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Unleashing the Potential of Field Bean Through Gamma Irradiation: A Study on Seed Germination, Mutation Frequency and Correlation Analysis

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ABSTRACT

A comprehensive experiment was undertaken to explore the frequency of mutations and the interrelationships among various parameters in a population of irradiated field beans (*Lablab purpureus* var. *lignosus* (L.) Prain). Cobalt (^{60}Co) was used as the radiation source, and field bean seeds from the highly renowned TFB-2 variety were sown in both petri plates and polybags after being exposed to several dosages of gamma radiation (10kR, 20kR, 30kR, 40kR, 50kR and 60kR). Untreated seeds were utilised as the control. After seven days, the germination rate in petri plates was evaluated. The percentage of germination ranged from 35.55 to 80.00% in petri plates and from 33.33% to 80.95% in polybags for various gamma radiation dosages. The percentage of germination in petri plates and polybags for the untreated control group was 93.33% and 93.65%, respectively. With an increase in irradiation dose, a gradual decrease in germination rate and seedling growth was seen. Probit analysis revealed that the LD50 value was 30kR. Further 1000 seeds were irradiated with 30kR and sown to raise the M1 generation along with the unirradiated seeds as control. Among the 1000 seeds only 392 plants survived and in these population mutation frequency and correlation were analyzed. Mutation frequency shows superiority of a plant parameter over mean of control. Varied mutation frequencies were observed in the irradiated plants on the basis of phenotypes. The mutants M_{205}, M_{184}, M_{169}, M_{76}, M_{258}, M_{317}, $M1_{55}$, M_{167}, M_{111}, M_{161}, M_{162}, M_{111}, M_{76}, M_{317} and M_{155} were chosen and advanced to M_2 generation for additional research as putative mutants after the data in M_1 generation revealed their superiority in terms of the number of primary branches per plant, pod and seed yield per plant. To decipher the intricate interplay of correlations, we examined associations between growth attributes and yield. These insights empower breeders to select plants with traits harmoniously aligned with pod yield per plant, fostering the cultivation of excellence. In conclusion, our research journey into field bean's hidden realms revealed captivating mutation frequencies, intriguing correlations, and valuable insights for breeders. Through the magic of irradiation, we unveiled nature's adaptability and harnessed its potential for enhanced cultivation.

Keywords Field bean, Mutation frequency, Correlation, Putative mutants, Probit analysis.

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INTRODUCTION

Field bean (*Lablab purpureus* var. *lignosus* (L.) *Prain*) has chromosome number 2n=22 and is a member of the Leguminosae (Fabaceae) family. It is commonly known as Dolichos bean, lablab bean, hyacinth bean, bonavist bean, Indian bean, and Egyptian kidney bean. It's origin in India and presently grown throughout the tropical regions of Asia, Africa and America (Deka and Sarkar 1990). It possesses anti-diabetic, anti-inflammatory, analgesic, anti-oxidant, anti-spasmodic effect and is also used for the treatment of iron deficiency anaemia (Esmail *et al.* 2017). Being predominantly a self pollinated crop (cleistogamous) its limited variability acts as an impediment to crop improvement as the genetic variability percentage is very low. Mutation breeding is one of the best options for supersizing the genetic base and enhancing the crop diversity Gamma rays have tremendous capacity to create variability (Chakraborthy and Parthasarathy 2003). Gamma rays are the most energetic form of electromagnetic radiation, their energy level is from ten to several hundred kilo electron volts and they are considered as the most penetrating compared to other radiations (Kovacs and Keresztes 2002). 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).

In pursuit of enhancing the genetic diversity of field bean, we conducted a study involving seed germination, seedling growth, gamma irradiation, and meticulous assessment of mutation frequency and correlation. Through this comprehensive exploration, we identified exceptional mutants with superior traits, advancing them to the esteemed M_2 generation as promising candidates for further investigation.

MATERIALS AND METHODS

Seeds underwent gamma radiation using source 60Co (Cobalt-60) at Bhabha Atomic Research Centre in Trombay, Maharashtra. Experiment conducted in late *kharif*–2019 at olericulture departmental block of College of Horticulture, Ananthrajupeta. Completely randomised design (CRD) was used for the initial experiment, and it was replicated thrice. Seeds of the field bean underwent with diverse dosages of gamma rays (0, 10, 20, 30, 40, 50 and 60kR) and were assessed for germination (%) in petri plates and polybags. In laboratory conditions, seeds were sown in petri plates and allowed to grow for ten days. On the other hand, seeds sown in polybags were carefully nurtured over the course of a month and different growth metrics meticulously observed and recorded. Subsequently, LD_{50} value of gamma radiation was calculated using germination percentage. Considering outcomes of the first experiment, 30kR assessed as LD_{50} value. Consequently 1000 seeds of cv. TFB-2 were exposed to 30kR and sown to generate M_1 generation. The data of each individual plant for growth, yield and quality traits were recorded.

RESULTS AND DISCUSSION

Varied levels of irradiation doses distinctly influenced the percentage of germination observed after seven days of seeding, both in petri plates and polybags containing field bean seedlings. Following a span of 30 days post-sowing, discernible variations emerged in various growth and biochemical traits. These distinctions were notably attributed to the application of gamma radiation treatment.

Germination (%)

Field bean seeds exposed to gamma rays significantly reduced the germination of the seeds in petri plates and polybags (93.33% and 93.65%, respectively) compared to the control (T_7). The T_1 showed notably the highest percentage of germination (10kR-80%) in the petri plates containing irradiated field bean seeds, while the T_6 had the lowest percentage of germination (60kR-35.55%). The polybags showed a similar pattern in terms of germination percentage; notably, the treatment T_1 (10kR-80.95%) had the highest germination percentage, while the treatment T_6 (60kR-33.33%) had the lowest. Based on the percentage of germination both in petri plates and polybags, the LD_{50} value was calculated using probit analysis, which observed probit values being 34.67kR and 33.11kR, respectively (Table 1).

Table 1. Impact of gamma radiation on various parameters of field bean in polybags at 30 Days after sowing.

Dosage	Germination in petri plates (%)	Germination in poly bags (%)
T_1	80.00	80.95
T_2	65.55	68.25
T_3	51.11	49.20
T_4	44.44	44.44
T_5	40.00	39.68
T_6	35.55	33.33
T_7	93.33	93.65
CD	1.92	1.81
SE(m)	0.63	0.59
SE (d)	0.89	0.83
CV	6.20	8.36

Gamma radiation exposure of seeds may have decreased their vigor, which in turn prevented them from germinating. The decrease in the quantity of internal growth regulators that control germination may be the reason of the reduction in the percentage of germination brought on by the high dose of radiation (Kiong *et al.* 2008).

Table 2. Percentage of mutants deviating from parent variety for various parameters.

Sl. No.	Character	Mean phenotypic value of control	Number of desirable mutants	Mutant freque-ncy
1	Plant height (cm)	87.57	35	8.92
2	Number of primary branches/plant	3.9	34	8.67
3	Internodal length (cm)	13.77	25	6.37
4	Days to 1st flowering	43.8	15	3.82
5	Days to 50% flowering	51.1	22	5.61
6	Number of flowers/ inflorescence	26.06	28	7.14
7	Length of inflorescence (cm)	24.54	37	9.43
8	Pod length (cm)	4.92	82	20.91
9	Pod width (cm)	1.93	42	10.71
10	Pod weight (g)	12.83	25	6.37
11	100 seed weight (g)	24.09	29	7.39
12	Number of inflorescence/plant	30.1	35	8.92
13	Number of pods/inflorescence	23.09	18	4.59
14	Pod yield/plant (g)	1001.77	51	13.01
15	Number of pods/plant	544.6	22	5.61
16	Number of seeds/pod	4.46	26	6.63
17	Seed yield(g)	575.4	60	15.30
18	Protein	7.87	23	5.86
19	SCMR	44.9	42	10.71

Mutation frequency

Mutation frequency shows superiority of a plant parameter over mean of control. Based on phenotypes, different mutation frequencies were seen in the irradiated plants. The data in M_1 generation is mentioned below (Table 2).

Plant growth characters

The irradiated plants showed different frequency of mutation for plant growth characters. As compared to the control mean, the plant height was superior in 35 mutants with a frequency of 8.92% whereas number of primary branches/plant and internodal length showed a frequency of 8.67% and 6.37%, respectively.

Floral characters

Frequency for different floral characters in the irradiated population were recorded which showed superiority over control mean. Days to first flowering was earlier in 15 mutants (3.82%) whereas days to 50% flowering was earlier in 22 mutants (5.61%). Frequency recorded for number of flowers/inflorescence and length of inflorescence was 7.14% and 9.43% respectively. However 20 mutants (5.1%) did not flower during the period of study.

Yield characters

Among the irradiated population superiority over the control mean was observed in 35 mutants (8.92%) for number of inflorescence/plant, 18 mutants (4.59%) for number of pods/inflorescence, 22 treated plants (13.01%) for number of pods/plant and 26 mutants (6.63%) for number of seeds/pod. However among the yield characters, highest frequency of mutation was observed for seed yield with 60 irradiated plants (15.30%) followed by pod yield/plant with 51 irradiated plants (13.01%).

Pod and seed characters

Mutation frequency recorded for pod length was 20.91% with 82 mutants, pod width was 10.71% with 42 mutants, whereas lower frequency was recorded

Table 3. Desirable mutant plants for yield and quality traits in M_1 population.

Parameter	Mutant plants
Number of primary branches/plant	M_{205}, M_{184}, M_{169}, M_{258}, M_{155}, M_{167} M_{111}, M_{161}, M_{162}, M_{290}, M_{106}, M_{317}, M_{76}
Days to first flowering	M_{128}, M_{392}, M_{131}, M_{323}, M_{328}, M_{386}, M_{107}, M_{115}, M_{129}, M_{241}
Pod yield (g)	M_{205}, M_{184}, M_{169}, M_{76}, M_{258}, M_{317}, M_{155}, M_{167}, M_{111}, M_{161},M_{162}
Seed yield (g)	M_{205}, M_{184}, M_{169}, M_{258}, M_{317}, M_{155}, M_{76}, M_{167}, M_{161}, M_{162},M_{162}
Protein (mg/100g)	M_{382}, M_{335}, M_{326}, M_{289}, M_{277}, M_{240}, M_{224}, M_{182}, M_{156}, M_{117}

*Total mutagenic population-392

for pod weight with 25 mutants (6.37%) and 100 seed weight (7.39 %) with 29 mutants in contrast to the control.

Biochemical parameters

From the data recorded the protein content showed superiority in 23 mutants (5.86%) and SCMR value showed superiority in 42 mutants (10.71%) as compared to the mean value of control in the respective parameters.

Putative mutants

According to the data recorded, the mutagenic population was found to be superior for a different yield and quality traits. From the M_1 mutant population the mutant plants which showed superiority over the control mean were carried out to M_2 generation for further studies and those mutant plants are listed in Table 3.

Mutants with high protein content and earliness are chosen from the mutant population to advance to the following generation. The mutants M_{205}, M_{184}, M_{169}, M_{76}, M_{258}, M_{317}, M_{155}, M_{167}, M_{111}, M_{161}, M_{162}, M_{111}, M_{76}, M_{317} and M_{155} showed superiority in number of primary branches/plant, pod yield and seed yield/plant. Hence these mutant plants have been selected for progressing to M_2 generation.

Correlation studies for different growth and yield characteristics

Correlation provides information on association of the growth attributes among themselves and to the yield. Estimates of association among the yield components traits and their effect on the yield are useful for designing an effective breeding programme. Correlation studies helps in identifying relationship between yield and yield attributes. The traits which are showing positive correlation with pod yield/plant will help breeders in practicing selection of desirable plants from a population. In the present study correlation coefficient was estimated for 20 yield and yield related traits in 392 mutagenic population (Table 4).

Plant height

The number of flowers/ inflorescence (r= 0.197), number of pods / inflorescence (r= 0.146) and days to pod harvest (r= 0.134) showed significantly positive correlation with plant height. However, SCMR values (r= -0.113) showed a significantly negative correlation with plant height.

Number of primary branches/plant

There was a significantly negative correlation of number of primary branches/plant was recorded with inflorescence length (r= -0.143).

Days to first flowering

Days to first flowering exhibited a significantly positive correlation with the days to 50 % flowering (r= 0.964), number of flowers/inflorescence (r= 0.127), number of pods/plant (r= 0.108), number of pods/ inflorescence (r= 0.115), seed yield (r= 0.146) and pod yield/plant (r=0.146).

Days to 50% flowering

Days to 50 % flowering showed a significantly positive correlation with the number of flowers/inflorescence (r=0.120), number of pods/inflorescence (r=0.108), seed yield (r=0.123) and pod yield/plant (r=0.123).

Internodal length

Internodal length showed a significantly negative correlation with the SCMR values value (r= -0.122).

Table 4. Character association among plant growth, yield and yield attributing traits.

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10
X1	1									
X2	-0.032	1								
X3	0.084	-0.010	1							
X4	0.085	0.004	0.964**	1						
X5	-0.082	0.057	0.015	0.020	1					
X6	0.092	-0.143**	0.098	0.084	-0.047	1				
X7	-0.007	-0.026	-0.062	-0.067	0.003	-0.005	1			
X8	-0.077	-0.086	-0.047	-0.057	-0.021	-0.007	-0.040	1		
X9	-0.098	0.013	-0.008	-0.005	-0.057	0.013	0.087	-0.073	1	
X10	0.060	0.008	0.068	0.066	0.099	-0.035	-0.057	0.043	-0.089	1
X11	0.134**	-0.026	0.010	0.031	-0.079	0.042	-0.022	-0.095	-0.030	0.015
X12	-0.113*	-0.066	-0.039	-0.047	-0.122*	-0.005	0.055	0.166**	0.006	-0.148**
X13	-0.009	-0.022	0.006	-0.017	0.073	0.034	-0.085	-0.014	0.043	0.007
X14	0.197**	-0.094	0.127*	0.120*	-0.047	0.106*	0.016	-0.034	-0.103*	0.106*
X15	-0.097	0.003	-0.004	-0.045	-0.063	-0.097	0.110*	0.085	0.067	-0.005
X16	0.064	-0.080	0.108*	0.075	-0.052	-0.030	0.081	0.048	-0.041	0.095
X17	0.146**	-0.073	0.115*	0.108*	-0.006	0.028	0.040	-0.008	-0.106*	0.141**
X18	0.069	-0.001	0.146**	0.123*	0.062	0.075	-0.018	0.043	0.023	-0.040
X19	0.004	0.049	-0.058	-0.073	0.028	0.000	-0.031	0.005	0.038	-0.063
X20	0.068	-0.004	0.146**	0.123*	0.062	0.079	-0.021	0.043	0.026	-0.038

Table 4.. Continued.

	X11	X12	X13	X14	X15	X16	X17	X18	X19	X20
X1										
X2										
X3										
X4										
X5										
X6										
X7										
X8										
X9										
X10										
X11	1									
X12	-0.017	1								
X13	0.074	-0.064	1							
X14	0.011	-0.264**	0.008	1						
X15	-0.009	0.104*	-0.023	-0.185**	1					
X16	-0.033	-0.118*	-0.031	0.713**	0.501**	1				
X17	-0.028	-0.240**	-0.032	0.952**	-0.081	0.805**	1			
X18	0.034	0.004	-0.042	0.108*	0.038	0.120*	0.125*	1		
X19	-0.121*	-0.025	0.018	-0.032	-0.052	-0.053	-0.039	0.034	1	
X20	0.038	0.004	-0.043	0.105*	0.040	0.119*	0.123*	0.998**	0.033	1

*, ** value is statistically significant at 0.05 % and 0.01 % probability levels respectively.

X1-Plant height. X2- Number of primary branches/plant. X3-Days to 1st flowering. X4-Days to 50% flowering .
X5-Internodal length. X6-Inflorescence length. X7- Pod length. X8-Pod width.
X9- Pod weight. X10- Number of seeds/pod. X11- Days to pod harvest. X12- SCMR.
X13- Protein %. X14- Number of flowers/inflorescence. X15- Number of inflorescence/plant. X16- Number of pods / plant.
X17-Number of pods/ inflorescence. X18- Seed yield. X19-100 seed weight. X20-Pod yield/plant.

Inflorescence length

Significantly positive correlation was shown by inflorescence length with the number of flowers/ inflorescence (r= 0.106).

Pod length

Pod length exhibited a significantly positive correlation with the number of inflorescence/plant (r= 0.110).

Pod width

Significantly positive correlation was shown by pod width with SCMR
(r= 0.166).

Pod weight

There was a negative and significant correlation for pod weight with number of flowers/inflorescence (r= -0.103) and number of pods/inflorescence (r= -0.106).

Number of seeds/pod

Significantly positive correlation was exhibited by number of seeds/pod with number of flowers/inflorescence (r= 0.106) and number of pods/inflorescence (r= 0.141) while number of seeds/pod showed a negative and significant correlation with SCMR (r= -0.148).

Days to pod harvest

Significantly negative correlation was exhibited by days to pod harvest with 100 seed weight (r= -0.121).

SCMR

SCMR exhibited significantly positive correlation with the number of inflorescence/plant (r= 0.104) whereas negative and significant correlation was shown by SCMR with number of pods/plant (r= -0.118) and number of pods/inflorescence (r= -0.240).

Protein content

Protein content did not show any significant correlation with the plant growth and yield parameters.

Number of flowers/inflorescence

A significantly positive correlation was exhibited by number of flowers/ inflorescence with number of pods/plant (r= 0.713), number of pods/inflorescence (r= 0.952), seed yield (r= 0.108) and pod yield (r= 0.105) while significant and negative correlation was shown by number of flowers/inflorescence with the number of inflorescences/plant (r= 0.185).

Number of inflorescences/plant

A significantly positive correlation was exhibited by number of inflorescences/plant with the number of pods per plant (r= 0.501).

Number of pods/plant

Number of pods/plant showed significantly positive correlation with number of pods/inflorescence (r= 0.805), seed yield (r= 0.120) and pod yield
(r= 0.119).

Number of pods/inflorescence

Significantly positive correlation was shown by number of pods/inflorescence with seed yield (r= 0.125) and pod yield/plant (r= 0.123).

Seed yield/plant

Seed yield showed a significantly positive correlation with pod yield/plant
(r= 0.998).

100 seed weight

Significantly negative correlation was exhibited by 100 seed weight with days to pod harvest (r= -0.121)

Pod yield/plant

A significantly positive correlation was shown by pod yield/plant with days to first flowering (r= 0.146), days to 50 % flowering (r= 0.123), number of flowers/ inflorescence (r= 0.105), number of pods/plant (r=

0.119), number of pods/inflorescence (r= 0.123) and seed yield (r= 0.998).

The vast majority of significant correlation estimates between yield and yield components were of a positive nature. 27 positive out of 39 significant correlation suggest that the improvement of those individual traits would simultaneously improve other pair wise traits because of correlated response. Traits which are significant with the pod yield per plant will also be useful in selection of desirable plants from the mutagenic population.

Conclusion

According to the results shown and described, the field bean underwent an induced mutation that resulted in an increase in leaf length, leaf width, phenol, and chlorophyll content while, germination, shoot length, root length, number of leaves, fresh weight, and dry weight were gradually decreased. There were specific advantages by induced mutation efficiently by gamma irradiation in the field bean by inducing changes at morphological, physiological and biochemical level. From this study it was ascertained that the plants grown from seeds subjected to 30kR (LD_{50}) irradiation showed superiority in number of primary branches/plant, pod yield and seed yield/plant.

Wide range of variation for different plant growth, yield and quality parameters exhibited in mutated population. A positive correlation was observed for most of the traits with the pod yield per plant. Putative mutants were selected based on the mutation frequency of the yield and quality traits and their character association. Among the mutant population the mutants M_{205}, M_{184}, M_{169}, M_{76}, M_{258}, M_{317}, M_{155}, M_{167}, M_{111}, M_{161},M_{162}, M_{111}, M_{76}, M_{317} and M_{155} which showed superiority in number of primary branches/plant, pod yield and seed yield/plant were selected for future studies.

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