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Induction of genetic variability for quantitative traits in horsegram (*Macrotyloma uniflorum*) through irradiation mutagenesis

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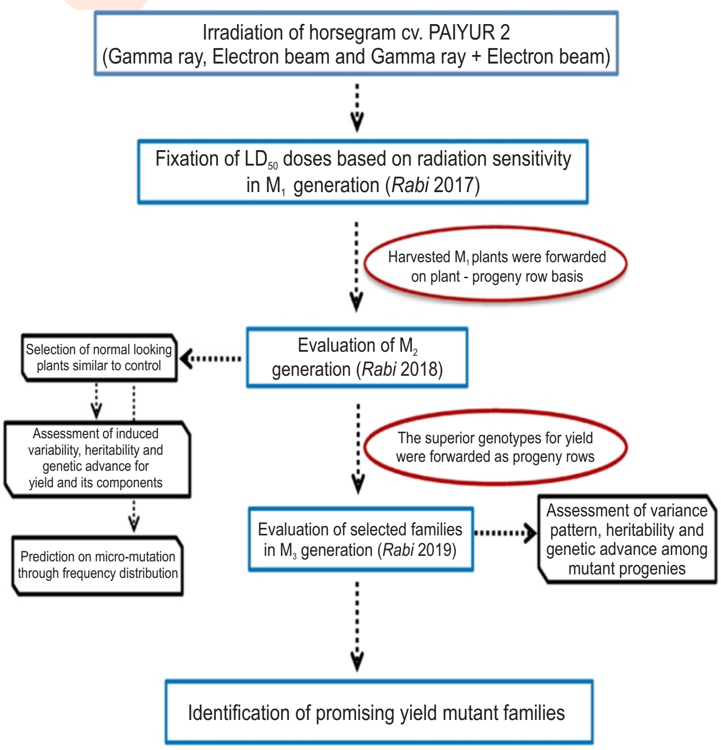
Abstract

Aim: The study was conducted to explore the magnitude of variability induced by gamma rays, electron beam and its combinations for quantitative traits in horsegram (*Macrotyloma uniflorum* (Lam) Verdc.).

Methodology: The horsegram cultivar PAIYUR 2 was irradiated with twelve combinations involving G, EB and G+EB. Desirable mutagenic doses were identified in M_1 based on plant injuries and forwarded to M2. Data on 11 yield component traits were recorded on normal looking M_2 plants for identification of micro-mutants. Promising mutants were forwarded to M_3 and the induced variability was ascertained using multivariate statistics.

Results: Significant transgressive variants were observed in M_2. The G+EB combination induced superior variants for yield. A positive shift in mean for yield and its attributing traits was observed in M_3. Most of the polygenic traits were governed by additive gene effects.

Interpretation: The increased mean for most of the polygenic traits in M_3 population proved that selection made on early generation was highly effective. The isolated high yielding mutant progenies of selected families will contribute towards yield improvement in horsegram.



Key words: Electron beam, Gamma rays, Horsegram, Induced variability

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Introduction

Horsegram (*Macrotyloma uniflorum* (Lam) Verdc.) is an autogamous crop under leguminous family with maturity duration of 120 - 180 days (Morris, 2008). It is well adapted to low rainfall areas with average temperature ranging from 18° to 27°C. The seeds are highly nutritious with 16.9 - 30.4% protein content (Patel *et al*., 1995), 51.9 - 60.9% carbohydrates and 0.58 - 2.06% low lipid content (Bravo *et al.,* 1999; Sodani *et al.,* 2004), lysine (Gopalan, 1989), molybdenum (Bravo *et al.,* 1999), phosphorous, iron, and vitamins *viz.,* carotene, vitamin C, riboflavin, niacin and thiamine (Sodani *et al.,* 2004). It is commonly known as poor man's pulse crop because of its drought tolerant nature with enriched health benefits satisfying the nutritional and economic requirement of marginal farmers. This multipurpose legume is primarily cultivated as a food crop in India, Mauritius, Malaysia, Myanmar, Sri Lanka and Nepal, while as fodder crop in Africa and Australia (Asha *et al.,* 2006). In India, horsegram occupies an area of 3.25 lakh ha with a production and productivity of 1.16 lakh tones and 355 kg ha^{-1}, respectively. Southern India is well thought-out as centre of origin for cultivated species of horsegram (Vavilov, 1951; Zohary, 1970).

The crop is highly photo-sensitive with limited productivity that has led to the replacement of horsegram cultivable areas with other crops. Scientific interventions in horsegram would highly facilitate the crop improvement thereby providing nutritional security in the developing countries. Exploitation of variability for yield, crop duration and ideal stature types would provide an opportunity for evolving superior agronomic types. Chahota *et al.* (2013) reported the existence of limited variability for desirable traits in Indian germplasm accessions. Moreover, hybridization followed by selection remains futile in horsegram because of small floral buds, extreme flower drop and low percentage of pod set (3-4%). Adoption of induced mutagenesis would ease crop improvement with shorter time period (Manjaya and Nandanwar, 2007). Horsegram being a diploid crop (2n=20 and 24), the probability of evolving mutants remains high providing maximum opportunities for selection. Earlier attempts were made on induced mutations in horsegram to explore the effectiveness of different physical (Bolbhat and Dhumal, 2009; Patel *et al.,* 2010) and chemical mutagens (Bolbhat *et al.,* 2012; Kulkarni and Mogle, 2013), but reports on electron beam and its combinations are meagre. Induction of variability through electron beam was found equally effective in relation with gamma rays in legume species (Joshi-Saha *et al.,* 2015; Souframanien *et al.,* 2016). With these views, the experimental study was undertaken to explore the effect of gamma rays (G), electron beam (EB) and combined treatment of gamma rays and electron beam (G+EB) in inducing variability for yield and its related components in horsegram.

Materials and Methods

A well adopted, horsegram cultivar "PAIYUR 2" released from Tamil Nadu Agricultural University (TNAU), India was utilized for inducing of variability through mutagenesis. Irradiations were carried out at Bhabha Atomic Research Center (BARC), Trombay, Mumbai, India. The mutagen treated seeds *viz.,* 100 Gy, 200 Gy, 300 Gy and 400 Gy of gamma rays, electron beam and G + EB were sown in a randomized block design with two replications, which constituted M_1 population. Assessment of plant injuries at M_1 generation a paved way in determining suitable doses for variability induction.

Each M_1 plant from 200 Gy and 300 Gy of gamma rays; 100 Gy and 200 Gy of electron beam and 100 Gy of G + EB was harvested individually and forwarded to M_2 generation following plant to progeny row method during 2018. Data on 12 biometrical traits *viz.,* days to first flowering, days to maturity, plant height (cm), pod length (cm), number of primary branches per plant, number of pods per cluster, number of clusters per plant, number of pods per plant, number of seeds per pod, hundred seed weight (g), biological yield (g) and single plant yield (g) were recorded for randomly selected 150 normal looking plants similar to control (PAIYUR 2) in each M_2 population. The selected individual genotypes on seed yield basis were forwarded to M_3 generation as progeny rows during 2019. Similar biometrical observations were recorded among the progenies of selected mutant families in M_3 generation. All the mutant generations were raised at experimental plot of Department of Pulses, TNAU during *Rabi* season.

Commercial package of practices was adopted with a spacial pattern of 30 × 15 cm in all generations. High order statistics *viz.,* skewness and kurtosis (Snedecor and Cochran, 1967) were performed in M_2 population for each mutagen to determine the magnitude of induced micro-mutations. The biometrical data of M_2 and M_3 population were utilized to estimate mean, range, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) (Burton, 1952), broad sense heritability (H^2) (Lush, 1940), genetic advance as percent of mean (GAM) (Johnson *et al.,* 1955) which provide information on nature and magnitude of variability present in the segregating population and the extent of trait improvement over selection. The estimation of first, second and high order statistics for segregating population are as follows:

First and second order statistics: The variance obtained from the control was taken as error variance (σ^2e). The occurrence of variance in mutant population was considered as phenotypic variance (σ^2p). The genotypic variance (σ^2g) was given by the difference between phenotypic and error variance.

Genotypic Coefficient of Variation (GCV)=$\sqrt{(\sigma^2g)}$/Mean × 100

Phenotypic Coefficient of Variation (PCV)=$\sqrt{(\sigma^2p)}$ / Mean × 100

Heritability (B.S) (H^2) = (σ^2g / σ^2p) × 100

Genetic Advance (GA) = (σ^2g/σ^2p)× k × $\sqrt{(\sigma^2p)}$ (k = selection differential)

High order statistics: The frequency distribution pattern was assessed in M_2 population by the estimates of skewness (β_1) and kurtosis (β_2) using SPSS software.

$$\text{Skewness} = \frac{(n\sum(Y-\mu)^3}{(n-1)(n-2)\sigma^3}$$

$$\text{Kurtosis} = \frac{n(n+1)\sum(Y-\mu)^4}{(n-1)(n-2)(n-3)\sigma^4} - \frac{3(n-1)^2}{(n-2)(n-3)}$$

n is the number of observations; Y is the individual observation; μ is the sample mean and σ is the standard deviation.

Results and Discussion

Improvement of yield and its related components were found promising in many legume species through induced mutagenesis. Datir (2016) reported the effectiveness of conventional mutagens (gamma rays, ethyl methane sulphonate, sodium azide and combinations) in generation of variants for polygenic traits in horsegram. The mutation induction through electron beam has proved to be promising because of its high absorbed dosage rate than gamma rays and other radiations (Zhu *et al.,* 2008). The high dosage rate results in increased double stranded DNA breaks leading to induction of several mutations in a population. Therefore, the present study was undertaken to explore the extent of variability induced by gamma rays, electron beam and its combinations in horsegram.

Statistical analyses on M_2 population would be highly beneficial in predicting the induction of micro-mutations (Scossiroli, 1977; Oladosu *et al.,* 2016). Most of the mutant population of M_2 generation exhibited low average mean compared to control for all quantitative traits, except days to first flowering, days to maturity and plant height (Table 1). The shift in mean towards negative direction resulted due to the deleterious effect of employed mutagens on yield component traits. The prolonged duration in flowering and maturity among mutant population has been reported earlier by Rudraswami *et al.* (2006) in horsegram and Manjaya and Nandanwar (2007) in soybean.

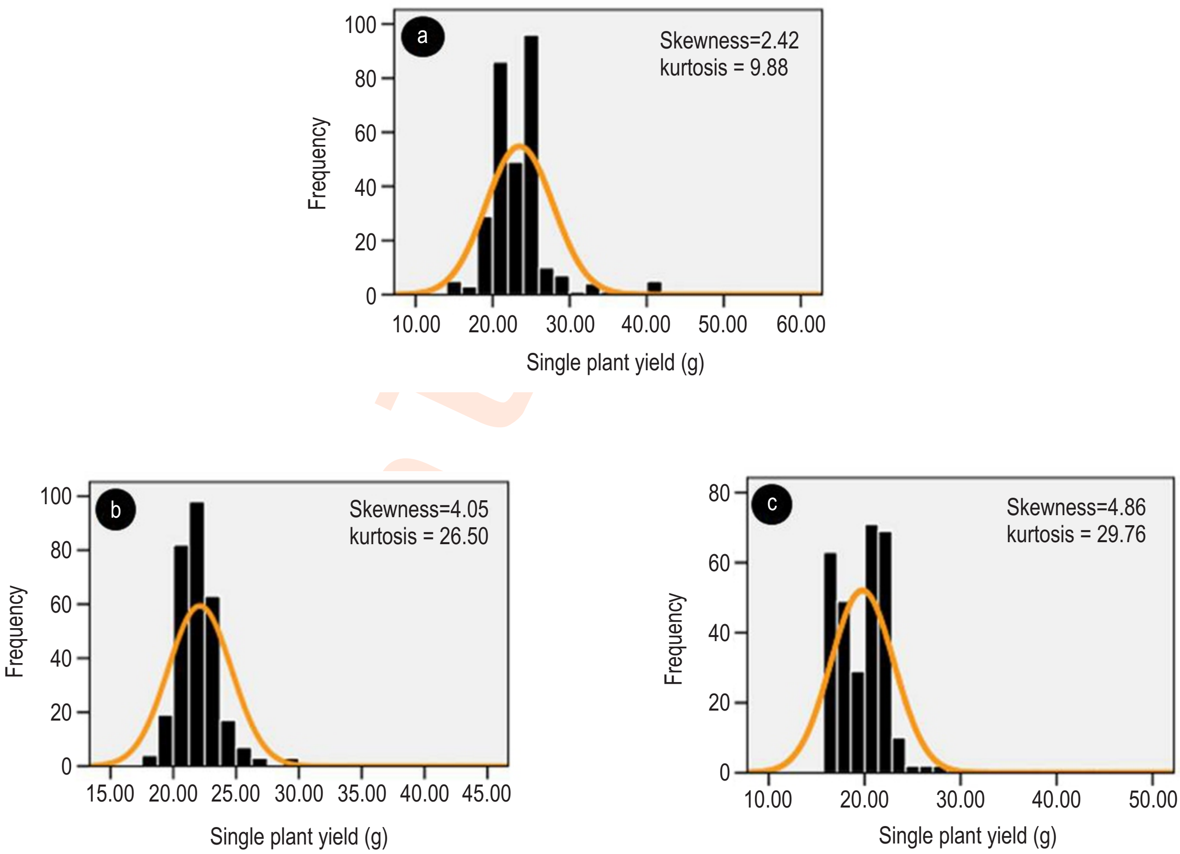

Fig. 1: Frequency distribution pattern of M_2 generation for single plant yield in the irradiated population of: (a) gamma rays; (b) electron beam and (c) gamma rays+electron beam.

Table 1: Mean, Shift in mean, range and variance for quantitative traits in M_2 population of horsegram cv. PAIYUR 2

Traits	Treatment	Mean	Shift in mean	Range	Variance	Traits	Treatment	Mean	Shift in mean	Range	Variance
	CONTROL	**51.17 ± 0.13**	**-**	**54.00 - 49.00**	**2.38**		**CONTROL**	**47.41 ± 0.23**	**-**	**58.00 - 44.00**	**7.73**
	G: 200 Gy	53.16 ± 0.21	1.99	59.00 - 50.00	6.77		G: 200 Gy	46.30 ± 0.47	- 1.11	55.00 - 36.00	32.60
Days to first flowering	G: 300 Gy	52.40 ± 0.23	1.23	59.00 - 43.00	7.99	**Number of clusters per plant**	G: 300 Gy	43.71 ± 0.54	- 3.70	66.00 - 28.00	43.22
	EB: 100 Gy	53.46 ± 0.21	2.29	61.00 - 47.00	6.63		EB: 100 Gy	41.97 ± 0.75	- 5.44	91.00 - 31.00	83.23
	EB: 200 Gy	53.83 ± 0.21	2.66	61.00 - 45.00	6.77		EB: 200 Gy	38.85 ± 0.49	- 8.56	61.00 - 21.00	37.38
	G+EB: 100 Gy	51.17 ± 0.17	0.00	61.00 - 47.00	4.30		G+EB: 100 Gy	31.14 ± 0.54	- 16.27	54.00 - 24.00	43.65
	CONTROL	**105.60 ± 0.15**	**-**	**110.00 - 102.00**	**3.38**		**CONTROL**	**111.47 ± 0.51**	**-**	**124.00 - 105.00**	**38.97**
	G: 200 Gy	108.23 ± 0.32	2.63	115.00 - 103.00	14.80		G: 200 Gy	70.85 ± 1.09	- 40.62	105.00 - 52.00	177.86
Days to maturity	G: 300 Gy	106.64 ± 0.27	1.04	116.00 - 92.00	10.51	**Number of pods per plant**	G: 300 Gy	69.30 ± 1.24	- 42.17	142.00 - 42.00	230.82
	EB: 100 Gy	107.74 ± 0.32	2.14	120.00 - 101.00	14.89		EB: 100 Gy	84.72 ± 1.01	- 26.74	157.00 - 34.00	152.12
	EB: 200 Gy	107.09 ± 0.30	1.49	120.00 - 94.00	13.62		EB: 200 Gy	68.55 ± 1.09	- 42.92	142.00 - 38.00	177.78
	G+EB: 100 Gy	108.44 ± 0.33	2.84	114.00 - 100.00	16.02		G+EB: 100 Gy	52.01 ± 1.14	- 59.46	162.00 - 28.00	194.36
	CONTROL	**72.29 ± 0.37**	**-**	**80.90 - 64.10**	**20.46**		**CONTROL**	**5.32 ± 0.02**	**-**	**5.70 - 4.68**	0.09
	G: 200 Gy	83.17 ± 0.85	10.88	102.30 - 60.20	108.34		G: 200 Gy	5.25 ± 0.04	- 0.07	6.00 - 4.33	0.22
Plant height (cm)	G: 300 Gy	87.00 ± 0.65	14.71	118.20 - 48.60	62.12	**Number of seeds per pod**	G: 300 Gy	5.43 ± 0.04	0.11	6.45 - 4.18	0.27
	EB: 100 Gy	74.40 ± 0.89	2.11	140.30 - 53.20	117.42		EB: 100 Gy	5.26 ± 0.04	- 0.06	6.00 - 4.00	0.21
	EB: 200 Gy	91.22 ± 1.11	18.93	161.20 - 51.30	182.88		EB: 200 Gy	5.63 ± 0.05	0.31	6.67 - 4.33	0.35
	G+EB: 100 Gy	76.84 ± 1.05	4.55	165.20 - 44.50	163.02		G+EB: 100 Gy	5.59 ± 0.05	0.26	6.67 - 4.00	0.32
	CONTROL	**5.12 ± 0.02**	**-**	**5.51 - 4.50**	**0.05**		**CONTROL**	**3.94 ± 0.01**	**-**	**4.11 - 3.81**	**0.01**
	G: 200 Gy	4.57 ± 0.04	- 0.55	5.63 - 3.18	0.25		G: 200 Gy	3.43 ± 0.02	- 0.50	4.54 - 3.06	0.04
Pod length (cm)	G: 300 Gy	4.65 ± 0.03	- 0.47	6.12 - 4.00	0.15	**Hundred seed weight (g)**	G: 300 Gy	3.68 ± 0.02	- 0.26	4.15 - 3.11	0.06
	EB: 100 Gy	4.74 ± 0.04	- 0.37	5.70 - 3.40	0.25		EB: 100 Gy	3.80 ± 0.01	- 0.13	4.49 - 3.11	0.03
	EB: 200 Gy	4.80 ± 0.03	- 0.31	5.73 - 3.70	0.13		EB: 200 Gy	3.88 ± 0.02	- 0.06	4.52 - 3.44	0.04
	G+EB: 100 Gy	4.81 ± 0.04	- 0.31	5.77 - 3.18	0.20		G+EB: 100 Gy	3.74 ± 0.01	- 0.20	4.15 - 3.32	0.02
	CONTROL	**8.62 ± 0.04**	**-**	**10.00 - 7.00**	**0.26**		**CONTROL**	**64.62 ± 0.24**	**-**	**69.00 - 61.00**	**8.93**
	G: 200 Gy	7.24 ± 0.08	- 1.38	11.00 - 5.00	1.06		G: 200 Gy	65.49 ± 0.43	0.87	82.00 - 55.00	27.63
Number of primary branches per plant	G: 300 Gy	7.40 ± 0.10	- 1.23	12.00 - 5.00	1.49	**Biological yield (g)**	G: 300 Gy	62.62 ± 0.55	- 2.00	112.00 - 51.00	45.38
	EB: 100 Gy	7.93 ± 0.08	- 0.69	10.00 - 6.00	0.94		EB: 100 Gy	66.70 ± 0.73	2.08	125.00 - 36.00	78.21
	EB: 200 Gy	7.02 ± 0.07	- 1.60	11.00 - 4.00	0.79		EB: 200 Gy	60.69 ± 0.52	- 3.93	70.00 - 47.00	39.60
	G+EB: 100 Gy	6.68 ± 0.08	- 1.95	10.00 - 4.00	0.98		G+EB: 100 Gy	62.86 ± 0.50	- 1.77	95.00 - 56.00	37.59
	CONTROL	**2.83 ± 0.01**	**-**	**2.92 - 2.51**	**0.02**		**CONTROL**	**27.86 ± 0.10**	**-**	**28.91 - 25.21**	**1.40**
	G: 200 Gy	2.25 ± 0.02	- 0.59	2.99 - 1.67	0.04		G: 200 Gy	23.52 ± 0.16	- 4.34	36.63 - 20.40	3.58
Number of pods per cluster	G: 300 Gy	2.01 ± 0.02	- 0.82	2.85 - 1.52	0.04	**Single plant yield (g)**	G: 300 Gy	23.59 ± 0.47	- 4.27	52.32 - 14.22	34.35
	EB: 100 Gy	2.13 ± 0.03	- 0.70	3.67 - 1.67	0.11		EB: 100 Gy	22.86 ± 0.23	- 5.00	42.86 - 18.53	7.50
	EB: 200 Gy	1.76 ± 0.02	- 1.07	2.67 - 1.33	0.04		EB: 200 Gy	21.36 ± 0.16	- 6.50	41.99 - 19.21	4.00
	G+EB: 100 Gy	2.09 ± 0.02	- 0.74	3.41 - 1.67	0.07		G+EB: 100 Gy	18.19 ± 0.29	- 9.68	47.13 - 16.31	12.42

G - Gamma rays; EB - Electron beam and SE - Standard Error

Table 2: Estimates of frequency distribution pattern in M_2 population of horsegram cv. PAIYUR 2

Traits	Treatments	Skewness	Kurtosis	Traits	Mutagen	Skewness	Kurtosis
	G	- 0.21	0.23		G	0.59*	-0.48
Days to first flowering	EB	- 0.44*	0.41	**Number of clusters per plant**	EB	2.14*	10.40*
	G + EB	- 0.49*	2.48*		G + EB	0.61*	-0.09
	G	0.38*	0.32		G	0.58*	1.06*
Days to maturity	EB	0.89*	2.17*	**Number of pods per plant**	EB	0.51*	5.19*
	G + EB	0.35	- 1.47*		G + EB	3.42*	25.10*
	G	- 0.18	0.94*		G	0.10	-0.89*
Plant height (cm)	EB	1.54*	5.42*	**Number of seeds per pod**	EB	-0.07	-0.51
	G + EB	3.71*	20.23*		G + EB	-0.47*	-0.18
	G	0.02	0.14		G	0.48*	0.41
Pod length (cm)	EB	- 0.30*	0.39	**Hundred seed weight (g)**	EB	0.18	2.79*
	G + EB	- 0.72*	0.74		G + EB	0.23	2.07*
	G	0.51*	1.21*		G	2.59*	15.60*
Number of primary branches per plant	EB	0.17	0.26	**Biological yield (g)**	EB	1.86*	15.73*
	G + EB	0.99*	0.91*		G + EB	1.97*	5.05*
	G	0.47*	1.36*		G	2.42*	9.88*
	EB	1.24*	2.39*	**Single plant yield (g)**	EB	4.05*	26.50*
	G + EB	0.83*	3.86*		G + EB	4.86*	29.76*

G - Gamma rays; EB - Electron beam; * Significant at 0.05%

Both positive (G: 300 Gy; EB: 200 Gy and G + EB: 100 Gy) and negative shift (G: 200 Gy and EB: 100 Gy) in mean value were noticed for number of seeds per pod among mutagenic treatments of PAIYUR 2. A decline in number of seeds per pod was observed earlier by Bolbhat and Dhumal (2012) in gamma rays and EMS treated population of horsegram. A similar trend was registered for single plant yield in all mutant population of PAIYUR 2 with the highest reduction at G + EB combination (-9.68 g). A decline in yield may be attributed to frequent occurrence of deleterious alleles in a population. However, several superior mutants were identified in all treatments with increased yield range than control. All the mutagenic treatments exhibited wide range of variation as compared to control (Table 1) indicating the occurrence of micro-mutants in a positive and negative direction for the studied traits. High order statistics *viz.,* skewness (β_1) and kurtosis (β_2) provide a better insight in predicting the magnitude and direction of micro-mutations in a population (Misra *et al.,* 2008; Kar *et al.,* 2019). Most of the mutant population exhibited significant skewness and kurtosis indicating the potentiality of employed mutagens in induction of variants (Table 2).

The positive skewed distribution (G: 2.42, EB: 4.05 and G+EB: 4.86) with leptokurtic curve (G: 9.88, EB: 26.50 and G+EB: 29.76) was registered for single plant yield in all irradiated treatments. It implies the occurrence of micro-mutants towards reduction in trait value with presence of extremities over the control range in a population (Fig. 1). Similarly, a leptokurtic curve with skewed pattern towards right was registered in all treated population for biological yield, number of pods per plant, number of pods per cluster and plant height. A sharp curve signifies the presence of outliers or extreme variants in the population providing scope for concerned trait improvement. Adoption of intense selection in mutant population will result in genetic gain for these traits (Roy, 2000).

The electron beam irradiated population exhibited non-significant skewness and kurtosis for number of primary branches per plant (β_1: 0.17 and β_2: 0.26) and number of seeds per pod (β_1: -0.07 and β_2: -0.51) indicating random occurrence of micro-mutants at both directions. Balint *et al.* (1968) reported equal distribution of micro-mutants in either direction for most of the polygenic traits. The induction of micro-mutants with increased trait value was noticed in electron beam (β_1: -0.44) and its combination (β_1: -0.49) treatment for days to first flowering. A flattened curve than normal (Platykurtic) was exhibited by G + EB (β_2: -1.47) mutant population indicating less frequency of superior mutants for maturing duration. The occurrence of variants with increased value for pod length was noticed in electron beam (β_1: -0.30) and its combination with gamma rays (β_1: -0.72). From the estimates, it is evident that mutagens were potential in inducing extreme variant types for most of the yield components. The occurrence of transgressive variants was analyzed to determine the scope of isolating mutants with improvement in yield component traits (Misra *et al.,* 2008).

The estimates provide clear picture on the extent of variation induced by the mutagens in comparison with control (Table 3). The genotype of M_2 population possessing value higher than the highest trait value of control was considered as positive transgressive variant (PTV) and the trait value lower than the lowest value of control as negative transgressive variant (NTV). The average and maximum transgressive variation were worked out for the PTV's and NTV's to identify the extent of deviations from the control. The negative transgressive variants were

Table 3: Frequency and magnitude of transgressive variants in M_2 population of horsegram cv. PAIYUR 2

Traits	Treatment	NTVF	NTVM	NTVA	MNTV	Traits	Treatment	PTVF	PTVM	PTVA	MPTV
Days to first flowering	G: 200 Gy	00	-	-	-	**Number of clusters per plant**	G: 200 Gy	00	-	-	-
	G: 300 Gy	02	44.00	5.00	6.00		G: 300 Gy	04	60.75	2.75	8.00
	EB: 100 Gy	02	47.00	2.00	2.00		EB: 100 Gy	04	80.00	22.00	33.00
	EB: 200 Gy	01	45.00	4.00	4.00		EB: 200 Gy	01	61.00	3.00	3.00
	G+EB: 100 Gy	06	47.50	1.50	2.00		G+EB: 100 Gy	00	-	-	-
Days to maturity	G: 200 Gy	00	-	-	-	**Number of pods per plant**	G: 200 Gy	00	-	-	-
	G: 300 Gy	01	92.00	10.00	10.00		G: 300 Gy	01	142.00	18.00	18.00
	EB: 100 Gy	01	101.00	1.00	1.00		EB: 100 Gy	02	152.50	28.50	33.00
	EB: 200 Gy	06	100.00	2.00	8.00		EB: 200 Gy	02	136.50	12.50	18.00
	G+EB: 100 Gy	02	100.50	1.50	2.00		G+EB: 100 Gy	05	159.67	35.67	38.00
Plant height (cm)	G: 200 Gy	08	61.14	2.96	3.90	**Number of seeds per pod**	G: 200 Gy	23	6.00	0.30	0.30
	G: 300 Gy	01	48.60	15.50	15.50		G: 300 Gy	31	6.13	0.43	0.75
	EB: 100 Gy	06	60.17	3.93	10.90		EB: 100 Gy	12	6.00	0.30	0.30
	EB: 200 Gy	02	51.71	12.39	12.78		EB: 200 Gy	41	6.23	0.53	0.97
	G+EB: 100 Gy	03	52.80	11.30	19.60		G+EB: 100 Gy	44	6.19	0.49	0.97
Traits	**Treatment**	**PTVF**	**PTVM**	**PTVA**	**MPTV**	**Traits**	**Treatment**	**PTVF**	**PTVM**	**PTVA**	**MPTV**
Pod length (cm)	G: 200 Gy	02	5.63	0.12	0.12	**Hundred seed weight (g)**	G: 200 Gy	01	4.54	0.43	0.43
	G: 300 Gy	02	5.90	0.39	0.61		G: 300 Gy	13	4.13	0.02	0.04
	EB: 100 Gy	09	5.60	0.09	0.19		EB: 100 Gy	05	4.41	0.30	0.38
	EB: 200 Gy	03	5.73	0.22	0.22		EB: 200 Gy	19	4.22	0.11	0.41
	G+EB: 100 Gy	04	5.59	0.08	0.26		G+EB: 100 Gy	04	4.15	0.04	0.04
Number of primary branches per plant	G: 200 Gy	01	11.00	1.00	1.00	**Biological yield (g)**	G: 200 Gy	38	71.11	2.11	13.00
	G: 300 Gy	01	12.00	2.00	2.00		G: 300 Gy	03	102.00	33.00	43.00
	EB: 100 Gy	00	-	-	-		EB: 100 Gy	19	81.60	12.60	56.00
	EB: 200 Gy	01	11.00	1.00	1.00		EB: 200 Gy	02	70.00	1.00	1.00
	G+EB: 100 Gy	00	-	-	-		G+EB: 100 Gy	23	74.83	5.83	26.00
Number of pods per cluster	G: 200 Gy	02	2.99	0.07	0.07	**Single plant yield (g)**	G: 200 Gy	02	32.92	4.01	7.72
	G: 300 Gy	00	-	-	-		G: 300 Gy	08	37.32	8.41	23.41
	EB: 100 Gy	02	3.50	0.58	0.75		EB: 100 Gy	06	32.73	3.82	13.95
	EB: 200 Gy	00	-	-	-		EB: 200 Gy	05	39.74	10.83	13.08
	G+EB: 100 Gy	01	3.41	0.49	0.49		G+EB: 100 Gy	10	41.81	12.90	18.22

G - Gamma ray; EB - Electron beam; **PTVF** - Frequency of positive transgressive variants; **PTVA** - Average positive transgressive variation; **PTVM** - Mean of positive transgressive variants; **MPTV** - Maximum positive transgressive variation; **NTVF** - Frequency of negative transgressive variants; **NTVM** - Mean of negative transgressive variants; **NTVA** - Average negative transgressive variation and **MNTV** - Maximum negative transgressive variation

Table 4: Identified desirable doses for the induction of transgressive variants in horsegram cv. PAIYUR 2

Characters	Mutagenic doses
Days to first flowering	G (300 Gy)
Days to maturity	EB (200 Gy)
Plant height (cm)	G+EB (100 Gy)
Pod length (cm)	G (300 Gy) and EB (200 Gy)
Number of primary branches per plant	-
Number of pods per cluster	EB (100 Gy)
Number of clusters per plant	EB (100 Gy)
Number of pods per plant	EB (100 Gy) and G+EB (100 Gy)
Number of seeds per pod	EB (200 Gy) and G+EB (100 Gy)
Hundred seed weight (g)	EB (100 Gy)
Biological yield (g)	EB (100 Gy)
Single plant yield (g)	G (300 Gy), EB (200 Gy) and G+EB (100 Gy)

G - Gamma rays; EB - Electron beam

considered for days to first flowering and days to maturity in this study. The effective doses were selected on the basis of high occurrence coupled with high average and maximum variation from control. The identified promising doses for extreme variants serve as a component in planning for improving of polygenic traits in horsegram (Table 4). Among the mutagens, G + EB: 100 Gy followed by G: 300 Gy induced high frequency (10 and 08) of superior types with high average (12.90 and 8.41) and maximum transgressive variation (18.22 and 23.41) for single plant yield. These mutagenic doses can be employed in future mutation breeding programmes to achieve extreme variants with enhanced yield (> 8 g) over control. The high frequency (06) of early duration types was noticed in 200 Gy electron beam irradiated population exhibiting variation range of 2 (NTVA) to 8 (MNTV) days over control. A wide variation was noticed among mutagenic treatments for occurrence of transgressive types. Among the mutagens, lower dose of electron beam (100 Gy) were found promising in inducing extreme variants for most of the studied yield component traits.

The estimates of mean ± standard error, shift in mean, range and variance of M_3 population are presented in Table 5. The shift in mean towards positive and negative direction was observed in mutagenic treatments which contributed towards increased variance over control. Among mutagens, combination of G + EB exhibited the highest shift in mean value for single plant yield (11.57 g), number of clusters per plant (38.33) and number of pods per plant (17.40). The increased plant height (146.37 cm) coupled with biological yield (121.26 g) in 300 Gy of gamma rays irradiated population seems to be useful for evolving mutant lines with superior fodder value. The extreme range than control was noticed for most of the yield component traits in mutant progenies of M_3 population. Implementation of selection on mutant progenies would assist in identifying promising lines with improved agronomic value. The mean of single plant yield increased from M_2 to M_3 generation with induction of superior mutants in all irradiated treatments. Imposing direct selection on yield basis in M_2 generation could be a reason for positive shift in mean value (Khan and Qureshi, 2006). The extreme mutants yielding more than 70 g (G: 300 Gy, EB: 200 Gy and G + EB: 100 Gy) were isolated in M_3 generation, which provide scope for yield enhancement in horsegram. A positive shift in mean was observed at all mutagenic treatments of M_2 and M_3 generations for plant height. The increase in mean value over control could be attributed to frequent induction of positive mutations for the trait (Khan and Wani, 2006).

The mean of hundred seed weight, number of clusters per plant, number of pods per plant and biological yield had shifted to positive direction in M_3 generation. A parallel report of positive shift in mean value for polygenic traits was given by Khan *et al.* (2004). Contrarily, decreased mean value was registered for number of primary branches per plant in both generations (M_2 and M_3) when compared against control. The induction of mutations with detrimental effects could be reason for reduced mean values in treated population (Singh *et al.,* 2000). The mean of days to first flowering and maturity declined in M_3 population compared to control with few exceptions. Mutants with reduced duration would escape from yield loss due to terminal drought as horsegram is cultivated with residual moisture after the end of North-East monsoon in Southern India. Similar early maturing genotypes were reported by Nilahayati *et al.* (2019) in gamma irradiated population of soybean. The shift in mean at both positive and negative directions was noticed in mutant population compared to control for number of seeds per pod. Similar report on quantitative traits was given by Mensah and Obadoni (2007) in groundnut. The extent of variability, heritability at broad sense (H^2) and genetic advance as percent of mean (GAM) were calculated for M_2 (Table 6) and M_3 populations (Table 7).

Low to moderate variability existed for most of the yield component traits in M_2 generation with few exceptions. The low extent of PCV and GCV was registered for days to first flowering and maturity at both generations. In general, the amount of variability increased in M_3 generation for most of the yield component traits in comparison with M_2 indicating the release of inherent variability. Traits *viz.,* single plant yield, biological yield, number of clusters per plant, number of pods per plant, number of pods per cluster and number of primary branches per plant exhibited high values of PCV and GCV in M_3 generation. Increase in variability estimate provides scope for realizing yield improvement through effective selection (Muduli and Misra, 2008). The estimates of heritability and genetic advance are highly useful in predicting the selection response on quantitative traits. In this study, the estimates of H^2 and GAM tend to increase in M_3 generation for most of the yield component traits with few exceptions. The enhanced heritability in subsequent generation could also be an indicative feature of increased homozygosity of genes for the concerned trait (Wani, 2011). The high H^2 coupled with high GAM indicates the governance of additive gene effects

Table 5: Mean, Shift in mean, range and variance for quantitative traits in M_3 generation of horsegram cv. PAIYUR 2

Traits	Treatment	Mean	Shift in mean	Range	Variance	Traits	Treatment	Mean	Shift in mean	Range	Variance
Days to first flowering	**CONTROL**	**52.11 ± 0.63**	**-**	**55.00 - 50.00**	**3.21**	Number of clusters per plant	**CONTROL**	**52.11 ± 1.16**	**-**	**58.00 - 46.00**	**10.77**
	G: 200 Gy	51.11 ± 0.52	- 1.00	55.00 - 48.00	4.54		G: 200 Gy	53.00 ± 2.52	0.89	69.00 - 31.00	108.00
	G: 300 Gy	49.98 ± 0.31	- 2.13	52.00 - 45.00	4.74		G: 300 Gy	83.16 ± 1.85	31.05	151.00 - 51.00	167.81
	EB: 100 Gy	52.02 ± 0.36	- 0.09	60.00 - 44.00	6.50		EB: 100 Gy	53.44 ± 2.82	1.33	125.00 - 31.00	390.17
	EB: 200 Gy	52.10 ± 0.41	- 0.01	61.00 - 47.00	8.13		EB: 200 Gy	58.18 ± 3.23	6.07	156.00 - 28.00	510.39
	G+EB: 100 Gy	51.56 ± 0.30	- 0.55	58.00 - 49.00	4.37		G+EB: 100 Gy	90.44 ± 3.05	38.33	182.00 - 45.00	454.85
Days to maturity	**CONTROL**	**106.11 ± 0.81**	**-**	**112.00 - 105.00**	**5.21**	Number of pods per plant	**CONTROL**	**119.59 ± 2.73**	**-**	**138.00 - 108.00**	**59.62**
	G: 200 Gy	106.78 ± 0.62	0.67	111.00 - 102.00	6.62		G: 200 Gy	121.22 ± 2.16	1.63	129.00 - 87.00	79.17
	G: 300 Gy	106.44 ± 0.59	0.33	116.00 - 101.00	16.81		G: 300 Gy	128.69 ± 3.85	9.09	281.00 - 98.00	726.02
	EB: 100 Gy	104.44 ± 0.49	- 1.67	123.00 - 99.00	11.85		EB: 100 Gy	119.94 ± 2.30	0.35	179.00 - 81.00	260.06
	EB: 200 Gy	106.00 ± 0.49	- 0.11	115.00 - 95.00	12.00		EB: 200 Gy	119.80 ± 3.78	0.20	261.00 - 60.00	699.46
	G+EB: 100 Gy	108.26 ± 0.51	2.15	116.00 - 100.00	12.67		G+EB: 100 Gy	136.99 ± 3.92	17.40	242.00 - 112.00	753.78
Plant height (cm)	**CONTROL**	**95.03 ± 1.58**	**-**	**101.20 - 88.60**	**19.99**	Number of seeds per pod	**CONTROL**	**5.18 ± 0.13**	**-**	**5.55 - 4.67**	**0.14**
	G: 200 Gy	97.29 ± 3.15	2.26	120.60 - 88.60	168.70		G: 200 Gy	4.70 ± 0.14	- 0.48	6.10 - 4.10	0.31
	G: 300 Gy	146.37 ± 2.45	51.33	208.60 - 110.30	294.15		G: 300 Gy	4.98 ± 0.08	- 0.20	6.00 - 4.11	0.28
	EB: 100 Gy	127.39 ± 2.67	32.35	169.40 - 62.50	349.00		EB: 100 Gy	5.07 ± 0.07	- 0.12	6.67 - 4.00	0.23
	EB: 200 Gy	120.49 ± 2.90	25.45	189.20 - 87.10	411.15		EB: 200 Gy	5.60 ± 0.08	0.42	6.67 - 4.33	0.32
	G+EB: 100 Gy	132.77 ± 3.72	37.73	220.50 - 76.40	678.51		G+EB: 100 Gy	5.59 ± 0.06	0.40	6.67 - 4.67	0.19
Pod length (cm)	**CONTROL**	**4.82 ±0.12**	**-**	**5.40 - 4.40**	**0.11**	Hundred seed weight (g)	**CONTROL**	**3.46 ± 0.07**	**-**	**3.81 - 3.21**	**0.03**
	G: 200 Gy	4.91 ±0.16	0.09	5.80 - 3.80	0.44		G: 200 Gy	3.51 ± 0.05	0.04	3.83 - 3.20	0.05
	G: 300 Gy	4.76 ±0.09	- 0.06	6.50 - 3.99	0.41		G: 300 Gy	3.55 ± 0.03	0.09	4.12 - 3.25	0.06
	EB: 100 Gy	4.78 ±0.07	- 0.04	6.11 - 3.25	0.23		EB: 100 Gy	3.70 ± 0.04	0.24	4.60 - 3.25	0.07
	EB: 200 Gy	4.94 ±0.09	0.12	6.30 - 3.50	0.38		EB: 200 Gy	3.91 ± 0.05	0.44	4.80 - 3.10	0.12
	G+EB: 100 Gy	4.67 ±0.08	- 0.15	6.30 - 3.14	0.32		G+EB: 100 Gy	3.75 ± 0.06	0.28	4.80 - 3.20	0.20
Number of primary branches per plant	**CONTROL**	**8.22 ± 0.22**	**-**	**10.00 - 8.00**	**0.40**	Biological yield (g)	**CONTROL**	**67.22 ± 0.68**	**-**	**71.00 - 64.00**	**3.73**
	G: 200 Gy	7.56 ± 0.33	- 0.67	10.00 - 5.00	1.80		G: 200 Gy	70.33 ± 1.27	3.11	82.00 - 64.00	27.33
	G: 300 Gy	7.02 ± 0.29	- 1.20	14.00 - 4.00	4.18		G: 300 Gy	121.26 ± 2.65	54.04	148.00 - 91.00	344.31
	EB: 100 Gy	6.38 ± 0.27	- 1.84	14.00 - 4.00	3.64		EB: 100 Gy	73.82 ± 3.28	6.60	222.00 - 51.00	526.07
	EB: 200 Gy	6.38 ± 0.26	- 1.84	12.00 - 4.00	3.32		EB: 200 Gy	70.20 ± 3.61	2.98	209.00 - 33.00	637.76
	G+EB: 100 Gy	6.40 ± 0.24	- 1.82	12.00 - 4.00	2.80		G+EB: 100 Gy	96.62 ± 3.44	29.40	189.00 - 49.00	579.88
Number of pods per cluster	**CONTROL**	**2.34 ± 0.05**	**-**	**2.52 - 2.00**	**0.02**	Single plant yield (g)	**CONTROL**	**24.56 ± 0.72**	**-**	**29.61 - 23.12**	**4.15**
	G: 200 Gy	2.23 ± 0.13	- 0.11	2.87 - 1.62	0.31		G: 200 Gy	25.94 ± 1.25	1.38	35.69 - 15.60	26.65
	G: 300 Gy	2.55 ± 0.07	0.21	3.67 - 1.33	0.27		G: 300 Gy	33.08 ± 2.00	8.52	74.64 - 14.36	195.99
	EB: 100 Gy	2.15 ± 0.09	- 0.19	3.33 - 1.33	0.37		EB: 100 Gy	27.36 ± 1.32	2.81	52.89 - 12.90	85.43
	EB: 200 Gy	2.48 ± 0.09	0.14	3.67 - 1.33	0.38		EB: 200 Gy	27.65 ± 1.81	3.09	72.74 - 13.40	160.37
	G+EB: 100 Gy	2.38 ± 0.09	0.04	3.33 - 1.33	0.40		G+EB: 100 Gy	36.13 ± 1.97	11.57	75.34 - 15.57	189.85

G - Gamma rays; EB - Electron beam and SE - Standard Error

Table 6: Estimates of variability parameters, heritability and genetic advance in M_2 population of horsegram cv. PAIYUR 2

Traits	Treatment	PCV (%)	GCV (%)	H^2 (%)	GAM (%)	Traits	Treatment	PCV (%)	GCV (%)	H^2 (%)	GAM (%)
Days to first flowering	G: 200 Gy	4.53	3.64	64.32	6.01	**Number of clusters per plant**	G: 200 Gy	12.30	10.73	76.14	19.29
	G: 300 Gy	4.74	3.87	66.56	6.51		G: 300 Gy	15.05	13.64	82.13	25.47
	EB: 100 Gy	4.47	3.57	63.70	5.87		EB: 100 Gy	21.80	20.77	90.76	40.75
	EB: 200 Gy	4.49	3.61	64.51	5.97		EB: 200 Gy	15.52	13.77	78.72	25.17
	G+EB: 100 Gy	3.77	2.52	44.66	3.47		G+EB: 100 Gy	21.28	19.32	82.39	36.12
Days to maturity	G: 200 Gy	3.57	3.13	77.21	5.67	**Number of pods per plant**	G: 200 Gy	18.85	16.66	78.06	30.32
	G: 300 Gy	3.05	2.51	67.87	4.26		G: 300 Gy	20.24	18.11	80.09	33.39
	EB: 100 Gy	3.59	3.16	77.35	5.72		EB: 100 Gy	14.60	12.60	74.42	22.39
	EB: 200 Gy	3.46	3.00	75.25	5.36		EB: 200 Gy	19.48	17.20	78.04	31.31
	G+EB: 100 Gy	3.68	3.26	78.69	5.97		G+EB: 100 Gy	26.87	24.02	79.95	44.25
Plant height (cm)	G: 200 Gy	12.47	11.23	81.02	20.82	**Number of seeds per pod**	G: 200 Gy	8.91	6.81	58.34	10.71
	G: 300 Gy	9.08	7.45	67.30	12.59		G: 300 Gy	9.55	7.76	66.09	13.00
	EB: 100 Gy	14.61	13.29	82.72	24.90		EB: 100 Gy	8.78	6.65	57.30	10.37
	EB: 200 Gy	14.87	14.02	88.90	27.23		EB: 200 Gy	10.54	9.07	74.10	16.09
	G+EB: 100 Gy	16.66	15.59	87.53	30.04		G+EB: 100 Gy	10.08	8.51	71.23	14.79
Pod length (cm)	G: 200 Gy	10.79	9.54	78.17	17.38	**Hundred seed weight (g)**	G: 200 Gy	6.06	5.31	76.91	9.59
	G: 300 Gy	8.33	6.70	64.67	11.10		G: 300 Gy	6.70	6.13	83.56	11.54
	EB: 100 Gy	10.56	9.38	78.88	17.16		EB: 100 Gy	4.75	3.95	69.34	6.78
	EB: 200 Gy	7.50	5.77	59.19	9.15		EB: 200 Gy	5.18	4.49	75.24	8.02
	G+EB: 100 Gy	9.21	7.87	73.02	13.86		G+EB: 100 Gy	3.42	2.13	38.92	2.74
Number of primary branches per plant	G: 200 Gy	14.28	12.41	75.55	22.22	**Biological yield (g)**	G: 200 Gy	8.04	6.62	67.75	11.22
	G: 300 Gy	16.49	14.97	82.42	28.00		G: 300 Gy	10.79	9.68	80.41	17.88
	EB: 100 Gy	12.28	10.45	72.44	18.32		EB: 100 Gy	13.30	12.52	88.64	24.29
	EB: 200 Gy	12.73	10.44	67.25	17.63		EB: 200 Gy	10.40	9.16	77.56	16.62
	G+EB: 100 Gy	14.85	12.72	73.42	22.46		G+EB: 100 Gy	9.77	8.54	76.30	15.36
Number of pods per cluster	G: 200 Gy	8.85	6.97	62.07	11.32	**Single plant yield (g)**	G: 200 Gy	8.06	6.29	60.88	10.10
	G: 300 Gy	10.13	8.10	63.89	13.33		G: 300 Gy	24.58	24.06	95.82	48.52
	EB: 100 Gy	15.37	14.25	85.99	27.23		EB: 100 Gy	12.02	10.84	81.40	20.15
	EB: 200 Gy	11.35	8.96	62.28	14.56		EB: 200 Gy	9.38	7.57	65.03	12.57
	G+EB: 100 Gy	12.04	10.52	76.28	18.93		G+EB: 100 Gy	19.43	18.30	88.75	35.51

PCV (%) - Phenotypic coefficient of variation; GCV (%) - Genotypic coefficient of variation; H^2 (%) - Heritability at broad sense; GAM (%) - Genetic advance as per cent of mean; G - Gamma rays and EB - Electron beam

Table 7: Estimates of variability parameters, heritability and genetic advance in M_3 population of horsegram cv. PAIYUR 2

Traits	Treatment	PCV (%)	GCV (%)	H^2 (%)	GAM (%)	Traits	Treatment	PCV (%)	GCV (%)	H^2 (%)	GAM (%)
Days to first flowering	G: 200 Gy	4.17	2.26	29.35	2.52	Number of clusters per plant	G: 200 Gy	19.61	18.61	90.03	36.37
	G: 300 Gy	4.36	2.47	32.27	2.90		G: 300 Gy	15.58	15.07	93.58	30.03
	EB: 100 Gy	4.90	3.49	50.61	5.11		EB: 100 Gy	36.96	36.45	97.24	74.04
	EB: 200 Gy	5.47	4.26	60.52	6.82		EB: 200 Gy	38.83	38.42	97.89	78.30
	G+EB: 100 Gy	4.05	2.09	26.48	2.21		G+EB: 100 Gy	23.58	23.30	97.63	47.43
Days to maturity	G: 200 Gy	2.41	1.11	21.27	1.06	Number of pods per plant	G: 200 Gy	7.34	3.65	24.69	3.73
	G: 300 Gy	3.85	3.20	69.00	5.47		G: 300 Gy	20.94	20.06	91.79	39.59
	EB: 100 Gy	3.30	2.47	56.02	3.80		EB: 100 Gy	13.45	11.80	77.07	21.35
	EB: 200 Gy	3.27	2.46	56.58	3.81		EB: 200 Gy	22.08	21.11	91.48	41.60
	G+EB: 100 Gy	3.29	2.52	58.89	3.99		G+EB: 100 Gy	20.04	19.23	92.09	38.02
Plant height (cm)	G: 200 Gy	13.35	12.53	88.15	24.24	Number of seeds per pod	G: 200 Gy	11.93	8.86	55.14	13.55
	G: 300 Gy	11.72	11.31	93.20	22.50		G: 300 Gy	10.65	7.56	50.37	11.06
	EB: 100 Gy	14.67	14.24	94.27	28.48		EB: 100 Gy	9.48	5.95	39.37	7.69
	EB: 200 Gy	16.83	16.41	95.14	32.98		EB: 200 Gy	10.02	7.48	55.63	11.49
	G+EB: 100 Gy	19.62	19.33	97.05	39.23		G+EB: 100 Gy	7.88	4.16	27.82	4.52
Pod length (cm)	G: 200 Gy	13.56	11.82	76.04	21.24	Hundred seed weight (g)	G: 200 Gy	6.24	3.37	29.09	3.74
	G: 300 Gy	13.52	11.58	73.40	20.44		G: 300 Gy	6.80	4.74	48.54	6.80
	EB: 100 Gy	10.04	7.26	52.23	10.80		EB: 100 Gy	7.11	5.36	56.78	8.32
	EB: 200 Gy	12.45	10.48	70.91	18.18		EB: 200 Gy	8.70	7.48	74.03	13.26
	G+EB: 100 Gy	12.16	9.87	65.91	16.51		G+EB: 100 Gy	12.00	11.07	85.15	21.05
Number of primary branches per plant	G: 200 Gy	17.77	15.70	78.08	28.58	Biological yield (g)	G: 200 Gy	7.43	6.91	86.36	13.22
	G: 300 Gy	29.12	27.71	90.55	54.32		G: 300 Gy	15.30	15.22	98.92	31.18
	EB: 100 Gy	29.89	28.22	89.13	54.88		EB: 100 Gy	31.07	30.96	99.29	63.55
	EB: 200 Gy	28.54	26.79	88.08	51.79		EB: 200 Gy	35.97	35.87	99.42	73.67
	G+EB: 100 Gy	26.15	24.23	85.89	46.26		G+EB: 100 Gy	24.92	24.84	99.36	51.01
Number of pods per cluster	G: 200 Gy	24.90	24.08	93.50	47.97	Single plant yield (g)	G: 200 Gy	19.90	18.29	84.44	34.61
	G: 300 Gy	20.24	19.46	92.47	38.55		G: 300 Gy	42.32	41.87	97.88	85.33
	EB: 100 Gy	28.28	27.50	94.57	55.10		EB: 100 Gy	33.78	32.95	95.14	66.20
	EB: 200 Gy	24.94	24.28	94.74	48.68		EB: 200 Gy	45.81	45.21	97.41	91.92
	G+EB: 100 Gy	26.58	25.90	94.98	52.00		G+EB: 100 Gy	38.14	37.72	97.81	76.84

PCV (%) - Phenotypic coefficient of variation; GCV (%) - Genotypic coefficient of variation; H^2 (%) - Heritability at broad sense; GAM (%) - Genetic advance as per cent of mean; G - Gamma rays and EB -

for the studied polygenic traits. Similar findings on quantitative traits were reported earlier by Sheeba *et al.* (2003) and Mensah *et al.* (2005). Traits *viz.,* days to first flowering and days to maturity exhibited low to moderate H^2 coupled with low GAM at both generations indicating preponderance of non - additive gene action. A significant improvement in mean of yield and its component traits was observed with increased H^2 and GAM values. It is evident that selection imposed on M_2 generation based on single plant yield was highly effective. Similar findings were reported by Solanki and Sharma (2002) in mutant population of lentils. Moreover, variability estimates increased for most of the yield component traits in M_3 generation indicating further scope of achieving desirable mutants in advanced generations through selection. In a nutshell, the mutagens used in the study were highly promising for inducing desirable variants in horsegram. The combination of G + EB was found desirable as it exhibited high amount of transgressive variants (PTVF: 10) coupled with improved genetic gain (GAM: 35.51%) in M_3 generation for single plant yield. The stability of isolated high yielding mutant progenies will be test verified in subsequent generation. Thus, the delineated superior mutants offer scope for yield realization in horsegram of Southern India.

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Add-on Information

Authors' contribution: S. Priyanka: Execution, Data compilation, analysis and manuscript preparation; **R. Sudhagar:** Conceptualization, methodology, execution, data analysis and supervision; **C. Vanniarajan:** Data execution, review and editing, fund acquisition; **K. Ganesamurthy:** Supervision, editing and resources **and J. Souframanien:** Methodology, review, editing, fund acquisition.

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