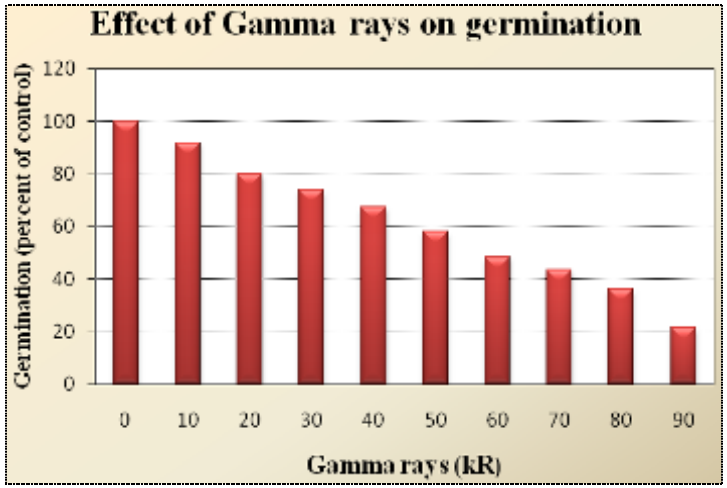
Mutagens (Dose/Conc.)	Germination percentage	Per cent over control	Per cent reduction
Gamma rays (kR)			
Control	93.00 (74.86)	100.00	-
10	85.33 (67.52)	91.75	8.25
20	74.22 (59.51)	79.81	20.19
30	68.56 (55.91)	73.72	26.27
40	62.56 (52.29)	67.27	32.73
50	54.00 (47.30)	58.06	41.94
60	45.00 (42.13)	48.39	51.61
70	40.33 (39.43)	43.37	56.63
80	33.64 (35.45)	36.17	63.83
90	20.02 (26.58)	21.53	78.47
Mean	**57.66 (50.09)**	**62.01**	**37.99**
SE(d) : 1.15 CD(0.05) : 2.42			
Ethyl Methane Sulphonate (mM)			
Control	92.21 (73.96)	100.00	-
30	84.00 (66.46)	91.09	8.91
40	72.00 (58.07)	78.08	21.92
50	68.00 (55.56)	73.74	26.26
60	55.00 (47.87)	59.65	40.35
70	42.67 (40.79)	46.27	53.73
Mean	**68.98 (57.11)**	**74.80**	**25.19**
SE(d) : 1.24 CD(0.05) : 2.78			

Within bracket showed transformed value (arc sin mean value)

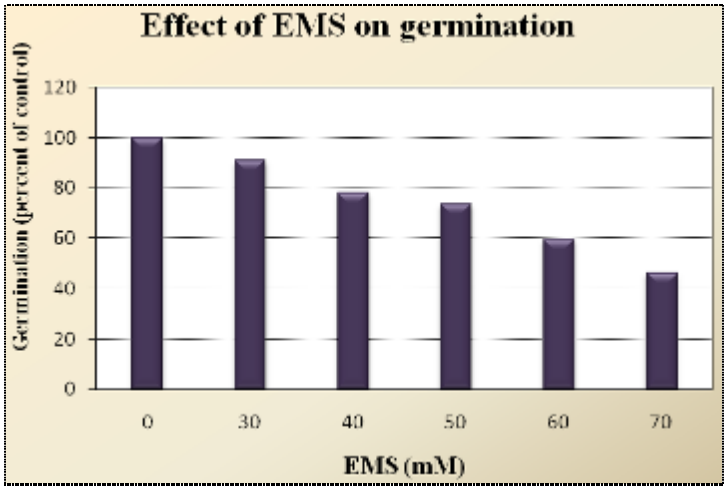
Table.2 Effect of mutagens on shoot and root length of Blackgram variety VBN 4 in M_1 generation

Mutagens (Dose/Conc.)	Shoot length (cm)	Per cent over control	Per cent reduction	Root length (cm)	Per cent over control	Per cent reduction
Gamma rays (kR)						
Control	25.92	100.00	-	6.01	100.00	-
10	24.29	93.71	6.29	5.67	94.34	5.66
20	22.04	85.03	14.97	5.02	83.52	16.48
30	21.80	84.10	15.90	4.80	79.86	20.14
40	20.13	77.66	22.34	4.03	67.05	32.95
50	18.75	72.33	27.67	5.31	88.35	11.65
60	16.20	62.50	37.50	4.82	80.19	19.81
70	13.33	51.42	48.58	4.75	79.03	20.97
80	10.40	40.12	59.88	3.60	59.90	40.10
90	8.54	32.94	67.06	1.57	26.12	73.88
Mean	**18.14**	**69.98**	**30.02**	**4.56**	**75.84**	**24.16**
SE(d) : 0.47 CD(0.05) : 0.99				SE(d) : 0.11 CD(0.05) : 0.23		
Ethyl Methane Sulphonate (mM)						
Control	25.72	100.00	-	6.75	100.00	-
30	21.33	82.93	17.07	5.94	88.00	12.00
40	22.05	85.73	14.27	5.20	77.03	22.97
50	17.45	67.84	32.16	3.70	54.18	45.82
60	14.45	56.18	43.82	2.80	41.48	58.52
70	7.30	28.38	71.62	1.20	17.78	82.22
Mean	**18.05**	**70.18**	**29.82**	**4.27**	**63.08**	**36.92**
SE(d) : 0.43 CD(0.05) : 0.96				SE(d) : 0.10 CD(0.05) : 0.22		

Fig.1 Effect of mutagens on germination percentage under laboratory condition

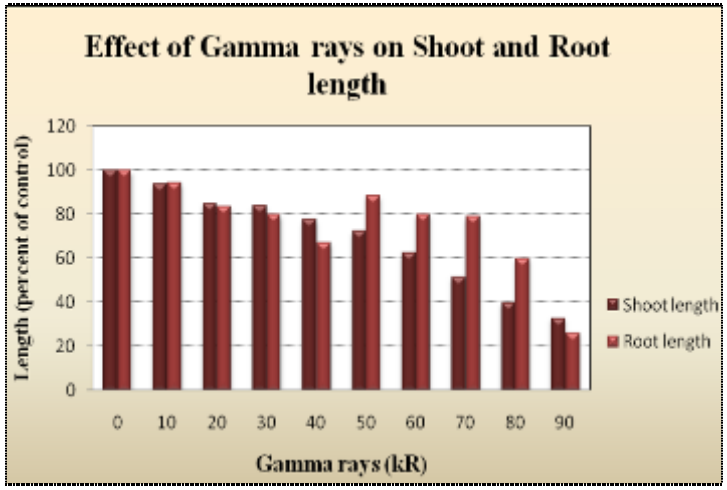


A

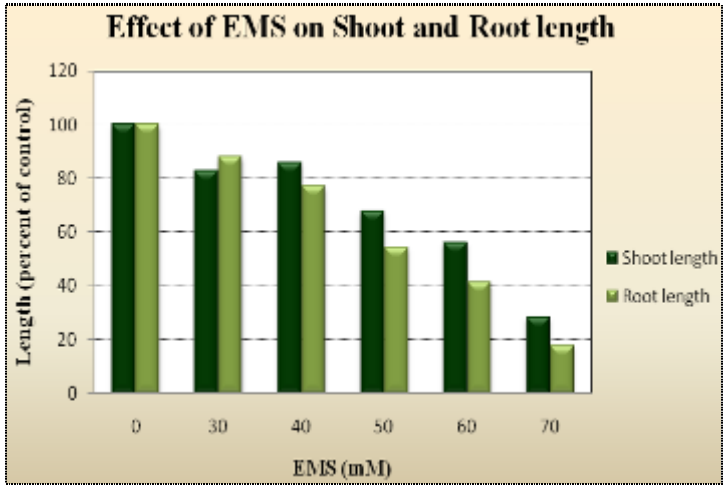


B

Fig. 2 Effect of mutagens on shoot and root length under laboratory condition

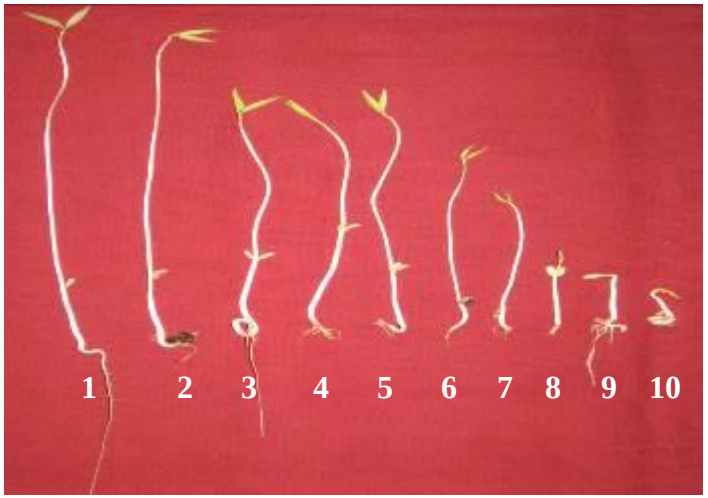


A



B

Plate.1 Determination of LD_{50} dose under laboratory condition



A) Gamma rays: 1) Control, 2) 10kR, 3) 20kR, 4) 30kR, 5) 40kR, 6) 50kR, 7) 60kR, 8) 70kR, 9) 80kR, 10) 90kR

B) EMS: 1) Control, 2) 30mM, 3) 40mM, 4) 50mM, 5) 60mM, 6) 70mM

The reduction in shoot and root length was attributed to the effects of mutagens on the physiological system (Gaul, 1977). Such a reduction in length of shoot and root arising out of mutagenic treatments was earlier observed in crop plants (Reddy and Gupta, 1989).

In conclusion, the current research was carried out to study the significant effect of Gamma rays and EMS on seed germination and seedling growth in blackgram variety VBN 4, under laboratory condition. Seed germination and seedling growth decreased with increase in dose / concentration of both the mutagens. Based on the seed germination percentage, LD_{50} dose was fixed as 50kR of gamma rays and 60mM of EMS treatments. It is recommended that in any mutation breeding programme on blackgram 50kR of gamma rays and 60mM of EMS may be used.

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