Indian J. Genet., 78(2): 261-269 (2018)
DOI: 10.5958/0975-6906.2018.00034.2

Effect of gamma irradiations on seed germination, seedling growth and mutation induction in Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.]

H. R. Mahla*, R. Sharma and R. K. Bhatt

ICAR-Central Arid Zone Research Institute, Jodhpur 342 003

(Received: January 2018; Revised: April 2018; Accepted: May 2018)

Abstarct

Cluster bean is an annual drought hardy legume crop mainly cultivated in arid regions receiving scanty (150-400 mm) and erratic rainfall during crop growth period. For the enhancement of genetic diversity in this locally adapted crop, three genotypes HG- 2-20 (hispid branched), CAZG-15-6 (branched glabrous) and CAZG 15-3 (single stem, hispid and regular bearing) were irradiated with gamma rays (100 -1600 gray) with 100 gray intervals. The mutation rate increased with increasing dose up to 300 gray, there after gradually reduced indicating decline in rate of recovery of mutations with respect to increasing M_1 damage. At higher dose of 800 gray, mutation efficiency drastically reduced with reduced survival and high level of pollen sterility in M_1 generation. Beyond 800 Gy dose all the seedlings died within a few days (15 d) of germination. Based on mutation efficiency and effectiveness a gamma radiation dose between wide ranges (200-700 Gy) was found appropriate for inducing variability. The mutagenesis has generated, a good amount of diversity for economically and agronomically important traits.

Key words: Gamma rays, mutation rate, mutagenic efficiency, effectiveness, sterility.

Introduction

Cluster bean or guar [*Cyamopsis tetragonoloba* (L.) Taub.] is an annual legume cultivated in north western parts of Indian arid zone covering states of Rajasthan (> 80 % area), Gujarat, Haryana and parts of Punjab over an area of 5.3 million hectares (Anonymous 2016). This crop has adaptability to hostile climatic conditions and can be sown even in delayed monsoon conditions where farmers have very limited options under rainfed conditions. However, productivity of the cluster bean in India fluctuated over the years (50-500 Kg/ha) due to scanty (150-400 mm) and erratic rainfall distribution (3-5 rain events during July – September) during crop growth period (Mahla et al. 2011). Most of the India's guar produce is exported with its share accounting to about 80% of the total global exports, however, returns are subject to unpredictable demand. In order to make the crop sustainable under fluctuating market demand and associated prices, we need to improve yield potential of the crop under deprived cultivation conditions.

The availability of genetic diversity conserved in the germplasm determines the success of any crop improvement program. However, on the basis of DNA markers, Kumar et al. (2017) reported the existence of poor genetic diversity in cluster bean. Further, exploitation of available diversity through hybridization is cumbersome due to small flower size and very poor seed setting in manually hybridized buds. Therefore, mutation breeding may be preferred choice in such situations for creating variability and isolating desirable mutants for specific purpose. Though, mutability of cluster bean has amply been demonstrated through various studies systematic work still lacks (Stafford 1989; Mittal et al. 1968; Vig 1965; Mahla et al. 2010; Arora and Pahuja 2008; Velu et al. 2007, 2012). The successful use of induced mutation depends on efficiency to create desirable changes with least undesirable effects (Harten 1998; Pathak 2015). There is thus need to generate, basic information for proper application of mutatgenesis in cluster bean improvement. A study to create useful genetic diversity in cluster bean was therefore conducted using gamma radiation.

*Corresponding author's e-mail: hrmahla2010@gmail.com
Published by the Indian Society of Genetics & Plant Breeding, A-Block, F2, First Floor, NASC Complex, IARI P.O., Pusa Campus, New Delhi 110 012; Online management by indianjournals.com; www.isgpb.org

Materials and methods

Mutagenic treatment

The seeds of three morphologically diverse genotypes namely HG-2-20, CAZG-15-6 and CAZG-15-3 were used for gamma irradiation. Three genotypes namely, HG-2-20 a highly branched and hispid released variety, CAZG-15-6 a branched glabrous and CAZG 15-3 a single stem hispid having regular bearing (on each node) were taken for study. Genetically pure and dried seeds having moisture content of about 9-12 % were irradiated with different doses of gamma rays (^{60}Co) ranging from 100 to 1600 Gray (Gy) with an increment of 100 Gy at Defence Research Laboratory (DRDO), Jodhpur, India. Non-irradiated dry seeds were used as control (untreated). A total of 800 untreated and irradiated seeds of each dose were sown at Central Research Farm of CAZRI, Jodhpur in 8 rows of 4 m length (100 seeds/row) spaced at 30 cm, during *kharif* 2015.

M_1 damage and determination of LD_{50} dose

The germination and survival was recorded in the field sown experiment in order to determine LD_{50} dose. Observations were recorded on 10 randomly selected seedlings for seedling height, plant fresh and dry weight on 21st day after sowing. Plants survived on 21st day of sowing and at harvest were recorded to calculate percent survival and LD_{50}. The frequency of plants showing partial or complete sterility and pollen sterility was recorded as a measure of damage for the calculation of mutation frequency, effectiveness and efficiency. Partially sterile plants bore a few small pods having 1-2 seeds per pod on some of the clusters, while plants with none or too few small pods (1-5 single seeded) were considered completely sterile. Three to five florets from mid portion of randomly chosen ten plants from each treatment were taken for the determination of pollen sterility. The aceto-carmine stained pollens were observed under microscope (Leica DM 3000) for identification of sterile non-staining pollen grains. M_1 seeds were harvested in bulk to evaluate the subsequent generations. The LD_{50} dose identified from field experiment was also verified through observations on genotype CAZG 15-3 in pot experiment. For this, control and treated seeds (100) for each dose were sown in separate pots (35 cm height and 30 cm diameter) with 25 seeds/pot.

Observations in M_2 generation

The bulk seed of each of M_1 population were planted to raise M_2 generation. In total, 1500 seeds for each treatment and untreated were space planted (45X15 cm) for efficient selection of mutants. The data were recorded on induced genetic variability for the growth parameters *viz*., plant height (PH), branches per plant (BRP), pods per plant (PDP), pod length (PDL), number of seeds per pod (SDPD) and seed yield per plant (SYP) on randomly selected 25 individual plants in each treatment at harvest to estimate variability. However macro-mutations like chlorophyll mutations, abnormal plants or morphological mutations and sterile plants were recorded through regular visits of field during entire crop growing period.

The mutation frequency was calculated on the basis of sterile mutations (plant basis) observed in M_2 plant population following the formula given by Konzak et al. (1965).

$$\text{Mutation frequency (\%)} = \frac{\text{Number of mutated (sterile) plants/}}{\text{Total number of } M_2 \text{ plants}} \times 100$$

$$\text{Mutagenic effectiveness} = \frac{\text{Mutation frequency (\%)/}}{\text{Dose of irradiation (Gy)}} \times 100$$

$$\text{Mutagenic efficiency} = \frac{\text{Mutation frequency (\%)/}}{\text{\% plants showing biological damage (sterility) in } M_1 \text{ generation}}$$

Results and discussion

The experiments with gamma induced mutagenesis in cluster bean generated interesting results both in M_1 and M_2 generations. A good amount of genetic diversity for economically and agronomically important traits has been generated.

Seed germination, seedling growth and LD_{50}

The untreated seed germination percent was 78.2, 73.4 and 72.2% for HG-2-20, CAZG-15-3 and CAZG-15-6, respectively. Compared to control, irradiated seeds exhibited a germination percentage ranging from 83.0 to 111.2% irrespective of genotypes under field conditions. Though, no trend was detected in germination with respect to irradiation treatments up to 400 Gy, it reduced above 600 Gy. Irradiation from 1000 to 1600 Gy delayed the germination with substantially less germination at 1400 and 1600 Gy (Fig. 1).

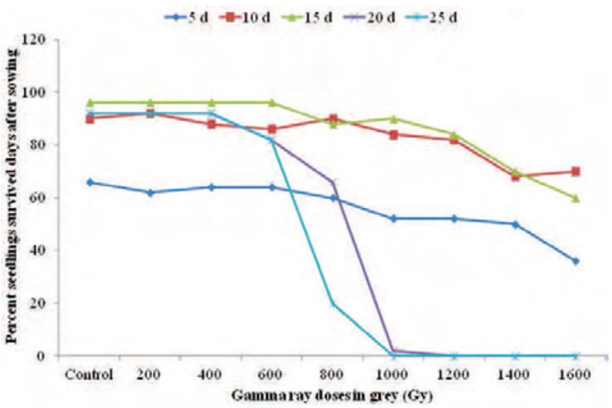

Fig. 1. Effect of gamma radiation on seed germination and seedling survival in cluster bean (CAZG-15-3) in pots experiment in M_1 generation

A separate experiment was laid out in pots to confirm the results on damage recorded in the field. Out of 100 seeds (CAZG 15-3) sown in four pots (25 seeds each), 36% (1600 Gy) to 66% (untreated) germination was recorded after 5 days of sowing. However, 10 days after sowing, a good level of germination was observed for all the treatments up to 800 Gy irradiation dose (Fig. 1). Similar to field observations, delay in germination in various treatments was also recorded in pot experiment. The survival of seedlings on 25th day was 92% at lower doses (200 and 400 Gy) while 82% survival of seedlings was detected at 600 Gy. An abrupt seedling death was exhibited at higher doses with merely 20% survival at 800 Gy. Complete mortality of seedlings was demonstrated above 1000 Gy (Fig. 1). Leaf yellowing was observed in both fields and pot experiment, 10 days after sowing at higher doses (800-1600 Gy) which ensued into seedling mortality (Fig. 2). Cluster bean seed germination and pattern of seedling mortality on irradiation has not yet been reported by other workers mostly because of their working with lower doses or failure to record observations (Yadav et al. 2004; Amrita et al. 2003; Mahla et al. 2010).

Gamma irradiated dicotyledonous seeds depict distinctive high germination and survival for long time probably with the support of cotyledonary leaves (Dubinin, 1964) providing a chance to repair to certain extent (Gaul 1958). Contrary to our observations an inverse relationship between survival and dose of mutagen have been reported in different leguminous crops with a gradual change in chickpea (Umavathi

Fig. 2. A view of yellowing of leaves after 15 days of sowing treated with 800 Gy

and Mullanainathan 2015), pigeon pea (Ariraman et al. 2014), cowpea (Horn et al. 2016), greengram (Vairam et al. 2014) and blackgram (Markeen et al. 2007). The drastic effect on seedling survival between 700 to 800 Gy places LD_{50} in a very narrow range of radiation treatment (Fig. 3). This could possibly be

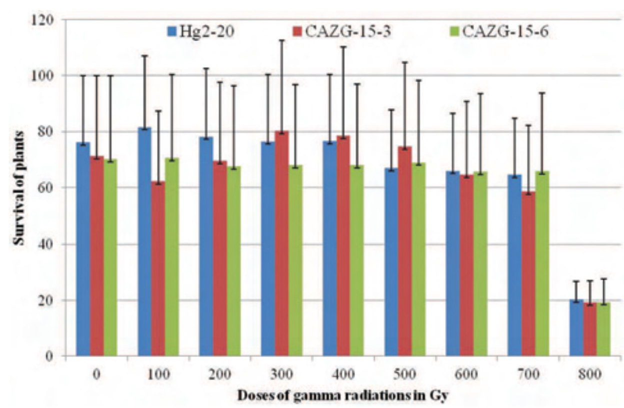

Fig. 3. Survival percent absolute and relative to control (error bars) based on 800 seeds sown under field conditions in M_1 generation

the reason for unavailability of information on LD_{50} in cluster bean (Chowdhury et al. 1975; Badami and Bhalla 1992; Mahla et al. 2010). The high-level of tolerance to gamma radiation with heavy abrupt damage at 800 Gy and beyond in guar may be indicative of some protective role of gummy endosperm that wraps around the embryo. Vig (1969) has also reported resistance of cluster bean chromosomes to radiation dose below 30 kR with no detectable effect.

Seedling growth parameters

The leaves per plant in control ranged from 3.1 (CAZG-15-3) to 4.1 (HG 2-20). Genotype HG-2-20 exhibited maximum fresh (11.63 g) and dry weight (2.02 g) while it was least in CAZG-15-3 (9.29 g and 1.53 g, respectively). Genotype HG-2-20 demonstrated maximum (6.42 cm) seedling height followed by CAZG-15-3 (5.88 cm) and CAZG-15-6 (5.65). Regardless of genotypes, number of leaves reduced progressively with increase in dose. An initial increase in CAZG-15-6 at 100 Gy and reduction in CAZG-15-3 indicated differential response of genotypes to gamma irradiation. Similar trend was followed by the plant height where genotypic response was less conspicuous and reduction was progressive starting from 100 to 800 Gy (Fig. 5, 6 A). Irrespective to genotypes, an increment in seedling weight was detected at 100 Gy which decreased gradually with increasing dose. Similar results were also reported by Mahla et al. (2010) while working with cluster bean var. RGC-936 and HG-365.

Plant and pollen sterility

A high frequency of sterile plants was observed in all three genotypes at 800 Gy (83%, 78% and 86% in HG-2-20, CAZG-15-6 and CAZG-15-3, respectively). Plant sterility was portrayed as complete sterility devoid of pods and female sterility was also portrayed by having smaller pods with 1-3 seeds only. Irrespective of genotypes, a gradual increase from 1% to 23% in sterile plant frequency was recorded with increasing dose (100 -700 Gy) of gamma ray (Fig. 4). Thus, genome damage in M_1 generation was reflected through seedling mortality and sterility. Pollen fertility was 100% in control which gradually reduced to 15% in sterile plants at 800 Gy. The increase in pollen sterility was more drastic from 700 to 800 Gy (Fig. 4). An increase in lethality, plant and pollen sterility was recorded by various workers in cluster bean and other crops with increasing radiations doses (Patil and Rane 2015; Bhosle and Kothekar 2010; Velu et al. 2007; Rampure et al. 2017) however, they used gamma radiation of lower range than taken in this study. Moreover, they failed to realize the drastic effect at 800 Gy and reported gradual change for the limited range of treatment doses usually between 100-500 Gy.

Sterile and morphological mutants in M_2 generation

In present study, naturally occurring sterile plants were not observed (untreated) in any of the three genotypes (Control). However, irradiated plants exhibited sterile plants a linear pattern with increasing dose of radiation. Maximum sterile plants (about 15%) in all the genotypes (15.31, 14.28 and 14.3% in HG-2-20, CAZG-15-6 and CAZG-15-3, respectively) were recorded at 800 Gy dose (Fig. 4). Sterile plants were taller with very long inflorescence and remained green for longer time (Fig. 8). Sporadic and very small pods with merely one to two seeds were observed in partially sterile plants (Fig. 6 D.2) while with long beaded inflorescence complete failure of pod formation and seed setting was recorded in fully sterile plants (Fig. 6 D.1). Out of 6235 plants (M_2 population) 42 complete sterile and 296 partially sterile plants were observed. Occasional appearance of partial or complete male sterile mutants has been reported in natural populations (Stafford 1989; Mittal et al. 1968). Alike our observation semi-sterile variants reported by Vig (1965) and Mittal et al. (1968) were taller with more racemes, less pods and remained green for prolonged period. Partial sterility in these variants was shown to have originated from reciprocal translocations. Similarly, Chaudhary et al. (1973) also reported chromosomal abnormalities such as anaphase bridges and laggards in addition to translocations in the progenies obtained from treated seeds. We observed gradual increase in recovery of partially sterile M_2 mutants with increase in dose of radiations (Fig. 4). Emergence of M_1 and M_2 partial sterile plants in high frequency could be a case of chromosomal aberrations and involvement of fragile loci (Huang et al. 2008) may not be ruled out. Availability of genetic male sterility would facilitate artificial hybridization in other wise a difficult to artificial emasculation guar crop for crop improvement. Induction of partially sterile mutants in high frequency in all the genotypes entails possibility of their induction in any genotype of interest in guar.

Other than sterile mutants, few morphological mutations having single leaf (2) instead of trifoliate leaf (Fig. 6 F), modified/deformed inflorescence (4) (Fig. 6 G), slender stem (10) (Fig. 6 E) and yellow leaves (6) (Fig. 6 B, C), branching in single stem type (8) were also observed among a total of 6235 plants over genotypes and treatments. Some morphological mutants were also reported earlier by Patil and Rane (2015), Bhosle and Kothekar (2010) and Mahla et al. (2010) in cluster bean.

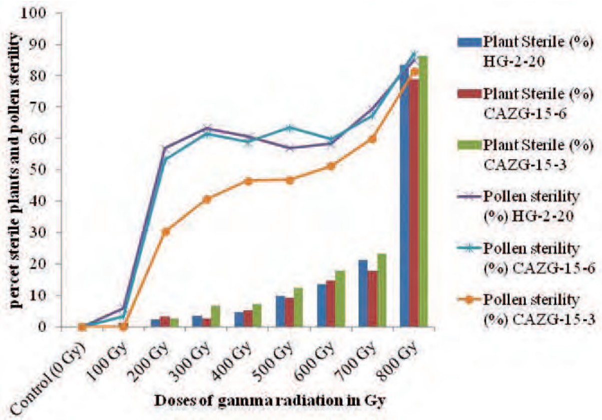

Fig. 4. Plant and pollen sterility observed in M_1 generation

and effectiveness of gamma treatment increased with increase in dose particularly at higher doses (500 to 800 Gy). The gradual increase in sterile plants indicated their inducibility in response to radiation treatment. The mutations with respect to M_1 damage first increased with increasing dose up to 300 Gy there after gradually reduced indicating decline in rate of recovery of mutations with respect to increasing M_1 damage. The three genotypes *viz*., CAZG-15-6 (0.18-1.78), HG-2-20 (0.18-1.23) and CAZG-15-3 (0.17-0.48) depicted comparative mutagenic efficiency. Further the efficiency declined abruptly with an increase of radiation dose from 700 Gy to 800 Gy in all the three genotypes due to higher proportion of sterile plants.

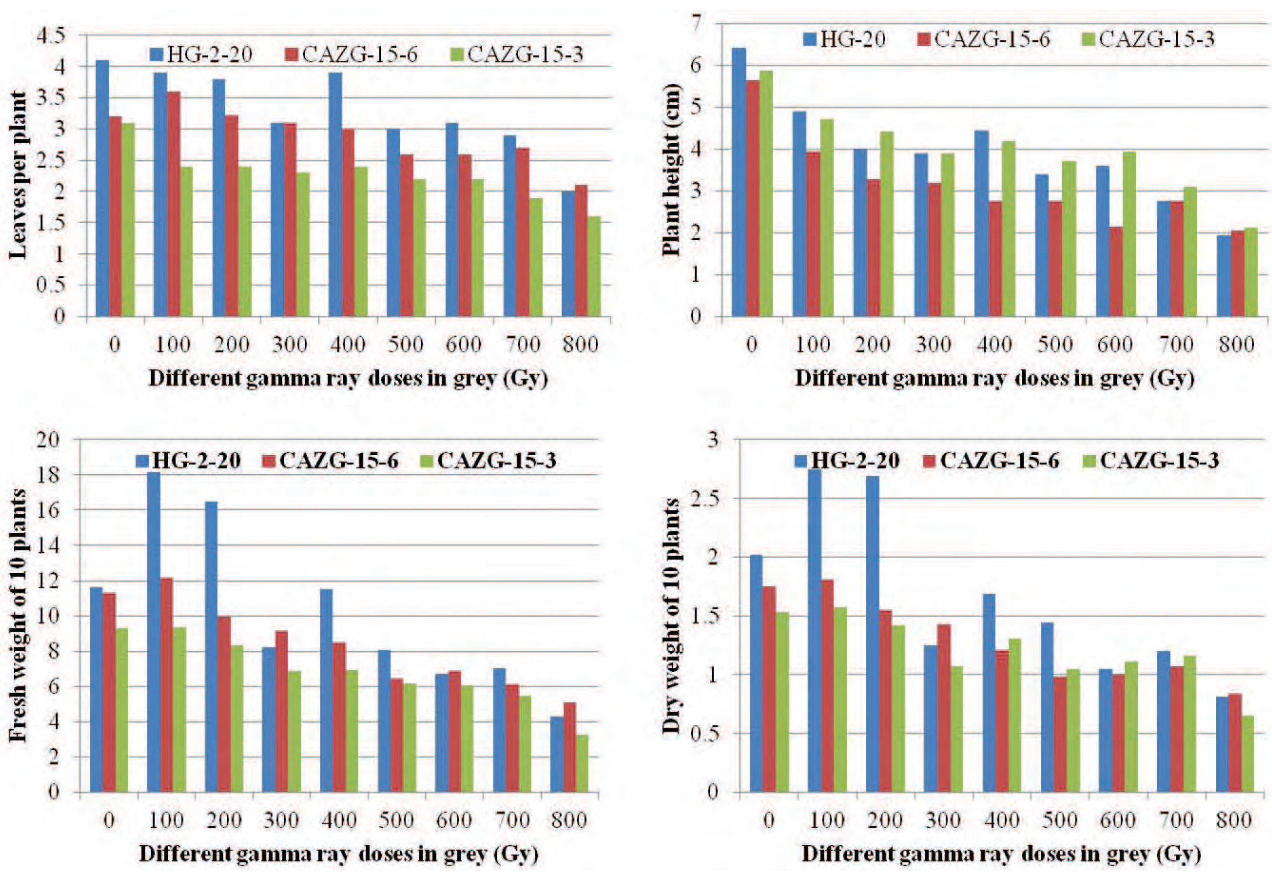

Fig. 5. Effect of different doses of gamma radiation on seedling growth parameters in M_1 generation

Frequency, effectiveness and Efficiency of gamma rays

The frequency, effectiveness and efficiency of treatment to induce male sterile mutations differed among genotypes while the trend followed with dose was comparable (Fig. 7). The frequency of mutants

Dose dependent mutation frequency was recorded while working with physical (gamma-rays) and chemical (EMS) mutagens in cluster bean by Badami and Bhalla (1992), Mahla et al. (2010), Velu et al. (2007, 2012) and Bhosle and Kothekar (2010).

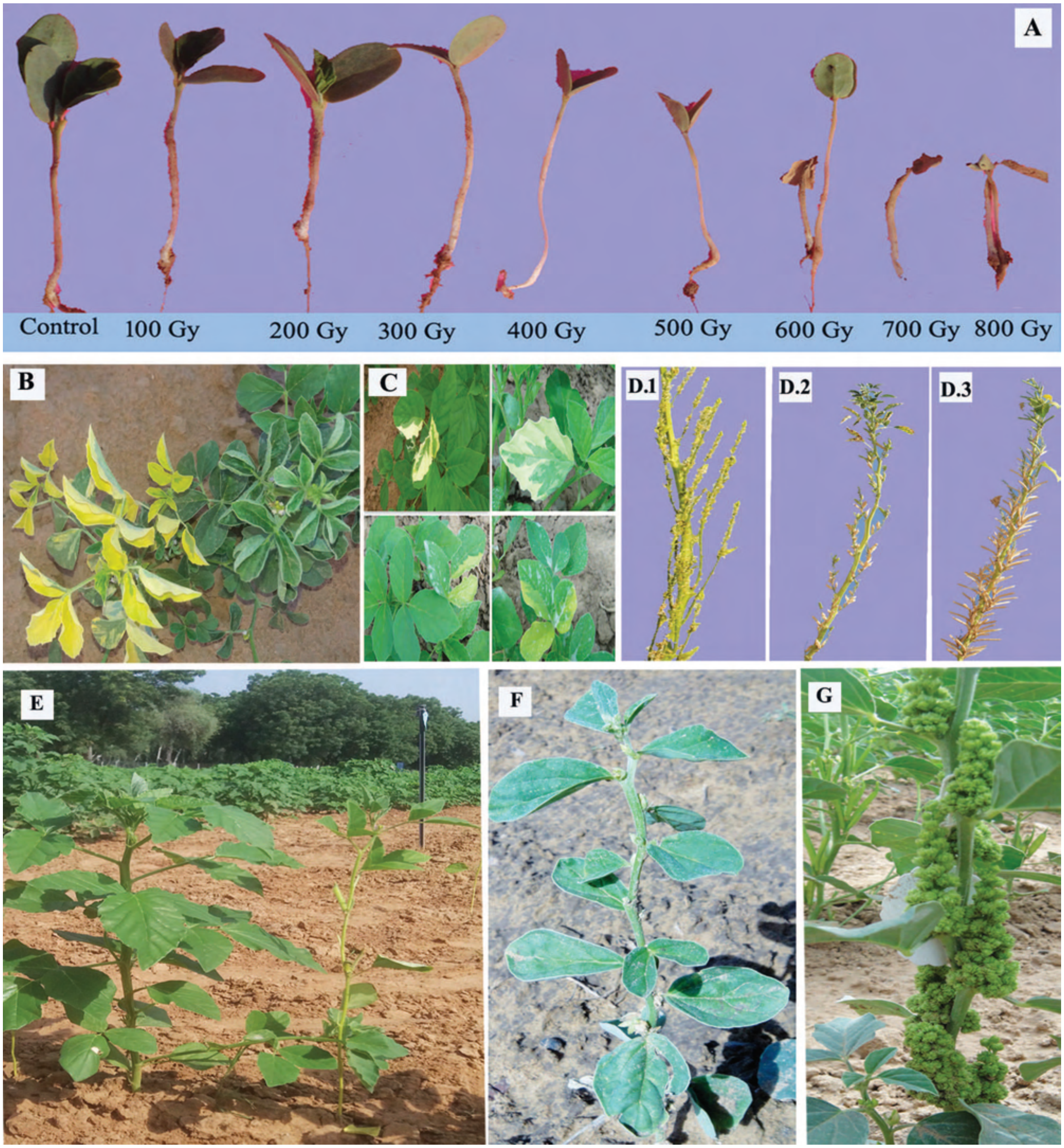

Fig. 6. A view of seedlings (A), chlorophyll deficient mutants (B, C), sterile (D.1), partial sterile (D.2) and normal plants (D.3), slender stem (E), simple leaves (F) and modified inflorescence (G) in M_2 generation

Yield and yield attributing traits

In order to estimate random effect of gamma radiation on yield and yield attributing traits, twenty-five randomly selected plants from each genotype treatment combination were taken for recording observations. Genotypic differences were conspicuous for all the traits and were high in variety HG-2-20 compared to other two genotypes (Table 1). Extended variability for plant height, branches per plant, cluster per plant, pods per plant and yield per plant were recorded in all the three genotypes without considerable reduction in mean. This provides opportunities for selection in

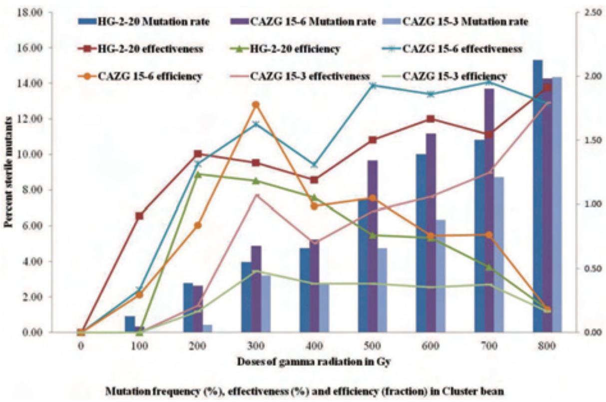

Fig. 7. Mutation frequency (%), effectiveness (%) and efficiency (fraction) in cluster bean M_2 generation

desirable direction for all the traits including yield. Induction of agronomical desirable mutants for seed per plant, protein and galactomannan were reported by Chaudhary et al. (1973). Rao and Rao (1982) recovered a mutant for high yield and early-flowering from Pusa Navbahar. Realizing obvious importance of determinate types in an otherwise indeterminate long duration cluster bean crop Singh et al. (1981) identified a determinate mutant with early flowering. Subsequently using gamma-rays and chemical mutagens Singh and Aggarwal (1986) identified many early flowering mutants of PLG143 having high and long pods, better yields and gum percentage.

Table 1. Mean and standard deviation (Mean ± SD) recorded in three genotypes of Cluster bean in M_2 generation

Treatments	Plant height (cm)	Branches/ plant (No.)	Clusters/ plant (No.)	Pods/plant (No.)	Pod length (cm)	Seeds/pod (No.)	Seed yield/ plant (g)
				HG-2-20			
Control	69.0 ± 6.6	8.2 ± 1.6	37.8 ± 6.8	135.3 ± 29.0	4.46 ± 0.36	6.11 ± 0.84	22.8 ± 5.1
100 Gy	69.5 ± 7.1	7.9 ± 1.2	34.9 ± 8.7	130.4 ± 33.2	4.44 ± 0.44	5.89 ± 0.89	21.8 ± 5.7
200 Gy	83.5 ± 7.2	7.6 ± 1.8	39.2 ± 8.2	142.6 ± 41.3	4.24 ± 0.45	5.67 ± 0.96	24.6 ± 7.2
300 Gy	67.1 ± 7.0	7.0 ± 1.6	27.9 ± 7.0	85.8 ± 26.8	4.17 ± 0.57	5.60 ± 0.98	13.0 ± 5.4
400 Gy	70.7 ± 11.3	7.4 ± 2.0	33.3 ± 10.9	111.6 ± 44.3	4.05 ± 0.56	5.25 ± 1.09	15.6 ± 6.9
500 Gy	64.8 ± 12.4	7.4 ± 2.0	32.5 ± 9.3	102.5 ± 35.1	3.93 ± 0.62	5.25 ± 1.28	14.1 ± 6.5
600 Gy	66.4 ± 9.5	6.6 ± 2.4	28.9 ± 8.9	96.7 ± 42.6	3.98 ± 0.52	5.38 ± 1.13	12.7 ± 5.3
700 Gy	72.4 ± 7.1	7.9 ± 2.0	33.1 ± 11.2	124.6 ± 55.0	3.86 ± 0.52	5.02 ± 1.25	15.9 ± 9.4
800 Gy	70.0 ± 8.3	6.9 ± 2.5	32.8 ± 13.9	106.6 ± 46.8	3.68 ± 0.80	4.74 ± 1.70	15.2 ± 10.2
				CAZG-15-6			
Control	51.6 ± 5.6	5.2 ± 1.1	22.9 ± 4.7	80.3 ± 19.9	4.72 ± 0.29	6.12 ± 0.46	12.0 ± 3.2
100 Gy	46.4 ± 5.8	4.6 ± 1.4	18.0 ± 5.6	63.5 ± 18.0	4.79 ± 0.27	6.30 ± 0.64	9.3 ± 3.4
200 Gy	51.0 ± 7.9	4.9 ± 1.9	20.5 ± 8.5	71.8 ± 31.0	4.85 ± 0.23	6.29 ± 0.71	11.2 ± 5.5
300 Gy	54.3 ± 10.5	4.3 ± 1.5	16.9 ± 7.5	58.6 ± 25.0	4.75 ± 0.37	6.28 ± 0.62	7.6 ± 2.8
400 Gy	56.3 ± 6.9	5.1 ± 1.6	23.8 ± 8.4	83.0 ± 27.0	4.79 ± 0.47	6.26 ± 1.06	9.9 ± 2.8
500 Gy	49.8 ± 8.6	4.7 ± 2.0	20.9 ± 9.3	70.2 ± 33.8	3.82 ± 0.68	4.75 ± 1.54	7.5 ± 4.1
600 Gy	53.5 ± 9.5	5.0 ± 1.8	22.2 ± 6.7	75.5 ± 31.8	3.70 ± 0.76	4.25 ± 1.46	7.3 ± 3.9
700 Gy	60.3 ± 10.6	5.8 ± 1.7	26.4 ± 8.1	97.0 ± 37.0	4.38 ± 0.59	5.13 ± 1.33	10.7 ± 5.1
800 Gy	55.7 ± 12.0	4.8 ± 2.1	21.1 ± 12.5	79.1 ± 51.8	4.38 ± 0.59	5.51 ± 1.26	9.2 ± 5.8
				CAZG-15-3			
Control	55.4 ± 6.0	-	13.0 ± 1.8	67.4 ± 14.8	4.42 ± 0.34	6.53 ± 0.56	10.5 ± 2.5
100 Gy	63.8 ± 7.3	-	11.8 ± 2.1	63.5 ± 19.1	4.36 ± 0.36	6.52 ± 0.79	9.5 ± 2.7
200 Gy	60.2 ± 8.0	-	12.3 ± 1.9	72.8 ± 18.5	4.29 ± 0.23	6.20 ± 0.52	11.1 ± 3.1
300 Gy	56.9 ± 5.7	-	11.9 ± 1.7	67.8 ± 16.3	4.19 ± 0.26	6.01 ± 0.61	10.0 ± 2.4
400 Gy	46.0 ± 7.1	-	10.3 ± 2.9	54.4 ± 22.4	4.27 ± 0.38	6.09 ± 0.89	7.6 ± 2.9
500 Gy	63.1 ± 15.1	-	13.1 ± 2.6	81.1 ± 19.8	3.87 ± 0.46	5.62 ± 1.06	11.5 ± 4.2
600 Gy	49.1 ± 8.6	-	11.4 ± 2.2	71.7 ± 34.3	4.58 ± 0.38	6.27 ± 0.98	9.5 ± 3.0
700 Gy	58.0 ± 19.6	-	11.6 ± 3.2	85.5 ± 44.8	4.37 ± 0.36	6.04 ± 0.96	11.2 ± 5.6
800 Gy	73.2 ± 15.8	-	13.1 ± 2.4	94.8 ± 36.3	4.14 ± 0.44	5.59 ± 1.15	11.3 ± 4.4

\- Unbranched genotype

Supporting the above M_1 parameters we recovered various types of morphological damages and mutants were also observed however, in a very low frequency to be of much use in optimization of radiation dose. Moreover, most such mutants were sterile inapt for significant use in further improvement. The present study has revealed peculiar behavior of cluster bean to gamma radiation treatments. A drastic damage inflicted by 800 Gray and higher doses without affecting germination has not earlier been reported. This could be because of presence of galactomannan a gummy polysaccharide in its endosperm providing some sort of protection and food supply during seed germination. Considering mutation rate, efficiency and effectiveness collectively any dose between 100-700 Gy may be considered for increasing the diversity for yield and related traits in cluster bean.

Authors' contribution

Conceptualization of research (HRM, RS, RKB), designing of experiment (HRM, RS), contribution of experimental material (HRM, RS), execution of field and pot experiment and data collection (HRM), analysis of data and interpretation (RS), preparation of manuscript (HRM, RS, RKB).

Declaration

The authors declare no conflict of interest.

References

Amrita, Ramakrishna K. and Jain U. K. 2003. Induction of variability through gamma irradiation in guar (*Cyamopsis tetragonoloba* (L.) Taub.). Progressive Agric., **3**: 121-122.

Anonymous. 2016. Agricultural Statistics at a Glance. Government of India. Ministry of Agriculture and Farmers Welfare, New Delhi.

Ariraman M., Gnanamurthy S., Dhanavel D., Bharathi T. and Murugan S. 2014. Mutagenic effect on seed germination, seedling growth and seedling survival of Pigeon pea (*Cajanus cajan* (L.) Millsp). Inter. Letters Nat. Sci., **16**: 41-49.

Arora R.N. and Pahuja S.K. 2008. Mutagenesis in guar [*Cyamopsis tetragonoloba* (L.)Taub.]. Plant Mutat. Rep., **2**: 7-9.

Badami P. S. and Bhalla J. K. 1992. Mutagenic effectiveness and efficiency of gamma rays, magnetic field and sodium azide in cluster bean. Adv. Plant Sci., **5**: 534-541.

Bhosale S. S. and Kothekar V. S. 2010. Mutagenic efficiency and effectiveness in clusterbean (*Cyamopsis tetragonoloba* (L.) Taub). J. Phytol., **2**: 21-27.

Chaudhary M. S., Ram H., Hooda, R. S. and Dhindsa K. S. 1973. Effect of gamma irradiation on yield and quality of guar (*Cyamopsist etragonoloba* L. Taub). Ann. Arid Zone, **12**: 19-22.

Chowdhury R. K., Chowdhury J. B. and Singh R. K. 1975. Induced polygenic variability in cluster bean. Crop Improv., **2**: 17-24.

Dubinin N. P. 1964. Problems of radiation genetics.Oliver and boyod, London, p. 445.

Gaul H. 1958. Present aspects of induced mutation in Plant Breeding. Euphytica, **7**: 275-289.

Harten A. M. 1998. Mutation Breeding Theory and Practical Applications. Cambridge University Press, New York. P. 353.

Horn L.N., Ghebrehiwot H.M. and Shimelis H.A. 2016. Selection of novel cowpea genotypes derived through gamma irradiation. Front. Plant Sci., **7**: 262-275.

Huang J., Ma L., Yang F., Fei S. Z. and Li L. 2008. 45S rDNA regions are chromosome fragile sites expressed as gaps in vitro on metaphase chromosomes of root-tip meristematic cells in *Lolium* spp. PLoS One, **3**: 2167.

Konzak C. F., Nilan R. A., Wanger J. and Foster R. J. 1965. Efficient chemical mutagenesis. Radiation Bot., **5**: 49-70.

Kumar S., Modi A. R., Parekh M. J., Mahla H. R., Sharma R., Fougat R. S., Yadav D., Yadav N. R. and Patil G. B. 2017. Role of conventional and biotechnological approaches for genetic improvement of cluster bean. Indus. Crops Prod., **97**: 639-648.

Mahla H. R., Kumar D., Henry A., Acharya S. and Pahuja S. K. 2011. Guar - present status and future prospects in arid zone. J. Arid Legumes, **8**: 133-137.

Mahla H.R., Kumar D. and Shekhawat A. 2010. Effectiveness and efficiency of mutagens and induced variability in cluster bean (*Cyamopsis tetragonoloba*). Indian J. agri. Sci., **80**: 1033-1037.

Markeen Kouser G., Suresh Babu and Lavanya G.R. 2007. Effect of mutagenesis on M_1 population in urdbean. J. Food Leg., **20**: 109-110.

Mittal S. P., Dabas B. S. and Thomas T. A. 1968. Male sterility in guar (*Cyamopsis tetragonoloba* (L.) Taub.). Current Sci., **37**: 357.

Pathak R. 2015. Cluster Bean: Physiology, Genetics and Cultivation. Springer,Singapore p. 151.

Patil B. M. and Rane G. M. 2015. Gamma radiation induced chlorophyll mutations in clusterbean (*Cyamopsis tetragonoloba* (L.) Taub) Var. NCB-12. Int. J. Allied Pract. Res. Rev., **2**: 75-85.

Rampure N. H., Choudhary A. D., Jambhulkar S. J. and Badore R. S. 2017. Isolation of desirable mutants in

Safflower for crop improvement. Indian J. Genet., **77**(1): 134-144. Doi: 10.5958/0975-6906.2017.00018.9.

Rao S. and Rao D. 1982. Studies on the effect of X-irradiation on *Cyamopsis tetragonoloba* (L.) Taub. Proc. Indian Natl. Sci. Acad., **48**: 410-415.

Singh V. P. and Aggarwal S. 1986. Induced high yielding mutants in cluster bean. Indian J. Agric. Sci., **56**: 695-700.

Singh V. P., Yadav R. K. and Chowdhury R. K. 1981. Note on a determinate mutant of cluster bean. Indian J. agric. Sci., **51**: 682-683.

Stafford R. E. 1989. Inheritance of partial male sterility in guar. Plant Breed., **103**: 43-46.

Umavathi S. and Mullainathan S. 2015. Physical and chemical induced mutagenesis study for identifying lethality dose in chickpea (*Cicer arietinum* L.) Var. Co-4. Int. Letters Nat. Sci., **8**: 1-5.

Vairam N., Ibrahim S. M. and Vanniarajan C. 2014. Frequency and spectrum of chlorophyll mutations in greengram [*Vigna radiata* (L.)Wilczek]. Asian J. Sci., **9**: 204-207.

Velu S., Mullainathan L. and Arulbalachandran D. 2012. Induced morphological variations in cluster bean (*Cyamopsis tetragonoloba* (L.) Taub). Int. J. Curr. Trends Res., **1**: 48-55.

Velu S., Mullainathan L., Arulbalachandran D., Dhanavel D. and Poonguzhali R. 2007. Effectiveness and Efficiency of gamma rays and EMS on cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.). Crop Res., **34**: 249-251.

Vig B. K. 1965. Effect of a reciprocal translocation on cytomorphology of guar. Sci. Cult., **31**: 531-533.

Vig B. K. 1969. Studies with 60Co-Radiated Guar (*Cyamopsis tetragonoloba*) (L.) Taub). The Ohio J. Sci., **69**: 18-31.

Yadav S. L., Singh V. V. and Ramkrishna K. 2004. Evaluation of promising M_3 progenies in guar (*Cyamopsis tetragonoloba* (L.) Taub.). Indian J. Genet., **64**: 75-76.