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Biological response, efficiency and effectiveness of gamma irradiation as mutagen on Pusa Navbahar cluster bean

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ABSTRACT

Cluster bean is a highly self- pollinated vegetable with a very narrow genetic base. Hence, mutation breeding is a viable option for generating variation. In the present study, effect of mutagen was studied in Pusa Navbahar variety of cluster bean using gamma rays from 50 Gy to 600 Gy. Preliminary evaluation at M_1 generation for germination and seedling growth parameters revealed at lower doses of 100 to 200 Gy germination and growth parameters showed improvement over control. Further pollens showed steady increase in sterility with increasing dose and mutated lines showed less survivability compared to control. Thus, 100-200 Gy gamma irradiation was found useful to improve the seed germination and seedling growth parameters in cluster bean. While evaluating M_2 generation, total 9 types of mutants (dark green, branched, dwarf, white flower, sterile, long pod, short pod, curve pod, small leaf) were observed and the mutation frequency was high at higher doses, whereas both mutagenic effectiveness and efficiency showed decreasing trend with increasing dose. Highest mutation effectiveness was observed at 50-100 Gy. Mutation efficiency based on pollen sterility was maximum at 50 Gy and based on lethality, it was maximum at 150 Gy.

Key words: *Cyamopsis tetragonoloba*, gamma rays, biological response, mutation efficiency and effectiveness

INTRODUCTION

Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub] is an underutilized legume vegetable of the family Fabaceae. In north-west India, it is grown extensively for its seeds (gum), whereas in south India, it is grown as a vegetable crop. Tender pods are nutritionally rich and being a good source of calcium, iron and dietary fiber, helps in supporting bone health, better blood circulation and bowel movement. It is also used as green manure as it fixes atmospheric nitrogen to the tune of 50-60 kg/ha/year and adds organic matter to soil (Lal, 11). In the present scenario high temperature and reducing water table due to climate change, it is an alternative for nutritional security in arid and semi-arid regions of India. Despite its potentials, the area under this vegetable is meagre due to non-availability of suitable vegetable type variety. Because it is a self-pollinated crop with a very narrow genetic base and lacks the required genetic variability for desirable traits. Creation of variation through manual hybridization is also very difficult owing to its small and delicate flower structure which makes the process of emasculation difficult. Moreover, a very less number of seeds per cross is another deterrent. Hence creation of variation through induced mutations and selecting the elite lines is a possible option in cluster bean. At present, very less conclusive information is available on relative efficiency and effectiveness of physical mutagens in cluster bean. Mutagenic effectiveness refers to the frequency of mutations induced by a unit dose of mutagen however, mutagenic efficiency informs about the proportion of mutations in relation to biological damage such as lethality and pollen sterility. Therefore, the present study has been undertaken to determine the above based on the biological response of plant to gamma irradiation.

MATERIALS AND METHODS

Cluster bean variety Pusa Navbahar was used for induced mutagenesis study as it is an old variety which has become susceptible to many biotic stresses over time and has reached a plateau in terms of productivity. Healthy and uniform sized seeds of Pusa Navbahar cluster bean were administered with 12 different doses of gamma rays i.e. 50 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, 350 Gy, 400 Gy, 450 Gy, 500 Gy, 550 Gy and 600 Gy in August 2018 at ^{60}Co gamma chamber at the ICAR- Indian Institute of Horticultural Research, Bengaluru. Out of 300 seeds from each treatment, 60 seeds were used for a laboratory experiment and the remaining seeds were grown along with control in randomized block design (RBD) in 3 replications in paired rows at a spacing 30 x 15cm between rows and plants (August 2018 to November 2018). In lab, seeds were

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allowed to germinate between moist germination paper in Completely randomized design (CRD) with 3 replications having 20 seeds per replication and kept in a walk-in germination chamber. Observations were recorded on number of seeds germinated (radical length more than 2 mm) per day up to ten days. Based on which following parameters were estimated. i) **Germination percentage**: taken on the 7th day after sowing ii) **Co-efficient of germination velocity (CGV)** ((Jones and Sanders, 5) iii) **Germination rate index (GRI)** (Esechie, 4). iv) **Germination index (GI)** (Arnold *et al*., 1). v) **Seedling height:** recorded on the 15th day after sowing. vi) **Seedling root length:** recorded on the 15th day after sowing. vii) **Seedling injury** (Myhill and Konzak, 13). In M_1 generation field experiment, observations were recorded on viii) **Pollen sterility (%):** determined by staining the pollen grains with 2 per cent acetocarmine dye and observed under a microscope. ix) **Survival of plants at maturity (%):** recorded by counting the number of plants survived and bore pods till maturity of seeds. At the end of M_1 generation seeds were harvested separately from each surviving plant. These harvested seeds were sown in field in plant to progeny row in M_2 generation (December - April 2019) along with control and the spectrum and frequency of viable mutations were observed in M_2 generation. Based on which the mutagenic effectiveness and efficiency of different mutagens were calculated according to the formulae suggested by Konzak *et al.* (8). Mutation rate was also estimated.

RESULTS AND DISCUSSION

Gamma rays belong to the group of ionizing radiations which interacts with atoms or molecules in the cell and produce free radicals. These free radicals in turn damage or, modify cell anatomy and physiology thus, produce altered biological response. Further, these responses depend on the irradiation level. Analysis of variance in M_1 generation for germination, seedling growth parameters, pollen sterility and survival at maturity showed significant differences among the treatments. The per cent seed germination was not affected up to the dose 550 Gy and were on par with the control and the lowest germination percentage (90 %) was recorded at 600 Gy (Fig. 1). Little effect of irradiation on germination per cent can be attributed to the presence of galactomannan in seed endosperm providing some sort of protection and food supply during seed germination as reported by Mahla *et al*. 2018. CGV showed decreasing trends with increasing dose of gamma rays (Fig. 1) because, CGV increases with increase in number of germinated seeds and decreases in time required for germination. Since control and lower dose of gamma rays showed 100 per cent germination within 5 days, they have higher value of CGV. Similarly, up to 100 Gy the GRI values

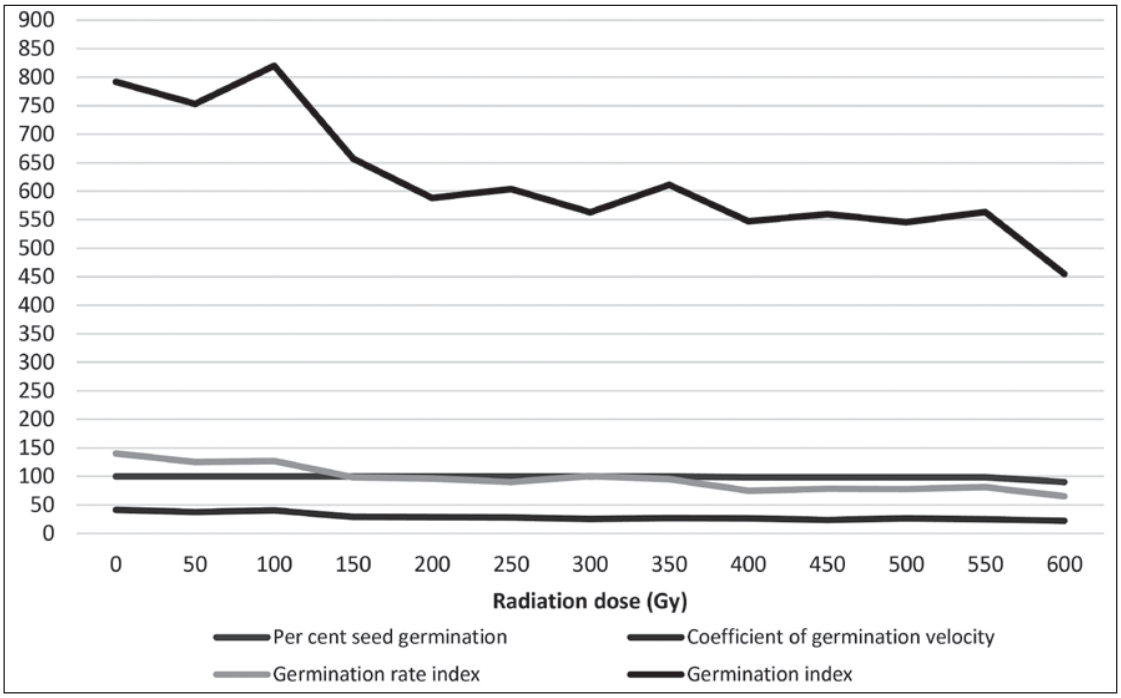

Fig. 1. Effect of gamma irradiation on germination parameters in Pusa Navbahar cluster bean.

were on par with control and at higher dose of 600 Gy the GRI was very low (Fig. 1). At lower doses higher value of GRI obtained as higher and faster germination was recorded in lower doses of gamma ray. Highest GI was recorded in 100 Gy (820) which is on par with control. Decreasing GI with increasing dose were observed (Fig. 1) because at higher dose, germination percentage and rate of germination were less when compared to control and lower doses. Similar results were reported by Kusmiyati *et al.* (10) as they also observed decreasing trend of seedling growth parameters at higher doses of gamma rays in soybean.

Seedling height increased compared to control at lower dose up to 150 Gy after which it followed a decreasing trend but height at 200 Gy was on par with the height obtained by 150 Gy, 250 Gy, 350 Gy and 300 Gy. At higher dose of 600 Gy least seedling height of 22.56 cm was recorded (Fig. 2). Lower level up to 200 Gy showed increase in root length compared to control (Fig. 2) and the lowest root length was observed at higher dose of gamma ray 600 Gy (12.98 cm) which was on par with control (12.75 cm). Here compared to control the mutated treatment showed increased length but among mutated seeds, the root length was decreased at higher dose. The decreasing seedling height and root length at higher dose can be attributed to the gross injury caused at cellular level due to gene controlled biochemical process or acute chromosomal aberrations or both (Kousar *et al*., 9). Mahla *et al.* (12), Shinde and More (14) and Dube *et al.* (3) also observed reduced plant height at increased dose of gamma irradiation. With respect to seedling injury the values between the treatments showed non-significant difference. The range was -19.15 to 7.31 per cent. Highest seedling injury was recorded in 600 Gy (7.31) and the lowest value was recorded at 200 Gy (-19.15 %). The seedling injury was more in higher doses compared to lower doses (Fig. 2) this is due to decrease in amylase and peroxidase activities. Similar results were found by Khan *et. al.* (7) in green gram, Khan and Wani (6) in mung bean.

Pollen sterility showed steady increase with increasing dose (Fig. 3). At higher dose of 600 Gy the highest pollen sterility (57.41%) was recorded and the lowest at 50 Gy which was on par with control (3.84%). The increased pollen sterility in mutated population has been attributed to chromosome abnormalities (Konzak *et al*., 8) and diverse phenomena like abortive pollen grains or embryo, sterile flowers and inhibition of growth. Similar trends were observed

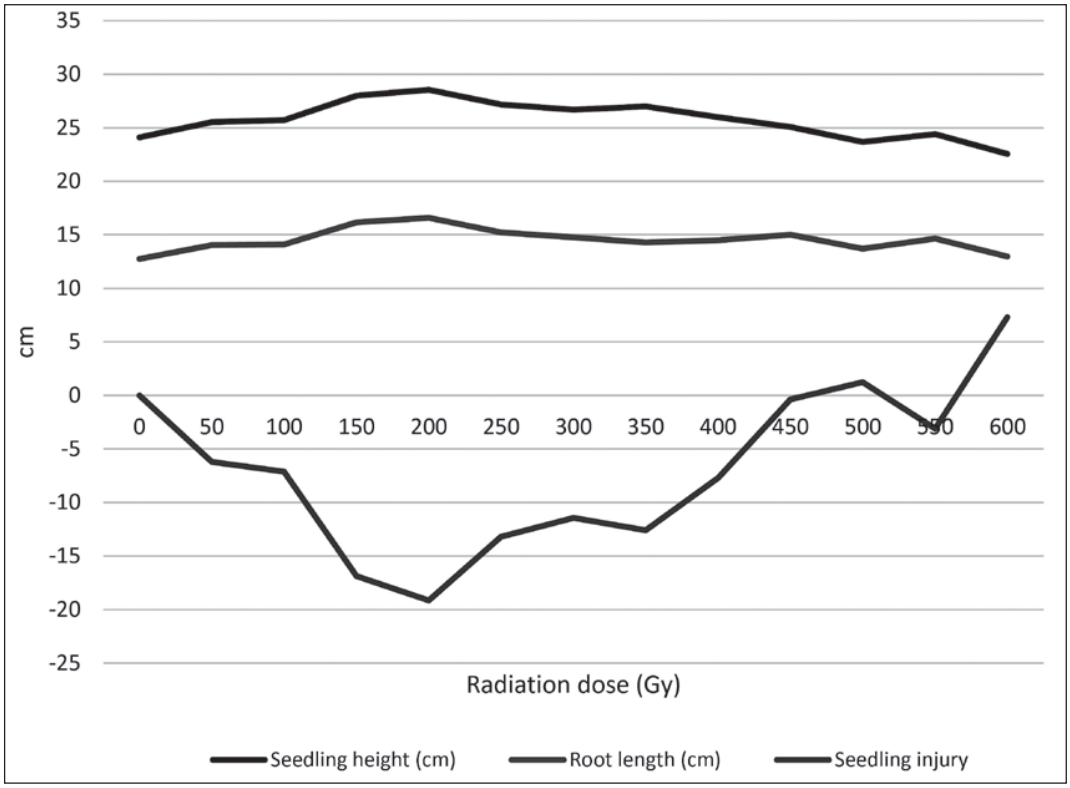

Fig. 2. Effect of gamma irradiation on seedling growth parameters in Pusa Navbahar cluster bean.

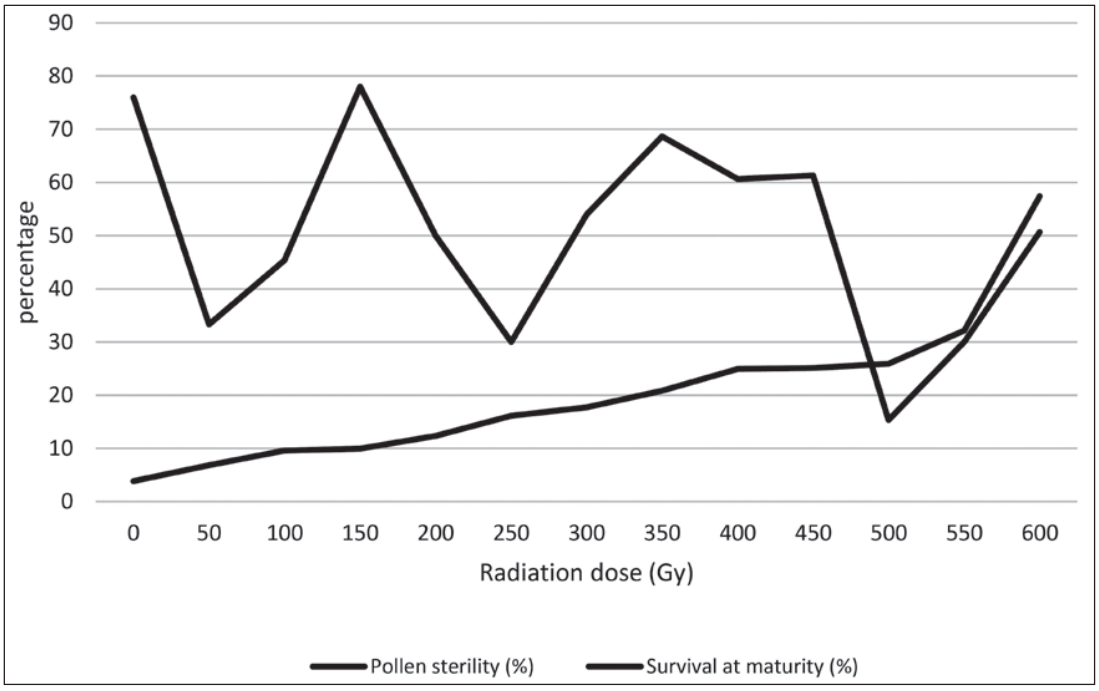

Fig. 3. Effect of gamma irradiation on pollen sterility and survival at maturity in Pusa Navbahar cluster bean.

by Shinde and More (14) and Bhosale and Kothekar (2) in cluster bean and Khan *et al*. (7) in green gram.

Highest percent of plants were survived in 150 Gy (78 %) which is on par with control (76 %) and 350 Gy (68.07 %). The lowest survival percentage was recorded at 550 Gy (15.33 %). No trend was observed with respect to survival percentage upon irradiation and wide fluctuation in the values was observed (Fig. 3). But compared to control in many mutated lines the lethality was more and it has been attributed to injury due to chromosomal aberrations or physical imbalance (Konzak *et al*., 8). For all the traits studied in M_1 generation, it was observed that though we have taken lower level of irradiation treatments still there is difference in response among them. In all the cases, the highest dose 600 Gy gave the most detrimental result and 100- 200 Gy gave the most beneficial responses over control. Thus, 100- 200 Gy gamma irradiation can be used to improve the seed germination and seedling growth parameters in cluster bean.

In M_2 generation total 9 types of mutants were observed namely dark green, branched, dwarf, white flower, sterile, long pod, short pod, curve pod and small leaf mutants. In the present study all the dose from 50 Gy to 600 Gy showed one or the other mutant (Table 1) and in 350 and 200 Gy majority of the mutants were observed. With the identified viable mutant the mutation frequency was calculated which was useful for estimating mutagenic effectiveness and efficiency (Table 2). In the present investigation the mutation frequency range was 2.90 to 5.31 per cent.

Highest mutation frequency was recorded in 250 Gy (5.31) followed by 500 and 600 Gy (5) whereas lowest mutation frequency was recorded at 100 Gy (2.9) followed by 350 Gy (2.99). No trend was followed with respect to mutation frequency but when compared to lower doses it was more in higher doses. But most of the mutants obtained were not useful. However, Velu *et al.* (15) observed highest mutation frequency at 200 Gy of gamma ray on M_2 plant basis.

Mutagenic effectiveness was calculated based on mutation frequency and in the present which ranged from 0.08 to 0.78. Highest value was recorded at lower doses i.e. 50 Gy (0.78) followed by 100 Gy (0.29) and the lower values were recorded at higher doses 550 and 600 Gy (0.08). With respect to Mutagenic effectiveness a decreasing trend was noticed with increasing dose. Bhosle and Kothekar (8) also reported reduction in the value of mutagenic effectiveness with the increased doses or concentration of mutagens in the cluster bean varieties GE-36 and HR. Based on mutation frequency in terms of biological injury like pollen sterility and lethality in M_1 generation, the mutagenic efficiency was calculated. With respect to pollen sterility, highest mutagenic efficiency was recorded

Table 1. Spectrum of viable mutants in M_2 generation of Pusa Navbahar cluster bean.

Treatment	Dose (Gy)	Chlorophyll mutants	Plant mutants		Flower mutants		Pod mutants			Leaf mutants	Total mutants	Total plants	Mutation frequency (%)
		Dark green	Branched	Dwarf	White flower	Sterile	Long pod	Short pod	Curve pod	Small leaf			
T_1	50	0	0	14	0	0	0	0	0	0	14	360	3.89
T_2	100	0	1	18	0	1	0	0	0	0	20	690	2.90
T_3	150	1	3	19	1	0	1	0	5	1	31	980	3.16
T_4	200	0	1	22	2	2	1	1	0	1	30	710	4.23
T_5	250	0	2	15	1	3	0	2	1	2	26	490	5.31
T_6	300	0	0	9	0	2	2	0	0	1	14	310	4.52
T_7	350	3	3	25	1	0	1	1	0	1	35	1170	2.99
T_8	400	1	2	8	0	2	0	0	1	0	14	320	4.38
T_9	450	0	1	10	0	1	0	0	0	0	12	260	4.62
T_{10}	500	0	0	10	0	1	0	1	0	0	12	240	5.00
T_{11}	550	0	0	9	0	0	0	2	0	0	11	240	4.58
T_{12}	600	0	1	11	1	1	2	0	0	1	17	340	5.00
Total		5	14	170	6	13	7	7	7	7	236	6110	

at lower dose of 50 Gy (0.57) followed by 100 Gy (0.34) and the lowest value was recorded at higher dose of 600 Gy (0.09). With respect to pollen sterility as dose increases mutagenic efficiency decreases but with respect to lethality no trend was noticed. The study on mutagenic effectiveness and efficiency was useful to identify the gamma ray dose at which viable and useful mutation will be more. Thus, it was concluded that at lower dose of gamma ray useful variation can be obtained in cluster bean.

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Table 2. Mutagenic effectiveness and efficiency of gamma irradiation on Pusa Navbahar cluster bean.

Treatment	Dose (Gy)	Mutation frequency (%)	Mutagenic effectiveness	Mutagenic efficiency (pollen sterility)	Mutagenic efficiency (lethality)	Mutation rate
T_1	50	3.89	0.78	0.57	0.06	
T_2	100	2.90	0.29	0.30	0.05	
T_3	150	3.16	0.21	0.32	0.14	0.20
T_4	200	4.23	0.21	0.34	0.08	
T_5	250	5.31	0.21	0.33	0.08	
T_6	300	4.52	0.15	0.26	0.10	
T_7	350	2.99	0.09	0.14	0.10	
T_8	400	4.38	0.11	0.18	0.13	
T_9	450	4.62	0.10	0.18	0.12	
T_{10}	500	5.00	0.10	0.19	0.06	
T_{11}	550	4.58	0.08	0.14	0.07	
T_{12}	600	5.00	0.08	0.09	0.10	

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