Mutagenic effectiveness and efficiency of gamma rays and sodium azide in M_2 generation of Cowpea [*Vigna unguiculata* (L.) Walp.]

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Abstract

Legumes play a pivotal role in combating the chronic hunger and malnutrition in the developing nations and are also ideal crops to achieve global food and nutrition security. In the era of climate change, erratic rainfalls, depleting arable land and water resource, feeding the rapidly growing population is a challenging task. Among breeding programs for crop improvement, induced mutagenesis has proven to be the viable, effective, robust and coherent tool to facilitate the process of creating varieties with improved yield. Like most other pulses, cowpea is a self pollinated, nutritious, versatile and widely adapted grain legume, but harbour a little accessible genetic variability. Lack of sufficient genetic variability and small size of flowers, traditional plant breeding methods are not enough to meet the demands of improvement of cowpea. Hence, induced mutagenesis was employed to induce significant genetic variability across a range of agro-economical traits in two cowpea varieties Gomati VU-89 and Pusa-578 from M_1 to M_4 generations. The success of induced mutagenesis largely depends on the selection of appropriate mutagen, its dose, effectiveness and efficiency. Hence present study was conduct to assess the effectiveness and efficiency of single and combined doses of sodium azide and gamma rays to set an appropriate protocol for induced mutagenesis experimentation in cowpea.

1. Introduction

Pulses are the edible grains of members of the Leguminosae family, and have been defined as “Leguminosae crops harvested wholly for their grain, including beans, lentils and cowpeas” by the Food and Agricultural Organization of the United Nations [1]. Pulses are considered as vital constituents in the diets of people and hence are most suitable crops to achieve the global food and nutrition security. The production of pulses in India is much

lower as compared to global pulse production and it may be due to the lack of high yielding varieties of pulses (Table 1). Additional there is a progressive decline in the per capita pulses consumption from 1951 (60.7 g/day) to 2017 (52.9 g/day) [2]. The low yielding potential of crops is one of the primary obstacles in achieving the desired goals of production. The common asynchronous and longer maturity period, more flower drop and less seed set, natural and anthropogenic pressures are other obstacles in achieving the higher production of pulses.

Table 1. Average yield of pulses in India and world compared during 2007-2016.

YEAR	PULSES YEILD (Q/ha)	
	INDIA	WORLD
2007	5.970	8.154
2008	6.042	8.607
2009	6.706	9.285
2010	6.496	9.043
2011	6.163	8.769
2012	6.460	9.381
2013	7.162	9.745
2014	7.056	9.436
2015	6.465	9.505
2016	6.644	9.929

Source: FAOSTAT | © FAO Statistics Division, 2017.

Among the ten primary pulse crops as recognised by FAO, Cowpea is an important member on the basis of its use, nutritive value and other desired qualities. It belongs to fabaceae family and is one among the most ancient pulse crops used by man [3]. It is nutritious, versatile and widely adapted grain legume to many areas of the humid tropics and temperate zones. The dry and immature pods serve as a rich source of dietary protein for millions of people in developing countries including India and are also used as fodder for livestock [4]. Cowpea has substantial tolerance to high temperatures and severe drought stress and could withstand both acidic and alkaline soils but is intolerant of frost [5]. It is suitable for cultivation in subtropical areas of the world and usually grown alone but can be intercropped with a range of other crops including sorghum, millet, maize, cassava and cotton [6]. Cowpea is considered as a vital constituent of traditional cropping system because of its capability to reinstate soil fertility for succeeding cereal crops grown in rotation with it and enhances soil fertility mostly in smallholder farming systems where very less or no fertilizer is used. Cowpea growing countries in the Asian region are India**,** Sri Lanka, Bangladesh, Myanmar, China, Korea, Thailand, Indonesia, Nepal, Pakistan, Philippines and Malaysia. According to FAO cowpea is cultivated annually on 12.3 million ha and the estimated production worldwide is about 7.69 million tonnes [7]. Globally Africa contributes about 95% of total world cowpea production (Fig 1). Nigeria is the top most producer of cowpea with an estimated 45% of the world cowpea production (Table 2). The objective of this study was to study effect of mutagens on physiological parameters and their mutagenic effectiveness and efficiency.

Fig 1. Production share of Cowpeas by region (Average 2005 – 2016).

Table 2: World's top 10 producers of Cowpea (Average 2016).

RANK	COUNTRY	PRODUCTION (mt)
1	Nigeria	3.02
2	Niger	1.98
3	Burkina Faso	0.60
4	Cameroon	0.19
5	United Republic of Tanzania	0.18
6	Sudan	0.16
7	Mali	0.14
8	Kenya	0.14
9	Myanmar	0.11
10	Sri Lanka	0.01

Source: FAOSTAT | © FAO Statistics Division, 2017.

2. Materials and methods

2.1. Experimental materials and Seed Irradiation

In the present study, M_0 seeds of two cowpea varieties viz., Gomati VU-89 and Pusa-578 were procured from NBPGR, New Delhi (S Table 1). Initially, LD50 was calculated, the doses of gamma rays and sodium azide were optimised by calculating values based on germination rate and survival percentage [6]. A set of 300 dry and healthy M_0 seeds were pre-soaked in the double distilled water for 9 hours and then treated with 0.01%, 0.02%, 0.03% and 0.04% concentrations of sodium azide (SA) solution for 6 hrs at 25 C at Mutation Breeding Laboratory, Department of Botany, Aligarh Muslim University, Aligarh, India. Another set of 600 M_0 seeds per dose were irradiated with 100, 200, 300 and 400 Gy at a dose rate of 11.58 Gy/min of gamma radiation using a Cobalt 60 (^{60}Co) gamma source under standard conditions at NBRI, Lucknow, India. Each set of irradiated 300 M_0 seeds were also treated with respective SA concentration to produce M_1 seeds. Each set of seeds were

thoroughly washed under tap water twice to get rid of any excess mutagen adhered to the seed surface before sowing in the field.

2.2. Experimental site and crop cultivation

The experimentation were performed at Agriculture field, Aligarh Muslim University, Aligarh (27º 29′ to 28º11′ N' 77º 29′ to 78º 38′ E). The net field size was 23.5 × 40 meter, with 13 blocks (each 3 × 1.8 m) with a 0.6 m (row to row) and 0.3 m (seed to seed) distance (S Fig 1). Cowpea M_1 seeds were sown in 10 replications of 30 seeds in a randomized complete block design (RCBD) to raise the M_1 generation during mid-april 2014-October 2014 along with respective controls. Field was irrigated at regular intervals after seed sowing and; weeding was carried out at seedling and flowering stage. All the M_1 plants were harvested separately at pod stage to collect M_2 seeds. During the mid-april 2015-October 2015, 10 healthy M_2 seeds collected from each M_1 plant were sown treatment wise for raising M_2 generation. In M_3 generation seven and four high yielding stable mutant lines of Gomati VU-89 and Pusa-578, respectively were identified and advanced to M_4 generation during the mid-april 2017-October 2017 [8]. All the recommended cultivation practices were taken care of; such as 15 - 20 N, 50 - 60 P_2O_5 and 50 - 60 K_2O kg/ha of fertilizers were added during seedbed preparation.

S1 Fig. Field plots layout of M_1 generations in a randomized complete block design (RCBD) for each cultivar (Source: Raina et al., 2020. http://creativecommons.org/licenses/by/4.0/).

2.3. Field Analysis

2.3.1. Seed germination

Seed germination was recorded after 15 days of seed sowing and the number of seeds germinated in each treated and untreated population was counted. After recording germination counts, the percentage of seed germination was calculated on the basis of total number of seeds sown in the field using the following formulla.

$$\text{Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds sown}} \text{ x } 100$$

2.3.2. Chlorophyll mutations

Chlorophyll mutants were observed in M_2 generation after 10-20 days of seed sowing in the field. The identification and categorisation proposed by Gustafsson [9] and Khan [10] was followed using the following formula. The influence of combination doses of gamma rays and sodium azide on the frequency of chlorophyll mutations was analyzed by calculating the Coefficient of interaction (k) proposed by Sharma [11].

$$\text{Mutation frequency (\%)} = \frac{\text{Number of mutant seedlings}}{\text{Total number of } M_2 \text{ seedlings}} \text{X } 100$$

$$\text{Coefficient of interaction (k)} = \frac{(a + b)}{(a) + (b)}$$

Where,

(a + b) = The frequency of mutation due to combined mutagen doses.

(a) + (b) = The frequency of mutation due to individual mutagen doses

k = Hypothetical interaction coefficient

If k = 1 additive interaction, and if it is less or more than 1 that indicates synergistic interaction.

2.4. Pollen fertility

The determination of pollen fertility was necessary to assess the pollen sterility based mutagenic effectiveness and efficiency. At the time of flowering, pollens were collected from 30 random plants per treatment and control in M_1 generation. Pollens were then placed on slides and stained with 1% acetocarmine solution. The uniformly stained pollens were counted as fertile, while as unstained pollens were counted as sterile.

2.5. Mutagenic effectiveness and efficiency

The mutation frequency induced by unit mutagen dose refers to mutagenic effectiveness whereas percentage of mutations in relation to biological damage refers to mutagenic efficiency. Mutagenic effectiveness and efficiency was calculated by following formulae given by Konzak *et al*. [12].

$$\text{Mutagenic effectiveness (Gamma rays)} = \frac{\text{Rate of mutation (Mp)}}{\text{Dose in Gray (Gy)}}$$

$$\text{Mutagenic effectiveness (SA)} = \frac{\text{Rate of mutation (Mp)}}{\text{Concentration} \times \text{duration of treatment}}$$

$$\text{Mutagenic effectiveness(Gamma rays + SA)} = \frac{\text{Rate of mutation (Mp)}}{\text{Dose of physical mutagen (Gy)} \times \text{concentration of chemical mutagen} \times \text{duration of treatment}}$$

$$\text{Mutagenic efficiency} = \frac{\text{Rate of mutation (Mp)}}{\text{* Biological damage in } M_1 \text{ generation}}$$

*Biological damage: For measuring the biological damage, three different criteria were used; Injury, Sterility and Meiotic abnormalities refers to %age decrease in seedling height (Mp/I), pollen fertility (Mp/S) and meiotic abnormalities (Mp/Me), respectively.

2.6. Morphological mutations

Data on twenty three qualitative and quantitative traits were recorded in M_2 generation of both the varieties. These traits include tall, dwarf, semi dwarf, compact/bushy, prostrate, semi dwarf spreading, one sided branching, axillary branching, broad leaf, narrow leaf, altered leaf architecture, elongated rachis, multiple flower, flower color, open flower, non flowering, late flowering, early maturity, small/narrow pods, bold seeded pods, coat color, coat pattern, shape and surface traits which were examined and modified on the basis of available cowpea descriptors from the International Union for the Protection of New Varieties of Plants [13] and International Board for Plant Genetic Resources [14]. The characteristics of each mutant is presented in results section. In the field, morphological mutant features for plant height variations, growth habit, leaf, flower, pod and seed were observed, recorded and analysed. The frequency (F) of the morphological mutant was evaluated using the following formula.

$$\text{F (\%)} = \frac{\text{No. of mutants}}{\text{Total number of plants studied}} \text{ x } 100$$

3. Results

3.1. Seed germination

In M_2 generation, compared to the control, the gamma irradiated and SA treated population reflected reduced seed germination (Table 3). In both the varieties, germination decreased with the increase in mutagen dose. In the var. Gomati VU-89, control showed a germination percentage of 93.33%, while it reduced from 88.33 to 80.67% in 100 - 400Gy

gamma rays and 87.33 to 81.33% in 0.01-0.04% SA and 87.00 to 77.67% in 100Gy + 0.01% SA - 400Gy+0.04% SA treatments. Variety Pusa-578 showed much reduced seed germination, the germination percentage for control was 92.00%, while it decreased from 85.00 to 80.33% in 100 - 400Gy gamma rays and 88.33 to 76.33% in 0.01 - 0.04% SA and 83.67 to 75.33% in 100Gy + 0.01% SA - 400Gy + 0.04% SA in gamma rays+SA treatments. As compared to M_1 generation, the %age of seed germination was improved in M_2 generation in both the varieties.

Table 3. Effects of gamma rays, SA and their combinations on seed germination and pollen fertility in M_2 generation two varieties of cowpea

Treatment	Gomati VU-89				Pusa-578			
	Seed germination	% inhibition	Pollen fertility (%)	% reduction	Seed germination	% inhibition	Pollen fertility (%)	% reduction
Control	**93.33**	**---**	**96.53**	**---**	**92.00**	**---**	**97.77**	**---**
100Gy γ rays	88.33	5.36	90.35	6.41	85.00	7.61	88.12	9.87
200Gy γ rays	86.00	7.86	89.11	7.69	83.33	9.42	86.88	11.14
300Gy γ rays	82.67	11.43	85.40	11.54	81.67	11.23	81.19	16.96
400Gy γ rays	80.67	13.57	81.19	15.90	80.33	12.68	77.23	21.01
Mean	**84.42**	**9.55**	**86.51**	**10.38**	**82.58**	**10.24**	**83.35**	**14.75**
0.01% SA	87.33	6.43	90.84	5.90	88.33	3.99	89.11	8.86
0.02% SA	86.00	7.86	87.13	9.74	84.00	8.70	84.65	13.42
0.03% SA	83.33	10.71	81.68	15.38	79.67	13.41	77.72	20.51
0.04% SA	81.33	12.86	77.23	20.00	76.33	16.67	75.00	23.29
Mean	**84.50**	**9.46**	**84.22**	**12.76**	**82.17**	**10.69**	**81.62**	**16.52**
100Gy γ rays +0.01% SA	87.00	6.79	91.58	5.13	83.67	9.06	87.13	10.89
200Gy γ rays +0.02% SA	83.67	10.36	86.14	10.77	80.67	12.32	84.41	13.67
300Gy γ rays +0.03% SA	80.33	13.93	83.66	13.33	77.33	15.94	76.49	21.77
400Gy γ rays +0.04% SA	77.67	16.79	74.50	22.82	75.33	18.12	72.03	26.33
Mean	**82.17**	**11.96**	**83.97**	**13.01**	**79.25**	**13.86**	**80.01**	**18.16**

192

193 ## 3.2. Pollen fertility

194 Variable degree of pollen fertility was recorded in different doses of mutagens
195 employed individually and in combination (Table 3). In the var. Gomati VU-89, per cent
196 pollen fertility reduction ranged from 6.41% in 100Gy to 15.90% in 400Gy in gamma rays
197 treatments, while as it ranged from 5.90% in 0.01% SA to 20.00% in 0.04% SA treatments
198 and in case of combination treatments it ranged from 5.13% in 100Gy+0.01% SA to 22.82%
199 in 400Gy+0.04% SA. In the var. Pusa-578, per cent pollen fertility reduction ranged from
200 9.87% in 100Gy to 21.01% in 400Gy in gamma rays treatments, while as it ranged from
201 8.86% to 23.29 in 0.01% SA-0.04% SA in SA treatments and in case of combination
202 treatments it ranged from 10.89% in 100Gy + 0.01% SA to 26.33% in 400Gy + 0.04% SA.
203 The individual mutagen treatments caused more reduction in pollen fertility than combination
204 treatments in both the varieties.

205 ## 3.3. Chlorophyll mutations: Frequency and Spectrum

206 Chlorophyll mutations are frequently employed for the determination of mutagenic
207 potency in inducing genetic variability as they are vital indicator in the assessment of induced
208 genetic changes of mutagenized population. In both the varieties six chlorophyll mutants viz.,
209 albina, chlorina, xantha, tigrina, viridis and xanthaviridis were observed in M_2 generation. A
210 concise account of chlorophyll mutants are presented in Table 4 and Fig 2. The spectrum and
211 frequency of the chlorophyll mutants are presented in Table 5. The chlorophyll mutant
212 frequencies were estimated on M_2 seedling basis. In both the varieties, except highest dose in
213 each treatment of gamma rays, SA and gamma rays+SA, mutation frequency increased
214 linearly with the increase in mutagen dose. The combination treatment induced higher

mutation frequency in comparison to individual treatments. SA treatments were more effective in inducing higher chlorophyll mutation frequency than gamma rays treatments in Pusa-578. In the var. Gomati VU-89, the chlorophyll mutation frequency ranged from 0.72 to 1.06%, 0.88 to 0.92% and 0.53 to 1.68% in gamma rays, SA and gamma rays+SA treatments, respectively. Var. Pusa-578 responded with the lesser mutation frequency compared to the var. Gomati VU-89. The chlorophyll mutation frequency ranged from 0.58 to 0.77% in gamma rays, 0.89 to 1.02% in SA treatment and the range of chlorophyll deficient mutants induced by the combined mutagens was 0.48 to 1.78% in the var. Pusa-578.

Table 4. Characteristic features of chlorophyll mutants and percentage of mutated plant progenies induced by SA, gamma rays and their combinations in M_2 generation of two varieties of cowpea.

Mutant types and their characteristics	Treatment	No. of M_1 plant progenies	No. of plant progenies segregating in M_2	% mutated plant progenies (Mp)
	Gomati VU-89			
	Control	270.00	0.00	0.00
1.Albina	100Gy γ rays	245.00	2.00	0.82
Lethal mutation with complete absence of pigments,	200Gy γ rays	223.00	11.00	4.93
white leaves; seedlings survive for 10-12 days after germination	300Gy γ rays	208.00	7.00	3.37
	400Gy γ rays	195.00	9.00	4.62
	Total	871.00	29.00	3.33
2. Chlorina	0.01% SA	235.00	2.00	0.85
Leaves are light green in colour; mostly seedlings	0.02% SA	218.00	5.00	2.29
survive for 20-25 days but few may survive till maturity	0.03% SA	203.00	5.00	2.46
	0.04% SA	183.00	6.00	3.28
3. Xantha	Total	839.00	18.00	2.15
Yellow colour leaves due to absence of chlorophyll;	100Gy γ rays + 0.01% SA	230.00	7.00	3.04
seedlings survive for 15-20 days	200Gy γ rays + 0.02% SA	210.00	8.00	3.81
4.Tigrina	300Gy γ rays + 0.03% SA	192.00	8.00	4.17
Leaves with yellow and green patches;	400Gy γ rays + 0.04% SA	178.00	10.00	5.62
seedlings survive for 15-25 days	Total		33.00	4.07
	Pusa-578			
	Control	260.00	0.00	0.00
	100Gy γ rays	235.00	1.00	0.43
5. Viridis	200Gy γ rays	213.00	5.00	2.35
Leaves possess viridine green colour;	300Gy γ rays	200.00	6.00	3.00
reduced seedling height survive till maturity	400Gy γ rays	185.00	6.00	3.24
	Total	833.00	18.00	2.16
	0.01% SA	225.00	1.00	0.44
	0.02% SA	208.00	5.00	2.40
	0.03% SA	193.00	4.00	2.07
	0.04% SA	173.00	6.00	3.47
6.Xanthavirdis	Total	799.00	16.00	2.00
Leaves possess viridine green colour.	100Gy γ rays + 0.01% SA	220.00	6.00	2.73
	200Gy γ rays + 0.02% SA	200.00	6.00	3.00
	300Gy γ rays + 0.03% SA	182.00	7.00	3.85
	400Gy γ rays + 0.04% SA	168.00	6.00	3.57
	Total	770.00	25.00	3.25

Table 5. Frequency and spectrum of chlorophyll mutants induced by gamma rays, SA and their combinations in M_2 generation of two varieties of cowpea.

Treatment	N	Chlorophyll mutant types						CMS	F (%)	K
		Albina	Chlorina	Xantha	Tigrina	Viridis	Xanthaviridis			
Gomati VU-89										
Control	3255.00	-	-	-	-	-	-	-	-	-
100Gy γ rays	3205.00	12.00	8.00	5.00	1.00	3.00	-	29.00	0.90	-
200Gy γ rays	3146.00	13.00	7.00	5.00	-	4.00	-	29.00	0.92	-
300Gy γ rays	3102.00	10.00	8.00	4.00	2.00	4.00	5.00	33.00	1.06	-
400Gy γ rays	3050.00	9.00	8.00	2.00	-	1.00	2.00	22.00	0.72	-
0.01%SA	3185.00	13.00	10.00	1.00	3.00	1.00	-	28.00	0.88	-
0.02%SA	3102.00	14.00	8.00	1.00	-	3.00	2.00	28.00	0.90	-
0.03%SA	3052.00	10.00	7.00	5.00	4.00	-	2.00	28.00	0.92	-
0.04%SA	2925.00	9.00	10.00	4.00	-	2.00	1.00	26.00	0.89	-
100Gy γ rays+0.01% SA	3025.00	11.00	-	-	1.00	-	4.00	16.00	0.53	0.30
200Gy γ rays+0.02% SA	2956.00	13.00	12.00	-	1.00	-	3.00	29.00	0.98	0.54
300Gy γ rays+0.03% SA	2854.00	15.00	15.00	6.00	4.00	6.00	2.00	48.00	1.68	0.85
400Gy γ rays+0.04% SA	2705.00	15.00	13.00	7.00	1.00	7.00	1.00	44.00	1.63	1.02
Pusa-578										
Control	3147.00	-	-	-	-	-	-	-	-	-
100Gy γ rays	3097.00	10.00	6.00	0.00	1.00	1.00	-	18.00	0.58	-
200Gy γ rays	3038.00	11.00	5.00	3.00	-	2.00	-	21.00	0.69	-
300Gy γ rays	2994.00	9.00	5.00	3.00	2.00	2.00	2.00	23.00	0.77	-
400Gy γ rays	2942.00	7.00	7.00	0.00	-	2.00	1.00	17.00	0.58	-
0.01%SA	3077.00	11.00	10.00	1.00	4.00	3.00	-	29.00	0.94	-
0.02%SA	2994.00	13.00	10.00	2.00	-	2.00	2.00	29.00	0.97	-
0.03%SA	2944.00	13.00	7.00	3.00	3.00	2.00	2.00	30.00	1.02	-
0.04%SA	2817.00	11.00	9.00	2.00	-	2.00	1.00	25.00	0.89	-
100Gy γ rays+0.01% SA	2917.00	10.00	-	-	1.00	-	3.00	14.00	0.48	0.32
200Gy γ rays+0.02% SA	2848.00	14.00	9.00	-	2.00	-	2.00	27.00	0.95	0.57
300Gy γ rays+0.03% SA	2746.00	16.00	14.00	6.00	5.00	6.00	2.00	49.00	1.78	1.02
400Gy γ rays+0.04% SA	2597.00	14.00	14.00	6.00	3.00	5.00	2.00	44.00	1.69	1.15

N= Number of M_2 seedlings, CMS= Chlorophyll mutated seedlings, F= Frequency, K= Coefficient of interaction based on % M_2 chlorophyll mutation frequency.

Fig 2. Chlorophyll mutants.

a Gomati VU-89 seedling (Control) showing green color of leaf.

b Albina mutant lacking both chlorophyll and carotenoid contents.

c Xantha mutant lacking chlorophyll content.

d Tigrina mutant with yellow and green patches.

e Pusa-578 Seedling (Control) showing green color of leaf.

f Chlorina mutant with light green leaves.

g Xanthaviridis mutant with yellow leaf apices.

h Viridis mutant with viridine green colour leaves.

The spectrum of chlorophyll mutations was albina> chlorina> xantha> viridis> xanthaviridis> tigrina in the var. Gomati VU-89, whereas it was albina> chlorina> viridis> xantha> tigrina> xanthaviridis in the var. Pusa-578 (Table 6). Among the mutants the occurrence of albina type of chlorophyll mutants was the highest in varieties Gomati VU-89 and Pusa-578. Among the doses the frequency of albina mutants was highest in individual and combination doses of gamma rays+SA, whereas SA treatments induced higher frequency of chlorina mutants in varieties Gomati VU-89 and Pusa-578 (Fig 3). The estimation of coefficient of interaction (k) showed synergistic effects of gamma rays and SA.

Fig 3. Comparative frequency and spectrum of chlorophyll mutations in cowpea based on pooled values of two varieties Gomati VU-89 and Pusa-578.

Table 6. Mutagen induced chlorophyll mutation frequency and spectrum in two varieties of cowpea.

Mutagen/ Variety	Comparative frequency of chlorophyll mutation spectrum						Total Frequency
	Albina	Chlorina	Xantha	Tigrina	Viridis	Xanthaviridis	
Gomati VU-89							
Gamma radiations	0.35	0.25	0.13	0.02	0.10	0.06	0.90
SA	0.38	0.29	0.09	0.06	0.05	0.04	0.90
Gamma radiations + SA	0.47	0.35	0.11	0.06	0.11	0.09	1.19
Average Frequency	0.40	0.30	0.11	0.04	0.09	0.06	1.00
Pusa-578							
Gamma radiations	0.31	0.19	0.05	0.03	0.06	0.02	0.65
SA	0.41	0.30	0.07	0.06	0.08	0.04	0.96
Gamma radiations + SA	0.49	0.33	0.11	0.10	0.10	0.08	1.21
Average Frequency	0.40	0.27	0.07	0.06	0.09	0.04	0.94

*Data based on pooled values of treatments

3.4. Mutagenic effectiveness and efficiency

The mutagen effectiveness and efficiency in a induced mutagenesis determines its usefulness and applicability for crop improvement. Its assessment has been evaluated on the basis of chlorophyll mutation frequency (Tables 7 and 8). The effectiveness and efficiency were highest at the intermediate treatments of mutagens, however lower doses of combined mutagen treatments were most effective and efficient in both the varieties. SA was effective than gamma rays. In both the varieties, effectiveness of single and combined mutagens treatments decreased progressively with the increase in dose in both the varieties. In the gamma irradiated population, the effectiveness ranged from 0.02 to 0.06 in the var. Gomati VU-89 and 0.01 to 0.03 in the var. Pusa-578. The effectiveness of SA treatments ranged from 25.00 to 41.66 and 16.66 to 41.66 in the varieties Gomati VU-89 and Pusa-578, respectively. The effectiveness in combination treatments fluctuated from 0.10 to 1.16 in the var. Gomati VU-89 and 0.06 to 1.00 in the var. Pusa-578. The effectiveness of mutagens was SA> gamma rays + SA> gamma rays.

Table 7. Effectiveness and efficiency of gamma rays, SA and their combinations in cowpea var. Gomati VU-89.

Treatment	(%) Seedling injury (I)	(%) Pollen sterlity (S)	% meiotic abnormalities (Me)	% Mutated plant progenies (Mp)	Mutagenic effectiveness	Mutagenic efficiency		
						Mp/I	Mp/S	Mp/Me
100Gy γ rays	10.91	6.41	3.19	2.00	0.02	0.18	0.31	0.63
200Gy γ rays	16.37	7.69	5.14	11.00	0.06	0.67	1.43	2.14
300Gy γ rays	18.88	11.54	5.47	7.00	0.02	0.37	0.61	1.28
400Gy γ rays	25.61	15.90	8.51	9.00	0.02	0.35	0.57	1.06
0.01%SA	17.40	5.90	1.63	2.00	33.33	0.11	0.34	1.23
0.02%SA	19.64	9.74	3.50	5.00	41.66	0.25	0.51	1.43
0.03%SA	22.31	15.38	6.02	5.00	27.77	0.22	0.33	0.83
0.04%SA	29.13	20.00	7.63	6.00	25.00	0.21	0.30	0.79
100Gy γ rays+ 0.01% SA	16.89	5.13	5.44	7.00	1.16	0.41	1.37	1.29
200Gy γ rays +0.02% SA	20.35	10.77	6.43	8.00	0.33	0.39	0.74	1.25
300Gy γ rays+0.03% SA	25.05	13.33	9.05	8.00	0.14	0.32	0.60	0.88
400Gy γ rays+0.04% SA	31.79	22.82	10.31	10.00	0.10	0.31	0.44	0.97

Mutagenic efficiency was evaluated on the basis of mutation frequency in relation to Mp/I, Mp/S and Mp/Me. Mp/I and Mp/S based maximum efficiency was noted at 200 Gy of gamma rays followed by 100 Gy+0.01% SA and 0.02% SA in both the varieties of cowpea. Mp/Me based efficiency was maximum at 200Gy gamma rays followed by 0.02% SA and 100Gy+0.01% SA in the var. Gomati VU-89. However, the trend was slightly altered in var. Pusa-578 as the maximum efficiency was achieved in 0.02% SA followed by 100Gy+ 0.01% SA and 100 Gy gamma rays. In both the varieties injury and sterility based mutagenic efficiency was found in the decreasing order i.e., gamma rays> gamma rays+ SA> SA. In var. Gomati VU-89 and Pusa-578, meiotic abnormalities based efficiency was higher in gamma rays + SA treatments gamma rays treatments and SA. Meiotic abnormalities based mutagenic efficiency was higher than injury and sterility based efficiency.

287 **Table 8.** Effectiveness and efficiency of gamma rays, SA and their combinations in cowpea var. Pusa-578.

Treatments	(%) Seedling injury (I)	(%) Pollen injury (S)	% meiotic abnormalities (Me)	% Mutated plant progenies (Mp)	Mutagenic effectiveness	Mutagenic efficiency		
						Mp/I	Mp/S	Mp/Me
100Gy γ rays	12.28	9.87	3.19	1.00	0.01	0.08	0.10	0.31
200Gy γ rays	17.17	11.14	5.77	6.00	0.03	0.35	0.54	1.04
300Gy γ rays	21.51	16.96	5.83	6.00	0.02	0.28	0.35	1.03
400Gy γ rays	23.47	21.01	8.48	6.00	0.02	0.26	0.29	0.71
0.01%SA	11.99	8.86	1.05	1.00	16.66	0.08	0.11	0.95
0.02%SA	14.84	13.42	3.56	5.00	41.66	0.34	0.37	1.41
0.03%SA	19.18	20.51	5.00	4.00	22.22	0.21	0.20	0.80
0.04%SA	20.12	23.29	6.62	6.00	25.00	0.30	0.26	0.91
100Gy γ rays+ 0.01% SA	13.11	10.89	4.05	5.00	1.00	0.38	0.46	1.23
200Gy γ rays+0.02% SA	17.72	13.67	4.90	6.00	0.25	0.34	0.44	1.22
300Gy γ rays+0.03% SA	21.12	21.77	5.63	7.00	0.13	0.33	0.32	1.24
400Gy γ rays+0.04% SA	26.88	26.33	8.43	8.00	0.06	0.30	0.30	0.95

3.5. Morphological mutations

A diverse range of mutagen induced morphological mutants were obtained in the mutagenized cowpea population in M_2 generation, many of which may be useful from yield improvement prospectives. These mutants revealed differences in the traits such as plant height, growth habit, leaf, flower pod and seed. The morphological mutations, detected at different growth stages of M_2 plants, were categorically inspected throughout the growing season. The maximum frequency was recorded in gamma rays+SA treatments (5.86 and 6.56%) and the minimum frequency with gamma rays treatments (4.49 and 5.05%), while the SA treatments induced intermediate frequency of morphological mutants (5.38 and 6.05%) in the varieties Gomati VU-89 and Pusa-578, respectively (Tables 9 and 10). The overall frequencies of morphological mutants were more in Pusa-578 than Gomati VU-89. The spectrum of morphological mutations was relatively wider in combined mutagen treatment than separate treatments (Fig 4). The most prominent morphological mutations were found to be associated with the seed and flower followed by growth habit, plant height and pod length (Tables 11 and 12). In general, the occurrence of tall, bushy, broad leaves, elongated rachis, multiple and open flower, bold podded and seeded mutants were found in more numbers at the lower doses of gamma rays and SA. The gamma rays+SA treatments produced significant effects on morphological mutations, however no additive effects were observed in both the varieties. A concise account of the morphological mutants isolated is discussed below.

Fig 4. Comparative percentage and spectrum of induced morphological mutations in cowpea based on pooled values of two varieties Gomati VU-89 and Pusa-578

Table 9. Frequency and spectrum of morphological mutants induced by gamma rays, SA and their combinations in M_2 generation of cowpea var. Gomati VU-89 at different stages of growth.

Treatment	N	Morphological mutant types						TMP	% Mutated plants	k
		Plant height	Growth habit	Leaf	Flower	Pod	Seed			
Control	3050.00	-	-	-	-	-	-	-	-	-
100Gy γ rays	3000.00	8.00	-	6.00	2.00	9.00	4.00	29.00	0.97	-
200Gy γ rays	2941.00	4.00	10.00	13.00	5.00	-	5.00	37.00	1.26	-
300Gy γ rays	2897.00	-	16.00	9.00	7.00	-	6.00	38.00	1.31	-
400Gy γ rays	2845.00	8.00	8.00	-	2.00	6.00	3.00	27.00	0.95	-
Total	**11683.00**	**20.00**	**34.00**	**28.00**	**16.00**	**15.00**	**18.00**	**131.00**	**4.49**	**0.00**
0.01%SA	2980.00	9.00	-	11.00	12.00	-	3.00	35.00	1.17	-
0.02%SA	2897.00	5.00	-	6.00	5.00	11.00	6.00	33.00	1.14	-
0.03%SA	2847.00	-	12.00	7.00	10.00	-	7.00	36.00	1.26	-
0.04%SA	2720.00	10.00	7.00	13.00	8.00	7.00	4.00	49.00	1.80	-
Total	**11444.00**	**24.00**	**19.00**	**37.00**	**35.00**	**18.00**	**20.00**	**153.00**	**5.38**	**0.00**
100Gy γ rays +0.01% SA	2820.00	6.00	-	-	15.00	-	9.00	30.00	1.06	0.44
200Gy γ rays+0.02% SA	2751.00	6.00	-	19.00	13.00	12.00	10.00	60.00	2.18	0.80
300Gy γ rays+0.03% SA	2649.00	-	16.00	9.00	-	-	4.00	29.00	1.09	0.37
400Gy γ rays+0.04% SA	2500.00	-	17.00	7.00	-	14.00	0.00	38.00	1.52	0.52
Total	**10720.00**	**12.00**	**33.00**	**35.00**	**28.00**	**26.00**	**23.00**	**157.00**	**5.86**	**2.13**

N = Number of M_2 plants, TMP = Total mutated plants, k = coefficient of interaction based on % M_2 mutated plants.

Table 10. Frequency and spectrum of morphological mutants induced by gamma rays, SA and their combinations in M_2 generation of cowpea var. Pusa-578 at different stages of growth

Treatments	N	Morphological mutant types						TMP	% Mutated plants	k
		Plant height	Growth habit	Leaf	Flower	Pod	Seed			
Control	2960.00	-	-	-	-	-	-	-	-	-
100Gy γ rays	2910.00	10.00	-	8.00	2.00	-	9.00	29.00	1.00	-
200Gy γ rays	2851.00	2.00	9.00	14.00	4.00	11.00	10.00	50.00	1.75	-
300Gy γ rays	2807.00	-	14.00	8.00	5.00	-	5.00	32.00	1.14	-
400Gy γ rays	2755.00	7.00	10.00	-	4.00	8.00	3.00	32.00	1.16	-
TOTAL	**11323.00**	**19.00**	**33.00**	**30.00**	**15.00**	**19.00**	**27.00**	**143.00**	**5.05**	-
0.01%SA	2890.00	10.00	-	11.00	12.00	-	4.00	37.00	1.28	-
0.02%SA	2807.00	7.00	13.00	6.00	5.00	9.00	8.00	48.00	1.71	-
0.03%SA	2757.00	-	-	8.00	10.00	9.00	9.00	36.00	1.31	-
0.04%SA	2630.00	11.00	8.00	10.00	10.00	-	7.00	46.00	1.75	-
TOTAL	**11084.00**	**28.00**	**21.00**	**35.00**	**37.00**	**18.00**	**28.00**	**167.00**	**6.05**	-
100Gy γ rays +0.01% SA	2730.00	8.00	-	-	14.00	-	10.00	32.00	1.17	0.46
200Gy γ rays+0.02% SA	2661.00	8.00	-	17.00	15.00	13.00	11.00	64.00	2.41	0.60
300Gy γ rays+0.03% SA	2559.00	3.00	15.00	11.00	-	2.00	5.00	36.00	1.41	0.48
400Gy γ rays+0.04% SA	2410.00	-	14.00	9.00	-	15.00	0.00	38.00	1.58	0.50
TOTAL	**10360.00**	**19.00**	**29.00**	**37.00**	**29.00**	**30.00**	**26.00**	**170.00**	**6.56**	**2.04**

N = Number of M_2 plants, TMP = Total mutated plants, k = coefficient of interaction based on % M_2 mutated plants.

Table 11. Frequency and spectrum of morphological mutants induced by various mutagens in M_2 generation of cowpea var. Gomati VU-89.

Mutant Type		Gamma rays		SA		Gamma rays + SA		Total		Grand Total	
		N	**F %**	**N**	**F%**	**N**	**F%**	**N**	**F%**	**N**	**F%**
Plant height	Tall	13.00	0.11	15.00	0.13	9.00	0.08	37.00	0.11	**56.00**	**0.17**
	Dwarf	5.00	0.04	8.00	0.07	2.00	0.02	15.00	0.04		
	Semi dwarf	2.00	0.02	1.00	0.01	1.00	0.01	4.00	0.01		
Growth habit	Compact/Bushy	8.00	0.07	7.00	0.06	12.00	0.11	27.00	0.08	**63.00**	**0.19**
	Prostrate	1.00	0.01	2.00	0.02	5.00	0.05	8.00	0.02		
	Semi dwarf Spreading	3.00	0.03	4.00	0.03	3.00	0.03	10.00	0.03		
	One sided branching	7.00	0.06	1.00	0.01	1.00	0.01	9.00	0.03		
	Axillary branching	3.00	0.03	2.00	0.02	4.00	0.04	9.00	0.03		
Leaf	Broad leaf	8.00	0.07	7.00	0.06	6.00	0.06	21.00	0.06	**59.00**	**0.17**
	Narrow leaf	5.00	0.04	5.00	0.04	7.00	0.07	17.00	0.05		
	Altered leaf architecture	2.00	0.02	2.00	0.02	5.00	0.05	9.00	0.03		
	Elongated rachis	4.00	0.03	6.00	0.05	2.00	0.02	12.00	0.04		
Flower	Multiple flower	4.00	0.04	3.00	0.03	3.00	0.03	10.00	0.03	**66.00**	**0.19**
	Colour	2.00	0.02	5.00	0.04	4.00	0.04	11.00	0.03		
	Open flower	7.00	0.06	7.00	0.06	6.00	0.06	20.00	0.06		
	Non flowering	1.00	0.01	1.00	0.01	2.00	0.02	4.00	0.01		
	Late flowering	6.00	0.05	6.00	0.05	2.00	0.02	14.00	0.04		
	Early maturity	4.00	0.03	2.00	0.02	1.00	0.01	7.00	0.02		
Pod	Small/Narrow pods	7.00	0.06	8.00	0.07	10.00	0.09	25.00	0.07		
	Bold pods	5.00	0.04	6.00	0.05	9.00	0.08	20.00	0.06	**45.00**	**0.13**
Seed	Coat colour	7.00	0.06	8.00	0.07	7.00	0.07	22.00	0.06		
	Coat pattern	6.00	0.05	9.00	0.08	8.00	0.07	23.00	0.07	**75.00**	**0.22**
	Shape and surface	10.00	0.09	11.00	0.10	9.00	0.08	30.00	0.09		
	Grand Total	**120.00**	**1.03**	**126.00**	**1.10**	**118.00**	**1.10**	**364.00**	**1.08**		

N= Number of mutants, F %= Mutant frequency of M_2 plants.

Table 12. Frequency and spectrum of morphological mutants induced by various mutagens in M_2 generation of cowpea var. Pusa-578.

Mutant Type		Gamma rays		SA		Gamma rays + SA		Total		Grand Total	
		N	**F %**	**N**	**F%**	**N**	**F%**	**N**	**F%**	**N**	**F%**
Plant height	Tall	10.00	0.09	11.00	0.10	8.00	0.08	29.00	0.09	**44.00**	**0.13**
	Dwarf	4.00	0.04	7.00	0.06	1.00	0.01	12.00	0.04		
	Semi dwarf	1.00	0.01	1.00	0.01	1.00	0.01	3.00	0.01		
Growth habit	Compact/Bushy	7.00	0.06	6.00	0.05	11.00	0.11	24.00	0.07	**57.00**	**0.17**
	Prostrate	4.00	0.04	1.00	0.01	6.00	0.06	11.00	0.03		
	Semi dwarf Spreading	2.00	0.02	3.00	0.03	2.00	0.02	7.00	0.02		
	One sided branching	5.00	0.04	2.00	0.02	1.00	0.01	8.00	0.02		
	Axillary branching	2.00	0.02	3.00	0.03	2.00	0.02	7.00	0.02		
Leaf	Broad leaf	7.00	0.06	6.00	0.05	5.00	0.05	18.00	0.05	**50.00**	**0.15**
	Narrow leaf	4.00	0.04	4.00	0.04	5.00	0.05	13.00	0.04		
	Altered leaf architecture	1.00	0.01	3.00	0.03	4.00	0.04	8.00	0.02		
	Elongated rachis	5.00	0.04	5.00	0.05	1.00	0.01	11.00	0.03		
Flower	Multiple flower	3.00	0.03	2.00	0.02	4.00	0.04	9.00	0.03	**64.00**	**0.20**
	Color	3.00	0.03	4.00	0.04	5.00	0.05	12.00	0.04		
	Open flower	6.00	0.05	8.00	0.07	5.00	0.05	19.00	0.06		
	Non flowering	2.00	0.02	2.00	0.02	1.00	0.01	5.00	0.02		
	Late flowering	5.00	0.04	5.00	0.05	1.00	0.01	11.00	0.03		
	Early maturity	3.00	0.03	4.00	0.04	1.00	0.01	8.00	0.02		
Pod	Small/Narrow pods	5.00	0.04	7.00	0.06	9.00	0.09	21.00	0.06	**45.00**	**0.14**
	Bold seeded pods	4.00	0.04	5.00	0.05	8.00	0.08	17.00	0.05		
Seed	Coat color	6.00	0.05	7.00	0.06	6.00	0.06	19.00	0.06	**75.00**	**0.23**
	Coat pattern	5.00	0.04	8.00	0.07	7.00	0.07	20.00	0.06		
	Shape and surface	9.00	0.08	10.00	0.09	8.00	0.08	27.00	0.08		
	Grand Total	**103.00**	**0.91**	**114.00**	**1.03**	**102.00**	**0.98**	**319.00**	**0.97**		

N= Number of mutants, F%= Mutant frequency of M_2 Plants

I. Plant height mutants (Fig 5)

(i) Tall mutants: Such mutants were induced with frequency of 0.11 and 0.09% of the total morphological mutations in the varieties Gomati VU-89 and Pusa-578, respectively at lower doses of gamma rays and SA in both the cowpea varieties. The mutants were much taller with increased length of internodes, reduced number of branches and pods than control plants. Plants attained a height 180-185 cm while, the height of control plants was 178-183 cm.

(ii) Dwarf mutants: Such mutants were mostly induced with a frequency of 0.04% of the total morphological mutations. These mutants were induced at higher doses of all the treatments except the combination treatments in the var. Gomati VU-89. These mutants possess shorter internodes, smaller and lesser leaves, reduced pod and seed size and poor yield. The plant height was severely reduced and it ranged from 110-115 cm.

(iii) Semi dwarf mutants: Such mutants were induced by the moderate doses of the mutagens at a frequency of 0.01% of the total morphological mutations in var. Gomati VU-89 and Pusa-578. These mutants were semi dwarf attaining height of 140-150 cm, harbour shorter internodes, reduced branching, less pods and lower yield in comparison to the untreated plants. Pod length was increased, however, the seeds were smaller in size.

II. Growth habit mutants (Fig 5)

(i) Compact/Bushy mutants: Such mutants are characterised with 150.55 cm average height, robust growth, increased branches, condensed internodes that imparted it a bushy appearance. The yield per plant was slightly higher and matured five days earlier compared to control plants. Such mutants were mostly induced by the moderate and higher treatments. The bushy mutants appeared at 0.08% of the total morphological mutations in the var. Gomati VU-89 and 0.07% in var. Pusa-578.

349 (ii) Prostrate mutants: Such mutants were flat on ground, branches spread several meters,
350 possess long internodes, and reflected trailing tendency at the soil surface. They were low
351 yielding with small sized pods, less number of pods containing 6-7 shriveled seeds with
352 rough and hard seed coat. In both the varieties of cowpea prostrate mutants were commonly
353 induced with combination treatments at a frequency of about 0.02% and 0.03% in the
354 varieties Gomati VU-89 and Pusa-578, respectively.

355 (iii) Spreading mutants: Such mutants were observed in 200Gy gamma rays treatment
356 (Gomati VU-89) and 0.03% SA treatment (Pusa-578). These mutants possess long internodes
357 and large canopy. The leaves were mostly broad and branches appeared spreading with wide
358 branch angle. Seed yield per plant was significantly reduced. Their frequency was 0.03% and
359 0.02% in varieties Gomati VU-89 and Pusa-578, respectively.

360 (iv) One sided branching mutants: Mutant plants had thick stems bearing branches on one
361 side and were late in flowering and maturing, low yielded with few pods and shriveled seeds.
362 These mutants were isolated in SA doses in both the cowpea varieties and in combination
363 treatment of 200Gy gamma rays + 0.02% SA in the var. Pusa-578. Their frequency was
364 recorded as 0.03% and 0.02% in the var. Gomati VU-89 and Pusa-578, respectively.

365 (v) Axillary branched mutants: Mutant plants with profuse axillary branching, condensed
366 internodes, small seeded pods and low seed set were mostly isolated from the moderate and
367 higher mutagen treatments. Their frequency was recorded as 0.03% and 0.02% in var.
368 Gomati VU-89 and var. Pusa-578, respectively.

369 **Fig 5.** Morphological mutations (plants with altered height and growth habit).

370 **a** Plant showing normal height and growth habit (Control).

371 **b** Tall mutant with less branches and long internodes.

c Dwarf mutant with stunted growth, shorter internodes and profuse branching.

d Semi dwarf mutant with sparse branching and short internodes.

e Spreading mutant with herbaceous branching and long vegetative phase.

f Bushy mutant with vigorous growth, profuse branching and condensed internodes.

g Axillary branched mutant.

h Prostrate mutant with horizontal growth habit bearing slender, soft and light green branches.

III. Leaf mutants (Fig 6-7)

(i) Broad/Giant leaves mutants: Such mutants were characterised by broad sized leaves, elongated ranchis, tall and vigorous growth. The yield of these mutant plants was found to be higher with the increase in mean number of pods than control plants. Moderate doses of gamma rays and SA treatments induced higher frequency of broad leaf mutants. Their frequency was 0.06% and 0.05% in the var. Gomati VU-89 and var. Pusa-578, respectively.

(ii) Narrow leaves mutants: Such mutants possess narrow leaves with pointed leaf tips. Compared to control, the yield was lower, small pods with few seeds per pod. Higher doses of mutagens induce higher frequency of mutants in both the cowpea varieties. Their frequency was recorded as 0.05% and 0.04% in the var. Gomati VU-89 and var. Pusa-578, respectively.

(iii) Altered leaf architecture mutants: This type of morphological mutations in plants showed wide variations in the leaf margins, apices leaf size and leaflet umber. Plants showed reduced height (about 95 cm) at maturity. Such mutants were induced by all the mutagen treatments

with the frequency of 0.03% and 0.02% in the var. Gomati VU-89 and var. Pusa-578 respectively.

(iv) Elongated rachis: Mutants possessed increased length of rachis with narrow leaflets. They appeared mostly at the lower mutagen doses with a frequency of 0.04% and 0.03% in the var. Gomati VU-89 and var. Pusa-578, respectively.

Fig 6. Morphological mutations (plants with altered leaf morphology).

a Trifoliate leaf of Control (Ovate).

b Broad leaf mutant (Obovate).

c Narrow leaf mutant (hastate).

d Elongated rachis mutant (hastate).

e Trifoliate mutant (Orbicular).

f Altered leaf architecture.

Fig 7. Morphological mutations (plants with altered leaflet number)

g Mutant showing incision of leaf.

h Tetrafoliate with fused terminal leaflets.

i Pentafoliate with variations in leaflet size and shape.

j Tetrafoliate with free leaflets.

k Broad tetrafoliate leaflets.

l Bifoliate leaf.

IV. Flower mutants (Fig 8)

(i) Multiple flower mutants: Such mutants were characterized by the peduncle bearing three/four flowers against two in control plants. Pods and seeds were small and shrivelled.

Mutants were late in maturity compared to the control. These mutants appeared at frequency of about 0.03% in both the varieties.

(ii) Flower colour mutants: Such mutants were characterized by white or blue coloured flowers compared to yellow coloured flowers in control. The 300 Gy gamma rays showed higher frequency of white or blue flower colour mutants. Blue colour flower mutants were observed at higher frequency than white colour flower mutants in the var. Gomati VU-89, whereas in the var. Pusa-578, white colour flower mutants were at higher frequency than the blue colour mutants. Their frequency was recorded as 0.03% in the var. Gomati VU-89 and 0.04% in the var. Pusa-578.

(iii) Open flower mutants: Such mutants harboured bell type flowers with wide open keel and wings and were induced at a frequency 0.06% of total mutation at moderate and higher doses of SA in var. Gomati VU-89 and Pusa-578. The anthers and stigmas were exposed and the flowers withered without forming pods.

(iv) Non-flowering/Vegetative mutants: These mutants were devoid of flowers throughout the life cycle, induced at a frequency of 0.01% and 0.02% in the var. Gomati VU-89 and var. Pusa-578, respectively. They possessed dark green leaves, profuse branching, petite internodes and big leaf. These mutants were noticed in SA and gamma rays+SA treatments.

(v) Late flowering mutants: In these mutant plants, the flowering as well as maturity period was delayed by 8-10 days in comparison to control plants. Higher doses of mutagens induced large number of late flowering mutants. Their frequency was 0.04% and 0.03% in the var. Gomati VU-89 and var. Pusa-578 respectively.

(vi) Early maturity mutants: Such mutants matured 10-15 days earlier than control and were induced at the lower mutagens doses used singly or in combination. The frequency of such mutants was 0.02% in both the varieties.

Fig 8. Morphological mutations (altered flower colour and number per peduncle).

a Yellow colour flowers (Control).

b Blue and yellow colour petals.

c Single peduncle bearing three and four flowers.

d Open flower mutant.

e Light blue colour flower mutant.

f White flowers mutant.

V. Pod mutants (Fig 9)

(i) Small/Narrow pods: Such mutants possessed small and /or narrow pods compared to long pods in control. The seed yield per plant was also reduced as compared to control plants. Higher doses of individual mutagens induced a broad spectrum of these mutants and no such mutants were observed in combined mutagen treatments. Their frequency was recorded as 0.07% and 0.06% in the var. Gomati VU-89 and var. Pusa-578, respectively.

(ii) Bold seeded pod mutants: The mutant plants are characterised by robust growth, normal height, long pods containing 14-15 seeds and were induced at a frequency of 0.06% and 0.05% in the var. Gomati VU-89 and Pusa-578 respectively. The bold size of pods led to significant increase in yield per plant as compared to control. Lower and moderate doses of individual and combined mutagens induced broad spectrum of these mutants.

Fig 9. Morphological mutations (altered pod size).

a Normal pod (control).

460 **b** Long and narrow pods.

461 **c** Broad pod with bold seeds.

462 **d** Broad pod with small seeds.

463 **e** Variations in pod width.

464 **f** Variations in pod length.

465 VI. Seed mutants (Fig 10)

466 (i) Seed coat colour mutants: Such mutants are characterized by the different seed coat color
467 observed after harvest. Seeds were brown and white colour in control plants of the var.
468 Gomati VU-89 and var. Pusa-578, respectively. The colour of seeds changed to dark red,
469 light red, and black in the treated population of both the varieties. These seed mutants
470 appeared at frequency of 0.06% in both the varieties.

471 (ii) Seed coat pattern mutants: The mutants exhibited marbled, dotted and spotted seed coat
472 patterns and such mutants occurred at frequency of 0.07% and 0.06% in the var. Gomati VU-
473 89 and var. Pusa-578, respectively.

474 (iii) Seed shape and surface mutants: Seeds of control plants of both the varieties were of
475 kidney shaped with rough seed surface and were induced at a frequency of 0.09% and 0.08%
476 in the var. Gomati VU-89 and Pusa-578, respectively. Mutants with globular shape and
477 smooth seed surface were isolated in the mutagen treated population.

478 **Fig 10. Morphological mutations (altered seed pattern, colour and shape)**

479 **a** Variations in the seed coat colour.

480 **b** Bold and small seed.

481 **c** Light and dark red seeds with smooth surface.

482 **d** Wrinkled seeds with irregular surface.

4. Discussion

A considerable decline in seed germination, seedling height, pollen fertility and plant survival of treated population was observed in mutagen treatments. However, the extent of diminution in the bio-physiological parameters differed among mutagen doses. The diminution in plant survival was recorded in all the mutagen doses, however, no direct correlation between the mutagen doses and the bio-physiological traits studied was observed which confirmed the fact that the mutagens interact independently with the genes governing a particular trait and that mutations occur in a random manner. These results are in line with earlier reports on several grain legumes [15], [16], [17], [18], [19], [20], [21]. However, the promoting effects of mutagen doses on bio-physiological parameters were also reported in several other crops [22], [23], [24], [25], [26].

5.1. Seed Germination

The assessment of seed germination gives an idea about the mutagenic potency, the dose at which germination is below 50 per cent is treated as lethal or undesirable. Since, the purpose of this present study was to develop large mutagenized population to facilitate effective screening of the high yielding mutants, therefore, the lethal doses of mutagens were discarded to ensure high frequency of desirable mutation. Decreased germination due to mutagen treatments may be the attributed to mutagen induced germination inhibition. The inhibition in seed germination was found to be more or less comparable in gamma rays and SA treatments, while the combined treatments induced higher seed germination inhibition in both the cowpea varieties, which may be attributed to the synergistic effect of the two mutagens. Chauhan and Singh [27] reported that gamma rays interact and cause disruption of

505 tunica zone that leads to the decrease in seed germination. Interference of the mutagens with
506 the auxin synthesizing enzymes may be another reason for seed germination inhibition [28].

507 In the present study, the decrease in pollen fertility has been noticed in the
508 mutagenized population. Pollen sterility was maximum in combined mutagen doses than
509 individual mutagen doses. This may be attributed to meiotic anomalies induced by gamma
510 rays, SA and their combination treatments leading to the formation of aberrant pollen grains.
511 Structure and physiology of the pollen grains is under genetic control and any irregular
512 meiotic cycle may induce significant variations in the pollen properties. Reduction in pollen
513 fertility after mutagen treatments have also been reported in lentil [29], [30], cowpea [31],
514 fenugreek [32], faba bean [33]. The pollen sterility percentage was found remarkably less in
515 M_2 than M_1 generation that depict the operation of recovery mechanism between the two
516 generations.

517 The survival at maturity was declined with increasing mutagen dose in both the
518 varieties of cowpea. These findings support the earlier reports in *Lathyrus sativus* [34] in
519 *Capsicum annum*, [35]. The reduction of plant survival at maturity may be attributed to the
520 disturbed physiological processes [36], [37] or cytological damages Manjaya [38]. The
521 observations recorded on several bio-physiological and cytological parameters revealed that
522 cowpea var. Pusa-578 was comparatively more sensitive towards the individual and
523 combination doses than Gomati VU-89. The differential response of varieties from the same
524 species towards the mutagens have also been reported earlier in *Cajanas cajan* [39], *Vigna*
525 spp [40], [41], [42], *Phaseolus vulgaris* [43], [44], *Jatropha curcus* [45] and *Lens culinaris*
526 [46]. Sparrow *et al.* [47] suggested that differential genotypic sensitivity towards mutagens
527 may be due to the gene loci mutated by one mutagen in one variety were not necessarily
528 mutated by the second mutagen in another variety. The two cowpea varieties viz., Gomati
529 VU-89 and Pusa-578, used in the present study, were from different races, therefore, it is

530 obvious that the two varieties have substantial variation in their genome and thereby the
531 heritable genetic identity of the two varieties is immensely different. Although the spectrum
532 of variations was more or less similar in both the varieties but the mutagen doses to achieve
533 that were not identical in various parameters studied. It can be concluded from the present
534 bio-physiological that mutagenesis induced a comparable spectrum of apparent variation
535 through random mutation in both the varieties of diverse origin with the variable treatments
536 of gamma rays and SA.

537 5.2. Chlorophyll mutations

538 In mutation breeding, the increase in mutation frequency is necessary for crop
539 improvement. In order to acquire desired mutations at highest rates, the picking of optimum
540 mutagen and appropriate dose is important. Both the varieties of cowpea showed a
541 differential response towards gamma rays, SA and gamma rays+SA treatments. The different
542 chlorophyll deficient mutants such as albina, chlorina, xantha, viridis, tigrina and
543 xanthaviridis were observed in M_2 generation were identified as per the categorization of
544 Gustafsson [9]. Gamma rays+SA doses induced higher frequency of chlorophyll mutations
545 than the gamma rays and SA. Intermediate doses showed the maximum frequency of
546 mutations compared to the lower and higher doses. Similar trends of higher chlorophyll
547 mutation frequency with intermediate mutagen doses were also reported in *Cicer arietinum*
548 [48], *Oryza sativa* [49], *Vigna mungo* [50] *Zingiber officinale* [51], *Lablab purpureus* [52].
549 The reduced chlorophyll mutation frequency at the higher mutagen doses may be due to
550 saturation of mutations which lead to the exclusion of the mutant cells during plant growth
551 and development. On contrary Pavadai *et al*. [53] in soybean, Barshile *et al*. [54] in chickpea,
552 Kumar *et al*. [55] in mungbean reported the linear increase in chlorophyll mutation frequency
553 with increase in doses of different mutagen treatments. In the present study, albina and

554 chlorina mutants were found more frequently in both the cowpea varieties. SA was found to
555 be more potent in the production of albina and chlorina mutants than gamma rays. The
556 gamma rays+SA treatments have induced maximum frequency of albina and chlorina
557 mutants than gamma rays and SA treatments employed individually. The synergistic effects
558 of gamma rays and SA in the combination treatments are very much evident from the higher
559 frequency of chlorophyll mutations. The possible reason for synergism might be that the
560 mutagen first applied may expose the accessible protected mutable sites to the second
561 mutagen, the repair enzymes may be rendered non functional by the second mutagen which
562 indirectly facilitates the fixation of mutations induced by first mutagen [11]. The synergism
563 between physical and chemical mutagens in combined mutagen treatments were earlier
564 documented in *Cicer arietinum* [56], *Phaseolus vulgaris* [57], *Vigna spp.*, [58], *Pisum*
565 *sativum* [59], *Linum usitatissimum* [60].

566 5.3. Mutagenic effectiveness and efficiency

567 Induced mutagenesis has been known as a vital tool for the improvement of wide
568 range of crops. The success of mutation breeding depends on enhancing the efficiency of
569 desired mutation. Further the mutagen choice is imperative to achieve the desired mutation
570 rate. Assessment of mutagenic effectiveness and efficiency provides an idea about the utility
571 of mutagens in inducing the desired mutation. Mutagenic effectiveness refers to mutation
572 frequency induced by the unit mutagen dose [12], [61]. Whereas mutagenic efficiency on the
573 contrary indicate the mutation induction (genetic damage) in M_2 generation in relation to the
574 biological damage viz., injury, sterility and meiotic aberrations induced in M_1 generation.
575 Henceforth, mutagenic effectiveness reveal genotypic sensitivity while as mutagenic
576 efficiency reveals mutagenic potency.

The SA doses were superior among mutagen doses employed in present study. On the basis of frequency of chlorophyll mutants, the order of mutagenic effectiveness was SA> gamma rays + SA> gamma rays. Similar results have been reported by Balai and Krishna [62] while studying the effectiveness of ethylmethane sulphonate, sodium azide and hydroxylamine on mungbean. Earlier workers such as Kaul and Bhan [63] in rice, Laskar and Khan [64] in lentil, Wani [65] and Shah *et al*. [66] in chickpea, Khan and Tyagi [67] in soybean and Dhulgande *et al*. [68] in pea have also reported the more effectiveness of chemical mutagens over physical mutagens. In both the varieties Gomati VU-89 and Pusa-578, intermediate doses of gamma rays and SA and the lower doses of the combined mutagen treatments were found to be more effective. The results were in close agreement with the results of Girija and Dhanavel [69] while studying the mutation breeding in cowpea. As per them, in order to attain higher effectiveness and efficiency, the mutation effect was more prominent in the cell that led to meiotic aberrations, physiological changes, which decreased cell survival and eliminated the mutation. The reduced effectiveness at the higher mutagen doses revealed that the mutations and their frequency are random events irrespective of the mutagen doses employed. Our results reflected a differential effectiveness in two varieties of cowpea which depict the genetic divergence and mutagenic sensitivity towards different mutagen doses.

The efficiency of the gamma rays, SA and gamma rays+SA treatments were assessed on the basis of three criteria such as Mp/I, Mp/S and Mp/Me. Mutagenic efficiency shows wide variation in the values depending on the criteria used for its evaluation. Each criterion revealed wide variation in the values of efficiencies for same mutagen dose, thereby reflecting the flexibility of using one or all at a time depending on the objectives of assessment. Lower doses showed enhanced mutagenic efficiency as compared to higher doses. Mp/Me based mutagenic efficiency was higher in comparison to Mp/I and Mp/S basis

602 in both the varieties. This may be due to low frequency of meiotic abnormalities than
603 seedling injury and pollen sterility in mutagen treatments. In the present study, the gamma
604 rays+SA treatments were more efficient than individual gamma rays and SA treatments and
605 this may be attributed to the higher frequency of chlorophyll mutants induced at combination
606 treatments. On contrary Zeerak [70] in brinjal reported that gamma rays+ EMS doses were
607 less efficient than the individual doses of gamma rays and EMS. In the present study, lower
608 and medium doses of gamma rays and SA and gamma rays+SA treatments were found to be
609 more efficient in both the varieties of cowpea. The results were in line with the earlier results
610 in *Lens culinaris* [64] *Glycine max* [67], *Vigna mungo* [71]. However, contrary results of
611 linear increase in mutagenic efficiency with the increase in the mutagen concentrations have
612 also been reported in black gram [72] and millet [73]. Makeen *et al*. [74] also reported higher
613 mutagenic efficiency at lower and medium doses in urdbean. The higher efficiencies of the
614 lower and medium doses may be due to the less biological damages as compared to higher
615 mutagen doses.

616 5.4. Morphological mutations

617 In the present study, several morphological mutants showing variations in characters
618 like altered plant height, growth habit, leaf architecture, flower colour and number, pod and
619 seed shape were isolated in M_2 generation of cowpea. Even though the majority of the
620 induced morphological mutants were uneconomical, however, these mutants can serve as a
621 source of valuable genes in hybridisation programmes. Toker [75] reported that the
622 morphological mutants are useful in gene mapping and phylogentic studies of crops. Mutagen
623 induced chromosomal anomalies or the pleiotropic effects of mutated genes could be the
624 probable reason for the induction of morphological mutants [76], [77]. The present study
625 morphological mutants revealed varying frequency and broader spectrum both between and

626 within the varieties at different mutagen doses reflecting the differential response of varieties
627 towards mutagen doses. Different mutagen doses and duration of treatment may be attributed
628 to the relative proportion of different mutation types [78]. In both the varieties Gomati VU-89
629 and Pusa-578, combined mutagen doses induced higher frequency of morphological
630 mutations followed by individual SA and gamma rays doses. Khursheed *et al*. [79] reported
631 highest frequency of morphological mutants in gamma rays and lowest with combined
632 treatments while investigating the frequency and spectrum of M_2 morphological faba bean
633 mutants. Previous reports of a high frequency and boarder spectrum of morphological
634 mutations in crops such as *Vigna mungo* [41], *Cicer arietinum* [80], [81], *Lens culinaris* [82],
635 *Phaseolus vulgaris* [83] *Glycine max* [67] also confirm that mutagens are effective in
636 inducing morphological mutations in wide range of crops that may serve as a source of
637 valuable genes for the crop improvement programs.

638 Some morphological mutants (early maturing, dwarf, bushy, bold seeded pods and
639 flower colour), screened in M_2 generation in the present study, can serve as source of
640 valuable genes or used as a parents in cross breeding programmes. Dwarf mutants, as
641 recorded in the present study, have been reported earlier in chickpea [21] barley [84],
642 mungbean spp. [77], lentil [85]. The decreased length of internodes and/or number of
643 internode could be attributed to the induction of dwarf and bushy mutants [86]. In our
644 experimentation dwarf and bushy mutants showed substantial decrease in internode length
645 but number of internodes were similar to that of control plants. The mutated genes that
646 control the altered plant height and growth habits are monogenic recessives [87]. Whereas
647 polygenes govern the semi dwarf trait in mutants in wheat [12] and triticale [88]. However,
648 Qin *et al*. [89] reported a dominant mutation in a single gene in dwarf mutants in rice. Both
649 dwarfness and bushy habit of a genotype ensure more plants per unit area, thereby,
650 significantly contribute to the production and productivity. The bold seeded pod mutants

651 induced are associated with high yield which may be valuable in increasing seed size that
652 lead to enhanced cowpea yield. Singh [90] reported that gene mutations govern the bold
653 seeded trait in *Vigna mungo*. Few early maturing mutants with seed yield comparable to the
654 controls were induced in the mutagenized population of both the cowpea varieties. The
655 mutants with modified phenotypes may be due to mutagen induced DNA breaks and altered
656 auxin, mineral and amino acids metabolism [91], [92], [41]. The morphological mutants
657 induced in present study were stable, non segregating and true breeding. Similarly, several
658 workers have also reported that the progenies of tall, dwarf, prostrate, bushy and bold seeded
659 bred true for the altered traits in subsequent generations [93], [94], [64]. The flower colour
660 trait is governed by multigene family, henceforth, flower colour mutants obtained in the
661 present study, can serve as genetic markers in mutation breeding experiments [95], [96], [97].

662 # Conclusions

663 Increase in mutagen doses lead to a progressive decline in the values of all bio-
664 physiological parameters. Combination treatments showed an enhanced effect on seed
665 germination, seedling height and pollen fertility compared to individual mutagen treatments.
666 A dose dependant increase in the frequency of chlorophyll mutations was observed in both
667 the varieties. The estimation of coefficient of interaction (k) did not show any additive effects
668 of mutagens. The spectrum of chlorophyll mutations in the var. Gomati VU-89 was albina>
669 chlorina> xantha> viridis> xanthaviridis> tigrina, whereas it was albina> chlorina> viridis>
670 xantha> tigrina> xanthaviridis in the var. Pusa-578. In general, mutagenic effectiveness and
671 efficiency was highest at the moderate doses of gamma rays and SA, however, lower doses of
672 gamma rays+SA were most effective and efficient in both the varieties. The effectiveness of
673 mutagens was found as SA> gamma rays + SA> gamma rays. Mp/I and Mp/S based

mutagenic efficiency was gamma rays> gamma rays+ SA> SA whereas Mp/Me based order was gamma rays + SA> gamma rays> SA. A diverse range of mutagen induced morphological mutants were obtained in the population of two varieties of cowpea in M_2 generation, many of which are useful from yield improvement prospective. Of all the mutant types, seed and flower mutants followed by growth habit, plant height and pod length were of maximum occurrence in the two varieties. The spectrum of morphological mutations induced by combined mutagens was relatively boarder than gamma rays and SA treatments in both the varieties. The highest frequency was noted in combination treatments of gamma rays+SA followed by SA and gamma rays treatments in both the varieties.

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Formal analysis: Aamir Raina.

Investigation: Aamir Raina.

Methodology: Aamir Raina.

Project administration: Aamir Raina.

Resources: Aamir Raina.

Software: Aamir Raina.

Supervision: Samiullah Khan.

Validation: Aamir Raina.

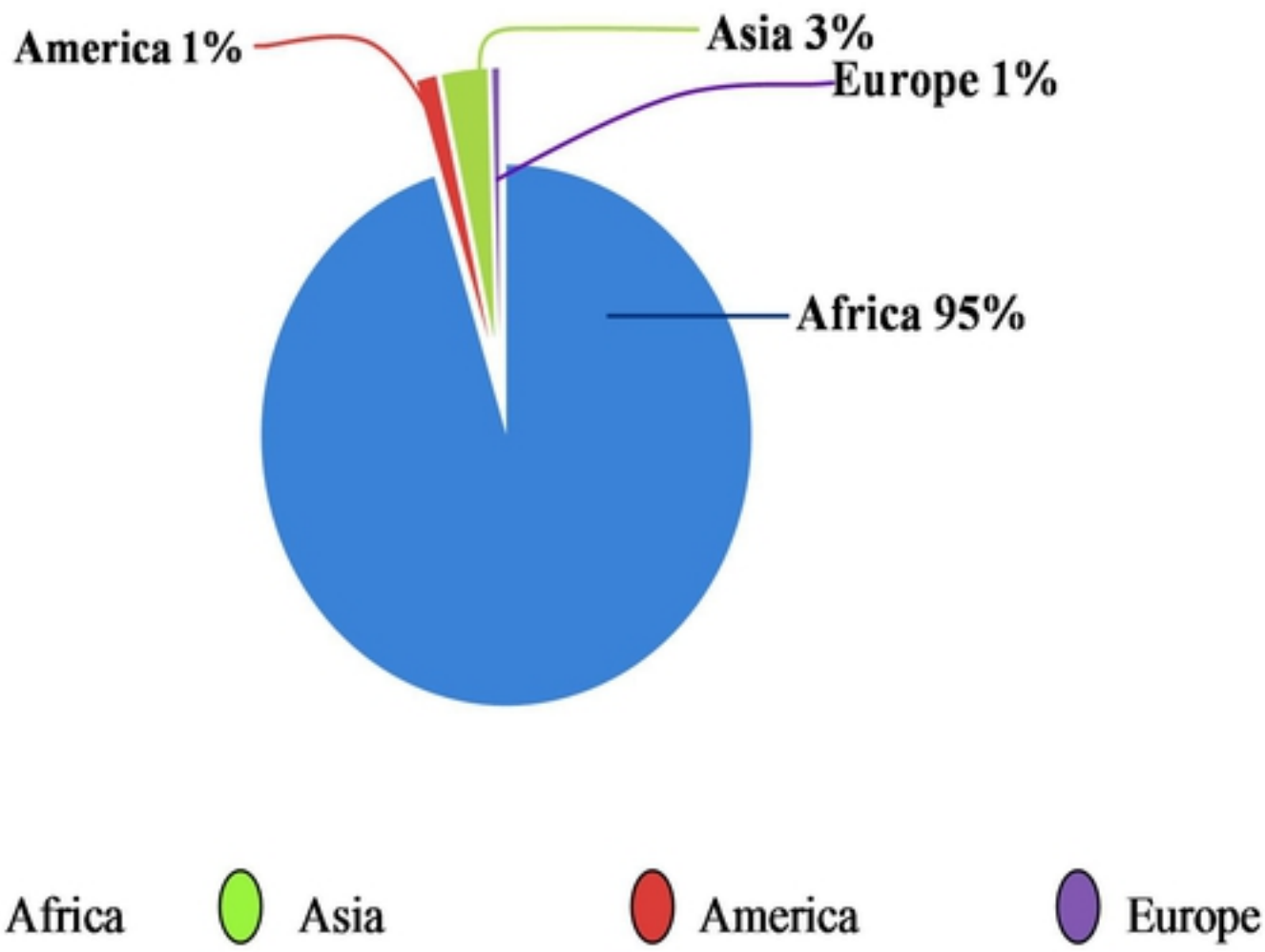
Visualization: Aamir Raina.

Writing - original draft: Aamir Raina.

Writing - review & editing: Aamir Raina.

Competing interests

The authors declare no competing interests.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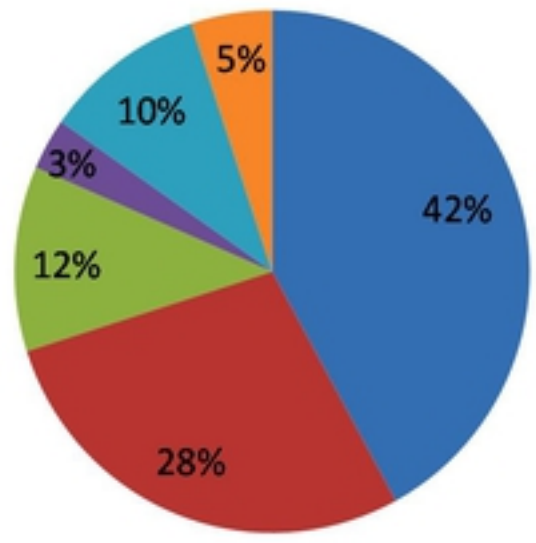

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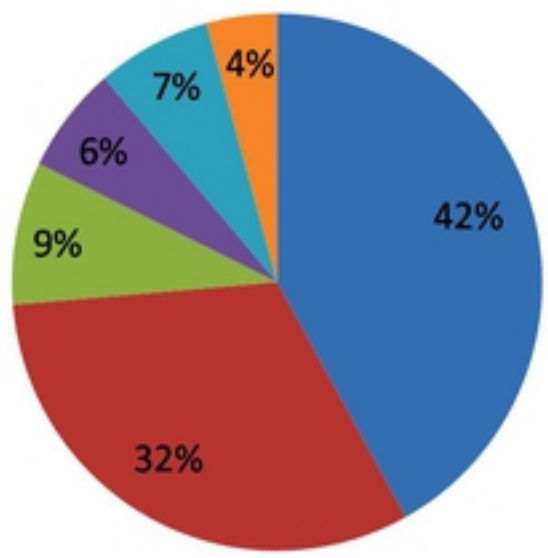
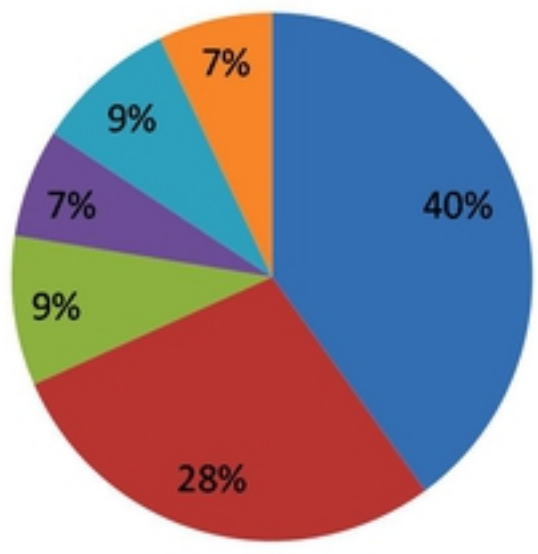


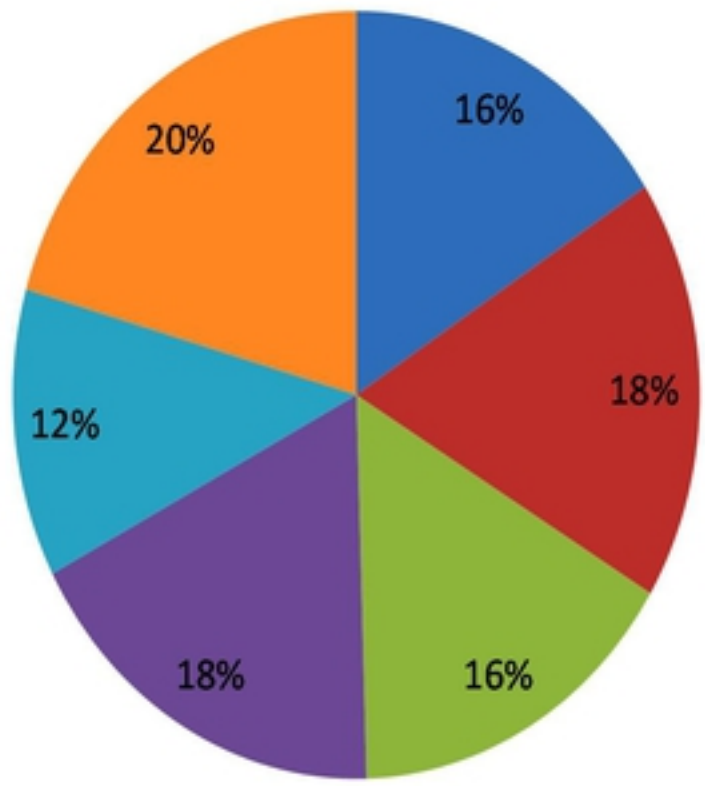
SA

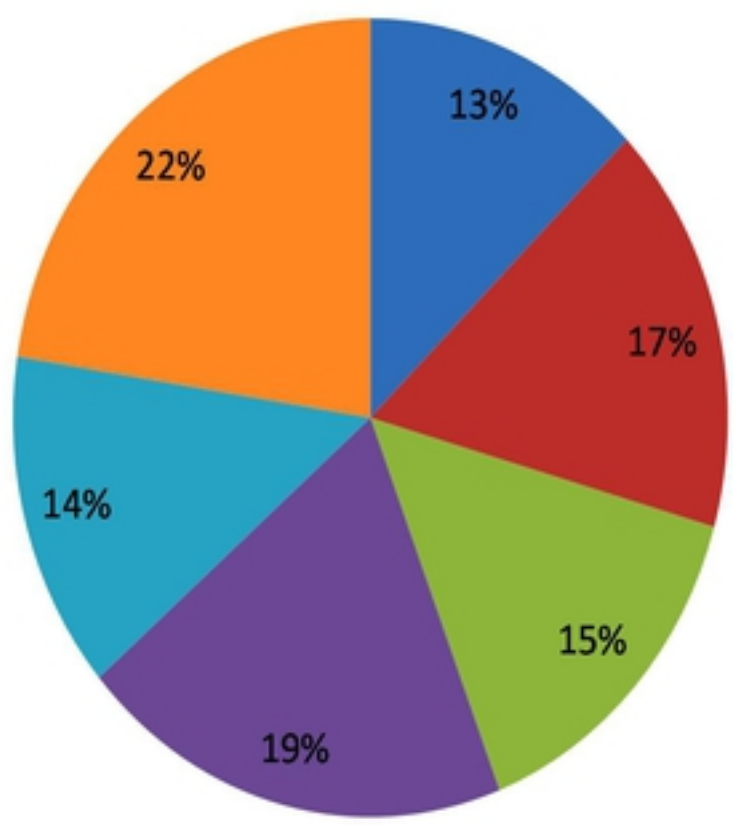


Gamma rays + SA

■ Albina ■ Chlorina ■ Xantha ■ Tigrina ■ Viridis ■ Xanthaviridis

Figure





Plant Size Growth habit Leaf Flower Pod Seed

Figure

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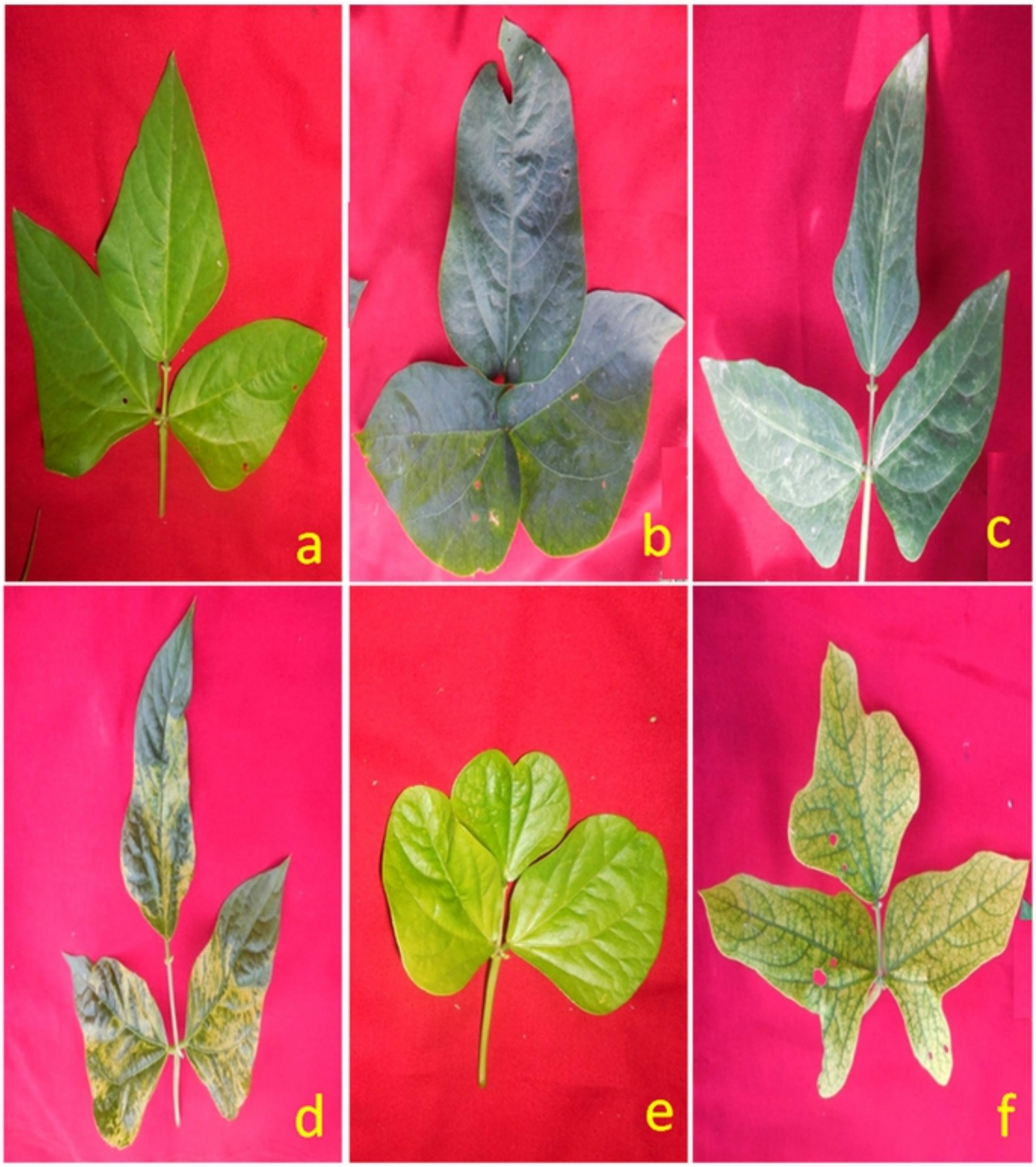

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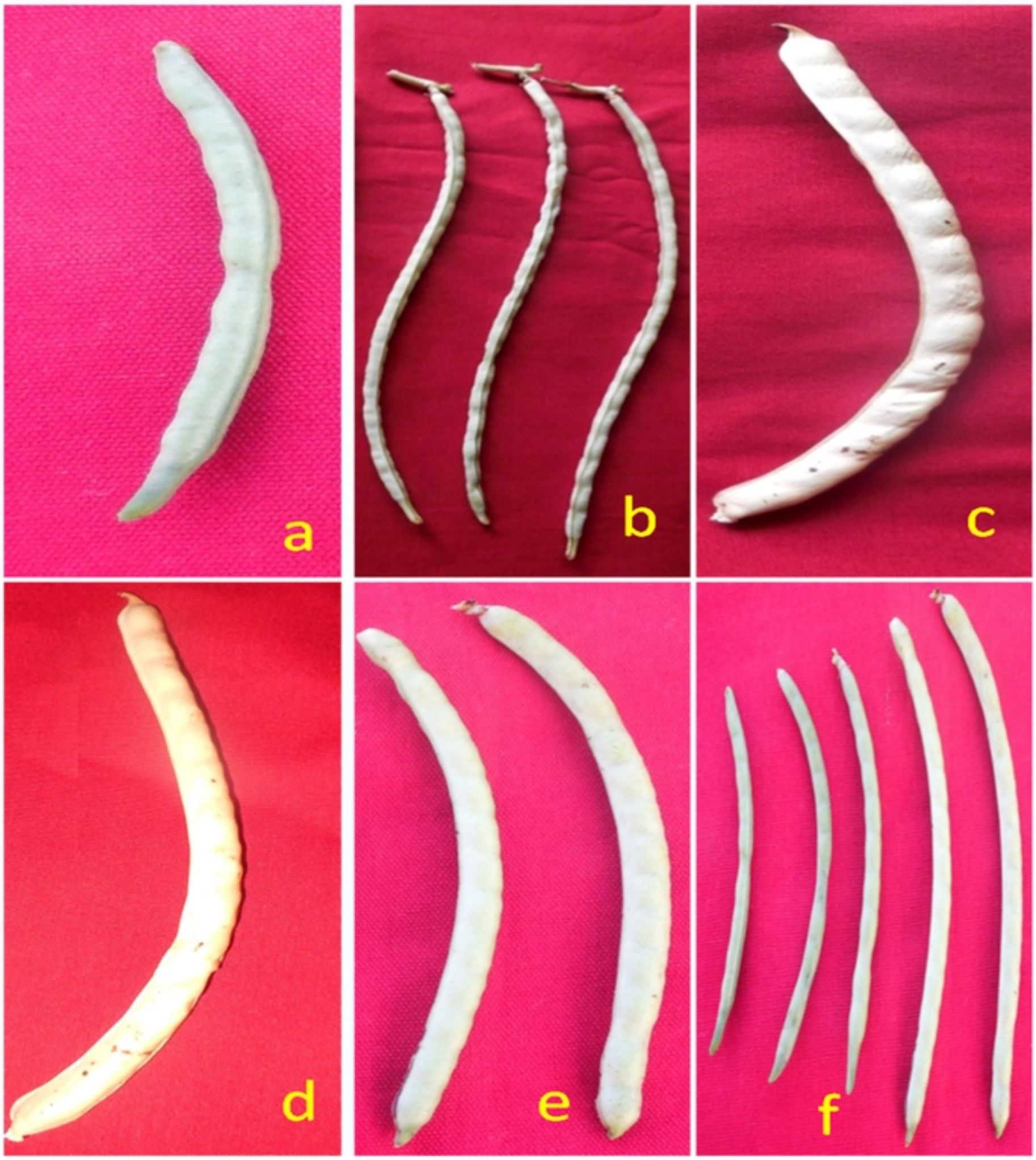

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