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# Studies on Mutagenic Effectiveness and Efficiency of Gamma Rays in Greengram [*Vigna radiata* (L.) Wilczek]

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**A B S T R A C T**

The mutagenic effectiveness and efficiency of gamma rays was studied on greengram breeding lines with dose of 20 kR and 100 kR for creation of desirable variability. These two parameters are the measures of usefulness of a particular mutagen for a particular crop genotype. Highest mutation frequency was found in the breeding lines of DGGV-2 × IPM-410-3 (11.42%) and DGGV-2 × SML-1815 (7.27%) with 100 kR. The mutagenic effectiveness was highest in the breeding lines of DGGV-7 × V-02-709 with 0.25 at the dose 20 kR, whereas the lowest (0.07) was observed in DGGV-2 × SML-1815 with 100 kR. The average mutagenic efficiency was highest in the breeding lines of DGGV-7 × V-02-802 (0.39). Hence, the mutagenic effectiveness was decreased with increased dose of mutagen, while the mutagenic efficiency was reported higher in lower dose of mutagen as the higher dose causes reduction in germination percentage and more lethal plants.

## Introduction

Greengram [*Vigna radiata* (L.) Wilczek] is a short duration grain legume crop occupied the position of third most important pulse crop after chickpea and pigeonpea. It can be grown in varying climatic conditions and having the ability to withstand drought. It can be cultivated during all three seasons*viz.*, *kharif*,

*rabi* and *summer* in different parts of the country as a sole crop or as an intercrop (green manure).

Greengram has easily digestible protein and contains approximately 25-28% protein, 62-65% carbohydrates and 3.5-4.5% fiber on dry weight basis (Gowda *et al.,* 2015). Lysine value in mungbean is comparatively high;

hence the protein is an excellent complement with rice for balance human diet.

Mutation is the sudden heritable changes in the organisms generally in the structural changes in the genes which are not caused due to any genetic recombination and crossing over. It is produced by changing the base sequence of genes either spontaneously or by induced mutation after treating with seeds or vegetative parts with mutagen. Induced mutation breeding has been recognized as a valuable supplement to conventional breeding in crop improvement. A number of chemical and physical mutagens are used in crop improvement programme for induction of useful mutants in a number of crops (Sange *et al.,* 2011 and Bashir *et al.,* 2013) and high yielding new varieties produced by gamma rays and EMS was observed by Khatri *et al.,* 2005. In plants, gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in the cells and tissues.

Mutagenic effectiveness and efficiency are the measures of usefulness of a particular mutagen for mutation breeding in a particular crop genotype. These two are the base parameters to predict the mutagenic potency of any mutagen. The prior information of comparative effectiveness and efficiency of various mutagens facilitate the selection, which is essential to recover high frequency of desirable mutations.

Although, effectiveness and efficiency of a particular mutagen are completely different properties but they together define the usefulness of any mutagen. It is not necessary that an effective mutagen shall be an efficient one also. The value of effectiveness and efficiency estimation depends on various factors like biological, environmental and

chemical that can modify mutation rate of different mutagens (Konzak *et al.,* 1965). A highly effective mutagen may not necessarily show high efficiency and vice-versa. The higher efficiency of a mutagen indicates relatively less biological damage (seedling injury, pollen sterility and ovule sterility etc.) in relation to the doses of mutagen (Tariq *et al.,* 2008). Hence, it is necessary to better understand the efficiency and effectiveness of different mutagens for creation of desirable variability with higher frequency and spectrum. Hence, it is essential to evaluate the efficiency, effectiveness and factor of effectiveness of gamma rays in green gram. Therefore, the present investigation was undertaken to assess the mutagenic effectiveness and efficiency oftwo different doses of gamma rays at different levels of morphological characters in greengram.

## Materials and Methods

The $F_2$ seeds derived from the following four different crosses are mentioned below in Table 1; were irradiated withgamma rays in *kharif*-2017 for creation of desirable variability and for driving the force of selection to desirable mutants and the field experiment was carried out at Experimental plot, AICRP on MULLaRP, Main Agricultural Research Station, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka, India.

## Gamma rays irradiation and development of $F_2M_1$ generation

The well dried, genetically pure, disease free and viable seeds from each cross were sent to Bhabha Atomic Research Centre, Mumbai; for gamma rays irradiation. The pedigree, number of seeds from each cross and the dose of irradiation is mentioned below in Table 1. The irradiated seeds were sown to develop the $F_2M_1$ generation during *kharif*-2017 along

with their respective checks. All the cultural practices including irrigation, weeding, manuring and plant protection practices were monitored to raise a good crop.In $F_2M_1$ generation; the germination reduction, reduction in seedling height and plant survivality was recorded and considered as Injury (I) and Lethality (L), (Sharma, *et al.,* 2005 and; Singh and Singh, 2001).The fertile $F_2M_1$ plants were harvested separately at maturity and seeds were sown in plant to row method to raise the $F_2M_2$ generation along with the controls.

## Observations recorded $F_2M_1$ generation

The observation for germination was taken on $7^{th}$, $15^{th}$ and $30^{th}$ day of sowing. Higher number of seeds was germinated at 20 kR as compared to 100 kR dose of gamma rays. The plants which survived beyond 30 days grew up to maturity, while a few plants died in between $15^{th}$ and $30^{th}$ day (Table 3).The following observations were recorded from the $F_2M_1$ generation during*kharif*-2017.

### Days to 50 percent germination count

The numbers of days from the date of sowing to the date of 50 per cent seeds germinated in each plot was counted and recorded.

### Plant count per plot after 15 days of germination

The number of plants survived after 15 days of germination count was counted and recorded in each plot to calculate field emergence.

### Plant count per plot after 30 days of germination

The number of plants survived after 30 days of germination was counted and recorded in each plot to calculate survival percentage.

## Field emergence percentage

Observation on field emergence was recorded on $15^{th}$ days after sowing. Field emergence percentage was calculated by using the following formula.

$$\text{Field emergence per cent (\%)} = \frac{\text{Number of plants emerged}}{\text{Total number of seeds sown}} \times 100$$

## Survival percentage

Seedlings survived on 30 days after sowing (DAS) were counted and survival per cent was calculated by using the following formula.

$$\text{Survival percentage (\%)} = \frac{\text{Number of seedlings survived up to 30 days}}{\text{Total number of seeds germinated}} \times 100$$

## Mutagenic effectiveness

The mutagenic effectiveness was calculated based on the formula given by Konzak *et al.,* (1965).

$$\text{Mutagenic effectiveness} = \frac{\text{Mutation frequency (MF)}}{\text{Dose of physical mutagen (kR)}} \times 100$$

Where,
MF = Mutation frequency
kR = unit of gamma radiation (kilo rad)

## Mutagenic efficiency

The mutagenic efficiency was determined by using the formula given by Konzak *et al.,* (1965).

$$\text{Mutagenic efficiency} = \frac{\text{Mutation frequency (MF)}}{\text{Biological damage in M}_1 \text{ generation}} \times 100$$

Where, Biological damage (L and I)

L = % of lethality in $M_1$ generation
I = % of seeding injury in $M_1$ generation

## Development of $F_2M_2$ generation

The seeds from $F_2M_1$ generation of *kharif*-2017 were harvested individual plant basis and sown in *summer*-2018 to develop the $F_2M_2$ population in an augmented design. Similar agronomical practices also followed to raise a healthy crop.The plants were screened for chlorophyll mutation (*eg.*-albina, xantha, chlorine and striata) along with the other viable morphological mutations.

## Results and Discussion

### Biological damages and survival per cent in $F_2M_1$ generation

In the present investigation, germination reduction and seedling lethality were recorded in $F_2M_1$ generation. After 7 days of sowing, the number of seeds germinated was counted in each mutant breeding lines and it was observed that the cross derivatives of DGGV-7 × V-02-802 have shown highest germination of 94.80 per cent, followed by the cross derivatives of DGGV-7 × V-02-709 which shown 87.60 per cent with dose 20 kR. Less than 50% germination was observed for the breeding lines of the cross DGGV-2 × SML-1815 (37.05 %) and DGGV-2 × IPM-410-3 (23.98 %) (Table 2).

The field emergence was highest for the cross derivatives of DGGV-7 × V-02-802 (81.6 %) at 20 kR. The cross derivatives of DGGV-7 × V-02-709, DGGV-2 × IPM-410-3 and DGGV-2 × SML-1815 have shown the field emergence percentage of 77.6, 18.9 and 30.94 per cent respectively.

The highest survival per cent was observed for the breeding lines of DGGV-7 × V-02-802 with 71.94%. This cross derivative was also shown highest field emergence. The breeding lines derived from the crosses DGGV-7 × V-02-709, DGGV-2 × IPM-410-3 and DGGV-2 × SML-1815 were shown 54.79, 59.32 and 62.5 per cent survival respectively with dose of 100 kR(Table 3).

The lowest germination reduction was recorded in the cross derivatives of DGGV-7 × V-02-709 (4.2 %) and DGGV-7 × V-02-802 (12.4 %) which were treated with 20 kR dose, whereas; the cross derivatives of DGGV-2 × IPM-410-3 and DGGV-2 × SML-1815 have recorded higher germination reduction of 76.01 and 62.94 per cent respectively with 100kR.

The lethality parentage was calculated by counting the dead seedlings and expressed as lethal plant percentage. Lowest lethality was observed for the cross derivative of DGGV-7 × V-02-802 (28.81 %). The lethality per cent was recorded for the cross derivatives of *viz*; DGGV-7 × V-02-709, DGGV-2 × IPM-410-3 and DGGV-2 × SML-1815 with 45.20, 40.67 and 37.50 per cent.

Highest mutation frequency was found in the breeding lines of DGGV-2 × IPM-410-3 (11.42 %) and DGGV-2 × SML-1815 (7.27 %) with 100 kR dose of irradiation but at 20 kR the mutation frequency was observed comparatively low *i.e*. 5.00 and 2.93 % for the lines of DGGV-7 × V-02-802 and DGGV-7 × V-02-709 respectively (Table 4). The result of mutation frequency in present study was in accordance with the results obtained by Kumar *et al.,* (2003) in lima bean.

The results obtained from the present study were reinforced with the findings of Bhosle and Kothekar (2010) in cluster bean and;Girija and Dhanavel (2009) on cowpea. The higher dose of mutagens generally causes increased lethality which is proved by the findings of the above. The probable cause for

increased pollen sterility and poor seed set might be due to meiotic irregularities.

**Mutagenic effectiveness and efficiency of gamma rays on greengram breeding lines in $F_2M_1$ generation**

The mutagenic effectiveness and efficiency of mutagen and their doses are prerequisites for induction and utilization of mutation (Sharma *et al.,* 2005). Mutagenic effectiveness is a measure of the frequency of mutations induced by the unit dose of the mutagen, while efficiency refers to the mutation rate in relation to biological damage such as seedling injury, pollen sterility, lethality in $M_1$ generation. Thus, mutagenic effectiveness and efficiency gives an idea to prior evaluation of a particular mutagen.

The mutagenic effectiveness was highest in the cross derivative of DGGV-7 × V-02-709 (0.25) at 20 kR whereas, the lowest mutagenic effectiveness (0.07) was observed from DGGV-2 × SML-1815 which was treated with 100 kR. The other two cross derivatives *viz*; DGGV-7 × V-02-802 and DGGV-2 × IPM-410-3 shown mutagenic effectiveness of 0.14 and 0.11 respectively (Table 5 and Fig 1). The results were supported by the findings of several other workers; Bhosle and Kothekar (2010) and Dube *et al.,* (2011) in cluster bean when treated with gamma rays. They also reported that the effectiveness goes on decreasing with increased dose of mutagen.

The average mutagenic efficiency was highest in the breeding lines of DGGV-7 × V-02-802 (0.39) and lowest was 0.15 in DGGV-7 × V-02-802. The cross derivatives of DGGV-7 × V-02-709 and DGGV-2 × IPM-410-3 have recoded 0.25 and 0.17 mutagenic efficiencies. The mutagenic efficiency with respect to germination reduction was found to be higher as compared to the lethal plants. The mutagenic efficiency in relevance to germination reduction was 0.40 and 0.69 in DGGV-7 × V-02-709 and DGGV-7 × V-02-802 with 20 kR, while it was 0.15 and 0.11in DGGV-2 × IPM-410-3 and DGGV-2 × SML-1815 with 100 kR respectively.

Compared to the lethal plant, the mutagenic efficiency was found 0.11 and 0.10 for the breeding lines of DGGV-7 × V-02-709 and DGGV-7 × V-02-802 with 20 kR, while 0.20 and 0.19 in DGGV-2 × IPM-410-3 and DGGV-2 × SML-1815 with 100 kR respectively (Table 5 and; Fig 2 and 3). Similar results were also reported by Bhosle and Kothekar (2010) in cluster bean, Mullainathan andUmavathi (2014) on chickpea,Patil *et al.,* (2015) on rice bean, Morel and Borkar (2016) on rajmas and;Rafiul and Samiullah (2017) on lentil. The findings of the above workers also showed conformity with the present investigation for the mutagenic effectiveness and efficiency of gamma rays on different crops.

**Table.1** Materials used for gamma rays irradiation

| Sl. No. | Pedigree | Doses of Mutagen (kR) | Number of seeds treated |
|---|---|---|---|
| 1. | DGGV-7 × V-02-709 | 20 | 500 |
| 2. | DGGV-7 × V-02-802 | 20 | 500 |
| 3. | DGGV-2 × IPM-410-3 | 100 | 492 |
| 4. | DGGV-2 × SML-1815 | 100 | 475 |

**Table.2** Germination counts on 7th DAS and plant count after 15th and 30th days of germination after gamma rays irradiation (*kharif* 2017)

| Sl. No. | Pedigree | Total number of seeds sown | Dose (kR) | First germination count (after 7 days after sowing) | Plant count per plot (after 15 days of germination) | Plant count per plot (after 30 days of germination) | Germination percentage |
|---|---|---|---|---|---|---|---|
| 1. | DGGV-7 × V-02-709 | 500 | 20 | 438 | 388 | 240 | 87.60 |
| 2. | DGGV-7 × V-02-802 | 500 | 20 | 474 | 408 | 341 | 94.80 |
| 3. | DGGV-2 × IPM-410-3 | 492 | 100 | 118 | 93 | 70 | 23.98 |
| 4. | DGGV-2 × SML-1815 | 475 | 100 | 176 | 147 | 110 | 37.05 |

*DAS- Days after sowing

**Table.3** Field emergence percentage and survival percentage in $F_2M_1$ generation after gamma rays irradiation (*kharif*-2017)

| Sl. No. | Pedigree | Field emergence percentage (%) | Survival (%) |
|---|---|---|---|
| 1. | DGGV-7 × V-02-709 | 77.6 | 54.79 |
| 2. | DGGV-7 × V-02-802 | 81.6 | 71.94 |
| 3. | DGGV-2 × IPM-410-3 | 18.9 | 59.32 |
| 4. | DGGV-2 × SML-1815 | 30.94 | 62.5 |

**Table.4** Mutation frequency, number of seeds not germinated and number of seedlings died in $F_2M_1$ generation after gamma rays irradiation (*kharif*-2017)

| Sl. No. | Pedigree | Number of seeds not germinated (R) | Number of seedlings died(L) | Putative mutants in $M_1$ | Mutation Frequency (MF %) | R (%) | L (%) |
|---|---|---|---|---|---|---|---|
| 1. | DGGV-7 × V-02-709 | 62 | 198 | 12 | 5.00 | 12.4 | 45.20 |
| 2. | DGGV-7 × V-02-802 | 21 | 138 | 10 | 2.93 | 4.2 | 28.81 |
| 3. | DGGV-2 × IPM-410-3 | 374 | 48 | 8 | 11.42 | 76.01 | 40.67 |
| 4. | DGGV-2 × SML-1815 | 299 | 66 | 8 | 7.27 | 62.94 | 37.50 |

Where, R %- Reduction of germination (Per cent of seeds not germinated), L %- Lethality per cent (Number of seedlings died)

**Table.5** Mutagenic effectiveness and efficiency of gamma rays in green gram in
$F_2M_1$ generation (*Kharif*-2017)

| Sl.No. | Pedigree | Mutagenics Effectiveness | Mutagenic Efficiencies | | |
|---|---|---|---|---|---|
| | | MF/kR | MF/R | MF/L | Average |
| 1. | DGGV-7 × V-02-709 | 0.25 | 0.40 | 0.11 | 0.25 |
| 2. | DGGV-7 × V-02-802 | 0.14 | 0.69 | 0.10 | 0.39 |
| 3. | DGGV-2 × IPM-410-3 | 0.11 | 0.15 | 0.20 | 0.17 |
| 4. | DGGV-2 × SML-1815 | 0.07 | 0.11 | 0.19 | 0.15 |

*MF- Mutation Frequency, kR- kilo Rad (Physical mutagen dose), R- Germination reduction or Number of seeds not germinated and L- Lethal plants or Number of seedlings died
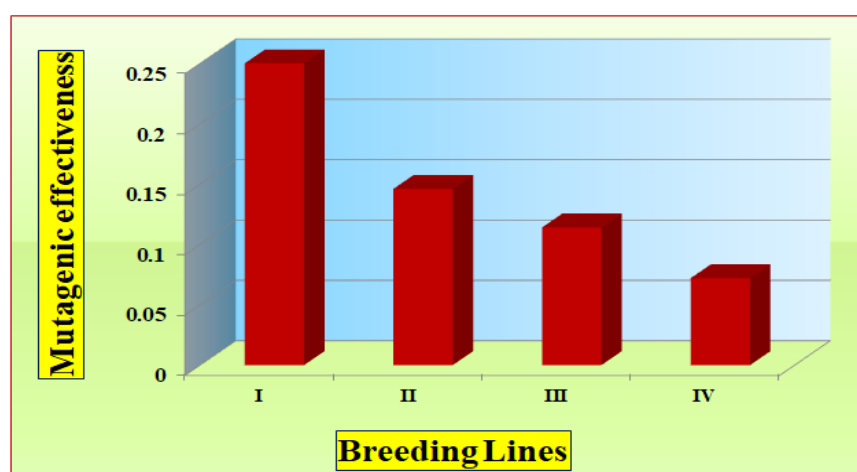


**Fig.1** Mutagenic effectiveness in $F_2M_1$-generation of greengram breeding lines [Roman    letter indicates; I- DGGV-7 × V-02-709 (20 kR), II- DGGV-7 × V-02-802 (20 kR),        III- DGGV-2 × IPM-410(100 kR) and IV- DGGV-2 × SML-1815) (100 kR)]
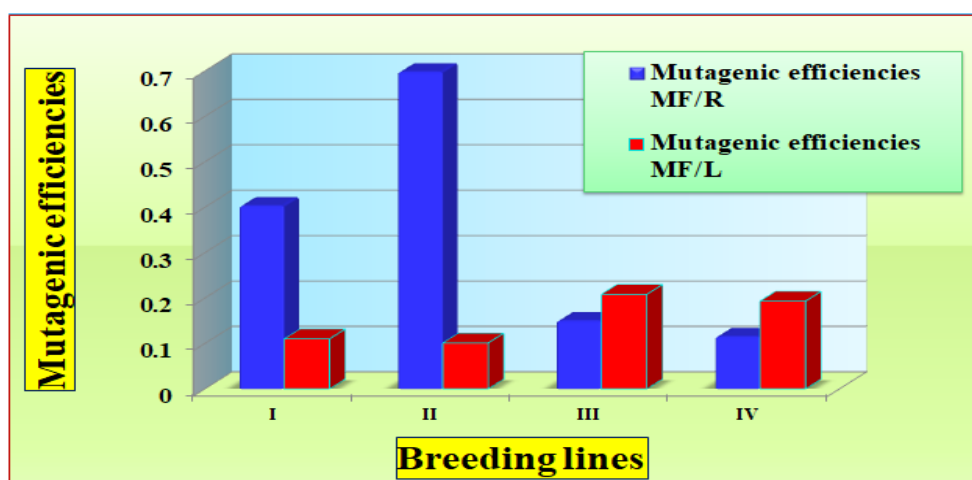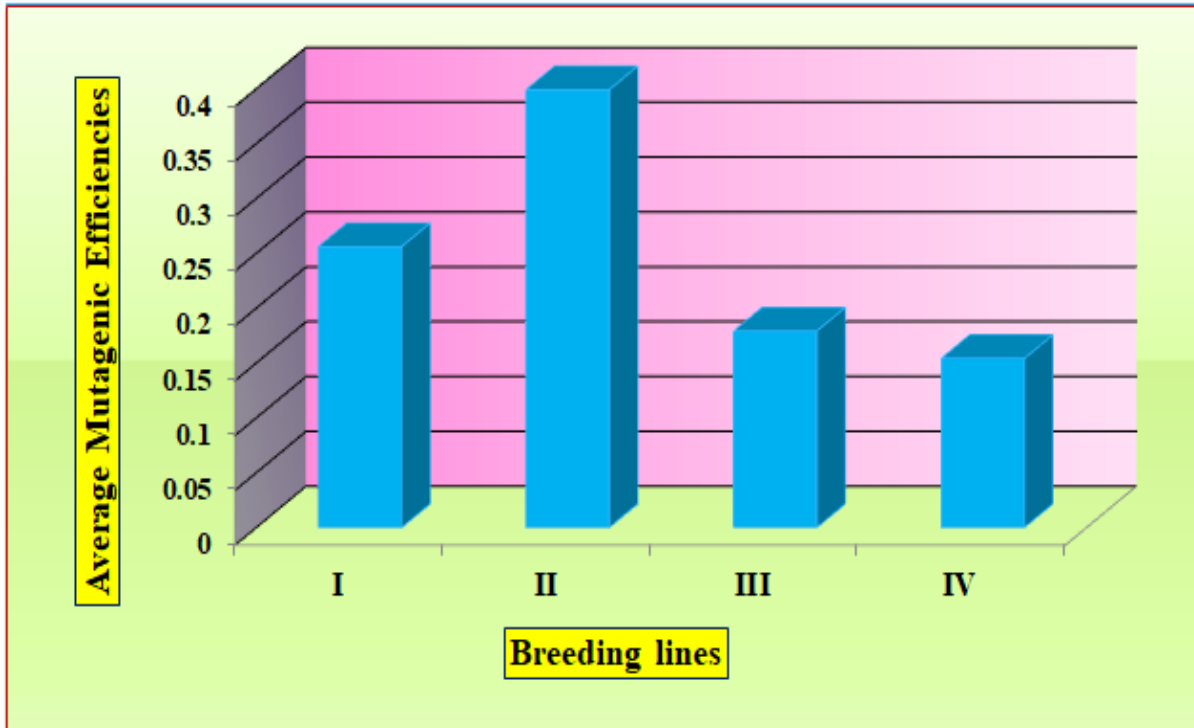


**Fig.2** Mutagenic efficiencies in $F_2M_1$- generation of greengram breeding lines [Roman    letter indicates; I- DGGV-7 × V-02-709 (20 kR), II- DGGV-7 × V-02-802 (20 kR),        III- DGGV-2 × IPM-410(100 kR) and IV- DGGV-2 × SML-1815) (100 kR)],     MF/R-Mutagenic frequency

w.r.t. germination reduction and MF/L- Mutagenic   frequency w.r.t. lethal plants
**Fig.3** Average Mutagenic Efficiencies in $F_2M_1$- generation [Roman letter indicates;
I- DGGV-7 × V-02-709 (20 kR), II- DGGV-7 × V-02-802 (20 kR), III- DGGV-2 ×
IPM-410(100 kR) and IV- DGGV-2 × SML-1815) (100 kR)]



| DGGV-7 × V-02-709 (20 kR) | DGGV-7 × V-02-802 (20 kR) |

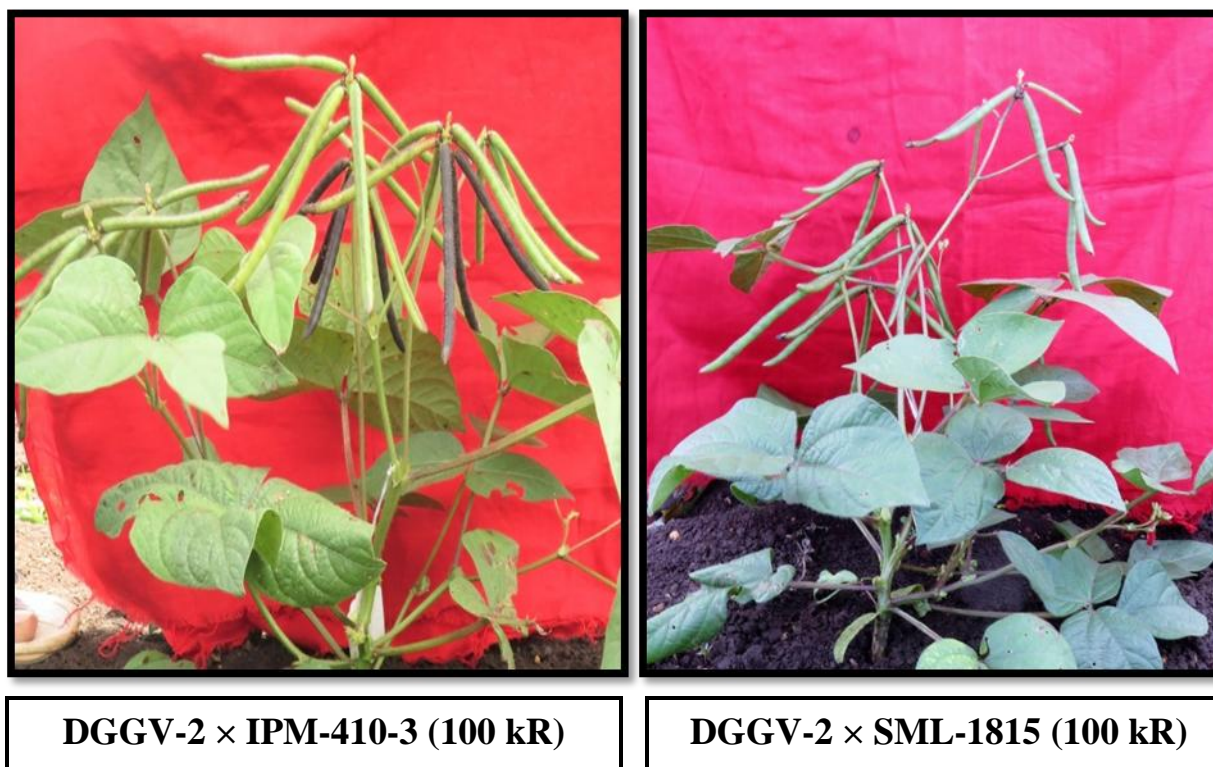| **DGGV-2 × IPM-410-3 (100 kR)** | **DGGV-2 × SML-1815 (100 kR)** |

**Fig.4** Superior putative mutants selected from $F_2M_1$ generation after gamma rays irradiation

In the present investigation, the mutagenic effectiveness was observed higher with 20 kR in the breeding lines of DGGV-7 × V-02-709 and DGGV-7 × V-02-802 as compared to the dose 100 kR. Even though the dose 100 kR was high for survival percentage, but it shown very vigorous plants as compared to 20 kR (Fig 4). The mutagenic effectiveness was decreased with increased dose of mutagen, while the mutagenic efficiency was reported higher in lower dose of mutagen as the higher dose causes reduction in germination percentage and more lethal plants. Thus, gamma ray is an efficient and effective for creation of genetic variability in greengram for crop improvement.

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