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RESEARCH ARTICLE

# Mutagenic effect of gamma rays on induced mutation and principal component analysis of yield characters on green gram in $M_2$ generation

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## Abstract

The present study was aimed to evaluate the effect of gamma irradiation on green gram through chlorophyll mutation frequency, leaf mutation frequency, mutagenic effectiveness and efficiency and principal component analysis. Eight different types of chlorophyll mutants, namely albino, aurea, striata, tigrina, xantha, chlorina, viridis, xanthaviridis and variegated were observed at different doses of gamma irradiation in $M_2$ generation. Albino showed the highest frequency percentage (0.229) followed by Viridis (0.163) and Xantha (0.131) and the highest frequency was noted at 500 Gray (Gy). Though, chlorophyll mutants can be lethal in nature, they increase genetic variability and induce new traits. Leaf mutant is another key indicator for induced mutations that induce leaf morphology changes and the highest frequency was noted in 500 Gy. Among the mutagenic treatments, the mutagenic effectiveness shows the maximum at 100 Gy and efficiency shows at 500 Gy. This indicated that low to moderate doses are more effective for induced mutation. This was also confirmed by Principal Component Analysis (PCA) results, which specified that gamma irradiation of 500 Gy indicated that the first five principal components were attributed to 70.82% total variability of traits studied. From this, the experimental finding evidently showed that 500 Gy of gamma irradiation, an optimum dose, resulted in considerable variation in all the parameters analyzed.

## Keywords

Gamma rays, Chlorophyll mutants, effectiveness, efficiency, Principal component analysis

## Introduction

In plant breeding, mutation breeding is a technique in which new crop varieties with desirable characteristics were created by the use of induced mutation. It includes high-energy radiation such as α, β and γ-rays, and certain chemicals can be employed, which initiate or inhibit the growth and differentiation of plant cells and organs to cause mutations and generate genetic variants from which desired mutants can be chosen (1). For the production of a high level of economically significant metabolites they can also modify the physiological characteristics of plants to create new mutants (2) and it is a persuasive technique for plant improvement (3). Genetic alterations in any organism including plants are mostly due to mutation or heritable changes, which produce new gene variants (4). Both the study of gene function and genetic diversity can benefit from mutation. Forward genetic studies (phenotype to the gene) or reverse genetic studies (gene to phenotype) of mutants can be used to determine gene function (5). Thus induced mutagenesis remains a useful technique for breeders as it is a rapid and inexpensive method for developing new alleles and phenotypes. In addition, new techniques will enable the identification of mutant alleles used in the development of successful mutant cultivars, shedding light on gene function and agricultural productivity. Gamma rays as a physical mutagen used to induce mutation which may cause higher incidence of genetic mutation in plants (6). It induces different type of variation as compared to other radiation (7). Gamma irradiation induces both direct and indirect damage on plants by changing their DNA structure and promotes the production of new varieties (8, 9). High yielding mutant varieties are developed through radiation with improved quality and are resistant to various stress factors (10). The fulfillment of mutation breeding mainly depends on choice of favorable mutants based on phenotypic characters (11).

Green gram is an important legume crop, cultivated in different agro ecological conditions of India. It is a vital ingredient of cuisines and is fed on as cooked complete seed, split grain, flour or as sprouts. It has a rich source of nutritional protein (24-28%), carbohydrate (59-65%), fiber (3.5-4.5%), mineral (4%) and ash (4.5-5.5%) (12). It is one of the widely distributed crops among the vigna species. Lysine and tryptophan, essential amino acids are mainly found in the green gram along with other proteins. It is relatively drought-tolerant and suited to various soil conditions, relatively drought-tolerant and suited to various soil conditions with limited irrigation (13). The green gram seeds were exposed to varying doses of gamma rays such as 100 Gy, 200 Gy, 300 Gy, 400 Gy, 500 Gy, 600 Gy, 700 Gy and 800 Gy based on the previous studies to find out the optimum dose. The optimum dose is determined to minimize the gamma irradiation's negative effects on seeds and mutation frequency is also high (14). The finding of chlorophyll and morphological mutants served as a primary index of induced mutation to generate the desirable mutants.

The genetic impact of the mutagen can be indicated with the aid of chlorophyll mutation, the first vital organic modification in any induced mutation (15). It is used as a marker in plant breeding for evaluating the gene action of mutagenic factors. Due to its lethal nature, it can be used to determine the threshold dose of mutagen and enhance genetic variability. The frequency of chlorophyll mutants is a primary index for estimating the genetic alteration of mutagenic treatment in plants and also reveals the presence of viable mutations in mutagen-treated populations. It is possible that the effect of gamma rays may cause nuclear gene mutations or chromosomal mutations which could lead to chlorophyll-deficient mutations (16). Chl metabolism and its regulation as well as the identification of gene function depend on chlorophyll mutations. The decrease in photosynthetic rate may be attributed to the mutation that occurs in the gene responsible for their production and based on the color pattern of leaves chlorophyll mutants were classified (17). The effectiveness and efficiency of mutagens are needed for the high frequency of desirable mutations (18). Mutagenic effectiveness is concerned with the dose of the mutagen to produce the rate of mutation (19), whereas efficiency means the rate of mutation related to damage. Chlorophyll mutants are being used as the primary index of the mutagenic effectiveness and efficiency (20). Principal component analysis (PCA) is a tool used to find out the characteristics which subsidize the yield and promising dose for maximum yield. It eliminates the repetition in data sets as morphological and physiological variations routinely occur. It has a strong inter correlation in a set of variables and each component explains percent (%) variation to the total variability. The first principal component (PC) is the major contributor to the total variation in the population followed by succeeding components. The first 3 principal components are often reflecting the variation patterns (21). By this identification, characteristics were done for the selected dose which could be utilized for crop development.

Thus this study aimed to assess the effect of gamma irradiation by screening the presence of chlorophyll and viable mutants, spectrum and frequency of macro mutations (chlorophyll and morphological mutations) to evaluate the mutagenic effectiveness and efficiency of gamma irradiation and principal component analysis to explain the relationships among the studied traits under all doses.

## Materials and Methods

### Seed material and source of mutagen

The dried healthy seeds of green gram variety "Vamban 2" were obtained from National Pulse Research Centre (NPRC), Vamban, Pudukkottai, Tamil Nadu, India and exposed to varying doses of gamma irradiation such as 100 Gy, 200 Gy, 300 Gy, 400 Gy, 500 Gy, 600 Gy, 700 Gy and 800 Gy from Cobalt-60 ($Co_{60}$) in gamma irradiation chamber at Bhabha Atomic Research Centre (BARC) Mumbai. The untreated seeds were used as a control.

### Raising of $M_1$ and $M_2$ generation

After completion of treatment, gamma irradiated seeds along with untreated seeds as control was sown in the field as in a randomized block design to rise M1 generation. Standardized agronomic practices such as irrigation, weeding and plant protection methods were carried out during the growth period of crop. The bulk seeds harvested from $M_1$ cultural practice were done during the period.

## Observation of mutants

### Chlorophyll mutants

$M_2$ generation seedlings were screened for chlorophyll mutants from seed germination for the first 4 weeks. Viable chlorophyll and morphological mutants were screened throughout the life span of the plant from germination to maturity of the crop. Chlorophyll mutants were classified into various types using the described method (22, 23).

$$\text{Chlorophyll mutation frequency (\%)} = \frac{\text{Total no of chlorophyll mutants}}{\text{Total no of M2 seedlings observed}} \text{X } 100$$

### Leaf mutants

Mutation frequency of the leaf mutants was calculated in $M_2$ generation using following formula

$$\text{Leaf mutation frequency (\%)} = \frac{\text{No of plants with leaf mutant characters}}{\text{Total no of plants survived}} \text{X } 100$$

### Mutagenic effectiveness and efficiency

In $M_2$ generation, the effectiveness and efficiency of gamma irradiation were calculated using the standard formula (19) based on the frequency of chlorophyll and leaf mutants and expressed as a %.

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

$$\text{Mutagenic efficiency} = \frac{\text{Mutation frequency (\%)}}{\text{Biological damage in M2 generation}}$$

*Biological damage was measured based on seedling injury (% of reduction in plant height) and lethality (% of survival reduction/reduction in germination).

### Quantitative characteristic and principal component analysis of $M_2$ generation

In $M_2$ generation, at maturity (75$^{th}$ day) plants from all the doses with control plants were separately harvested and the following morphological parameters such as Plant height (cm), number of branches per plant, number of leaves per plant, days to first flowering, days to 50% flowering, number of fruit cluster per plant, number of pods per plant, number of seeds per plant, 100 seed weight (gm) and seed yield per plant were characterized and PCA analysis was investigated for traits under treated doses by XLSTAT software.

## Result

### *Frequency and spectrum of chlorophyll mutation*

Chlorophyll mutations are the first important biological alterations induced by the mutagens. It seems to be a very useful method for evaluating the effect of gamma irradiation doses in creating genetic variation. It also called as leaf color mutation or chlorophyll deficiency mutation. Different types of chlorophyll mutants such as albino, aurea, striata, tigrina, xantha, chlorina, viridis, xanthaviridis and variegated were noted in $M_2$ plant progenies. It was recorded in the field during the first 15 days at seedling stage and it was grouped into lethal and viable mutants based on the intensity of pigmentation. The characteristics of these mutations

| Chlorophyll mutants | Description |
|---|---|
| ***Albina*** | Seedlings shows white in color because of lacking chlorophyll and it survives only for few days and it is a lethal mutant. |
| ***Xantha*** | Seedling shows yellowish color leaf because of the presence of carotenoids and the absence of chlorophyll. It can survive up to 3-4 leaf stages (8-10 days) and it is a lethal mutant. |
| ***Chlorina*** | Seedling shows a light green color leaf and survives till maturity. It is a viable mutant. |
| ***Viridis*** | Seedling shows light yellowish-green color (viridine green) leaves at starting stages of growth and it is slow growing. Seedling survived to maturity and it is viable mutants. |
| ***Xanthaviridis*** | Seedling shows light yellow to dark green color leaves and it survive upto maturity. It is a viable mutant. |
| ***Aurea*** | Seedling shows a golden yellow color leaf and it is a viable mutant. |
| ***Striata*** | Seedling show longitudinal strips of various colors on their leaves and it is viable mutant. |
| ***Tigrina*** | Seedlings show yellow color at the edges of leaves sometimes green patches are also seen and survive upto 3-4 weeks. |
| ***Variegated*** | Seedling shows yellow, white and green color stripes on the leaf lamina and it is a viable mutant. |

were classified with some modifications:

In the present assessment, the frequency of chlorophyll mutants was calculated based on the appearance of chlorophyll mutants on a 900 M2 plant basis and it was shown in Fig. 1. It shows that the frequency has been increased with increasing doses but at the maximum doses it has decreased. The maximum frequency was observed in 500 Gy (1.553%) followed by 300 Gy (0.991%) and 400 Gy (0.969%) of gamma irradiation doses. The least frequency was recorded at 700 Gy (0.589%). Among the different types of chlorophyll mutants observed, Albino showed the highest frequency % (0.229) followed by Viridis (0.163) and Xantha (0.131) and the frequency was given in Table 1.

**Table 1.** Frequency of chlorophyll mutant spectrum in gamma irradiation treatment of $M_2$ generation

| Chlorophyll mutants | Gamma irradiation | | | | | | | | | Total number (Gy) |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Control** | **100 Gy** | **200 Gy** | **300 Gy** | **400 Gy** | **500 Gy** | **600 Gy** | **700 Gy** | **800 Gy** | |
| No of plants studied | **900** | **828** | **825** | **807** | **825** | **837** | **681** | **678** | **624** | **6105** |
| Albino | - | 3 | | 3 | 1 | 3 | 3 | | 1 | 14 (0.229) |
| Aurea | - | | 1 | | 1 | | | | | 2 (0.032) |
| Striata | - | | | 1 | 1 | 1 | 1 | | 1 | 5 (0.081) |
| Tigrina | - | | 2 | | | 1 | | 1 | | 4 (0.065) |
| Xantha | - | 1 | 1 | | 2 | 2 | | 1 | 1 | 8 (0.131) |
| Chlorina | - | | 1 | | 1 | 2 | | 1 | | 5 (0.081) |
| Viridis | - | 2 | | 2 | 1 | 3 | 1 | | 1 | 10 (0.163) |
| Xanthaviridis | - | | 1 | | 1 | | | 1 | | 3 (0.049) |
| Variegated | - | 1 | 1 | 2 | | 1 | | | | 5 (0.081) |
| **Chlorophyll mutation frequency (%)** | - | **0.845** | **0.848** | **0.991** | **0.969** | **1.553** | **0.734** | **0.589** | **0.640** | **0.912** |

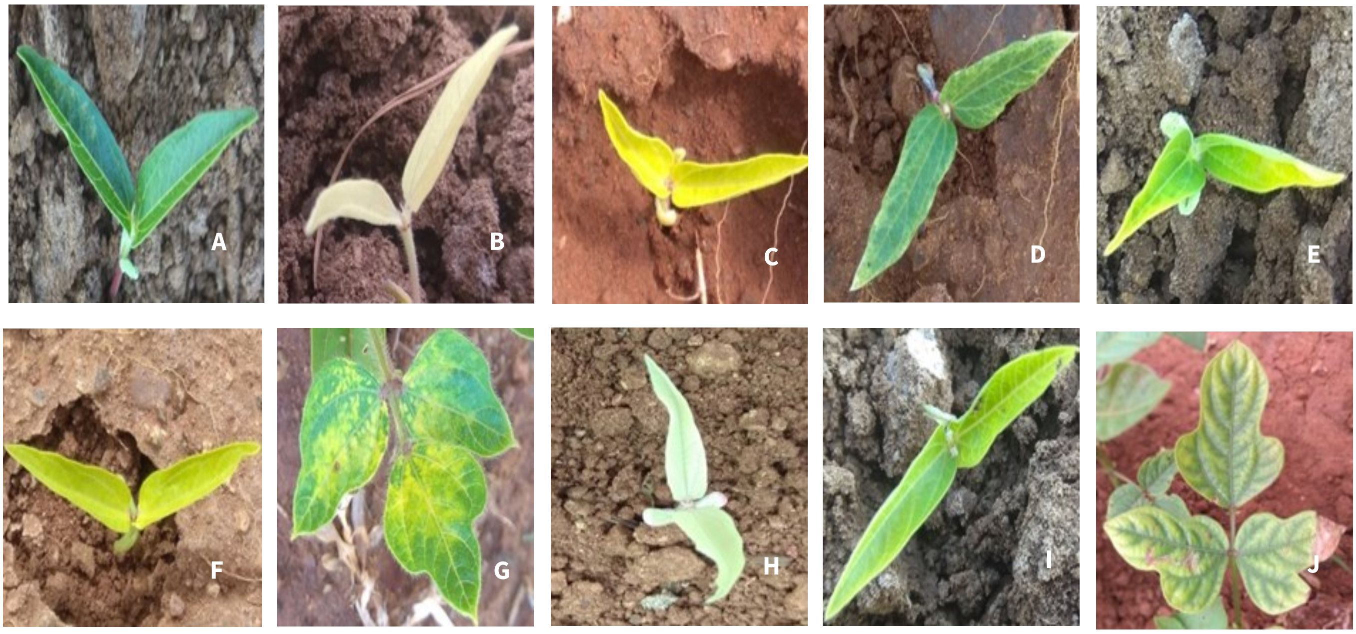


**Fig. 1.** Chlorophyll mutants in $M_2$ generation under the effect of gamma irradiation.
A- Control B- Albino C- Aurea D- Striata E- Tigrina F- Xantha G- Chlorina H- Viridis I- Xanthaviridis J- Variegated.

### Frequency and spectrum of leaf mutants

Twenty-three types of mutants affecting cotyledonary leaf (unifoliate, trifoliate primary, quadrifoliate and penta foliate) and leaf (ovate, bilobed, obcordate, trifoliate with abnormal, lyrate, ligulate, oblong, shrinked, pentagon-shaped hooked leaf tip, mucronate, ovate with two-lobed, deeply lobed, lorate, subglobose, cuneate, hastate, round shape with deeply lobed, deltoid with obtuse leaf apex, trifoliate with unequal leaf) were recorded in M2 generation and it was shown in Fig. 2 The frequency of theses mutants shows the highest in 500 Gy (2.38%) and lowest was observed in 700 Gy (1.02%) and it was shown in the Table 2.

### Effectiveness and efficiency of mutagenic treatment

Mutagenic effectiveness and efficiency determination can be helpful to select the optimum dose of mutagen. Among the different doses of gamma irradiation, mutagenic effectiveness shows a maximum of 100 Gy (0.030) and a minimum was 800 Gy (0.002). Mutagenic

**Table 2.** Frequency of Leaf mutant in $M_2$ generation under the effect of gamma irradiation

| Leaf mutant | Gamma irradiation | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 100 Gy | 200 Gy | 300 Gy | 400 Gy | 500 Gy | 600 Gy | 700 Gy | 800 Gy |
| **Total number of plants studied** | **828** | **825** | **807** | **825** | **837** | **678** | **681** | **624** |
| Ovate | 1 | - | 1 | - | 1 | - | - | 1 |
| Bilobed | 2 | - | - | 2 | - | 2 | 1 | - |
| Obcordate | - | 1 | - | - | - | - | - | - |
| Trifoliate with abnormal | 1 | - | 2 | - | 1 | | 1 | |
| Lyrate | 1 | | | | 1 | | | |
| Ligulate | | 1 | | | 1 | | | |
| Oblong | | | | 1 | | 1 | | 1 |
| Shrinked | 2 | 1 | | | 2 | 1 | | |
| Pentagon shaped hooked leaf tip | 2 | | 2 | 1 | 2 | | 1 | 1 |
| Mucronate | 1 | 2 | | | 1 | | | |
| Ovate with two lobed | 1 | 1 | 2 | | 1 | | 1 | |
| Deeply lobed | 1 | 1 | | 1 | | | | |
| Lorate | | 1 | 1 | | | | | |
| Sub globose | 1 | | 2 | | 2 | | | 1 |
| Cuneate | | | | | 1 | | | |
| Unifoliate | | 1 | | 1 | | | | |
| Trifoliate primary | 1 | 1 | 1 | | 1 | 1 | 1 | 1 |
| Quardrifoliate | | | 1 | | | | | 1 |
| Penta foliate | 1 | 1 | | 2 | | | | |
| Hastate | | | | | | 1 | | |
| Round shape with deeply lobed | 1 | | | 1 | 2 | | 1 | 1 |
| Deltoid with obtuse leaf apex | 1 | | 1 | 1 | 1 | | 1 | 1 |
| Trifoliate with unequal leaf | 1 | 1 | 2 | | 3 | 1 | | 1 |
| **Total** | **18** | **12** | **15** | **10** | **20** | **7** | **7** | **9** |
| **Mutation frequency** | **2.1** | **1.45** | **1.85** | **1.21** | **2.38** | **1.03** | **1.02** | **1.44** |

effectiveness was more efficacious in lower doses when compared to higher doses because the mutagen induced was more effective in increasing doses of gamma irradiation. Mutagenic efficiency was calculated

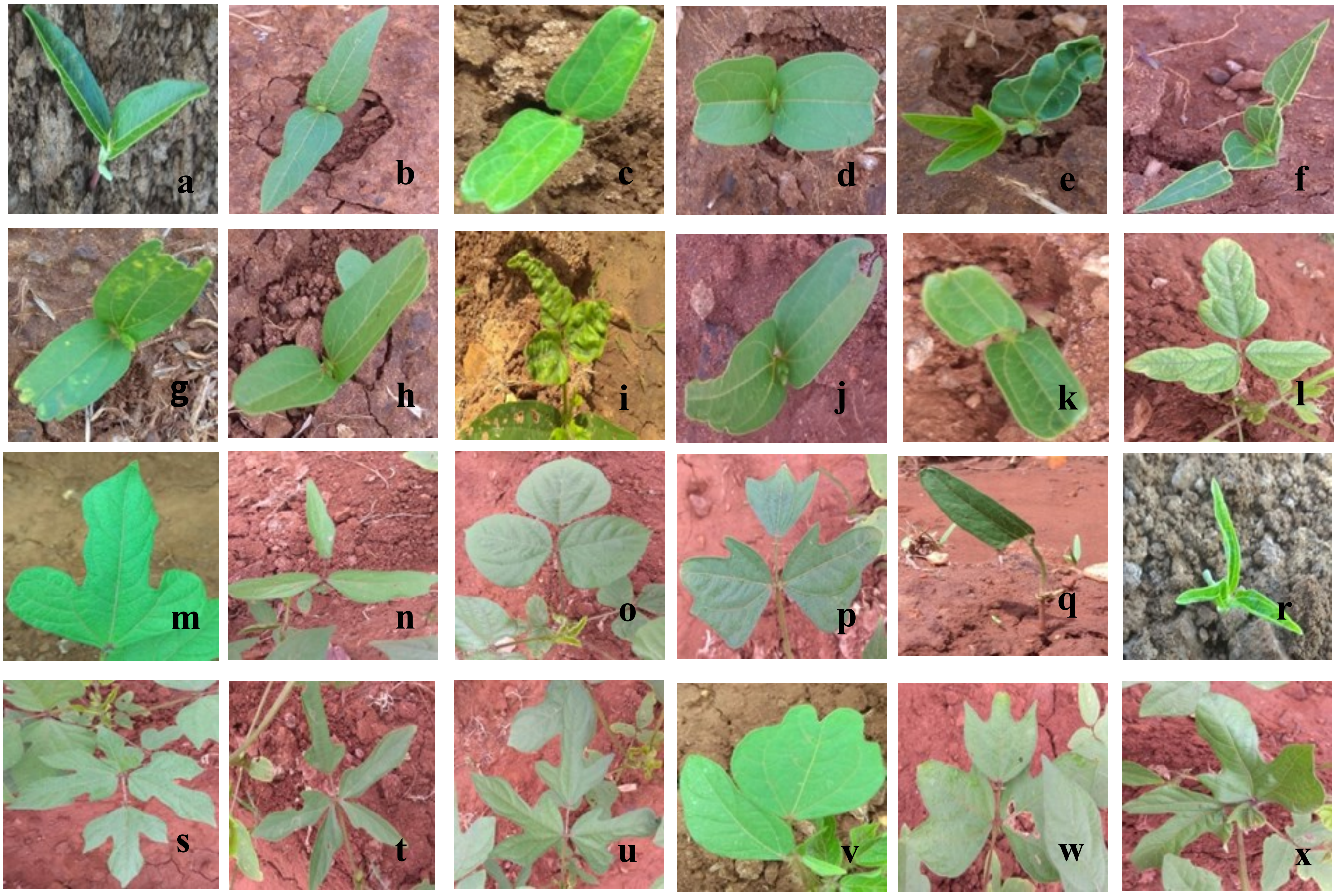


**Fig. 2.** Leaf mutants in $M_2$ generation under the effect of gamma irradiation.

**a** - Control **b** - Ovate **c** - Bilobed **d** - Obcordate **e** - Trifoliate with abnormal **f** – Lyrate **g** - Ligulate **h** - Oblong **i** - Shrinked **j** - Pentagon shaped hooked leaf tip **k** - Mucronate **l** - Ovate with two lobed **m** - Deeply lobed **n** - Lorate **o** - Sub globose **p** – Cuneate **q** - Unifoliate **r** - Trifoliate primary **s** - Quardrifoliate **t** - Penta foliate **u** - Hastate **v** - Round shape with deeply lobed **w** - Deltoid with obtuse leaf apex **x** - Trifoliate with unequal leaf.

based on the biological damage caused by seedling lethality and seedling injury. Efficiency related to lethality was increased in 500 Gy (0.477) and decreased in 700 Gy (0.065) and related to injury it showed an increased value in 100 Gy (0.903) and decreased value in both 700 and 800 Gy (0.070). It shows that the moderate dose of gamma irradiation (500 Gy) was more efficient compared to other doses and this was given in Table 3.

### *Quantitative characteristic analysis of M2 generation*

In M2 generation, high significant reduction was noted in 800 Gy for all parameters studied and it was given in Fig. 3. The plants of M2 generation flowered on the 31$^{st}$ day in control and this was increased with an increased dose in 800 Gy as on the 34$^{th}$ day. This similar trend was also shown for the days taken for 50 % flowering which was highest in 800 Gy (43$^{rd}$ day) as compared to the control (45$^{th}$ day). Plant height among the treatments was found to

**Table 3.** Effectiveness and efficiency of gamma irradiation in $M_2$ generation

| Doses | Survival reduction (%) (L) | Height reduction (%) (I) | Mutation frequency (%) (M) | Effectiveness M/Gy | Efficiency | |
|---|---|---|---|---|---|---|
| | | | | | M/L | M/I |
| **100 Gy** | 8.00 | 3.33 | 3.01 | 0.030 | 0.376 | 0.903 |
| **200 Gy** | 8.33 | 6.18 | 2.30 | 0.011 | 0.276 | 0.372 |
| **300 Gy** | 10.33 | 10.13 | 2.85 | 0.009 | 0.275 | 0.281 |
| **400 Gy** | 8.33 | 13.33 | 1.69 | 0.004 | 0.202 | 0.126 |
| **500 Gy** | 7.00 | 12.82 | 3.34 | 0.006 | 0.477 | 0.260 |
| **600 Gy** | 24.33 | 19.29 | 1.76 | 0.002 | 0.072 | 0.091 |
| **700 Gy** | 24.66 | 23.13 | 1.62 | 0.003 | 0.065 | 0.070 |
| **800 Gy** | 30.66 | 29.66 | 2.08 | 0.002 | 0.067 | 0.070 |

have a significant difference in 800 Gy (47.66±0.85) as compared to the control (67.77 ± 0.58). The branches per plant show a reduction in 800 Gy (1.1±0.05) as compared to the control (1.73 ± 0.08). A significant difference was found in the number of leaves which shows negative effects on 800 Gy (22.8±0.78) as compared to the control (45.9±0.81). The yield contributing characters such as fruit cluster/ plant (5.70±0.26), pods/plant (20.80±1.13), seeds/plant (7.27±0.35), 100 seed weight (3.25±0.10) and seed yield/ plant (7.12±0.24) were reduced in 800 Gy as compared to a number of fruit cluster/plant (17.03± 0.18), pods/plant (41.73± 0.98), seeds/plant (12.20± 0.15), 100 seed weight (3.51± 0.03) and seed yield/plant (11.16± 0.23) of control plants.

### *Principal component analysis*

In this present study, PCA was performed for variable traits and traits contributing towards yield in different doses of gamma irradiation. It reveals that out of eleven, only 6 principal components (PCs) exhibit more than 1.00 eigenvalues and showed 79.03% total variability of traits studied under 300 Gy followed by 400Gy which shows five principal components with 74.34% of the total variance, in 200 Gy 5 principal component with 71.56% of the total variance and in 500 Gy which exhibits 5 principal components with 70.82% of the total variability. Based on the eigenvalue and total variability studies 500 Gy shows a positive response to yield characters with 5 PCs and it was given in Fig. 4.

The first component (PC1) shows 22.60 % of the variation and the traits such as plant height, no of branches, no of fruit clusters, pod length, no of seeds per pod, 100 seed weight, days to first flowering and days to 50 % flowering showed the positive and no of leaves, no of pods, seed yield per plant shows the negative one. 14.40% of the variation was explained by the 2nd principal component (PC2) and the highest value was scored by the variables such as plant height, no of leaves, no of pods, pod length, no of seeds per pod, seed yield per plant, days to 1st flowering and days to 50% flowering whereas lowest values were seen in no of branches, no of fruit and 100 seed weight. A variance of the 3rd principal component was 12.48% and the factors loading with the high score was only in plant height, no of branches, no of fruit cluster, 100 seed weight and seed yield per plant showing the positive score and

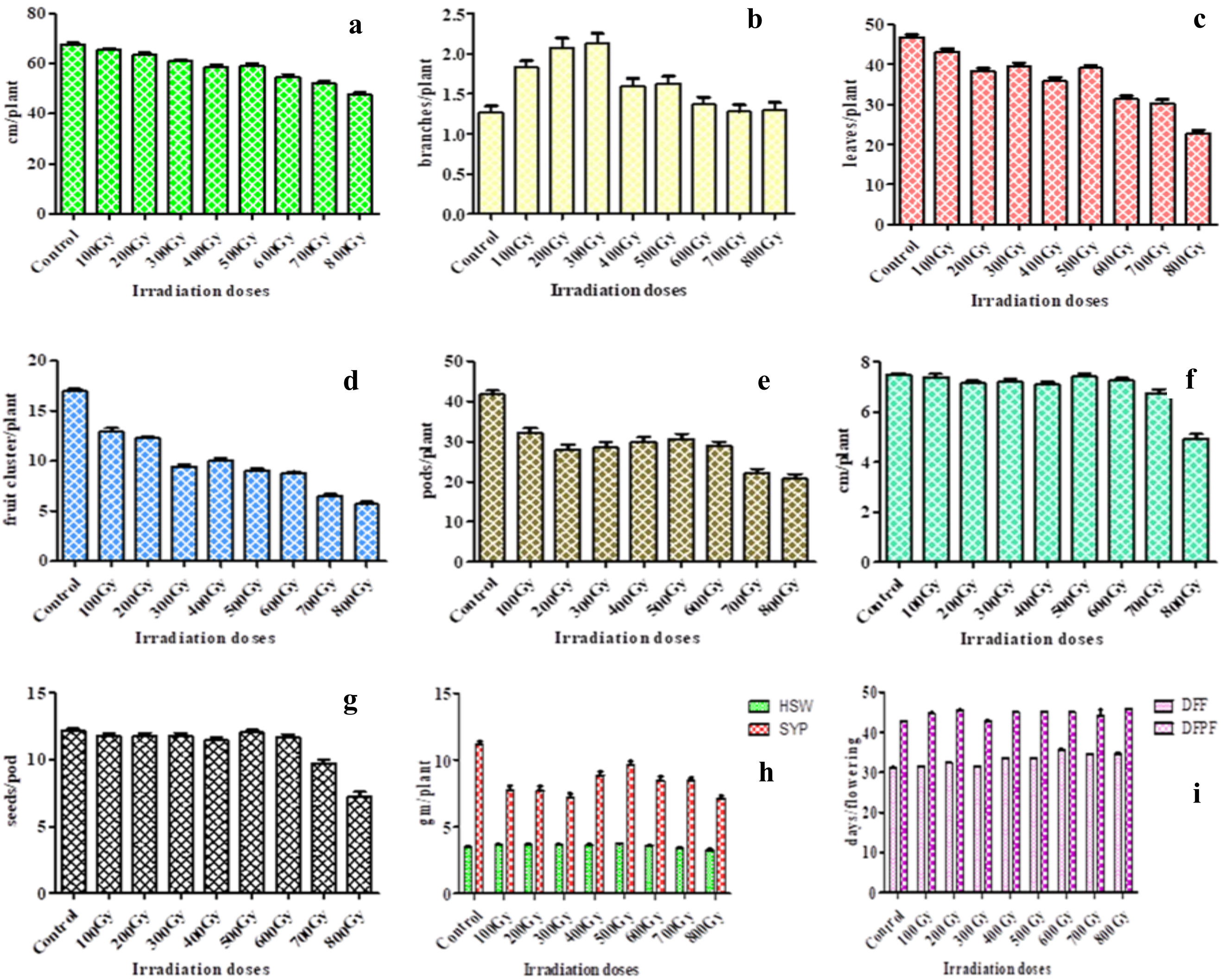


**Fig. 3.** Quantitative characteristics analysis of $M_2$ generation.

a) Plant height b) number of branches c) number of leaves d) number of fruit cluster e) Number of pods f) pod length g) number of seeds h) hundred seed weight and seed yield per plant i) days to first flowering and days to fifty percent flowering

| 100 Gy | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 | PC9 | PC10 | PC11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Eigenvalue | 2.573 | 1.686 | 1.576 | 1.426 | 0.992 | 0.974 | 0.802 | 0.333 | 0.261 | 0.215 | 0.162 |
| Variability (%) | 23.387 | 15.330 | 14.328 | 12.960 | 9.016 | 8.857 | 7.288 | 3.027 | 2.376 | 1.953 | 1.477 |
| Cumulative % | 23.387 | 38.717 | 53.044 | 66.005 | 75.021 | 83.878 | 91.167 | 94.194 | 96.570 | 98.523 | 100.000 |
| 200 Gy | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 | PC9 | PC10 | PC11 |
| Eigenvalue | 2.286 | 1.811 | 1.579 | 1.131 | 1.065 | 0.857 | 0.752 | 0.536 | 0.443 | 0.385 | 0.155 |
| Variability (%) | 20.786 | 16.461 | 14.352 | 10.283 | 9.681 | 7.791 | 6.836 | 4.874 | 4.025 | 3.499 | 1.413 |
| Cumulative % | 20.786 | 37.246 | 51.598 | 61.881 | 71.562 | 79.353 | 86.189 | 91.062 | 95.087 | 98.587 | 100.000 |
| 300 Gy | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 | PC9 | PC10 | PC11 |
| Eigenvalue | 1.915 | 1.744 | 1.438 | 1.351 | 1.169 | 1.077 | 0.755 | 0.640 | 0.387 | 0.339 | 0.186 |
| Variability (%) | 17.405 | 15.853 | 13.070 | 12.285 | 10.626 | 9.793 | 6.863 | 5.819 | 3.514 | 3.085 | 1.687 |
| Cumulative % | 17.405 | 33.258 | 46.329 | 58.614 | 69.239 | 79.032 | 85.895 | 91.713 | 95.228 | 98.313 | 100.000 |
| 400 Gy | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 | PC9 | PC10 | PC11 |
| Eigenvalue | 2.815 | 1.553 | 1.411 | 1.218 | 1.176 | 0.811 | 0.698 | 0.522 | 0.346 | 0.300 | 0.150 |
| Variability (%) | 25.591 | 14.122 | 12.830 | 11.071 | 10.691 | 7.373 | 6.349 | 4.741 | 3.142 | 2.726 | 1.365 |
| Cumulative % | 25.591 | 39.713 | 52.543 | 63.613 | 74.304 | 81.677 | 88.026 | 92.767 | 95.909 | 98.635 | 100.000 |
| 500 Gy | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 | PC9 | PC10 | PC11 |
| Eigenvalue | 2.487 | 1.585 | 1.373 | 1.264 | 1.082 | 0.884 | 0.819 | 0.734 | 0.386 | 0.230 | 0.156 |
| Variability (%) | 22.605 | 14.408 | 12.482 | 11.487 | 9.840 | 8.034 | 7.442 | 6.675 | 3.513 | 2.094 | 1.420 |
| Cumulative % | 22.605 | 37.013 | 49.495 | 60.982 | 70.822 | 78.856 | 86.298 | 92.973 | 96.486 | 98.580 | 100.000 |
| 600 Gy | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 | PC9 | PC10 | PC11 |
| Eigenvalue | 2.338 | 1.870 | 1.209 | 1.190 | 1.002 | 0.941 | 0.776 | 0.747 | 0.388 | 0.364 | 0.175 |
| Variability (%) | 21.251 | 17.004 | 10.994 | 10.822 | 9.106 | 8.555 | 7.053 | 6.788 | 3.523 | 3.313 | 1.591 |
| Cumulative % | 21.251 | 38.256 | 49.250 | 60.072 | 69.178 | 77.733 | 84.786 | 91.574 | 95.097 | 98.409 | 100.000 |
| 700 Gy | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 | PC9 | PC10 | PC11 |
| Eigenvalue | 2.639 | 1.473 | 1.466 | 1.343 | 0.970 | 0.903 | 0.831 | 0.511 | 0.414 | 0.288 | 0.162 |
| Variability (%) | 23.987 | 13.392 | 13.331 | 12.205 | 8.820 | 8.210 | 7.557 | 4.647 | 3.764 | 2.615 | 1.473 |
| Cumulative % | 23.987 | 37.379 | 50.710 | 62.915 | 71.735 | 79.945 | 87.502 | 92.149 | 95.912 | 98.527 | 100.000 |
| 800 Gy | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 | PC9 | PC10 | PC11 |
| Eigenvalue | 2.464 | 1.561 | 1.346 | 1.206 | 1.068 | 0.964 | 0.698 | 0.623 | 0.455 | 0.313 | 0.301 |
| Variability (%) | 22.401 | 14.192 | 12.233 | 10.960 | 9.708 | 8.767 | 6.349 | 5.665 | 4.139 | 2.848 | 2.738 |
| Cumulative % | 22.401 | 36.593 | 48.827 | 59.786 | 69.494 | 78.261 | 84.610 | 90.275 | 94.414 | 97.262 | 100.000 |

**Fig. 4.** Eigen values, variability percentage and cumulative percentage for 11 traits under gamma irradiation treatment.

**PC1-** Plant height , **PC2**- No of branches, **PC3**- No of leaves, **PC4-**No of fruit cluster, **PC5**- No of pods **PC6**- Pod length, **PC7**- No of seeds per pod, **PC8**-100 seed weight, **PC9**- Seed yield per plant, **PC10**- Days to first flowering **PC11**-Days to 50% flowering

no of leaves, no of pods, pod length, no of seeds per pod, days to first flowering and days to 50% flowering showed the negative score. The $4^{th}$ PC shows 11.48% and the traits no of fruit cluster, no of pods, pod length, no of seeds per pod, 100 seed weight, seed yield per plant and fays to first flowering shows the plus value and plant height, no of branches, no of leaves shows the minus value. The $5^{th}$ principal component shows 9.84% with the positive response of the traits such as plant height, no of leaves, no of seeds per pod, 100 seed weight, days to first flowering and negative response with no of branches, no of fruit cluster, no of pods, pod length, seed yield per plant, days to 50% flowering. The remaining PCs explained the 8.03%, 7.44%, 6.67%, 3.51%, 2.09% and 1.42% of total variance individually. Scree plot corroborates the percentage of variance associated with each principal component by a graph between eigenvalue and principal component number and it was shown in Fig. 5. Biplots were constructed based on the gamma irradiation doses and quantitative characters analysis which showed the observed characters correlation with the particular dose and it was given in Fig. 6.

## Discussion

The selection of the optimum dose of gamma irradiation can be useful to produce the high frequency of desirable mutants. It can be done by evaluating the basic alteration which can be produced by inducing gamma irradiation on a particular crop. In this study, green gram seeds were exposed to different doses of gamma irradiation such as 100, 200, 300, 400, 500, 600,700 and 800 Gy. Chlorophyll

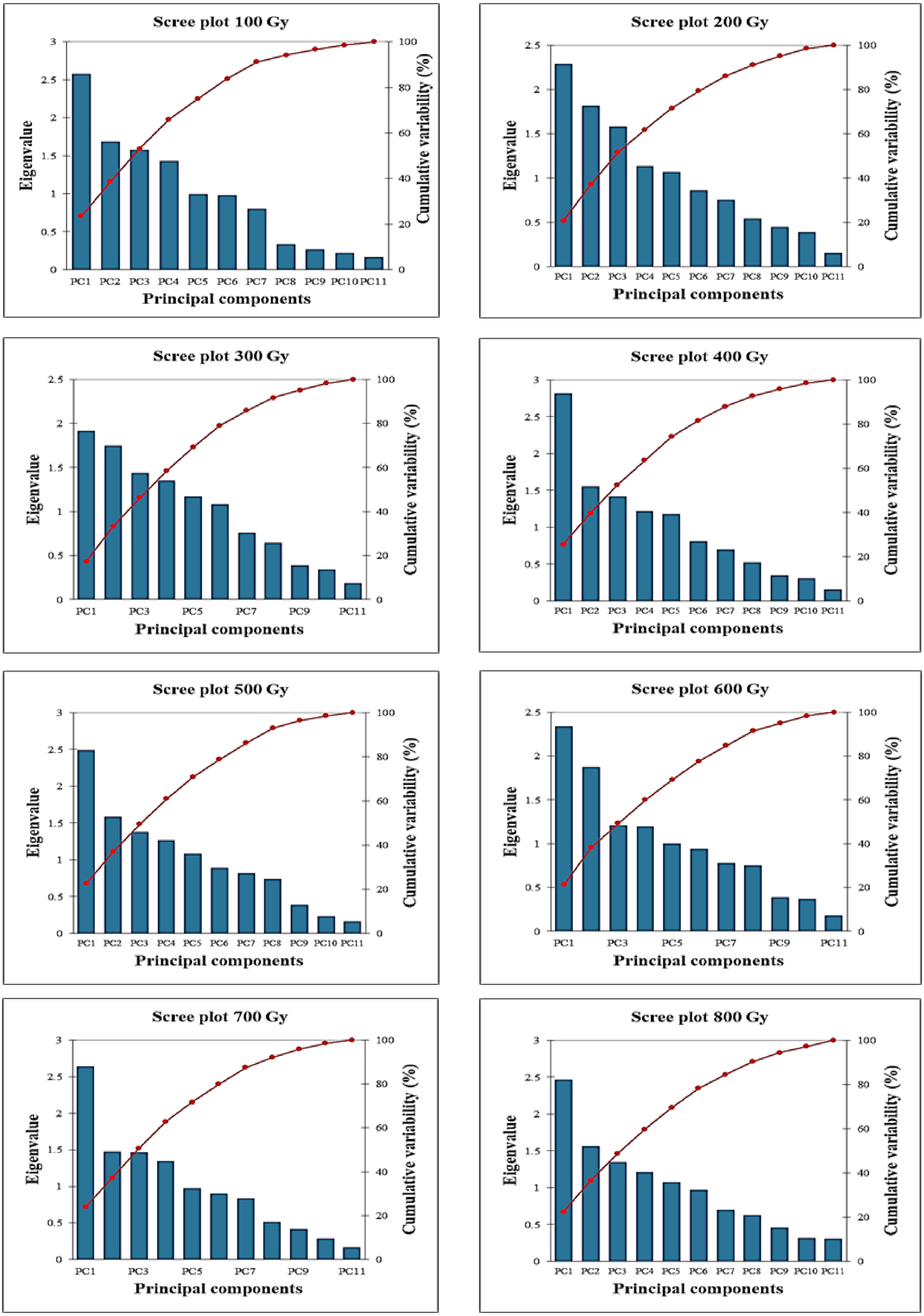


**Fig. 5.** Scree plot constructed using 11 PCs for gamma irradiation treatment.

mutants were found almost in all the irradiation doses and the frequency of chlorophyll mutation was highest in 500 Gy which was supported by the previous study on *Zingiber officinale* (24).The appearance of chlorophyll mutants such as albino, aurea, striata, tigrina, xantha, chlorina, viridis, xanthaviridis and variegated were found inM2 plant basis and it accordance with green gram (25-29) and in black gram (30). The occurrence of chlorophyll-deficient mutants in the treated gamma irradiation could not survive for a long period. These mutants are of no economic value but their frequency was deliberated to measure the effectiveness and efficiency of induced mutation by gamma irradiation and also served as a primary index for evaluating the genetic alteration which was caused by the mutagens. A wide spectrum of leaf mutants was found in gamma-irradiated treatment and has been reported earlier in green gram (31) and in cowpea (32). The effectiveness and efficiency of mutagens are important parameters to exploit induced mutagenesis for crop improvement. The mutagenic effectiveness was found to be increased in lower doses (100 Gy) and decreased with increasing doses. The efficiency of gamma irradiated treatment was high in 500 Gy based on lethality and in 100 Gy based on the seedling injury and it indicates less biological damage. Similar results were worked out by so many workers in *Vigna unguiculata* L. (33), *Pisum sativum* L. (34) in green gram (35), lentil (36) and cluster bean (37) under physical, chemical and in combination treatment.

Quantitative characters such as days to 1$^{st}$ flowering, days to 50% flowering, plant height, number of branches, number of leaves and yield characters such as no of fruit clusters, no of pods, pod length, no of seeds per pod, 100 seed weight, seed yield per plant showed significant difference among the gamma-irradiated doses. In this, all the parameters show the decreased content with increased doses and the maximum was noted in 800 Gy. Due to the disturbance of biochemical pathways, the flowering may be delayed, which supports the synthesis of flower-inducing substances (38). Days to 50 % flowering were delayed in gamma-irradiated seeds when compared to control and this was reported in cowpea (39) and in black gram (40). Plant height was used widely as an index to regulate the genetic effects of physical mutagens, such as gamma rays and it was reported in black gram (41) and in green gram (42). It can cause effects on variability in plant height and based on this selection could be made for both tall and dwarf varieties (43). The number of branches reduced might be due to mutagenic treatment like auxin destruction and chromosomal impairment associated with physiological changes (44). Similar results of reduction have been reported in black gram (45) and decreasing consequence on the number of leaves was reported in *Lepidium sativum* (46).

Yield parameters were decreased might be due to the disturbance in meiosis which was affected by the frequency of spores and it affects the traits directly. Similar results have been reported in cowpea (47), black gram (48), chickpea (49), pigeon pea (50), soybean (51) and in Sunflower (52). Thus, in this study it revealed that the reduction of quantitative parameters was prominent in increased doses. To find out the important trait that means to assess the useful morphological characters, principal component analysis is used in this study. From the quantitative analysis of M2 generation plants, it showed that the yield component can be used as consistent criteria to improve the seed yield of green gram induced by gamma irradiation. This can be proved by PCA results sketched that the 500 Gy shows the optimum dose which shows that the first five principal components attributed to 70.82% total variability of traits studied. Factors analysis reduced eleven variables into 5 factors which show the positive as well as negative efforts on the yield parameters. Similar results were noted for quantitative characters and qualitative characters (53) and other reports are on the first 5 components show nearly 79% of the total variation in green gram (54). Therefore, it was identified that gamma irradiation of 500 Gy resulted in considerable variation for most of the yield characters analyzed.

## Conclusion

In the present study, it was inferred that there were dose-dependent changes in all the parameters analyzed. Among the chlorophyll mutants, albino occurred more frequently, followed by viridis and xantha and the frequency of occurrence was highest in 500 Gy as compared to other doses. Leaf mutant as a part of viable mutant resulted in gamma irradiation treatment of 500 Gy at the highest frequency. Mutagenic effectiveness was high at 100 Gy and efficiency was high at 500 Gy compared to other doses, and it also indicates that both mutagenic effectiveness and efficiency levels were high from low to moderate doses. Based on these results, the quantitative characters of $M_2$ generation were exposed to PCA analysis and 500 Gy shows a positive correlation with yield characters, with 70.82% of the total variation. From the above findings it was concluded that among the different doses of gamma irradiation, 500 Gy was an optimum dose to increase the genetic variability and economically useful mutants were segregated into further generations.

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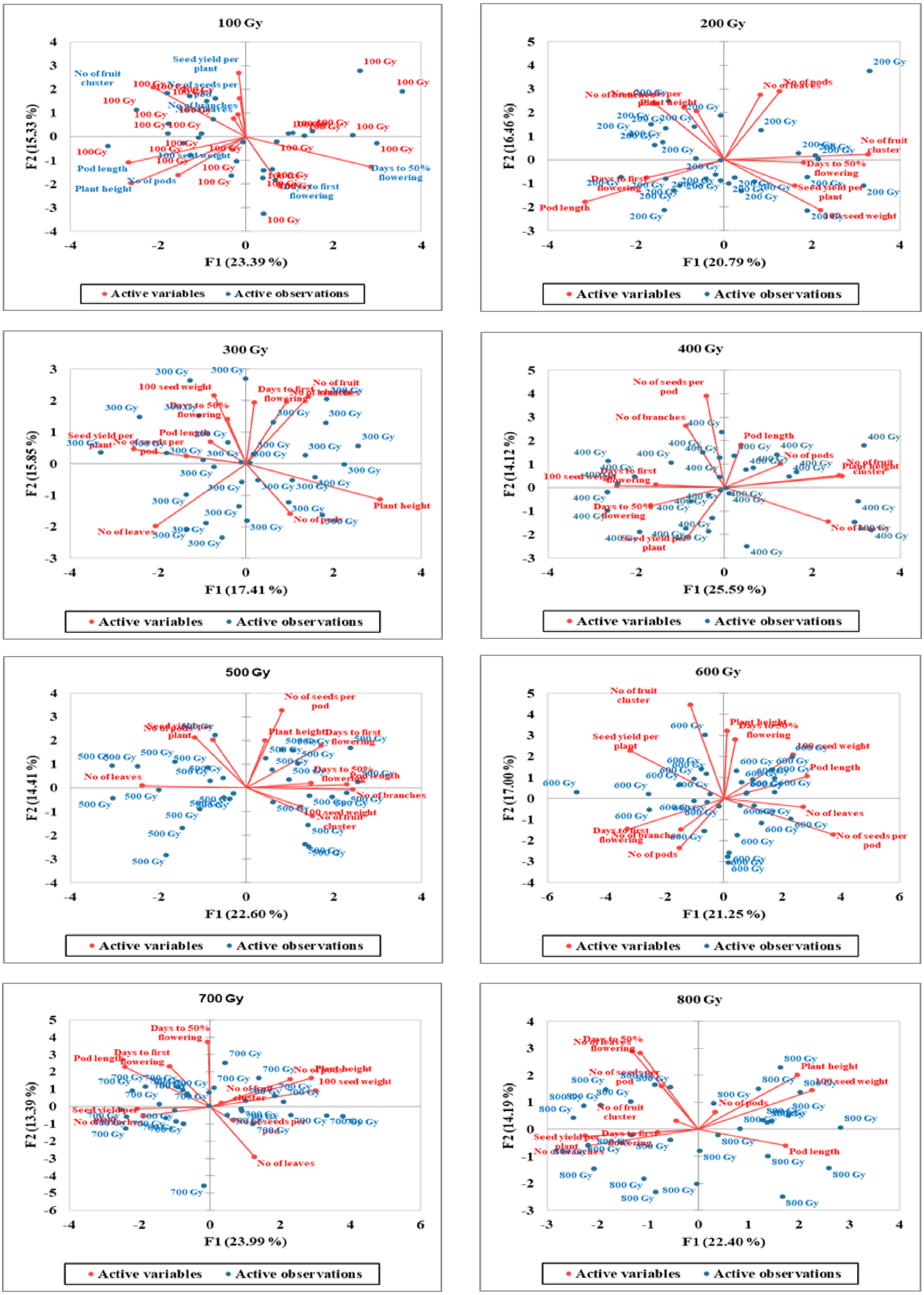


**Fig. 6.** Biplots for all observed parameters assessed in green gram from different doses of gamma rays. Red color indicated the parameters studied and blue color indicated the different doses of gamma irradiation

## Authors contributions

Laboratory experiment, analysis of data, interpretation and statistical analysis was conducted by DAB and VS; Fieldwork and data collection for VS; composing a manuscript VS, DAB, AR, SG, KY, GB and BV.

## Compliance with ethical standards

**Conflict of interest**: The authors declare no conflict of interest.

**Ethical issues**: None.

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