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## ASSESSING IMPACT OF GAMMA IRRADIATION IN FIELD BEAN AND SELECTION OF PUTATIVE MUTANTS THROUGH MUTATION FREQUENCY ANALYSIS

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### ABSTRACT

In order to improve field bean growth and production, an experiment was carried out at the College of Horticulture (COH), Anantharajupeta, with the goal of identifying the ideal radiation quality and dose range. Using cobalt-60 ( $Co^{60}$ ) as the radiation source, seeds of the well-known field bean variety TFB-2 were treated to several dosages of gamma rays (10, 20, 30, 40, 50 and 60kR). After that, the irradiated seedlings were sown in polybags and petri plates, with untreated seeds acting as the control. After seven days for seeds on petri plates and thirty days for seeds in polybags, the germination percentages were evaluated. For the various gamma ray dosages, the germination rates varied from 35.55% to 80% on petri plates and from 33.33% to 80.95% in polybags. On the other hand, the untreated control seeds germination rates were 93.65% and 93.33% in polybags and petri plates, respectively. With an increase in radiation dosage, a progressive decrease in seedling growth and percentage germination was seen.  $T_6$  (60kR) had the lowest seedling growth, measuring 26.56 cm, whereas  $T_1$  (10kR) showed the highest seedling growth, measuring 46.56 cm. The  $LD_{50}$  value, according to Probit analysis, was 30kR. After that, 1000 seeds were exposed to 30 kR of radiation and seeded to produce the  $M_1$  generation. In addition, unirradiated seeds were used as a control. The mutagenic population outperformed the control in a number of quantitative and biochemical measures. Based on the mutation frequency, to go on to the next generation,  $M_{184}$ ,  $M_{169}$ ,  $M_{205}$ ,  $M_{161}$ ,  $M_{162}$ ,  $M_{76}$ ,  $M_{258}$ ,  $M_{167}$ ,  $M_{111}$ ,  $M_{76}$ ,  $M_{317}$  and  $M_{155}$ , which displayed superiority in number of primary branches plant<sup>-1</sup>, pod yield and seed yield plant<sup>-1</sup>, hence these mutant plants have been selected for progressing to  $M_2$  generation.

**Key words :** Gamma ray, Germination, Mutagenic population, Probit analysis.

### Introduction

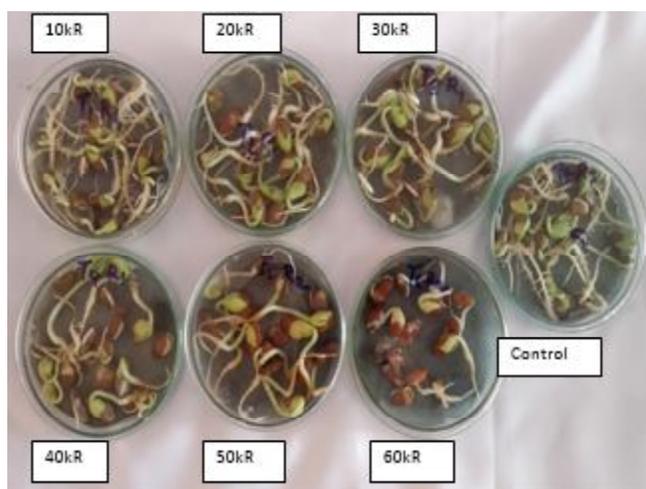
Field bean [*Lablab purpureus* var. *lignosus* (L.) Prain] is one among the Fabaceae family ( $2n=22$ ). It's common names are Egyptian kidney bean, Dolichos bean, lablab bean, hyacinth bean and bonavist bean. It's origin in India and presently grown throughout the tropical regions of Asia, Africa and America (Deka and Sarkar, 1990). The crop's restricted diversity, resulting from its cleistogamous nature, hinders crop development because

its genetic variability percentage is quite low. One of the finest methods for increasing crop variety and supersizing the genetic basis is mutation breeding. Gamma rays have tremendous capacity to create variability (Chakraborty and Parthasarathy, 2003). Gamma rays have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the radiation dose (Ashraf *et al.*, 2003). Hence with an intention to increase the variability in the field bean,

mutation study was carried out. In which  $LD_{50}$  value through probit analysis was done based on germination and seedling growth. Classification based the quantitative and biochemical parameters in  $M_1$  mutagenic population was done when seeds were treated 30kR ( $LD_{50}$  dose). The frequency of mutations indicates a plant parameter's superiority above the control mean, based on which the mutants selected to go for next generation.

### Materials and Methods

In the late *kharif* season of 2018–19, the experiment was carried out at College Of Horticulture, Anantharajupeta, Andhra Pradesh. For this experiment, field bean seeds of the genotype Tirupati Field Bean-2 (TFB-2) were obtained. The seedlings were exposed to  $Co^{60}$  (Cobalt-60) gamma irradiation at the Bhabha Atomic Research Centre in Trombay, Maharashtra. TFB-2 seedlings treated with doses of 10, 20, 30, 40, 50, 60 kR and untreated seeds served as the control group. Completely Randomised Design (CRD) with three replications and seven treatments was used for the first experiment. Thirty plants made up each replications, and fertiliser at the prescribed amount of 75:100:100 kg/ha was applied. Seeds were sown in petri plates under laboratory conditions, and after ten days the percentage of germination was recorded in order to calculate the  $LD_{50}$  value of gamma irradiation. Similar to this, seeds were sown in polybags, and the percentage of germination was recorded. In polybags during a month-long period, the plants were observed for several growth and biochemical traits. Based on the percentage of germination in both petri plates and polybags, the  $LD_{50}$  value was calculated using probit analysis; the findings revealed that the probit values were 34.67kR and 33.11kR, respectively.



**Plate 1 :** Effect of different doses of gamma irradiation on germination percentage of field bean cv. TFB-2 in petri plates.



T<sub>1</sub>: 10kR

T<sub>2</sub>: 20kR



T<sub>3</sub>: 30kR

T<sub>4</sub>: 40kR



T<sub>7</sub>: 0kR (Control)

**Plate 2 :** Effect of different doses of gamma irradiation on germination percentage of field bean in cv. TFB-2 polybags.

As a result, it was determined that 30kR was the ideal gamma irradiation dosage for field beans. Next, 1000 seeds were planted next to unirradiated seeds as a control after being treated to 30 kR of radiation in order to generate the  $M_1$  generation. Every single plant was given a set of data in  $M_1$ , which were then categorised using both quantitative and biological factors.

### Results and Discussion

The radiation dosages given to field bean seeds significantly affected the percentage of germination, which is observed in both petri plates and polybags after seven days of sowing. Different growth and biochemical traits indicated substantial differences after 30 days after seeding, which were altered by the gamma radiation treatment. The germination % was calculated using the

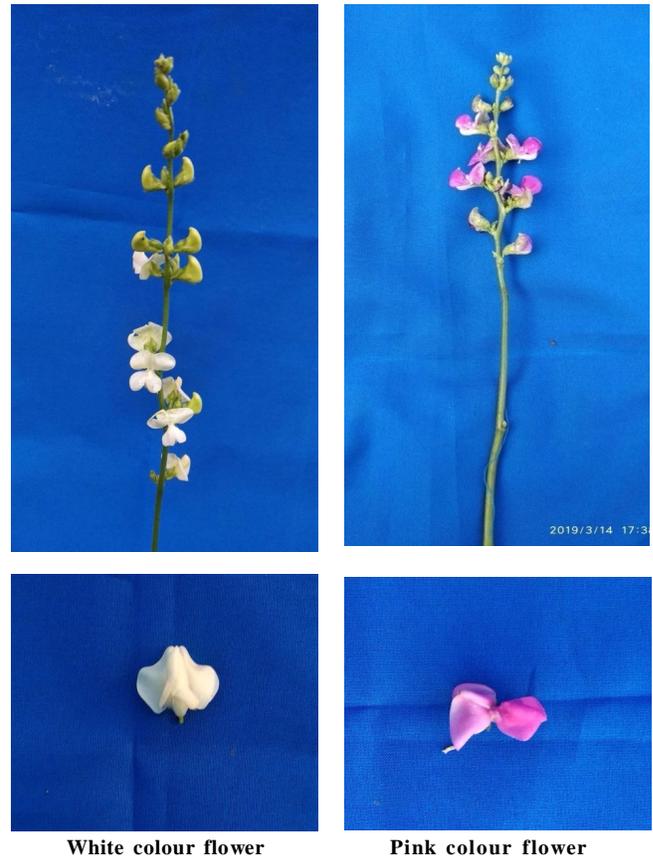
formula

$$\text{Germination \%} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

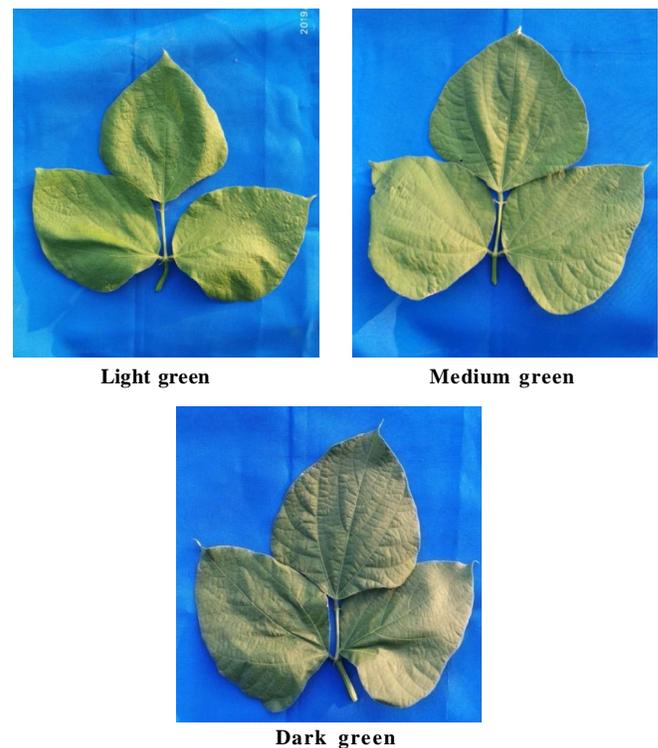
In both petri plates and polybags, the seeds treated with gamma rays showed a substantial decrease in seed germination compared to the control ( $T_7$ ) (93.33% and 93.65%, respectively). Of the irradiated seeds placed in the petri plates,  $T_1$  (10kR-80%) showed the considerably highest percentage of seed germination, while  $T_6$  (60kR-35.55%) showed the significantly lowest percentage. The germination % of the seeds in the polybags showed a similar pattern; the treatment  $T_1$  (80.95%) recorded the highest germination percentage, while the treatment  $T_6$  (60kR-33.33%) recorded the lowest. Based on the percentage of germination in both petri plates and polybags, the  $LD_{50}$  value was calculated using probit analysis, with the probit value coming out at 34.67kR and 33.11kR, respectively (Table 1), In accordance with this, 30kR was determined to be the ideal gamma irradiation dose for field beans. A decrease in vigour may have resulted from seeds being physically exposed to mutagen gamma rays, which ultimately causes germination to fail. Kiong *et al.* (2008) mentioned that a decrease in the quantity of internal growth regulators, which regulate germination, may be the reason of the drop in the germination % brought on by the high radiation dosage. 1000 seeds were irradiated with 30 kR and placed alongside the unirradiated seeds as a control in order to produce the  $M_1$  generation. Merely 392 plants emerged from the 1000 seeds, allowing for the categorization of the  $M_1$  mutagenic population according to several quantitative and biochemical criteria.

At 30 days after sowing, five healthy seedlings were chosen at random to observe the shoot length. The measurement of the shoot length was taken in centimeters (cm) from the collar area to the cotyledon attachment site. With a length of 21.53 cm,  $T_1$  (10 kR) had the longest shoots of all the irradiated plants, while  $T_6$  (60 kR) had the shortest, measuring 17.46 cm. The results indicated that  $T_7$  (unirradiated plants) had a maximum shoot length (22.80 cm). There were notable variations across the dosages, and it displayed that shoot length tended to progressively decrease as radiation exposure increased (Table 1).

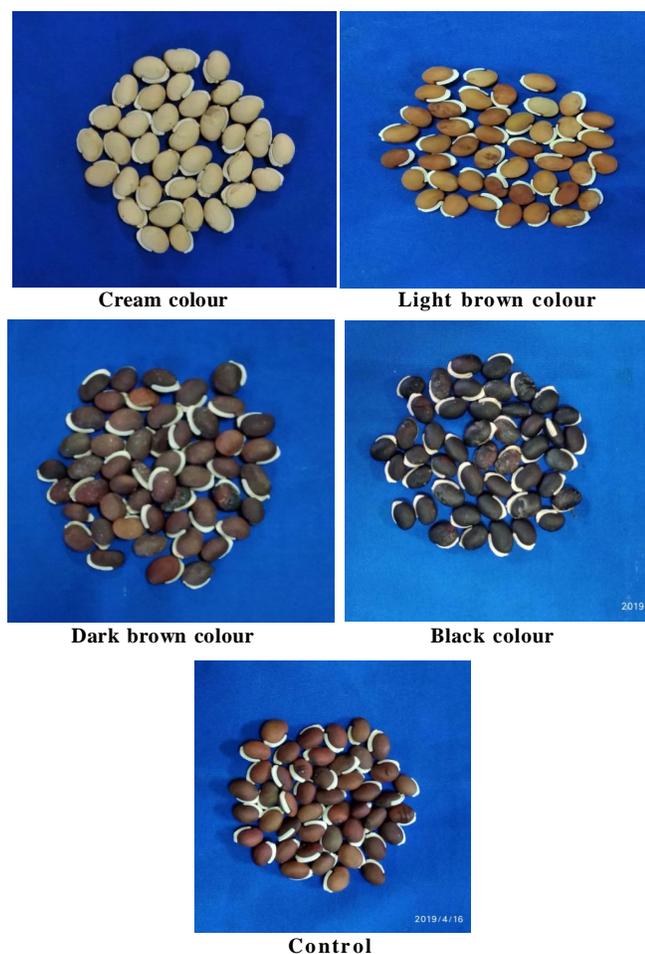
After 30 days of sowing, five healthy seedlings were chosen at random for each treatment. From the collar area to the tip of the root, the length of the root was measured. The seedlings' average root length was calculated and computed in centimetres. The superior root length was found in  $T_1$  (10kR-25.33 cm), whereas  $T_6$  (60kR-9.10 cm) showed the lowest root length among



**Plate 3 :** Effect of gamma irradiation on flower colour of field bean.



**Plate 4 :** Effect of gamma irradiation on leaf colour of field bean cv. TFB-2.



**Plate 5 :** Effect of gamma irradiation on seed colour of field bean.

irradiated seeds. The greatest root length of 25.40 cm was seen in the  $T_7$  (control) (Table 1). Gamma-ray dosages that were lower showed a stimulatory impact on root length. The activation of growth hormones like auxin as well as cell division rates were theorised to be the cause of these irradiation-induced stimulations (Zaka *et al.*, 2004).

By counting the leaves on seedlings at 30 days after sowing, the number of leaves was determined. The highest number of leaves (19.10) displayed by control group. Of the irradiated population, number of leaves recorded highest in  $T_1$  (10kR-18.0) and  $T_6$  (60kR-15.43) displayed lowest number of leaves (Table 1). With increasing dosage of irradiation reduced number of leaves was observed. The results were concurrent to study of Jagajantham *et al.* (2012) in okra. It is also hypothesised that, by slowing down the rate of physiological processes, enzymes necessary for leaf initiation at higher gamma irradiation levels may have prevented and halted cell division (Akhtar, 2014). The reduction in leaf count may be ascribed to alterations in plant shape and function brought about by gamma irradiation, which are influenced



**Plate 6 :** Measurement of inflorescence and pod length.

by different dosages and exposure times (Piri *et al.*, 2011).

For leaf length randomly 5 leaves from five healthy seedlings were collected for each treatment. The length was measured in centimetres (cm) from the apex of the blade to the base of the petiole and average was calculated. For leaf length,  $T_7$  (control plants) displayed average of 5.73 cm. Of the irradiated plants  $T_4$  (40kR-6.06 cm) displayed maximum leaf length while  $T_2$  (20kR-3.66 cm) showed lowest leaf length (Table 1). Except in  $T_3$  and  $T_2$ , among treatments reduction in leaf length over control was observed. The results coincided with the observations in muskdana (Priyanka and Animesh, 2012) and in okra (Maryum and Kasimu, 2016). Whereas, Asare *et al.* (2017) in okra observed negative trend.

Five leaves from five healthy seedlings were chosen at random for each treatment for leaf width and measured at the widest point perpendicular to the longitudinal axis of the leaf in centimetres (cm).  $T_4$  (40kR-5.9 cm) and  $T_2$  (20kR-3.5 cm) showed highest and lowest leaf width respectively, among irradiated plants while  $T_7$  (control) showed 5.53 cm. With the exception of  $T_3$  and  $T_4$ , there was a significant decrease in leaf breadth in irradiated plants when compared to control (Table 1). Higher radiation dosages than 40 kR led to a considerable reduction in leaf size. There has been evidence of a progressive reduction in leaf size in brassica species as radiation intensities rise (Rafiullah and Hasan, 1994). As the irradiation dosage increased from 10 kR to 40 kR, the leaf length and leaf breadth gradually increased in comparison to the untreated control. Reduced radiation

**Table 1 :** Impact of gamma irradiation on various traits after 30 days of sowing.

Dosage	G1(%)	G2(%)	SL (cm)	RL (cm)	NOL	LL(cm)	LW(cm)	Phl.(mg/100g)
T <sub>1</sub> (10kR)	80.00	80.95	21.53	25.03	18.00	3.66	5.13	516.25
T <sub>2</sub> (20kR)	65.55	68.25	19.53	23.06	17.16	5.50	3.50	587.56
T <sub>3</sub> (30kR)	51.11	49.20	19.43	24.53	16.93	5.93	5.73	683.88
T <sub>4</sub> (40kR)	44.44	44.44	18.80	20.96	16.83	6.06	5.90	747.46
T <sub>5</sub> (50kR)	40.00	39.68	18.03	15.13	16.26	4.83	4.66	786.40
T <sub>6</sub> (60kR)	35.55	33.33	17.46	9.10	15.43	4.36	4.20	824.46
T <sub>7</sub> (Controle)	93.33	93.65	22.60	25.40	19.10	5.73	5.53	536.40
C.D.	1.92	1.81	2.04	2.04	0.92	0.24	0.27	2.73
SE(m)	0.63	0.59	0.66	0.66	0.30	0.07	0.09	0.89
SE(d)	0.89	0.83	0.94	0.73	0.42	0.11	0.12	1.26
C.V.	6.20	8.36	5.88	4.38	3.05	2.64	3.17	0.23

RT- Root length

SL- Shoot length

LL-Leaf length

G1- Germination in petri plates

NOL- Number of leaves

Phl- Phenol

G2- Germination in polybags

LW- Leaf width

levels resulted in a stimulatory effect that increased leaf growth. Studies in jatropha by Nayak *et al.* (2015) had similarity with the results.

The phenol was estimated based on the method developed by Singleton *et al.* (1965). The phenol level of the untreated control plants was 536.40 mg/100g. T<sub>6</sub> had the greatest phenol level among the irradiated plants (60 kR-824.46 mg/100g), whereas T<sub>1</sub> had the lowest phenol content (10 kR-516.25 mg/100g) (Table 1). A correlation was observed between the dosage of gamma irradiation and the quantity of phenols. Oufedjikh *et al.* (2000) in red saunders. Phenolic molecules are essential for the biotic and abiotic stress tolerance of plant secondary metabolites. It is well known that phenylalanine ammonia lyase (PAL), which acts as precursor for production of polyphenolic chemicals, becomes more active under stress from gamma irradiation. By interacting with free radicals, these polyphenolic chemicals can also protect DNA damage caused by gamma radiation (Akshatha *et al.*, 2016).

#### Classification of mutagenic population (392 plants) based on ranges of quantitative and biochemical parameters

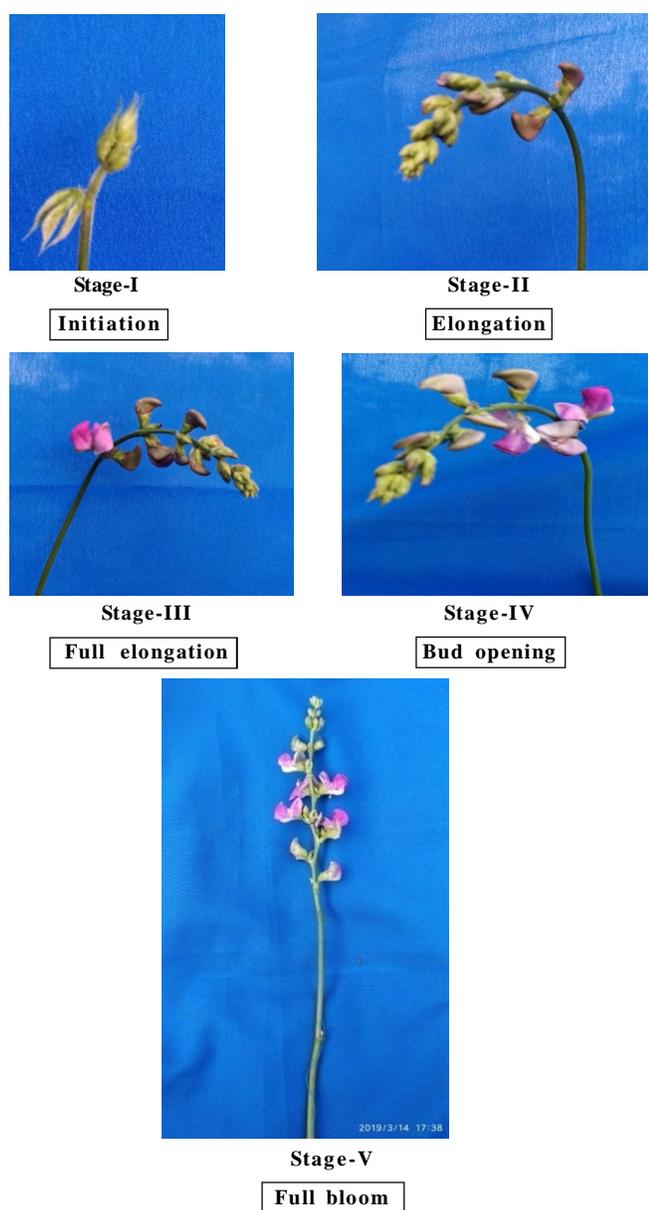
The range for plant height varied from 75.8 to 87.57 cm. For the untreated control, the average plant height was 87.57 cm. Among the plants whose seeds were subjected to 30kR irradiation, M<sub>39</sub> recorded a maximum height of 93.2 cm while M<sub>1</sub> recorded the minimum height of 75.80 cm. Among the irradiated plants 28 mutants (7.14%) were classified as tall, 257 mutants were classified as medium tall (65.56%) and 107 mutants were

grouped as short (27.29%).

Number of primary branches plant<sup>-1</sup> for control recorded was average of 3.9. Of the irradiated plants, 34 mutants recorded a maximum count *i.e.*, 4, while 225 irradiated plants recorded 2. The range recorded was 2 to 4. Among the mutant population, 57.39 % plants (225 mutants) showed less, while more number of branches plant<sup>-1</sup> were observed in 42.6% plants (167 mutants).

Days to first flowering in the untreated control were measured, and the mean was 43.8 days. While M<sub>110</sub>, M<sub>152</sub>, M<sub>198</sub>, M<sub>222</sub>, M<sub>256</sub> and M<sub>280</sub> had late flowering (56 days), M<sub>128</sub> and M<sub>392</sub> displayed early flowering (41 days) among the irradiated plants. Days to first blooming in the mutagenic population varied from 41 to 56 days. Based on the range, early flowering was recorded in 54 mutants (13.77%), whereas 246 mutants (62.5%) showed mid season flowering and 93 mutants (23.72%) recorded late flowering.

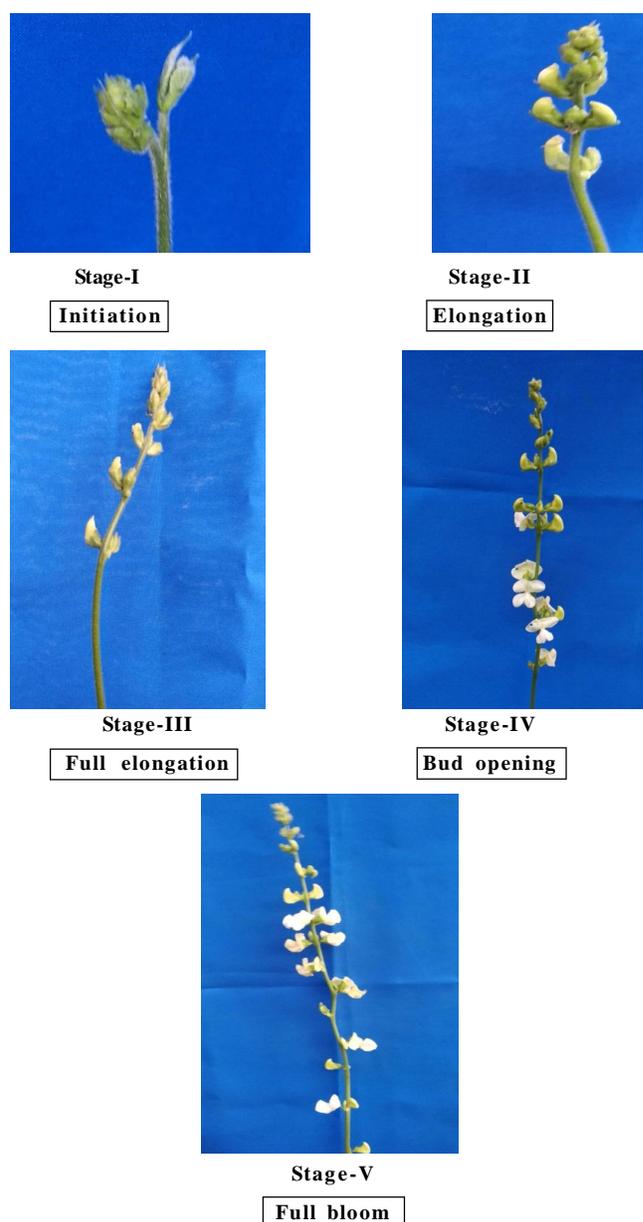
Among mutagenic population, days to 50% flowering was observed within 48 days (early) in M<sub>128</sub>, whereas it took 63 days (late) in M<sub>110</sub>, M<sub>152</sub>, M<sub>198</sub>, M<sub>222</sub>, M<sub>256</sub> and M<sub>280</sub>. The data obtained for days to 50% blooming in the mutant population varied from 48 to 63 days, according to the categorization based on ranges. Based on the range recorded, 47 mutants (11.98%) took less days to 50% flowering whereas 246 mutants (62.75%) recorded medium number of days taken to 50% flowering (53.57 days), while more number of days were taken for 50% flowering in 79 mutants (20.15%). Whereas, the control plants took 51.1 days for 50% flowering. However, 20 mutants (5.1%) did not flower during the entire crop duration.



**Plate 7 :** Developmental stages of inflorescence with pink flowers.

The control mean for the number of flowers inflorescence<sup>-1</sup>, based on the reported data, was 26.06. Among the gamma ray induced plant population  $M_{98}$  displayed the highest number of flowers inflorescence<sup>-1</sup> (29.12) while the lowest number of flowers inflorescence<sup>-1</sup> was recorded in  $M_4$  (8.34). The data secured for number of flowers inflorescence<sup>-1</sup> showed a range of 8.34 to 29.12. Of the irradiated plants highest number of plants were grouped under medium range with 200 mutants (51.02%) while the low range was recorded in 96 mutants (24.48%) and 76 mutants (19.38%) showed a high range.

The mean length of the inflorescence in control was 24.54cm. Among the mutant population the maximum inflorescence length was displayed in  $M_{112}$  (27.9 cm)



**Plate 8 :** Developmental stages of flowers of control plants.

and minimum inflorescence length was observed in  $M_6$  (10.66 cm). Range for the length of inflorescence recorded was 10.66 - 27.9 cm. Based on the recorded range, highest mutant population were grouped under medium inflorescence length *i.e* 211 mutants (53.82%), whereas 138 mutant plants (35.2%) were grouped as having lengthy inflorescence.

The general mean recorded for control plants for the number of inflorescences plant<sup>-1</sup> was as 30.10. Among the mutagenic population maximum number of inflorescences plant<sup>-1</sup> was displayed in  $M_1$  (41), while the minimum number of inflorescences plant<sup>-1</sup> was recorded in  $M_{191}$  (13). From the data observed the range for the number of inflorescences plant<sup>-1</sup> recorded in the mutant

plants was 13 to 41. Among the irradiated plants 235 (59.94%) plants were classified as having medium number of inflorescences plant<sup>-1</sup>, whereas 122 irradiated plants (31.12%) were classified as having less number of inflorescences plant<sup>-1</sup>.

M<sub>205</sub> (26.08) recorded the maximum number of pods inflorescence<sup>-1</sup>, while the minimum number of pods inflorescence<sup>-1</sup> were recorded in M<sub>316</sub> (5.77), while the control plants recorded 23.09 pods inflorescence<sup>-1</sup>. The range for number of pods inflorescence<sup>-1</sup> in mutant plants varied from 5.77 to 26.08. Based on the range recorded, 176 irradiated plants (44.89%) were grouped as having medium number of pods per inflorescence. 139 irradiated plants (35.45%) were classified as having less number of pods inflorescence<sup>-1</sup>.

The number of pods plant<sup>-1</sup> was maximum in M<sub>258</sub> (769) and minimum in M<sub>316</sub> (98), while the control plants recorded a mean of 544.6 pods per plant<sup>-1</sup>. Number of pods plant<sup>-1</sup> in mutants recorded a range of 98 to 769. Data obtained showed that 192 mutants (48.97 %) were classified under the medium group followed by 159 irradiated plants which recorded less number of pods plant<sup>-1</sup> (40.56%), whereas 21 irradiated plants (5.31%) showed a high range.

Among the irradiated plant population maximum pod yield per plant<sup>-1</sup> was recorded in M<sub>205</sub> (2157.67 g), whereas minimum pod yield per plant<sup>-1</sup> was recorded in M<sub>143</sub> (156.92 g). The control plants recorded a average pod yield of 1001.77 g per plant. Data recorded for pod yield per plant<sup>-1</sup> showed a range of 156.92g to 2157.67 g. Based on the range recorded among the irradiated plants 70.40% (276 mutant plants) were classified as low yielding while 21.93% (86 mutant plants) were grouped as the medium yielding and 2.5% (10 mutant plants) were classified as high yielding group.

The maximum number of seeds pod<sup>-1</sup> were recorded in M<sub>29</sub>, M<sub>35</sub>, M<sub>68</sub>, M<sub>100</sub>, M<sub>154</sub> (4.46) and minimum were recorded in M<sub>234</sub> and M<sub>184</sub> (3.12). The control plants recorded an average of 4.46 seeds per pod. A range of 3 to 4.7 was observed in the mutant population. Based on the ranges plotted for mutants, highest number of plants were grouped as having less number of seeds per pod with 208 mutants (53.06 %), whereas 164 mutants (41.83%) showed high range for number of seeds pod<sup>-1</sup>.

The data observed for the seed yield per plant<sup>-1</sup> showed a mean of 575.40 g in control plants. Where as in the mutagenic population the maximum seed yield was recorded in M<sub>205</sub> (1341.92 g), while the minimum seed yield was recorded in M<sub>143</sub> (90.6 g). From the recorded data range plotted for seed yield varied from 90.60 to



**Plate 9:** Effect of gamma rays on root and shoot length of field bean cv. TFB-2 at 30 DAS.

1341.92 g. Among ranges plotted, 274 irradiated plants (69.89%) were grouped as having low seed yield. 89 irradiated plants (22.70%) were grouped as those having medium seed yield whereas in high seed yield was recorded in 9 irradiated plants (2.29%).

The untreated control plants recorded a average pod length of 4.92 cm. Among the mutagenic population the maximum pod length was recorded in M<sub>2</sub> (5.6 cm) and minimum pod length was recorded in M<sub>22</sub> (4.1 cm). A range of 4.10 to 5.60 cm was recorded for pod length in the mutagenic population. Based on these ranges, 214 plants (54.59%) were classified as having less pod length while 158 plants (40.38%) were classified as having high pod length.

Among the irradiated plant population maximum pod width was recorded in 27 mutagenic plants (2.1 cm), while minimum pod length was recorded in 10 mutagenic plants (1.4 cm). The pod width of untreated control was 1.93 cm. In mutant population, the range recorded for pod width varied from 1.4 to 2.1cm. Based on these ranges, 60.20% (236 plants) were classified as having more pod width, while 34.69% (136 plants) showed low range.

The general mean for pod weight in untreated control plants was 12.84 g. Among the mutagenic population maximum pod weight was recorded in M<sub>1</sub> (14.2 g), while minimum pod weight was recorded in M<sub>68</sub>, M<sub>131</sub> and M<sub>173</sub> (8.98 g). From the recorded data the range for pod weight was 8.98 to 14.2 g. Among the irradiated plants 204 (52.04%) mutants were classified under low pod weight while 168 mutants (42.85%) were grouped as having high pod weight.

Among the mutagenic population maximum 100 seed weight was recorded in M<sub>200</sub> (28.09 g), while minimum 100 seed weight was recorded in M<sub>40</sub> (18.78 g). The 100

**Table 2 :** Mutants (%) deviating from parent for various traits.

S. no.	Traits	Mean value of control	Desirable mutants count	Mutant frequency
1	Plant height (cm)	87.57	35	8.92
2	Number of primary branches plant <sup>-1</sup>	3.9	34	8.67
3	Days to 1 <sup>st</sup> flowering	43.8	15	3.82
4	Days to 50% flowering	51.1	22	5.61
5	Number of flowers inflorescence <sup>-1</sup>	26.06	28	7.14
6	Length of inflorescence (cm)	24.54	37	9.43
7	Pod length (cm)	4.92	82	20.91
8	Pod width (cm)	1.93	42	10.71
9	Pod weight (g)	12.83	25	6.37
10	Number of inflorescence plant <sup>-1</sup>	30.1	35	8.92
11	Number of pods inflorescence <sup>-1</sup>	23.09	18	4.59
12	Pod yield plant <sup>-1</sup> (g)	1001.77	51	13.01
13	Number of pods plant <sup>-1</sup>	544.6	22	5.61
14	Number of seeds pod <sup>-1</sup>	4.46	26	6.63
15	Seed yield(g)	575.4	60	15.30
16	Protein	7.87	23	5.86

**Table 3 :** Suitable mutant plants for the M<sub>1</sub> population in terms of yield and quality attributes.

Trait	Mutant plants
Pod yield (g)	M <sub>184</sub> , M <sub>169</sub> , M <sub>205</sub> , M <sub>161</sub> , M <sub>162</sub> , M <sub>76</sub> , M <sub>258</sub> , M <sub>167</sub> , M <sub>111</sub> , M <sub>76</sub> , M <sub>317</sub> and M <sub>155</sub>
Seed yield (g)	M <sub>205</sub> , M <sub>184</sub> , M <sub>169</sub> , M <sub>258</sub> , M <sub>317</sub> , M <sub>155</sub> , M <sub>76</sub> , M <sub>167</sub> , M <sub>161</sub> , M <sub>162</sub>

\*Total mutagenic population-392

seed weight in the control plants recorded a mean of 24.09 g. For 100 seed weight recorded, the range plotted in mutant population was 18.78g to 28.09g. Among irradiated plants 55.86% (219 plants) were classified as medium while 38.01 % (149 plants) of mutagenic population were classified as low whereas high range for 100 seed weight was recorded in 4 mutants (1.02%), which was lowest among the mutant population

Among the mutagenic population the maximum protein content in M<sub>382</sub> (8.06 mg/100 g) and minimum in M<sub>4</sub> (6.08 mg/100 g) was recorded, while the control recorded 7.87 mg/100 g. Recorded data for protein content in seeds of field bean was ranged from 6.08 to 8.06 mg/100 g. Among mutagenic population, 221 plants (56.37 %) were classified as having low protein while 151 plants (38.52 %) were classified as having high protein.

**Mutation frequency :** The mutation frequency for induced visible mutants were calculated in percentage for each character as suggested by Gaul (1958) by using the following formula.

$$\text{Mutation frequency \%} = \frac{\text{Number of visible mutant scored}}{\text{Total plant population in treatment}} \times 100$$

The frequency of mutations indicates a plant parameter's superiority above the control mean. Different mutation rates were observed in the irradiated plants based on their phenotypes (Table 2). 35 mutants (8.92%), 18 mutants (4.59%) for the number of pods inflorescence<sup>-1</sup>, 22 treated plants (13.01%) for number of pods plant<sup>-1</sup>, and 26 mutants (6.63%) for the number of seeds pod<sup>-1</sup> were found to have superiority above the control mean within the irradiation population. Pod yield plant<sup>-1</sup> with 51 irradiated plants (13.01%) and seed yield with 60 irradiated plants (15.30%) showed the highest frequency of mutation among the yield characteristics. According to the results, 23 mutants (5.86%) had higher protein content than the control group's mean value. The mutant plants from the M<sub>1</sub> mutant population that outperformed the control mean were transferred to the M<sub>2</sub> generation for additional research. To go on to the next generation, M<sub>184</sub>, M<sub>169</sub>, M<sub>205</sub>, M<sub>161</sub>, M<sub>162</sub>, M<sub>76</sub>, M<sub>258</sub>, M<sub>167</sub>, M<sub>111</sub>, M<sub>76</sub>, M<sub>317</sub> and M<sub>155</sub> which displayed superiority in number of primary branches plant<sup>-1</sup>, pod yield and seed yield plant<sup>-1</sup>, thus seeds of these mutant plants selected for progressing to next generation (Table 3).

## Conclusion

Based on the provided and discussed data, it can be inferred that the field bean underwent an induced mutation that resulted in decline in germination, shoot length and

root length, number of leaves, leaf length and width while showed progress in phenol content. Moreover, field beans may be effectively exposed to gamma radiation to bring about morphological, physiological, and biochemical alterations. The LD<sub>50</sub> value under petri plates and polybags, respectively, was found to be 34.67kR and 33.11kR, based on the germination percentage and seedling development. In accordance with this, 30kR was determined to be the ideal gamma irradiation dose for field beans. In comparison to the unirradiated seeds used as a control, the mutagenic population exhibited superiority in a number of metrics, when 1000 seeds were irradiated with 30kR and planted to produce the M<sub>1</sub> generation. A categorization of the mutagenic population (392 plants) was carried out using a variety of quantitative and biochemical characteristics. Based on the frequency of mutations in yield and quality traits as well as the correlation between those traits and character, putative mutants were chosen (M<sub>184</sub>, M<sub>169</sub>, M<sub>205</sub>, M<sub>161</sub>, M<sub>162</sub>, M<sub>76</sub>, M<sub>258</sub>, M<sub>167</sub>, M<sub>111</sub>, M<sub>76</sub>, M<sub>317</sub> and M<sub>155</sub>), which displayed superiority in pod yield and seed yield plant<sup>-1</sup> were selected for next generation.

#### Authors contribution

Conceptualization and designing of the research work: S. Sadarunnisa, Execution of field/lab experiments and data collection: K.N. Mahesha and K. Harish Reddy, Analysis of data and interpretation: K.N. Mahesha, S. Sadarunnisa, P. Syamsundar Reddy, C. Madhumathi and B. Tanuja Priya, Preparation of manuscript: K.N. Mahesha and S. Sadarunnisa.

#### Declaration

None of the authors have conflict of interest.

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