Effect of gamma radiation on germination and seedling growth in field bean [Lablab purpureus var. lignosus (L.) Prain] cv. TFB-2

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Abstract
Induced mutations play a significant role in the crop improvement of vegetable crops. An experiment was conducted at College of Horticulture, Anantharajupeta with an objective to establish the radiation quality and dose range for improvement of field bean. The seeds of TFB-2, a popular variety of field bean were treated with different doses of gamma rays viz., 10kR, 20kR, 30kR, 40kR, 50kR and 60kR using cobalt (Co60) as a source of radiation. The irradiated seeds along with untreated control were sown in both petri plates and polybags. Germination percentage in petri plates were assessed after seven days. The germination percentage ranged from 35.55% to 80% in petri plates and 33.33% to 80.95% in polybags for different doses of gamma rays. In case of untreated control the germination percentage in petri plates and polybags was 93.33% and 93.65% respectively. The growth of the seedlings in polybags was assessed after 30 days. A decrease in the germination and retarded seedling growth (roots and shoot length) in irradiated plants as compared to control was observed. A gradual decline in germination percentage and seedling growth was recorded with increase in irradiation dose. Lowest seedling growth (26.56 cm) was recorded in T6 (60kR) while the highest seedling growth (46.56 cm) was recorded in T1 (10kR).

Keywords: Gamma irradiation, LD50, germination percentage, field bean

Introduction
Field bean [Lablab purpureus var. lignosus (L.) Prain] belongs to the family Leguminosae (Fabaceae), with the chromosome number 2n= 22. The wild form of lablab is believed to have originated from India. The area under beans is 230 thousand hectares with production of 2278 thousand MT (NHB. 2017). Mutation breeding appears to be one of the well standardized, efficient and cost-effective breeding techniques that can be exploited for the creation of high yielding and high quality varieties. The past several decades have documented a large number of salient works regarding the utilization of radiation with special emphasis on γ-rays for evolution of eminent varieties of agricultural crops of economic importance. Hence, the present investigation was carried out to determine the lethal dose of gamma rays on germination and growth of the field bean seedlings.

Material and Methods
The experiment was conducted at college of horticulture, Anantharajupeta during late kharif 2018 to find out the effect of gamma irradiation on germination percentage of field bean variety TFB-2 and also to identify the lethal dose (LD50) value of gamma radiation. Dry and healthy seeds of locally grown field bean variety TFB-2 (Tirupati Field Bean-2) were procured for the present investigation. At Bhabha Atomic Research Centre, Trombay, Mumbai, Maharashtra, the seeds were subjected to gamma irradiation treatments with Co60 (Cobalt-60) as a source. The field bean seeds after irradiation with different doses of gamma rays viz., 10, 20, 30, 40, 50 kR and healthy untreated seeds as control were checked for the germination percentage in both petri plates and poly bag conditions. The experimental design was completely randomized design (CRD) with 3 replications. LD50 was determined by probit analysis. The seeds which were sown in petri plates were observed for the percentage of germination seven days after sowing. Under polybag conditions, seeds were sown in polybags.
Results and Discussion

Exposure of field bean seeds to different doses of irradiation significantly influenced the germination percentage recorded at seven days after sowing in both petriplates and polybags. The growth and biochemical parameters of seedlings also showed significant difference after 30 days of sowing.

Germination (%)

The field bean seeds treated with the gamma rays recorded significantly low seed germination compared to control in both petriplates (T1-93.33%) and in polybags (T1-93.65%). Among the irradiated seeds of field bean sown in the petriplates, significantly maximum germination percentage was observed in T1 (10kR-80%) and minimum seed germination percentage was observed in T6 (60kR-35.55%). Similar trend was also observed in the germination percentage of seeds in the polybags, significantly maximum germination percentage was noted with the treatment T1 (10kR-80.95%) and the treatment T6 (60kR-33.33%) the minimum seed germination percentage. The LD50 value was determined at 34.67kR and 33.11kR respectively based on probit analysis. Exposure of seeds to physical mutagen gamma rays might have caused a reduction in vigour, which in turn lead to the failure of germination. Kiong et al. (2008) [7] stated that the reduction in the percentage of germination caused by the high dose of radiation might be a result of the reduction in the amount of internal growth regulators which control germination. Shah et al. (2008) [15] opined that processes like auxin destruction, changes of the ascorbic acid content, physiological and biochemical disturbances could induce the inhibition of plant germination and development.

Shoot length (cm)

Among the irradiated plants highest shoot length was noticed in T1 (10kR-21.53 cm) where as the minimum shoot length was observed in T6 (60kR-17.46 cm). However, in the T7 (unirradiated control plants) maximum shoot length of 22.80 cm was recorded (Table. 2 and Fig. 2). It was observed that shoot length tended to decrease progressively with increasing dose of irradiation with significant disparity among the doses. Decrease in the shoot length with an increase in the irradiation dose might be attributed to the biochemical and physiological changes induced by gamma irradiation (Melki and Marouani, 2010).

Root length (cm)

T1 (10kR-25.33 cm) recorded the superior root length while minimum root length was observed in T6 (60kR-9.10 cm). However, in the T7 (control) maximum root length was recorded (25.40cm) (Table. 2 and Fig. 2). The stimulatory effect on root length was observed in the lower doses of gamma rays. The hypothetic origin of these stimulations by irradiation was due to the cell division rates as well as an activation of growth hormone, such as auxin (Zaka et al., 2004) [17].

Leaf length and leaf width (cm)

Control (T1) plants showed a mean leaf length of 5.73 cm. Among the irradiated plants maximum leaf length was observed in T4 (40kR-6.06 cm) where as lowest leaf length was recorded in T2 (20kR-3.66 cm) (Table. 4 and Fig. 4). There was a reduction in leaf length among treatments over control except in T1 and T2. The results corroborated with the findings of Priyanka and Animesh (2012) [13] in muskdana and Maryum and Kasimu (2016) [9] in okra. Where as a negative trend with respect to leaf length was observed in studies of Asare et al. (2017) [3] in okra. Among the irradiated plants highest leaf width was recorded in T4 (40kR-5.9 cm) whereas T2 (20kR-3.5 cm) recorded the lowest leaf width. Leaf width of 5.53 cm was observed in T1 (control). A significant reduction in leaf width was observed in irradiated plants compare to control except in T1 and T2. The results showed similarity with the findings of Priyanka and Animesh (2012) [12] in muskdana.

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Biochemical parameters
Phenols (mg/100g)
The untreated control plants recorded a phenol content of 536.40 mg/100g. Among the irradiated plants highest phenol content was recorded with T₆ (60kR-824.46 mg/100g) where as T₁ recorded (10 kR-516.25 mg/100g) the lowest phenol content (Table. 5 and Fig. 5). An increase in amount of phenols was recorded with increase in dose of gamma irradiation. Similar results were obtained by Oufedjikh et al. (2000) [11] in Pterocarpus santalinus and Akshatha et al. (2016) [2] in Terminalia arjuna.

For plant secondary metabolites phenolic compounds are important for biotic and abiotic stress tolerance. The stress caused by exposure to gamma irradiation is known to increase the activity of phenylalanine ammonia lyase (PAL) a precursor for the synthesis of polyphenolic compounds. These polyphenolic compounds in turn are capable of preventing the DNA damage induced by gamma rays by reacting with free radicals (Akshatha et al., 2016) [2].

Chlorophyll content (SCMR)
A significant difference was recorded in the chlorophyll content of irradiated plants. Irradiated treatment T₃ (30kR-37.70) showed highest SCMR value which was statistically on par with T₂ (20kR-37.53) followed by T₁ (10kR-37.36) where the lowest chlorophyll content was observed in T₅ (50kR-33.96) (Table. 5 and Fig. 5). The data clearly showed an increase in SCMR values in plants irradiated with 10kR to 30kR compared to the non irradiated control.

The concomitant observations were recorded by Sharma et al. (2010) [16] in pea and in cowpea by Girija and Dhanavel (2013) [4]. A gradual decline in the SCMR values was recorded in Orthosiphon stamineus when the gamma rays dosage was increased from 40kR to 60kR (Kiong et al., 2008) [7]. Kim et al. (2000) opined that the significant increase in the chlorophyll content can be correlated with stimulated growth at low doses of irradiation. Kiong et al. (2008) [7] reported that reduction in chlorophyll was due to a more selective destruction of chlorophyll biosynthesis or degradation of chlorophyll precursors.

Conclusion
From the results presented and discussed, it can be concluded that there was decrease in germination, shoot length, root length, number leaves, fresh weight dry weight and increase in leaf length, leaf width, phenol and chlorophyll content by induced mutation in the field bean. Further, gamma irradiation in field bean may be efficiently used to induce changes at morphological, physiological and biochemical level. Based on the germination percentage and seedling growth the LD₅₀ value was determined as 34.67kR and 33.11kR under petri plates and polybags respectively. Correspondingly, it was decided as 30kR as optimum dosage of gamma irradiation in field bean for future studies.

Table 1: Effect of gamma irradiation on germination of field bean cv. TFB - 2 at seven DAS under controlled conditions.

| Dosage | Germination % in petri plates | Germination % in polybags |
|--------|-------------------------------|--------------------------|
| T₁ (10kR) | 80.00 | 80.95 |
| T₂ (20kR) | 65.55 | 68.25 |
| T₃ (30kR) | 51.11 | 49.20 |
| T₄ (40kR) | 44.44 | 44.44 |
| T₅ (50kR) | 40.00 | 39.68 |
| T₆ (60kR) | 35.55 | 33.33 |
| T₇ (Control) | 93.33 | 93.65 |

C.D. 1.92 | 1.81
SE(m) 0.63 | 0.59
SE(d) 0.89 | 0.83
C.V. 6.20 | 8.36

Fig 1: Effect of gamma irradiation on germination of field bean at seven DAS in petri plates and polybags
Table 2. Effect of gamma irradiation on field bean shoot length, root length and root to shoot ratio at 30 DAS in polybags.

| Treatment | Shoot length (cm) | Root length (cm) | Root : Shoot |
|-----------|-------------------|------------------|--------------|
| T1 (10kR) | 21.53             | 25.03            | 1.16         |
| T2 (20kR) | 19.53             | 23.06            | 1.18         |
| T3 (30kR) | 19.43             | 24.53            | 1.26         |
| T4 (40kR) | 18.80             | 20.96            | 1.11         |
| T5 (50kR) | 18.03             | 15.13            | 0.83         |
| T6 (60kR) | 17.46             | 9.10             | 0.52         |
| T7 (Control) | 22.60               | 25.40            | 1.11         |

C.D. 2.04 SE(m) 0.66 SE(d) 0.94 C.V. 5.88

Table 3: Effect of gamma irradiation on fresh and dry weight of field bean seedlings at 30 DAS.

| Dosage   | Fresh weight (g) | Dry weight (g) |
|----------|-------------------|----------------|
| T1 (10kR) | 4.00              | 0.68           |
| T2 (20kR) | 3.97              | 0.65           |
| T3 (30kR) | 3.88              | 0.58           |
| T4 (40kR) | 2.91              | 0.41           |
| T5 (50kR) | 2.37              | 0.26           |
| T6 (60kR) | 1.09              | 0.19           |
| T7 (Control) | 5.18               | 1.24           |

C.D. 0.26 SE(m) 0.08 SE(d) 0.12 C.V. 4.45

Table 4: Effect of gamma irradiation on leaf characters of field bean in polybags at 30 DAS.

| Dosage   | No. of leaves | Leaf length(cm) | Leaf width (cm) |
|----------|---------------|-----------------|-----------------|
| T1 (10kR) | 18.00         | 3.66            | 5.13            |
| T2 (20kR) | 17.16         | 5.50            | 3.50            |
| T3 (30kR) | 16.93         | 5.93            | 5.73            |
| T4 (40kR) | 16.83         | 6.06            | 5.90            |
| T5 (50kR) | 16.26         | 4.83            | 4.66            |
| T6 (60kR) | 15.43         | 4.36            | 4.20            |
| T7 (Control) | 19.10           | 5.73            | 5.53            |

C.D. 0.92 SE(m) 0.08 SE(d) 0.12 C.V. 3.05

Table 5: Effect of gamma irradiation on biochemical parameters (phenols and chlorophyll) in field bean in polybags at 30 DAS.

| Dosage   | Phenols (mg/100g) | Chlorophyll (SCMR) |
|----------|-------------------|-------------------|
| T1 (10kR) | 516.25            | 37.36             |
| T2 (20kR) | 587.56            | 37.53             |
| T3 (30kR) | 683.88            | 37.70             |
| T4 (40kR) | 747.46            | 36.76             |
| T5 (50kR) | 786.40            | 33.96             |
| T6 (60kR) | 824.46            | 34.86             |
| T7 (Control) | 536.40           | 37.26             |

C.D. 2.73 SE(m) 0.89 SE(d) 1.26 C.V. 0.23

References
1. Akhtar N. Effect of physical and chemical mutagens on morphological behaviour of tomato (Lycopersicon...
1. Akshatha KR, Chandrashekar HM, Somashekarappa J, Souframanien. Effect of gamma irradiation on germination, growth, and biochemical parameters of *Terminalia arjuna* Roxb. Radiation Protection and Environment. 2016; 36(1):38-44.

2. Asare AT, Mensah F, Acheampong S, Bediako E, Armah J. Effects of Gamma Irradiation on Agromorphological Characteristics of Okra (*Abelmoschus esculentus* L. Moench.). Advances in Agriculture. 2012; 2(4):56-58.

3. Girija M, Dhanavel D. Effect of gamma rays on quantitative traits of cowpea in M1 generation. International Journal of Research in Biological Science. 2013; 3(2):84-87.

4. Jagajanantham N, Dhanavel D, Pavadaip P, Chidambaram AA. Growth and yield parameters using gamma rays in bhendi (*Abelmoschus esculentus* (L.) Moench) va. Arka Anamika. International Journal of Research in Plant Science. 2012; 2(4):56-58.

5. Kim JS, Lee EK, Back MH, Kim DH, Lee YB. Influence of Low dose γ radiation on the physiology of germinative seed of vegetable crops. Korean Journal of Environmental Agriculture. 2000; 19:58-61.

6. Kiong ALP, Lai GA, Hussein SN, Harun AR. Physiological responses of *Orthosiphon stamineus* plantlets to gamma irradiation. American-Eurasian Journal of Sustainable Agriculture. 2008; 2(2):135-149.

7. Majeed A, Khan AR, Ahmad H, Muhammad Z. Gamma irradiation effects on some growth parameters of *Lepidium sativum* L. ARPN Journal of Agricultural and Biological Science. 2008; 5(1):39-42.

8. Maryam AH, Kasimu AA. Effect of combined doses of gamma ray and sodium azide (mutagenic agents) on the morphological traits of some varieties of okra (*Abelmoschus esculentus*). African Journal of Agriculture. 2016; 11(32):2968-2973.

9. Nayak D, Patil NS, Behera LK, Jadeja DB. Effects of gamma rays on germination and growth in *Jatropha curcas* L. Journal of Applied and Natural Science. 2015; 7(2):964-969.

10. Oufedjikh H, Mahrouz M, Amiot MJ, Lacroix M. Effect of gamma irradiation on phenolic compounds and phenylalanine and phenylalanine ammonia lyase activity during storage in relation to peel injury from peel of *Citrus clementina* hort. Ex. Tanaka Journal of Agricultural Food Chemistry. 2000; 48(2):559-65.

11. Priyanka DK, Animesh DK. Induced mutagenesis in *Abelmoschus moschatus* (L.) Medik. International Research Journal of Pharmacy. 2012; 3(5):432-435.

12. Rafiullah S, Hassan S. Effect of gamma irradiation on morphology of brassica species. International Nuclear Information System. 1994; 10(2):169-174.

13. Shah TM, Mirza JJ, Haq MA, Atta BM. Radio sensitivity of various chickpea genotypes in M1 generation I-Laboratory studies. Pak. J Bot. 2008; 40:649-665.

14. Sharma A, Plaha P, Rathour R, Katoch V, Singh Y, Khalsa GS. Induced mutagenesis for improvement of garden pea. International Journal of Vegetable Science. 2010; 16:60-72.