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KILL CURVE ANALYSIS AND RESPONSE OF FIRST GENERATION BLACK GRAM (*VIGNA MUNGO* (L.) HEPPER) CULTIVARS TO GAMMA RAYS AND ELECTRON BEAM RADIATION

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ABSTRACT
The present study was carried out to study the impact of mutagens on biological parameters such as seed germination, shoot and root length and to determine the lethal dose (LD_{50}) of gamma ray, electron beam and combined effect of gamma ray + electron beam on two black gram cultivars ADT-3 and CO 6. Black gram seeds were treated with different doses (200, 300, 400 Gy) of gamma rays, electron beam and combined treatment. Radiation induced morphological changes such as reduced germination, decreased shoot and root length was observed compared to control. In black gram variety ADT 3, LD_{50} was noticed at 309.75 Gy, 289.81 Gy and 317.61 Gy for gamma rays, electron beam and combined treatment respectively. Similarly, LD_{50} was noticed at 301.06 Gy, 283.99 and 311.24 Gy for gamma rays, electron beam and combined treatment respectively for CO 6 variety. Decrease in the germination percent with increase in dose was observed in the present study. Optimal mutagenic doses were calculated based on germination percentages which were considered useful in developing black gram mutagenized population to create genetic variability for various qualitative and quantitative traits.

INTRODUCTION

Black gram (*Vigna mungo*) is an important pulse crop occupying unique position in Indian agriculture. Among the pulses, it stands fourth in production and acreage (Deepalakshmi, and Anandakumar, 2004). Genetic variability is low in this crop due to cleistogamous nature and narrow genetic base among the released cultivars (Gupta *et al*. 2005). As genetic variability is essential for any crop improvement programme, induced mutations provide an important source for variability (Kumari *et al.,* 2013). Mutation induction has become an establishment tool in plant breeding to supplement existing germplasm and to improve cultivars in certain specific traits (Kumar and Ramesh, 2004). Induction of mutation forms an important part of breeding programme as it widens the gene pool through creation of genetic variability. The genetic variability is the basic requirement for making progress in crop breeding (Appala Swamy and Reddy, 2004). Radiation, including γ-rays, x-rays, fast neutrons, thermal neutrons etc. It has been widely applied to induce mutations and made great progress in plant breeding. More than 3,000 mutant crop varieties have been produced over the past 60 years (http://www-mvd.iaea.org). Most of those varieties were developed using ionizing radiation mainly γ-rays (64%) followed by x-rays (22 %) (Souframanien et al. 2016). Among different ionizing radiations, gamma rays have been commonly used, and numerous mutants have been produced in black gram (Souframanien and Pandey, 2006). In India there are seven mutant varieties of black gram released by both physical and chemical mutagens (Natarajan, 2005). Apart from the conventional electromagnetic radiations, like x-rays and gamma rays, electron beam is now an alternative source of energy to induce mutation. Both gamma rays and electron beams have low LETs of around 0.2KeV/µm. However, electron beam has a higher dose rate compared to γ-rays and is administered as short pulses while gamma irradiation is continuous (Souframanien *et al.,* 2016). Electron beams are produced from particle accelerators capable of accelerating electrons to near the speed of light (~190,000 miles/ second). This electron beam generator uses commercial electricity as an energy source and can be simply switched on or off.

In order to exploit induced mutagenesis for crop improvement, the basic studies on effectiveness and efficiency of a mutagen in a crop are necessary to recover high frequency of desirable mutations. The choice of effective and efficient mutagens is very essential to recover a high frequency and spectrum of desirable mutations. The success of mutation using the mutagens depends on use of appropriate mutagen and dose plays a critical role in induction of mutation. The dose of a mutagen that achieves the optimum mutation frequency with the least possible unintended damage is regarded as the optimal dose for induced mutagenesis (Mba *et al.,* 2010). From the past to the present, doses that lead to 50% lethality (LD_{50}) or a dose lower than LD_{50} have often been chosen for induction of mutation. The LD_{50} is different between species and varieties in a species. For example, the LD_{50} of K-851 mungbean cultivar is 54.06 kR and for Sona is 53.20 (Tah, 2006). LD_{50} for Pea is 200 Gy (Aney, 2013). 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 present investigation was undertaken to study the impact of gamma rays, electron beam and combined treatment on biological materials (ADT 3, CO 6) in M_1 generation such as germination, shoot length and root length and to determine the $LD_{50.}$ These mutant population may be use for identifying new genetic variability and will be utilized in future breeding programme to improve the yield and quality traits in blackgram.

MATERIALS AND METHODS

The seeds of two black gram varieties namely ADT 3and Co 6 for the induction of mutation treatment were obtained from Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Madurai, India. Black gram seeds were packed in butter paper covers and placed in the Gamma chamber, it was exposed to gamma irradiation from the Cobalt 60 gamma source for appropriate time for each dose based on the half-life of the source in the gamma chamber installed at Bhabha Atomic Research Centre, Mumbai. Non-irradiated dried seeds were taken as control. Seeds of ADT 3 and Co 6 were treated at 3 different doses (200, 300 and 400 Gy). For electron beam irradiation, seeds of ADT 3 and Co 6 were treated at 3 different doses (200, 300 and 400Gy) with 10 MeV electron beam from electron accelerator facility at Electron Beam Centre, Bhabha Atomic Research Centre, Kharghar, Navi Mumbai, India. For combined treatment, black gram seeds of ADT 3 and CO 6 were exposed with Electron beam and then same seeds were treated with gamma rays. The treated seeds with control were sown in germination trays at germination room, Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Madurai for working out the LD_{50} and probit analysis was done using the germination percent (Sharma, 1998).

After mutagenic treatment, seeds were sown immediately in the field in a randomized block design along with control (untreated seeds). All M_1 plants were grown with a spacing of 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. Probit analysis was carried out to determine the lethal dose (LD_{50}) of gamma rays and electron beam and combined treatment (electron beam and gamma ray) in both varieties of black gram *viz.,* ADT 3 and CO 6.

Germination

Seeds were germinated on petri dishes (9 cm) on 4 layers of Whatman No. 1 filter pa-per, moistened with distilled water. Petri dishes were placed at 25º ± 2ºC under a 16:8 h (day: night) photoperiod in an incubator (Farooq *et al.,* 2008). Germination percent (GP) was calculated 7 days after germination, according to Dezfuli *et al.* (2008) using following formula:

$$\text{Germination Percentag} = \frac{\text{No.of seeds germinated at 7 days after germination}}{\text{No. of seeds sown}} \times 100$$

Shoot length

The length of the shoot from the cotyledonary node to the tip of the shoot was measured on ten randomly selected seedlings on the seventh day and expressed in centimetre.

Root length

The root length from the cotyledonary node to the tip of the primary root was measured on ten randomly selected seedlings and expressed in centimetre.

RESULTS AND DISCUSSION

Mutation induction is a random process determined by probability, use of appropriate mutagen and dose plays a critical role in induction of mutation. It has been clearly shown in a number of plant species that the effect induced varies with the varying mutagens and with variation in mutagen dose (Goyal and Khan, 2010). LD_{50} is of great importance to know the sensitivity of different genotypes to the critical dose of mutagens causing 50 per cent mortality. A gradual reduction in the germination was noticed corresponding to an increase in the dosage of gamma rays, electron beam and combined treatment (electron beam + gamma ray) for both the varieties ADT 3 and CO 6. Probit analysis was done using the seed germination values in both the varieties and LD_{50} dose was determined. LD_{50} was noticed for the variety ADT 3 at 309. 75 Gy of gamma rays and 289.81 Gy of electron beam and 317.61

Table 1: Probit analysis for calculating LD_{50} in ADT 3

Treatment	Log10 of doses	Observed mortality percentage	Corrected mortality percentage	Empirical probit unit	LD_{50} value
Gamma rays (Gy)					
200 Gy	2.30	18.86	18.0	4.09	309.75
300 Gy	2.48	34.57	33.9	4.59	
400 Gy	2.60	49.13	48.6	4.97	
Electron beam (Gy)					
200 Gy	2.30	24.54	23.8	4.29	289.81
300 Gy	2.48	36.65	36.0	4.64	
400 Gy	2.60	54.38	53.9	5.10	
Electron beam (Gy)+ Gamma rays (Gy)					
200 Gy +200 Gy	2.30	17.86	17.0	4.05	317.61
300 Gy +300 Gy	2.48	24.57	23.8	4.29	
400 Gy +400 Gy	2.60	52.13	51.6	5.04	

Table 2: Probit analysis for calculating LD_{50} in CO 6

Treatment	Log 10 of doses	Observed mortality percentage	Corrected mortality percentage	Empirical probit unit	LD_{50} value
Gamma rays (Gy)					
200 Gy	2.30	18.67	18.67	4.08	301.06
300 Gy	2.48	31.83	31.83	4.51	
400 Gy	2.60	54.54	54.54	5.10	
Electron beam (Gy)					
200 Gy	2.30	20.76	20.0	4.16	283.99
300 Gy	2.48	42.04	41.5	4.78	
400 Gy	2.60	56.37	55.9	5.15	
Electron beam (Gy) + Gamma rays (Gy)					
200 Gy + 200 Gy	2.30	19.54	18.7	4.11	311.24
300 Gy + 300 Gy	2.48	22.86	22.1	4.23	
400 Gy + 400 Gy	2.60	55.13	54.7	5.12	

Table 3: Impact of gamma rays, electron beam and combined treatment (electron beam + gamma rays) on M_1 generation studies in ADT 3

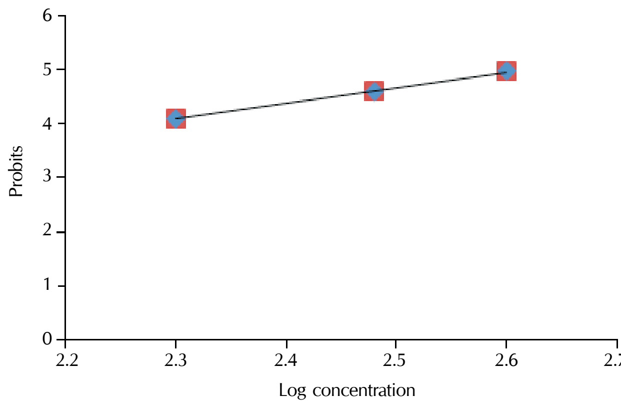
Treatment	Germinatin reduction over control (%)	Shoot length reduction over control (%)	Root length reduction over control (%)
Gamma rays (Gy)			
Control	100.00	100.00	100.00
200 Gy	70.74	67.67	67.97
300 Gy	50.20	50.51	50.68
400 Gy	42.57	40.36	44.81
Electron beam (Gy)			
Control	100.00	100.00	100.00
200 Gy	71.56	66.52	61.05
300 Gy	48.89	46.72	46.17
400 Gy	43.95	39.88	39.85
Electron beam (Gy) + Gamma rays (Gy)			
Control	100.00	100.00	100.00
200 Gy + 200 Gy	69.22	72.21	70.08
300 Gy + 300 Gy	55.95	53.28	52.48
400 Gy + 400 Gy	41.73	43.32	42.26

Table 4: Impact of gamma rays, electron beam and combined treatment (electron beam + gamma rays) on M_1 generation studies in CO 6

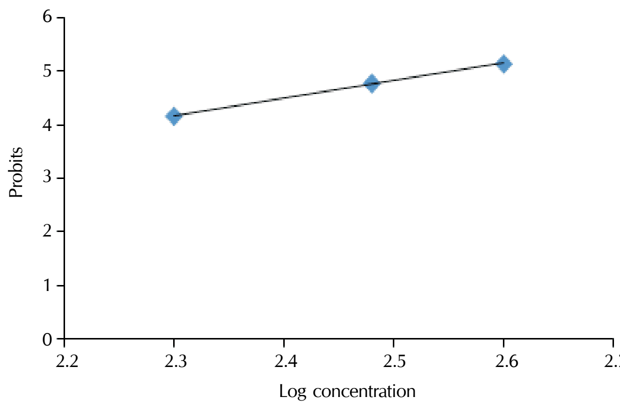
Treatment	Germination reduction over control (%)	Shoot length reduction over control (%)	Root length reduction over control(%)
Gamma rays (Gy)			
Control	100.00	100.00	100.00
200 Gy	68.62	71.35	71.22
300 Gy	49.02	50.80	51.91
400 Gy	42.61	44.64	43.63
Electron beam (Gy)			
Control	100.00	100.00	100.00
200 Gy	67.01	65.02	71.35
300 Gy	48.13	45.97	44.42
400 Gy	44.46	36.84	38.24
Electron beam (Gy) + Gamma rays (Gy)			
Control	100.00	100.00	100.00
200 Gy + 200 Gy	72.75	74.06	71.75
300 Gy + 300 Gy	51.48	52.91	53.22
400 Gy + 400 Gy	42.12	41.88	39.55

Gy for combined treatment (electron beam + gamma rays) (Table 1, Fig 1a). The gamma irradiation of 301.06 Gy and 283.99 Gy of electron beam and 311.24 Gy of combined treatment (electron beam + gamma ray) were recorded as LD_{50} for CO 6 (Table 2, Fig 1b).

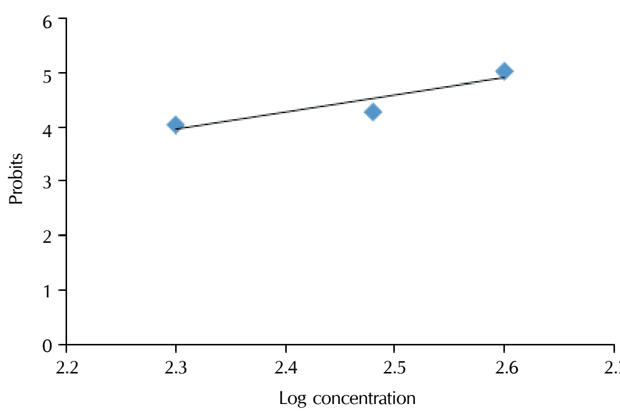
Decrease in germination % with the increase of treatment doses was observed in this study. It indicated that germination percentage was reduced under the influence of mutagenic treatment with increasing doses. Similar results were reported in black gram (Thilagavathi and Mullainathan 2011) and mung



Gamma rays

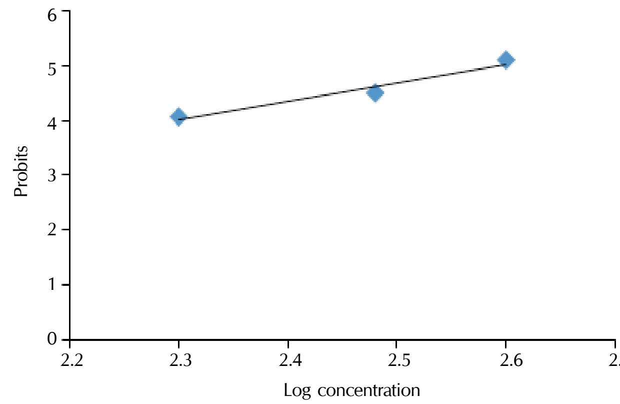


Electron beam

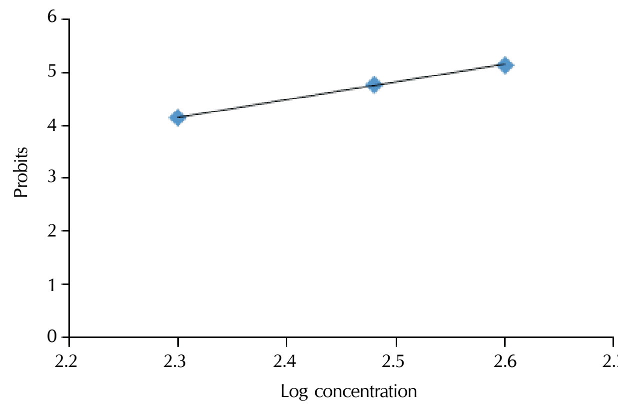


Electron beam + Gamma rays

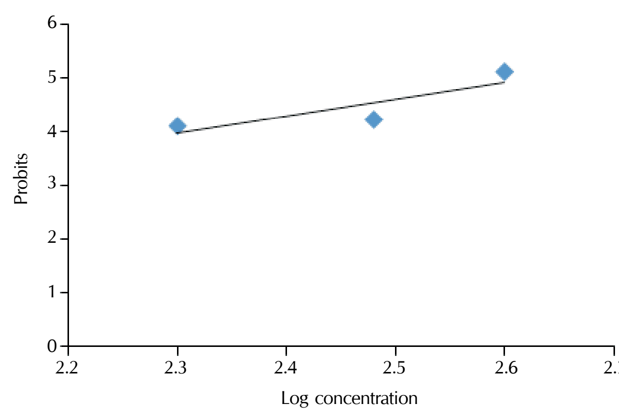
Figure 1a: Plots of Log doses versus probits for calculation of LD_{50} in ADT 3



Gamma rays



Electron beam



Electron beam + Gamma rays

Figure 1b: Plots of Log doses versus probits for calculation of LD_{50} in CO 6

bean (Borah 1992). LD_{50} is of great importance to know the sensitivity of different genotypes to the critical dose of mutagens causing 50% mortality. Radio-sensitivity varied with black gram genotype and was associated with seed testa texture, thickness and seed weight. As the present varieties considered in this experiment showed LD_{50} almost in the similar range and whereby it may be concluded that both genotypes were equally sensitive to gamma rays, electron beam and combined treatment (electron beam + gamma rays) irradiation.

Seed germination

Effect of gamma radiation, electron beam and their combined treatment (electron beam + gamma rays) on seed germination, shoot length and root length of plants are presented in Table 3 and 4. The data presented in table 3,4 reveal that the seed germination in field was reduced with increasing dose of gamma rays, electron beam and their combinations (electron beam + gamma rays). The seed germination was decreased from 70.74 % to 42.57 % for gamma rays and 71.56 % to

48.89 % for electron beam and 69.22% to 41.73 % for combined treatment (electron beam +gamma ray) in ADT 3 over control. In black gram variety CO 6, germination reduction observed from 68.62 % to 42.61 % for gamma rays, 67.01 % to 44.46 % for electron beam and 72.75% to 42.12% for combined treatment (electron beam + gamma rays) over control. This shows significant influence of mutagen on germination. Similar observations were reported previously in blackgram (Khan & Wani 2006; Sagade and Apparao, 2011; Thilagavathi and Mullainathan, 2011; Ramya, *et al*., 2014) and rice (Rajarajan *et al*., (2016) and Lavanya *et al*. (2011) in greengram. The survival of plants to maturity depends on the damage nature and extent of chromosomal damage. Increasing frequency of chromosomal damage with increasing radiation dose may be responsible for less germination and reduction in plant growth and survival Kiong *et al.,* (2008).

Shoot length and root length

In both cultivars, all the treatments showed significant differences for shoot and root length reduction per cent over control for both mutagenic treatments (Table 3 and 4). The higher per cent reduction in shoot length 40.36 % and 44.64 % was observed in - ADT 3 and CO 6 respectively. The higher percent of root length reduction of 44.81 % and 43.63 % was observed in ADT 3 CO 6 respectively. The higher reduction of shoot and root length was recorded in higher dose of gamma rays (400 Gy). In electron beam treatments, higher dose (400 Gy) showed decreased shoot (39.88 % in ADT 3 and 36.84 % in CO 6) and root length (39.85 % in ADT 3 and 38.24 % in CO 6) reduction per cent over control. The higher per cent reduction in shoot length (43.32 % - ADT 3 and 41.88 % – CO 6) and root length (42.26 % - ADT 3 and 39.55 % – CO 6) was recorded in higher dose of combined treatment (electron beam + gamma rays) (400 Gy). Similar reduction in shoot and root length was reported in mungbean (Kamini and Akhaury 1988) and black gram (Surender *et al*. 2014) and Lentil (Ram narayan ahirwar *et al*. 2014). Baojiang et al. (1989) have also reported dose-dependent chromosomal aberration of root tip cells of electron beam irradiated dry *Vicia faba* seeds. Mutagens can cause physiological damages mainly manifested as growth retardation and death is generally not restricted in M_1 generation (Mak *et al.,* 1986). Gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cells and tissues (Gunckel and Sparrow 1961). In earlier studies, eelectron beam radiation showed less physiological damage in M_1 generation and wide mutation frequency in M_2 generation (Rui *et al*., 1995). In the present study, there is not much difference in the reduction in germination percent, shoot and root length of both black gram varieties ADT 3and Co 6 caused by gamma rays, electron beam and combined treatment.

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