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Comparative effectiveness and efficiency of electron beam and ^{60}Co γ-rays in induction of mutations in black gram [*Vigna mungo* (L.) Hepper]

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ABSTRACT

Dry seeds of black gram cultivar TU94-2 was treated with different doses (200, 300, 400 and 500 Gy) of electron beam and γ-rays to study the mutagenic effectiveness and efficiency on frequency and spectrum of chlorophyll and morphological mutation. A wide spectrum of chlorophyll and morphological mutations was observed in M_2 generation of γ-rays and electron beam treated population. Xantha was the most frequent chlorophyll mutant in both γ-rays (2.3%) and electron beam (4.3%) treated populations. Spectrum of morphological mutations observed were common for both γ-rays and electron beam treated populations except early seedling vigour, terminal pod bearing, three primary leaves and partial sterile mutants which were specifically observed in electron beam treated M_2 population. However, overall morphological mutation frequency was higher (9.6%) in electron beam treated population in comparison to γ-rays treated population (6.6%). Frequency of pod and root mutants were high (0.32% and 0.45%) in electron beam treated population in comparison to γ-rays treated population (0.23% and 0.35%). There was a dose dependent increase in the spectrum and frequency of chlorophyll and morphological mutations in both γ-rays (upto 400 Gy) and electron beam (upto 500 Gy) treated M_2 population. The electron beam was found to have higher effectiveness, efficiency and mutation rate than γ-rays of similar doses. Overall effectiveness was higher (0.061) in electron beam treatments when compared to γ-rays treatments (0.034). Mutation rate based on chlorophyll and morphological mutants was higher (8.3 and 2.4) in electron beam in comparison to gamma rays treated M_2 population (4.1 and 1.7). This study shows that electron beam can be used as an effective mutagen for induction of mutations in black gram.

Keywords: Electron beam, Effectiveness, Efficiency, Gamma rays, Mutation frequency

Radiation, including γ-rays, X- rays, fast neutrons, thermal neutrons etc. 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 %). 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 (Park and Vestal 2002).

Different kinds of radiation have different energy transfer patterns. The mutation effect of radiation are known to be a function of their linear energy transfer (LET), which is defined as the energy deposited to the target material when ionizing radiations pass through it. LET is usually expressed in kilo electron volt per micrometer (KeV/μm). Both γ-rays and electron beams have low LETs of around 0.2KeV/ μm (Magori *et al.* 2010). However, electron beam has a higher dose rate compared to γ-rays and is administered as short pulses while gamma irradiation is continuous. Absorbed dose-rate exhibits its strong influence on relative biological effectiveness (RBE), which was called dose-rate effect relationship (Hoglund *et al*. 2007). The higher dose-rate, greater is the RBE. The absorbed dose rate of electron beam on biomaterials may reach 10^{10} $Gy.s^{-1}$, which is much higher than that of γ-rays (usually under 60 $Gy.s^{-1}$) and those of other radiation methods (Zhu *et al.* 2008). Compared with γ-rays or X-rays, the electron beam is limited to treating relatively thin packages because of the low penetrating power (< 2 inches) of electrons.

Black gram (*Vigna mungo*) is a small annual plant and is commonly known in India as Urdbean. It is an important food grain legume and rich in protein. 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. 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. 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 (Makeen and Babu 2010). Mutagenic effectiveness is a measure of the frequency of mutations induced with application of unit dose of a mutagen, whereas mutagenic efficiency indicates the proportion of mutations in the M_2 generation in relation to the biological damage such as gross chromosomal aberrations, lethality and sterility caused in M_1 (Konzak *et al.* 1965).

Among different ionizing radiations, γ-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) first reported that 2 MeV electron beam radiation induced high mutation rate and wide mutation spectrum. Electron beam produces lower damage in M_1 and higher mutagenic efficiency, and wider mutation spectrum than ^{60}Co γ-rays in rice (Baojiang *et al.* 1982). Electron beam was used for induction of mutation in rice, barley, soybean, azuki bean and peas (Shu *et al.* 1996; Xu *et al.* 1983; Rui *et al.* 1995; Li *et al.* 1988; Lu *et al.* 1995; Luo *et al.* 2012, Chen *et al.* 1995). However, the use of electron beam for inducing mutations in black gram is not reported. In the present study, we report the use of electron beam for induction of chlorophyll and morphological mutants in black gram and also their effectiveness and efficiency in comparison to γ-rays in black gram.

MATERIALS AND METHODS

Genetically pure, uniform and dry seeds (12% moisture content) of black gram variety TU94-2 was irradiated with four different doses (200, 300, 400 and 500 Gy) of ^{60}Co γ-rays at Bhaba Atomic Research Centre, Trombay, India. For electron beam irradiation, seeds were treated with 7.5 MeV electron beam from electron accelerator facility at Raja Ramanna Centre for Advanced Technology (RRCAT), Indore, India. Aluminium scatterer was used to achieve low dose ~8 Gy/s in a field width of 15 x 15 cm. Treated seeds along with control variety (TU94-2) were sown in the experimental field facility at Trombay, to raise M_1 generation. Observations on injury and lethality were recorded. M_1 plants were harvested separately to raise plant progenies rows in M_2 generation. M_2 generation was raised as per recommended package of practices. Observations on chlorophyll and viable morphological mutations were recorded from the day of emergence to till the plants attained physiological maturity. Frequency of chlorophyll mutation and viable mutations was calculated on M_2 plant basis. Data on biological abnormalities such as injury and lethality in M_1 generation and mutation frequencies in M_2 generation were used to determine the mutagenic effectiveness and efficiency according to the formulae suggested by Konzak *et al.* (1965). Mutagenic efficiency was calculated in two different ways using a) chlorophyll mutation frequency in M_2 and b) viable morphological mutation frequency in M_2.

$$\text{Mutagenic effectiveness} = \frac{\text{Mutation frequency}}{\text{Dose (Gy)}}$$

Where, Gy = dose of mutagenic treatment in Gray (1 Gray = 0.1 kilo rad).

$$\text{Mutagenic efficiency} = \frac{\text{Mutation frequency}}{\text{Biological damage}}$$

Mutation rate was calculated by the following formula

$$\text{Mutation rate} = \frac{\text{Sum of values of effectiveness or efficiency of particular mutagen}}{\text{Number of treatments of a particular mutagen}}$$

RESULTS AND DISCUSSION

Chlorophyll mutations

Chlorophyll mutations provide one of the most dependable indices for the evaluation of genetic effects of mutagenic treatments and have been reported in black gram (Lal *et al.* 2009, Usharani and Anandakumar 2013; Ramya *et al.* 2013). Gaul (1964) reported that, chlorophyll mutations are employed as markers for the evaluation of gene action of mutagenic factors in inducing mutation. It was reported that about 250-300 loci might be involved for breakdown of the chlorophyll apparatus in barley (Swami Nathan 1957). Hence, the probability of occurrence of such category of mutation is obvious in all mutagen treatments. In this study, a wide spectrum of chlorophyll mutations were observed in mutagen treated populations of TU94-2 in M_2 generation which included albino, xantha, chlorina and viridis. No chlorophyll mutations were observed in untreated population (control). The frequency of chlorophyll mutations in γ-rays and electron beam treated populations varied from 3.1 % to 5.7% and 4.8% to 11.3% respectively (Table 1). Among different types of chlorophyll mutations, xantha was the most frequent (2.3% and 4.3%) followed by chlorina and albino in both γ-rays and electron beam treated populations. Previous studies using γ-rays showed xantha as the most frequently observed chlorophyll mutations in black gram (Lal *et al.* 2009, Makeen *et al.* 2013)

The overall frequency of chlorophyll mutations in the electron beam treated population was higher (33.0%) in comparison to that of γ-rays treated population (16.5%) suggesting higher efficiency of electron beam treatment (Fig. 1). Similar trend was observed in chickpea (Joshi-Saha *et al*. 2015). Viable chlorophyll mutations (chlorina and viridis) and lethal mutants (albino and xantha) were produced more at higher doses of electron beam in comparison to γ-rays. Chlorophyll mutation frequency rate was high (8.3) in electron beam treated population than that of γ-rays (4.1) treated population (Fig. 2). There was a dose dependent increase in the spectrum and frequency of chlorophyll mutations in both γ-rays (except 400 Gy) and electron beam treated M_2 population. Similar trend in progressive increase in mutation frequency of chlorophyll mutations was observed with increasing doses in black gram (Makeen *et al*. 2013) and *Vigna* species (Ignacimuthu and Babu 1988). Khan and Tyagi (2010) also reported reduction and increase in chlorophyll mutation frequency in two different varieties of soybean treated with same mutagen and dose. The increased chlorophyll mutation frequency at higher doses may be attributed to the chromosomal aberrations or saturation in the mutational events which may result in the elimination of mutant cells during growth (Brock 1965).

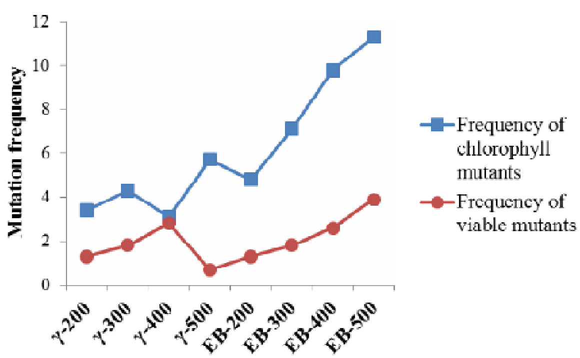

Figure 1. Frequency of chlorophyll and viable mutants induced by γ-rays and electron beam

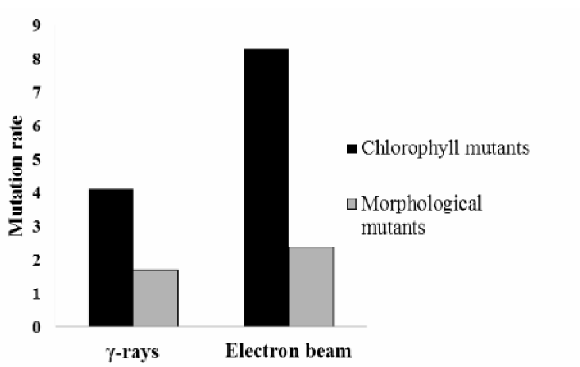

Figure 2. Mutation rate in terms of effectiveness in M_2 generation of TU94-2 black gram treated with γ-rays and electron beam.

Viable morphological mutations

A wide spectrum of morphological mutations was observed in M_2 generation of γ-rays and electron beam treated population. No morphological mutations were observed in untreated control TU94-2 variety. The frequency of morphological mutations in γ-rays and electron beam treated populations varied from 0.7% to 2.8% and 1.3% to 3.9% respectively (Table 1). Overall morphological mutation frequency was higher (9.6%) in electron beam treated population in comparison to that of γ-rays (6.6%) treated population (Fig. 1). Spectrum of morphological mutations (dwarf, tall, leaf size and shape, increased branch number, branch position, stem girth, early and late flowering, increased pod number, pod and seed colour, increased primary and lateral root length mutants) observed were common in both γ-rays and electron beam treated populations except for early seedling vigour, terminal pod bearing, three primary leaves and partial sterile mutants which were specifically observed in electron beam treated M_2 population (Fig. 3). Frequency of mutations affecting plant height (tall, dwarf) was similar in both γ-rays (0.53%) and electron beam (0.51%) treated populations. More dwarf plants were observed in γ-rays (400 Gy) treated M_2 population in comparison to the similar dose applied as electron beam. Frequency of pod and root mutants was high (0.32% and 0.45%) in electron beam treated population

Table 1. Effectiveness of different doses of γ-rays and electron beam in TU94-2 black gram.

Treatment	M_2 plants studied	Number of chlorophyll mutants	Number of viable mutants	Chlorophyll mutation frequency (%) (M_c)	Viable mutation frequency (%) (M_v)	Effectiveness		
						M_c/Gy	M_v /Gy	Average
γ- 200 Gy	1720	59	22	3.4	1.3	0.017	0.006	0.011
γ-300 Gy	1705	74	30	4.3	1.8	0.014	0.006	0.01
γ-400 Gy	1735	53	49	3.1	2.8	0.007	0.007	0.007
γ-500 Gy	1680	95	12	5.7	0.7	0.011	0.001	0.006
Over all γ	**6840**	**281**	**113**	**16.5**	**6.6**	**0.049**	**0.02**	**0.034**
Mutation rate	**-**	**-**	**-**	**4.1**	**1.7**	**0.012**	**0.005**	**0.008**
EB-200 Gy	1710	82	22	4.8	1.3	0.024	0.006	0.015
EB-300 Gy	1675	119	30	7.1	1.8	0.024	0.006	0.015
EB-400 Gy	1640	161	43	9.8	2.6	0.025	0.007	0.016
EB-500 Gy	1580	178	61	11.3	3.9	0.023	0.008	0.015
Over all EB	**6605**	**540**	**156**	**33.0**	**9.6**	**0.096**	**0.027**	**0.061**
Mutation rate	**-**	**-**	**-**	**8.3**	**2.4**	**0.024**	**0.007**	**0.015**

Figure 3. Morphological mutants induced by electron beam in M_2 generation of black gram. a) Top pod bearing mutant (500 Gy), b) Extreme dwarf mutant (400 Gy), c) Partial sterile mutant and d) Long root mutant (400 Gy).

than that of to γ-rays treated population (0.23% and 0.35%) (Data not shown). Luo *et al*. (2012) also recorded mutants with increased pod number along with other mutants in electron beam treated M_2 population of azuki bean. There was a dose dependent increase in the spectrum and frequency of morphological mutations in both γ-rays (till 400 Gy) and electron beam treated M_2 population indicating a positive relationship between dose of mutagenic treatment and frequency of morphological mutations. However, in γ-rays frequency of morphological mutations reduced after 400 Gy due to increased sterility. Differential induction of morphological mutation in different doses of γ-rays has been reported in mungbean (Mishra and Singh 2014) and chickpea (Kharkwal 2000). The frequency of morphological mutations increased with higher doses of electron beam. Similar trend was observed in electron beam treated M_2 population of azuki bean (Luo *et al*. 2012). Viable morphological mutation frequency was higher (2.4%) in electron beam in comparison to γ-rays (1.7%) treated population (Fig.2). Similar mutation rate has been observed in cockscomb seeds treated with electron beam (Wang *et al*. 2006).

Effectiveness and efficiency of mutagen treatments

The relative effectiveness and efficiency of different mutagenic treatments are presented in Table 1 and 2. In γ-rays treatments, 200 Gy was the most effective followed by 300 Gy and 400 Gy. In electron beam treatments, 400 Gy was the most effective followed by 200 Gy and 300 Gy. However, based on morphological mutations 500 Gy of electron beam was the most effective (0.008) as compared to that of similar dose of γ-rays (0.001). Similarly 500 Gy of electron beam was found to be most effective in azuki bean (Luo *et al*. 2012). Overall effectiveness was higher (0.061) in electron beam treatments when compared to γ-rays treatments (0.034).

The mutagenic efficiency varied depending on the criteria selected. The efficiency estimated on the basis of seedling injury was generally higher compared with lethality. This observation was consistent with the findings of Mishra and Singh (2014); Wani (2009) and Khan and Tyagi (2010). The lethality and injury was the highest when higher doses of mutagenic treatments were used. The efficiency of γ-rays and electron beam treatments declined considerably with the increase in the dose of mutagens except for 500 Gy electron beam treatment. The decrease in efficiency at higher doses may be attributed to the failure in recovery of viable mutations proportionate to the dose of mutagens. This result was consistent with the findings of Mishra and Singh (2014). Solanki and Sharma (1994) opined that higher efficiency of a mutagen indicates relatively less biological damage (*i.e.,* lethality, seedling injury, sterility etc.) in relation to mutation induced. The higher mutagenic efficiency, mostly at lower and

Table 2. Efficiency of different doses of γ-rays and electron beam in TU94-2 black gram.

Treatment	Chlorophyll mutation frequency (%) (M_c)	Viable mutation frequency (%) (M_v)	Plant survival reduction (%) in M_1 (L)	Seedling height reduction (%) in M_1(I)	Efficiency based of chlorophyll mutation frequency			Efficiency based on viable mutation frequency		
					M_c/L	M_c/I	Average	M_v/L	M_v/I	Average
γ- 200 Gy	3.4	1.3	8.8	7.3	0.39	0.47	0.43	0.15	0.18	0.17
γ-300 Gy	4.3	1.8	14.0	10.7	0.31	0.40	0.36	0.13	0.17	0.15
γ-400 Gy	3.1	2.8	20.6	17.1	0.15	0.18	0.17	0.14	0.16	0.15
γ-500 Gy	5.7	0.7	29.5	24.4	0.19	0.23	0.21	0.02	0.03	0.03
Over all γ	**16.5**	**6.6**	**72.9**	**59.5**	**1.04**	**1.28**	**1.17**	**0.44**	**0.54**	**0.5**
Mutation rate	**4.1**	**1.7**	**-**	**-**	**0.26**	**0.32**	**0.3**	**0.11**	**0.14**	**0.13**
EB-200 Gy	4.8	1.3	7.4	2.0	0.65	2.40	1.53	0.18	0.65	0.42
EB-300 Gy	7.1	1.8	9.6	5.9	0.74	1.20	0.97	0.19	0.31	0.25
EB-400 Gy	9.8	2.6	16.2	8.3	0.60	1.18	0.89	0.16	0.31	0.24
EB-500 Gy	11.3	3.8	19.9	10.7	0.57	1.06	1.63	0.19	0.36	0.28
Over all EB	**33.0**	**9.6**	**53.1**	**26.9**	**2.56**	**5.84**	**5.02**	**0.72**	**1.63**	**1.19**
Mutation rate	**8.3**	**2.4**	**-**	**-**	**0.64**	**1.5**	**1.3**	**0.18**	**0.41**	**0.29**

intermediate doses of viable mutants than higher doses were previously reported for γ-rays in black gram (Deepalakshmi and Ananda Kumar 2004; Usharani and Anandakumar 2013; Surendar *et al*. 2014). Some previous studies have revealed that electron beam radiation holds the virtue of small physiological damage in M_1 generation and wide mutation frequency in M_2 generation (Jia *et al*. 2005, Rui *et al*. 1995).

In the present study, electron beam was found to have higher effectiveness and efficiency than those of γ-rays of similar doses. Similar results were reported in chickpea (Joshi-Saha *et al*. 2015) and peas (Chen *et al*. 1995). Baojiang *et al*. (1982) have also reported a relatively higher mutagenic efficiency and a broader mutagenic spectrum of electron beam irradiation as compared to γ-rays on rice seeds. Mutation rate was higher in electron beam treated population when compared to γ-rays. Such high mutation rate and increased mutation frequency in the electron beam treated population is attributed to dose rate. Electron beam has a higher dose rate of 8Gy/sec as compared to γ-rays (0.83Gy/sec) administered in this study. With such high dose-rate, electron beam can produce high density free radicals in a very short time, resulting in a large number of DNA double strand breaks, bringing mutation effects with high mutation efficiency and wide variety (Zhu *et al*. 2008). Baojiang *et al*. (1989) have also reported dose-dependent chromosomal aberration of root tip cells of electron beam irradiated dry *Vicia faba* seeds. Various morphological mutants identified in this study needs to be studied for their true breeding nature and yield parameters. Some of the economical mutants with increased pod number and root length will serve as source of variability and will be utilized in future breeding programmes. The dwarf and root mutants can also be used in basic studies to understand development and morphogenesis.

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