



P-ISSN: 2349-8528

E-ISSN: 2321-4902

www.chemijournal.com

IJCS 2020; 8(4): 2527-2531

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Received: 25-05-2020

Accepted: 27-06-2020

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International Journal of Chemical Studies

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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DOI: <https://doi.org/10.22271/chemi.2020.v8.i4ac.10014>

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 (^{60}Co) 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 Co_{60} (Cobalt-60) as a source. The field bean seeds after irradiation with different doses of gamma rays viz., 10, 20, 30, 40, 50, 60kR 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

filled with sand, soil and manure in 1:1:1 proportion and bags were irrigated daily with rose can. In both petri plate and polybags the germination percentage was observed for seven days to ascertain the LD₅₀ value of gamma irradiation. The plants grown in polybags were maintained for one month and observations on various growth parameters *viz.*, shoot length, root length, root to shoot ratio, number of leaves per plant, fresh and dry weight and biochemical parameters *viz.*, phenol content and SCMR were recorded.

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 petri plates (T₇-93.33%) and in polybags (T₇- 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].

Root to shoot ratio

A significant difference was recorded for root to shoot ratio. Among irradiated plants, highest root to shoot ratio was recorded in T₃ (30kR-1.26) where as lowest root to shoot ratio was recorded in T₆ (60kR-0.52) (Table. 2 and Fig. 2).

Fresh and dry weight (g)

In irradiated plants, maximum fresh weight was recorded in T₁ (10kR-4.0 g) where as lowest fresh weight was recorded in T₆ (60kR-1.09 g). Dry weight was maximum in T₁ (10kR-0.68 g). T₆ (60kR-0.19 g) recorded minimum dry weight whereas the control plants recorded a fresh and dry weight of 5.18 g and 1.24 g respectively (Table. 3 and Fig. 3). Reduced plant stature or reduced moisture content in plants due to radiation stress might be the reason for reduction in the fresh and dry weight of plants (Majeed *et al.*, 2010).

Number of leaves

The plants which were not irradiated recorded the maximum number of leaves (19.10). Among the irradiated plants, maximum number of leaves were recorded in plants exposed to T₁ (10kR-18.0) and lowest number of leaves were recorded in T₆ (60kR-15.43) treatments (Table.4 and Fig. 4). The number of leaves in field bean cv. TFB-2 showed a decline with increasing dose of irradiation. These results coincided with the findings of Jagajantham *et al.* (2012) [5] in okra. The decrease in the number of leaves might be attributed to the changes caused by gamma irradiation in plant morphology and function, as affected by various dosage and duration of exposure time (Piri *et al.*, 2011) [12]. It is also predicted that enzymes essential for leaf initiation at higher intensities of gamma irradiation might have stopped and blocked the cell division by decreasing the rate of physiological processes (Akhtar, 2014) [1].

Leaf length and leaf width (cm)

Control (T₇) 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 T₃ 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 T₇ (control). A significant reduction in leaf width was observed in irradiated plants compare to control except in T₃ and T₄. The results showed similarity with the findings of Priyanka and Animesh (2012) [13] in muskdana.

The irradiation doses higher than the 40kR significantly reduced the leaf size *i.e.*, both leaf length and leaf width. A gradual decrease in the leaf size with increased intensities of radiation was reported in brassica species by Rafiullah and Hasan (1994) [14]. The leaf length and leaf width showed a gradual increase compared to untreated control, with increase in irradiation dose from 10kR to 40kR. Lower doses of irradiation produced a stimulatory effect in increasing the leaf size. These results were in conformity with the findings of Nayak *et al.* (2015) [10] in jatropha.

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
T ₁ (10kR)	80.00	80.95
T ₂ (20kR)	65.55	68.25
T ₃ (30kR)	51.11	49.20
T ₄ (40kR)	44.44	44.44
T ₅ (50kR)	40.00	39.68
T ₆ (60kR)	35.55	33.33
T ₇ (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

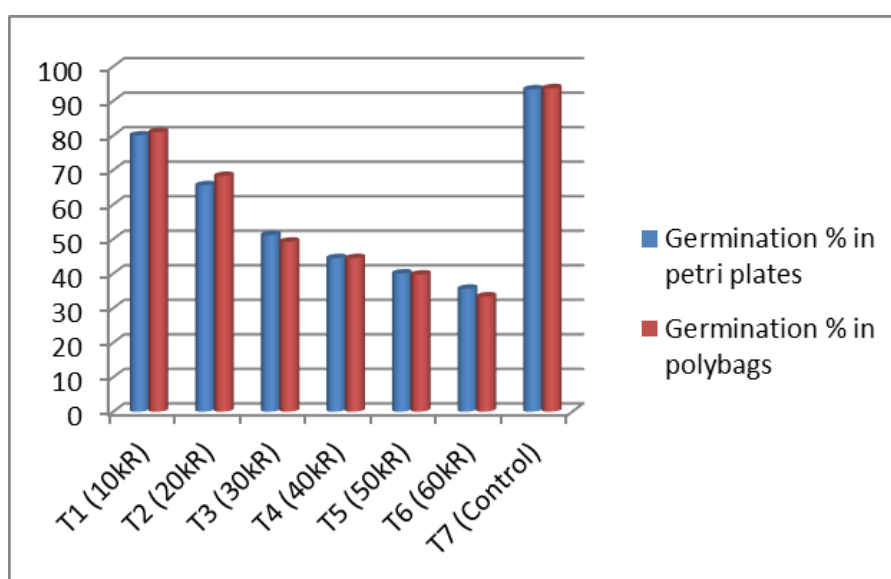
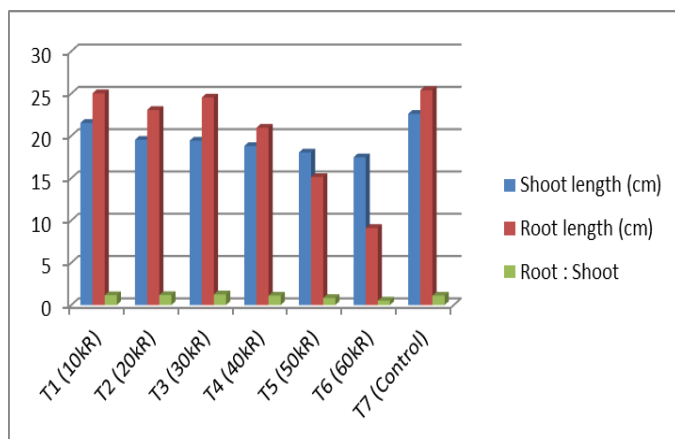


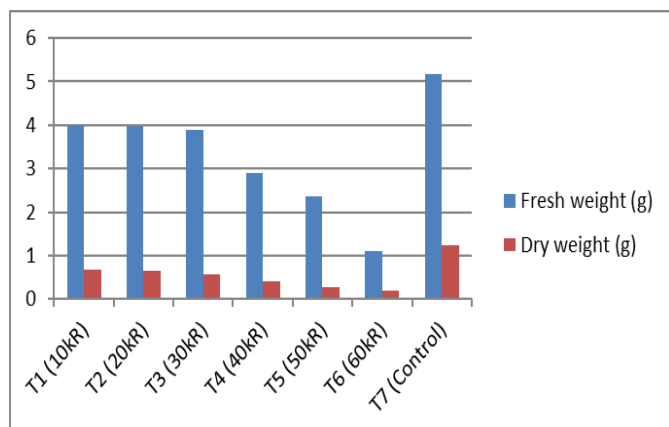
Fig 1: Effect of gamma irradiation on germination of field bean at seven DAS in petri plates and polybags

Table 2. Effect of gamma irradiation on field bean shoot length, root length and root to shoot ratio at 30 DAS in polybags.

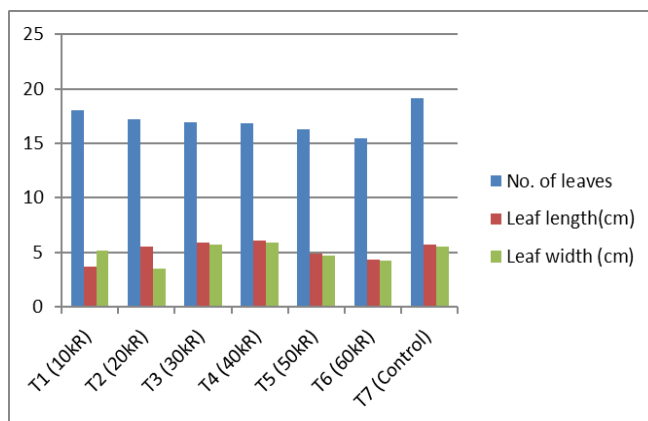
Treatment	Shoot length (cm)	Root length (cm)	Root : Shoot
T ₁ (10kR)	21.53	25.03	1.16
T ₂ (20kR)	19.53	23.06	1.18
T ₃ (30kR)	19.43	24.53	1.26
T ₄ (40kR)	18.80	20.96	1.11
T ₅ (50kR)	18.03	15.13	0.83
T ₆ (60kR)	17.46	9.10	0.52
T ₇ (Control)	22.60	25.40	1.11
C.D.	2.04	2.04	0.15
SE(m)	0.66	0.66	0.05
SE(d)	0.94	0.73	0.07
C.V.	5.88	4.38	8.75

**Fig 2:** Effect of gamma irradiation on field bean shoot length, root length and root to shoot ratio at 30 DAS in polybags**Table 3:** Effect of gamma irradiation on fresh and dry weight of field bean seedlings at 30 DAS.

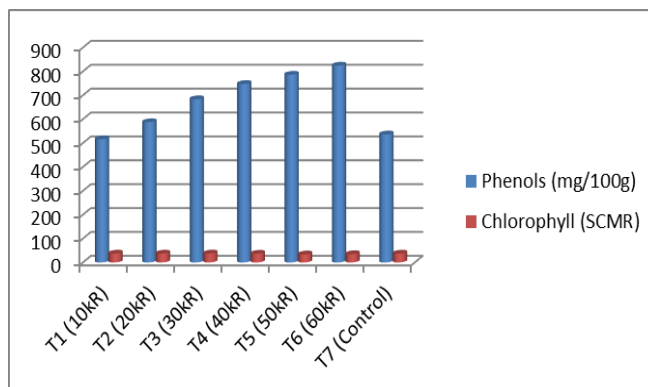
Dosage	Fresh weight (g)	Dry weight (g)
T ₁ (10kR)	4.00	0.68
T ₂ (20kR)	3.97	0.65
T ₃ (30kR)	3.88	0.58
T ₄ (40kR)	2.91	0.41
T ₅ (50kR)	2.37	0.26
T ₆ (60kR)	1.09	0.19
T ₇ (Control)	5.18	1.24
C.D.	0.26	0.26
SE(m)	0.08	0.08
SE(d)	0.12	0.12
C.V.	4.45	26.48

**Fig 3:** Effect of gamma irradiation on fresh and dry weight of field bean seedling at 30 DAS.**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)
T ₁ (10kR)	18.00	3.66	5.13
T ₂ (20kR)	17.16	5.50	3.50
T ₃ (30kR)	16.93	5.93	5.73
T ₄ (40kR)	16.83	6.06	5.90
T ₅ (50kR)	16.26	4.83	4.66
T ₆ (60kR)	15.43	4.36	4.20
T ₇ (Control)	19.10	5.73	5.53
C.D.	0.92	0.24	0.27
SE(m)	0.30	0.07	0.09
SE(d)	0.42	0.11	0.12
C.V.	3.05	2.64	3.17

**Fig 4:** Effect of gamma irradiation on leaf characters at 30 DAS in field bean in polybags**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)
T ₁ (10kR)	516.25	37.36
T ₂ (20kR)	587.56	37.53
T ₃ (30kR)	683.88	37.70
T ₄ (40kR)	747.46	36.76
T ₅ (50kR)	786.40	33.96
T ₆ (60kR)	824.46	34.86
T ₇ (Control)	536.40	37.26
C.D.	2.73	0.81
SE(m)	0.89	0.26
SE(d)	1.26	0.37
C.V.	0.23	1.26

**Fig 5:** Effect of gamma irradiation on biochemical parameters (phenols and chlorophyll) at 30 DAS in field bean in polybag

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