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Research Note

Probit analysis and effect of electron beam and gamma rays in blackgram (*Vigna mungo* (L.) Hepper)

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Abstract
An experimental investigation was carried out to determine the lethal dose (LD_{50}) of electron beam and gamma rays on two black gram varieties *viz*., MDU 1, VBN (Bg) 4. The lethal dose was determined by probit analysis. Dry seeds of both the varieties were treated with electron beam and gamma rays on different dosage *viz*., 100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy for Gamma rays and 200 Gy, 300 Gy, 400 Gy, 500 Gy and 600 Gy for electron beam along with the control (non-treated). LD_{50} dose for Electron beam on MDU 1 and VBN (Bg)4 was 304.91Gy and 302.21 Gy whereas, LD_{50} dose for gamma rays on MDU 1 and VBN (Bg) 4 was 324.89 Gy and 318.12 Gy respectively. The germination percentage and survival percentage of both varieties were gradually reduced with increasing doses of electron beam and gamma rays. The shoot length, root length, plant height at 30^{th} day, pollen fertility and seed fertility were also decreased gradually with increasing doses of electron beam and gamma rays. The LD_{50} values determined for the different blackgram varieties could be useful in blackgram varietal improvement programme in future.

Key words
Electron beam, Gamma rays, LD_{50} value, and germination

In Tamil Nadu 3.55 lakh ha is affected by sodicity Black gram is an important pulse crop extensively grown in India. It is the cheapest source of protein for millions of Indians. It contains about 26% protein, which is almost three times that of cereal. The essential amino acid composition of black gram is tryptophan, lysine, methionine, phenyl alanine, threonine, valine, leucine and isoleucine. Black gram also plays in important role in sustaining soil fertility by improving soil physical properties and fixing atmospheric nitrogen. Being a drought resistant crop, it is suitable for dry land farming and predominantly used as an inter crop. For any successful breeding programme, the variability is the basic tool on which the selection is exercised for genetic improvement. Creation of variability through pollination and artificial hybridization is very difficult in this crop as the flowers are cleistogamous and delicate to handle. Even if hybridization is carried out the seed set is less than 5%. Also, this crop lacks proper male sterility system commercially to be utilized for hybridization (Selvam *et al*., 2010).

In order to improve yield and other polygenic characters, mutation breeding can be effectively utilized. 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 essential in order to realize response to selection pressure as the estimates of genetic parameters of variation are specific for a particular population and the phenotypic expression of the quantitative character may be altered by environmental stress that affect plant growth and development. Therefore, an attempt has been made to study the magnitude of variability through induction of mutation using both gamma rays and electron beam.

Mutation breeding is expected to make a contribution primarily as an important adjunct to the conventional breeding approach. A large number of desirable varieties have been developed through mutation breeding in field of Agricultural and Horticulture crops. The artificial induction of mutation by gamma rays provides a tool to overcome the limitation of variability in black gram. As genetic variability is essential for any crop improvement programme, induced mutations provide an important source for variability. Selecting the appropriate mutagen and the treatment methods are desirable to not only induce a higher mutation rate in a target trait, but also to have less of an effect on the remaining genetic background. Radiation breeding induce plant mutation; by X-ray, γ-ray, ion beam, laser beam, neutron and electron beam, which result in gene mutation and chromosome aberration, and then gain new variety. However, mutation is regarded as random and success of obtaining desired mutant trait depend on three factors such as efficiency of mutagenesis, the starting plant material and mutant screening (Hase *et al*., 2012). The frequency with which the desired mutants appear depends on the efficiency of the mutagenesis. New effective methods with higher mutation induction ability are highly desired for the success of the mutation breeding. Apart from the conventional electromagnetic radiations, like X-ray and γ-ray, electron beam is now an alternative source of energy to induce mutation. 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.

Among different ionizing radiations, gamma rays have been commonly used, and numerous mutants have been produced in black gram (Souframanien and Pandey, 2006). 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). Calaldecatt (1955) reported that barley were treated with 2 MeV electrons beam and showed that electron beam induced high mutation rate and wide mutation spectrum. Most of research reports of electron beam radiation breeding were published in China; the earliest is in the 1980s. Some researches revealed that electron beam radiation holds small physiological damage in M_1 generation and wide mutation frequency in M_2 generation. High energy pulse electron (HEPE) beam radiation had little influence on the function of plasma membrane and protein, while it could induce much DNA damage of single strand breaks (SSB) and double strand breaks (DSB) that were required for gene mutation. The G-value for DSB formation of HEPE beam radiation in aqueous solution was 5.7 times higher than that caused by ^{60}Co gamma rays. HEPE can be a new effective method for induced mutation breeding and deserves further research in the future (Zhu *et al*., 2008). The success of mutation using the radiations depends on its dose, rate of mutation, the number of screened plants and the mutation efficiency. Higher doses of radiation inevitably bring about mortality, high pollen and seed sterility and deleterious mutations. To avoid excessive loss of actual experimental materials, radio-sensitivity tests must be conducted to determine LD_{50}. LD_{50} of the crops is varied depending on the species, varieties and part of the mutation and the water content of the material. Though there are few reports on LD_{50} dose of black gram, there were no reports on LD_{50} dose using electron beam. Hence the present investigation was undertaken to determine LD $_{50}$ and to study the impact of gamma rays and electron beam on biological materials in M_1 generation.

The varieties MDU 1 and VBN (Bg) 4 of blackgram was employed as experimental materials during the present study. These varieties were irradiated with 100 Gy, 200Gy, 300Gy, 400 Gy and 500 Gy doses of gamma radiation from ^{60}CO source fixed in the gamma chamber at Tamil Nadu Agricultural University, Coimbatore. For electron beam irradiation, seeds were irradiated with 200 Gy, 300 Gy, 400 Gy, 500 Gy and 600 Gy doses using 10 MeV electron beam from electron accelerator facility at Electron Beam Centre, Bhabha Atomic Research Centre, Kharghar, Navi Mumbai, India. After mutagenic treatment seeds were sown in the field in a randomized block design along with control (the untreated seeds) during 2013-2014. Number of seeds germinated on 7th day was counted and the germination percent was calculated and the plants those survived from germination till the maturity were counted and recorded. The shoot length and root length of 25 seedlings from each treatment was recorded on the 14th day from the day of soaking in paper cup and compared with the control. Pollen sterility was tested for each treatment by using two per cent freshly prepared potassium iodide solution and examined under stereomicroscope. Dark stained and normal size pollen grains were considered as fertile and those of irregular shape and size with light or no stain were considered as sterile. The number of plants survived till maturity were scored from each treatment and recorded as % survival and compared with the control.

Statistical Analysis: Probit analysis: The LD_{50} values of gamma radiation and Electron beam for both the genotypes were determined based on the Probit analysis (Finney, 1971 and Finney, 1978). The probit function is the inverse cumulative distribution function (CDF) or quartile function associated with the standard normal distribution. The procedure for determination of LD_{50} using probit analysis is as follows.

1) Mutagen dose values were transformed into $\log_{10}$ value.
2) Mortality percentage of seeds due to treatment doses were worked out and rounded to the nearest whole number.
3) Corrected mortality percentage was calculated by using Abbott's formula given below and rounded to the nearest whole number.

$$\text{Corrected mortality } (\%) = \frac{M_{\text{observed}} - M_{\text{control}}}{100 - M_{\text{control}}} \times 100$$

4) The corrected values were converted to the probit transformation.
5) Probit values were graphed (Y-axis) against Log_{10} concentration (X-axis) and a straight line passing through most of the plotted points were drawn to estimate the Log_{10} concentration associated with a probit of 5.
6) Antilog to the Log_{10} value corresponding to the probit 5 was calculated to find out the LD_{50} for the particular mutagen under study.

Determination of lethal dose: Two blackgram varieties namely MDU 1 and VBN (Bg)4 were chosen to study the effect of different doses of electron beam and gamma rays to determine LD_{50}.

LD_{50} values were determined with the help of probit analysis for the physical mutagens used based on their germination of both varieties. Optimum dose is the dose that cause maximum of mutation with minimum of damage to the plant. LD_{50} for electron beam on MDU 1 and VBN (Bg) 4 was 304.91Gy and 302.21 Gy respectively. Whereas, LD_{50} dose for gamma rays on MDU 1 and VBN (Bg) 4 was 324.89 Gy and 318.12 Gy respectively (Table. 1 and 2). This clearly revealed that, the LD_{50} is fixed more or less same in both varieties for both mutagens. This was consistent with the findings of Ramya *et al*., (2014) and Surnder and Vanniarajan (2014) in blackgram. Therefore, LD_{50} dose observed in this study is the optimum dosage for mutagenizing the blackgram seeds of different varieties to induce mutations to produce viable mutants and maintenance of population for mutation breeding.

Biological injury in M_1 generation: Effect of electron beam and gamma rays on germination and survival: In this present study impact of different doses of gamma rays and electron beam 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. Germination percentage was found to decrease with increase of mutagens (Table. 3 and 4). The seed germination was decreased from 75.60% to 36.50% for gamma rays and 78.30% to 31.20% for gamma rays in MDU 1 over control. In blackgram variety VBN (Bg)4, gamma irradiation has range of germination reduction from 78.41% to 27.50% and electron beam varied from 67.85% to 27.56% over control. This shows significant influence of mutagen on germination. Electron beam possess lower damage in M_1 and higher mutagen frequency, and wider mutation spectrum than ^{60}Co γ-rays in rice.

In the present study, survival per cent reduction increased with increase of mutagens in both varieties (Table 3 and 4). Significant influence of mutagen was noticed in all the treatments. This might have been due to the effect of mutagens on merismatic tissues of seed (Ramya *et al*., 2014). The decrease in survival of plants at maturity is due to rapid infusion mutagen and their ability to produce chromosomal aberrations. The maximum reduction in survival 33.99% (MDU1) and 36.74% (VBN (Bg)4) was recorded in higher dose (500 Gy) of gamma rays. In electron beam, higher dose (600 Gy) showed increased survival 35.12% (MDU1) and 35.39% (VBN (Bg)4) reduction per cent over control. The survival percentage and mean value of M_1 generation decreased with increase in dose of treatments (Pavadai *et al.*, 2010) in soybean.

Effect of electron beam and gamma rays on shoot length, root length, plant height at 30 DAS, pollen fertility and seed fertility: A drastic and prominent reduction on germination per cent was noticed in electron beam compared to gamma rays. 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 percent reduction in shoot length (30.78%-MDU 1 and 31.55%-VBN (Bg) 4) and root length (45.019%- MDU 1 and 39.17%- VBN (Bg) 4) was recorded in higher dose of gamma rays (500 Gy). In electron beam treatments, higher dose (600Gy) showed increased shoot (27.13%-MDU 1 and 29.21%-VBN (Bg) 4) and root length (24.45 % - MDU 1 and 31.40 % - VBN (Bg) 4) reduction per cent over control.

Seedling injury is broadly used as an index of determining biological effects of various physical mutagens in M_1 generation. Plant height was also found to be significantly reduced in higher doses of physical mutagenic treatments. All the treatments resulted retardation in height of plants. The maximum plant height reduction on 30 DAS (66.84%) was observed in higher dose of electron beam (600 Gy) than gamma rays (76.80%) in MDU 1 (Table 3 and 4). In VBN (Bg)4, the maximum plant height reduction was found in gamma rays (66.84%) than electron beam (76.46%). The differential sensitivity of biological material to different mutagen may be due to the metabolic processes affected at embryonic level. The higher efficiency of a mutagen indicates relatively less biological damage (*i.e.,* lethality, seedling injury, sterility etc.) in relation to mutation induced. Gamma rays treatment show disturbed mitotic behavior in mung bean. The sticky chromosome, fragments and ring chromosome at metaphase and the laggards and bridges at anaphase were noticed. The chromosomal aberrations combined treatment enhanced chromosomal aberrations. The meiotic abnormalities like misorientation at metaphase, bridge at anaphase, fragmentation and multinucleolate condition were observed in gamma rays. 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. Similar observations were previously reported in blackgram (Surender and Vanniarajan, 2014). In most cases, meiotic abnormalities are responsible for pollen fertility reduction in chickpea.

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 / indirect utilization in successful breeding programme. LD_{50} dose is the optimum dosage for mutagenizing of mutagens with minimum injury and maximum viable mutants. This study revealed actual injury to biological material caused by physical mutagens of gamma rays and electron beam. Comparing the electron beam and gamma rays, the overall injury is less in Electron beam than gamma rays. Optimum doses of mutagens to induce the desirable mutants in maximum level. Hence, this is useful for further research in mutation breeding.

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Table 1. Determination of lethal doses by gamma rays and electron beam in MDU 1 Probit analysis

Gamma rays					
Group	Dosage	Log dose	% Dead	% Corrected	Probits
1.	100 Gy	2.00	21.59	14.20	3.93
2.	200 Gy	2.30	35.90	29.90	4.47
3.	300 Gy	2.50	49.98	45.20	4.88
4.	400 Gy	2.60	68.90	66.00	5.41
5.	500 Gy	2.70	72.50	69.90	5.52
Electron beam					
Group	Dosage	Log dose	% Dead	% Corrected	Probits
1.	200 Gy	2.30	44.36	39.50	4.73
2.	300 Gy	2.48	66.79	63.90	5.36
3.	400 Gy	2.60	70.34	67.80	5.46
4.	500 Gy	2.70	92.56	91.90	6.40
5.	600 Gy	2.78	94.87	94.40	6.59

Table 2. Determination of lethal doses by gamma rays and electron beam in VBN (Bg) 4 Probit Analysis

Gamma rays					
Group	Dosage	Log dose	% Dead	% Corrected	Probits
1.	100 Gy	2.00	11.90	10.70	3.76
2.	200 Gy	2.30	26.90	25.90	4.35
3.	300 Gy	2.48	50.90	50.30	5.01
4.	400 Gy	2.60	60.75	60.20	5.26
5.	500 Gy	2.70	75.90	75.90	5.69
Electron beam					
Group	Dosage	Log dose	% Dead	% Corrected	Probits
1.	200 Gy	2.03	32.15	30.70	4.49
2.	300 Gy	2.48	50.90	49.80	5.00
3.	400 Gy	2.60	62.90	62.10	5.31
4.	500 Gy	2.70	74.10	73.50	5.63
5.	600 Gy	2.78	78.60	78.10	5.78

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Table 3. Impact of gamma rays and electron beam on biological parameters of MDU1 in M_1 generation

Treatment	Germination (%) over control	Shoot length (%) over control	Root length (%) over control	Plant height on 30th DAS (%) over control	Survival on 30th DAS (%) over control	Pollen fertility over control (%)	Seed fertility over control (%)
Gamma rays							
100 Gy	83.66	83.13	85.77	86.76	64.78	77.31	84.01
200 Gy	72.78	57.39	71.13	84.99	57.50	54.45	80.60
300 Gy	53.3	45.91	51.05	81.45	51.19	49.23	73.71
400 Gy	43.89	38.26	45.019	77.34	36.18	36.12	72.81
500 Gy	35.23	30.78	57.74	76.80	33.99	30.55	71.91
Electron beam							
200 Gy	66.93	81.39	67.78	87.43	67.90	72.2	89.39
300 Gy	53.68	52.1	51.88	84.99	56.07	53.44	82.84
400 Gy	45.81	48.52	45.19	81.67	48.20	46.56	76.52
500 Gy	37.06	40.00	42.68	78.26	38.74	34.4	71.91
600 Gy	33.9	27.13	28.45	66.84	35.12	27.57	66.02

Table 4. Impact of gamma rays and electron beam on biological parameters of VBN (Bg) 4 in M_1 generation

Treatment	Germination (%) over control	Shoot length (%) over control	Root length (%) over control	Plant height on 30th DAS (%) over control	Survival on 30th DAS (%) over control	Pollen fertility over control (%)	Seed fertility over control (%)
Gamma rays							
100 Gy	76.4	86.6	85.95	90.89	65.89	72.11	84.23
200 Gy	65.2	69.21	66.94	88.10	57.71	56.89	80.43
300 Gy	55.2	49.45	49.92	85.97	50.92	46.41	76.98
400 Gy	41.5	39.04	44.17	73.27	39.01	35.02	70.81
500 Gy	38.8	31.55	39.17	66.84	36.74	30.67	66.10
Electron beam							
200 Gy	67.97	82.82	81.65	92.15	70.88	72.27	85.63
300 Gy	54.52	50.69	49.59	88.61	57.04	52.46	81.65
400 Gy	45.99	47.77	45.45	85.06	48.53	50.22	73.81
500 Gy	37.5	39.38	40.5	81.52	38.52	35.02	69.84
600 Gy	33.77	29.21	31.4	76.46	35.39	29.05	68.86