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Mutagenesis induced variability through gamma rays, EMS and combination treatments in chickpea genotypes

Vishal Dinkar, Anju Arora, RK Panwar, SK Verma and Rohit

Abstract
Mutation breeding is an effective tool for creation of genetic variability in chickpea crop due to its self-pollinated nature and narrow genetic base. The seeds of three desi varieties released from Pantnagar were mutagenized with physical mutagen (gamma rays at 300 and 400 Gy doses) and chemical mutagen (ethyl-methane sulphonate @ 0.5%) and their combination treatments. M_1 generation showed gradual decrease in germination with increase in mutagen strength in addition to this, combination treatments caused more biological damage than individual mutagen treatment. In M_2 generation, variance for varieties revealed significant differences for thirteen agronomic traits except two *i.e.* number of primary branches per plant and pod length. However, all kinds of mutagenic treatments and variety x treatment variance were significant for eight traits including yield per plant. Different viable mutants were identified for early and late maturity, stem and leaf character, mutants with high number of primary branches or secondary branches and double mutants. In general, combined treatments 300 Gy + 0.5% EMS and 400 Gy + 0.5% EMS were observed to be most effective in inducing variability for most of the characters under study. But, frequency of viable mutants increased with increase in dose of gamma ray, whereas EMS alone gave comparatively higher frequency than combined treatments in all the three varieties. The isolated mutants suitable for mechanical harvesting and ideal plant type with early flowering can be used as genetic stocks and utilized in future crop improvement programs.

Keywords: Mutation, mutagenesis, chickpea, EMS (ethyl methane sulphonate), gamma rays, mutagenic treatments

Introduction
Chickpea (*Cicer arietinum* L.) is a self-pollinated diploid (2n=2x=16) legume crop that belongs to the family *Fabaceae*. It has a genome size of ~738Mb (Varshney *et al.* 2013) [29]. Chickpea was originated in south-eastern Turkey and adjoining regions of Syria. India is the largest producer of chickpea with a share of about 66% area and about 65% in production of chickpea in the world. In India chickpea is cultivated in an area of about 10.76 milllion hectare with a total production of 11.16 million tons (Directorate of Economics and Statistics 2018) [5]. Genetic variability is the prerequisite for crop improvement program followed by appropriate selection procedure. Due to self-pollinated nature of crop and small flower size, conventional methods of plant breeding are tedious and costly. Further narrow genetic base of chickpea makes improvement limited through hybridization techniques. Mutation breeding is an effective tool for a plant breeder to generate variability by upgrading a specific character without altering the original genetic make-up of the cultivar. Mutation offers the possibility of inducing desired attributes that either cannot be found on nature or lost during evolution (Novak and Brunner, 1992). Spontaneous mutations occur at a very low frequency (10^{-7} to 10^{-9}) and consequently cannot be expected to serve for crop improvement effectively. However, induced mutation is considered as a rapid and effective tool in plant breeding for creating variability. Artificial mutations can be induced using treatments with physical (X-rays, gamma rays, fast and slow neutrons etc.) and/or chemical *viz*., ethyl methane sulphonate (EMS), methyl methane sulphonate (MMS), sodium azide, base analogs, arcidine dye etc. agents, called as mutagens. Brock (1965) [4] proposed the hypothesis of induction of quantitative variability through mutagenic treatment. Induced mutations are of two types, one is macro mutations which include large changes and the other is micro mutations which involve changes in quantitative traits and can be measured using various statistical parameters, and thus these are of great importance for a plant breeder. Such mutations could be useful for improving quantitatively inherited traits such as yield, without disturbing the major part of the genotype and the phenotypic architecture of the crop. Induced mutations have played a great role in increasing world food security.

There are hundreds of examples worldwide related to use of new varieties, derived directly or indirectly from mutants in several crops. In recent years, a number of attempts to assess mutagen-induced genetic variability in quantitative traits of pulses were elucidated out. In India, mutation breeding has yielded considerable dividends both in enhancing our knowledge on various mutagenesis processes relevant to crop improvement and for developing improved varieties. It may be inferred that creation of genetic variability in chickpea as well as isolation of useful mutants would be of great importance for developing high yielding genotypes suitable for different agro-climatic conditions.

Material and Methods

Seeds of three desi chickpea varieties *viz.* Pant Gram 114, Pant Gram 186 and Pant Gram 3 released from Pantnagar were mutagenized with gamma rays (300 Gy & 400 Gy doses), ethyl-methane sulphonate (EMS 0.5%) and their combination treatments (300 Gy gamma ray + 0.5% EMS & 400 Gy gamma ray + 0.5% EMS). Two thousand seeds of each of the three varieties were exposed to gamma radiation at Bhabha Atomic Research Centre (BARC), Trombay, Mumbai. Cobalt-60 (Co^{60}) isotope was used as the source of gamma rays. The gamma rays treated seeds were divided into two equal parts. First part comprised of only physical mutagen treatment and the other part was used for EMS treatment which constituted the combination of gamma ray followed by EMS treatment. On the other side, a fresh sample of 1000 healthy and dry seeds with moisture content of 10-12% was taken from normal non-irradiated seed lot for the purpose of EMS treatment. The seeds from both gamma ray treatments (300 Gy and 400 Gy) and normal lot were pre-soaked in distilled water for 12-16 hours. These seeds were then soaked in freshly prepared 0.5% EMS solution for 3 hours in a dark and cool place (0.5% EMS was prepared by dissolving 10 ml of EMS in 2 liters of 100 mM phosphate buffer of pH 7). The seeds were washed thoroughly to remove residual chemical, then dried on blotting paper and sown on the same day.

A total of 15 treatments (5 doses x 3 genotypes) were space planted in separate plots without any replication to grow M_1 generation. Non-treated seeds were sown as control. Number of seeds germinated in each treatment was counted after one month from date of sowing. Throughout crop season, mutants for various traits were identified visually and tagged. From each treatment plot, 20 plants showing or expected to be a mutant were selected and harvested separately. In M_1 plants germination percentage was calculated for each treatment.

The M_1 harvest of individual mutant plants constituted M_2 seeds. The M_2 seeds were sown in individual plant to progeny rows of each treatment under three replications. Each replication had 10 rows of 2 meters along with all controls (three non-treated varieties).

Analysis of variance was carried out in M_2 generation to test the significance of differences among effects for different treatments on different quantitative characters. In M_2 generation, number of mutant plants in segregating progenies was counted and spectrum of qualitative mutations was recorded in the field when the seedlings were 15 to 20 days old. The mutants which reached to flowering stage and produced seeds were scored as viable mutants and observations were recorded for different quantitative traits i.e. yield and its attributing components from randomly selected 3 plants from each replication and plant progeny row. The variability parameters such as mean, range and variance were also estimated.

In M1 and M2 generation, frequency of different qualitative mutations was estimated using the method suggested by Gaul (1961) [6]. Percentage of M1 plant progenies segregating for mutations in M2 generation in each treatment is equal to the ratio of number of segregating progenies to the total number of progenies scored and number of mutants per 100 plants equal to the ratio of number of mutants to the total number of plants scored.

Results and Discussion

Analysis of Genetic variability

Analysis of variance was conducted in M_2 generation for 15 different yield related traits for different treatments (Table 1). Effects of different mutagenic treatments were found significant for various traits *viz.* plant height, days to maturity, number of secondary branches per plant, cumulative length of petiole and rachis, pods per plant, pod length, seeds per pod and yield per plant. Varietal effects were also found significant for all the traits except for number of primary branches per plant and pod length. The interaction effect was also found significant for almost all the traits except for internode length, cumulative length of petiole and rachis, leaf width, pods per plant, seeds per pod, pod length, and 100 seed weight. Interaction effect was found significant along with significant effects of varieties indicating that significant varietal differences were significantly affected by different mutagenic treatments.

Table 1: Analysis of variance (ANOVA) of three varieties and five mutagenic treatments for different characters in M_2 generation

Source of variation	D.f.	Mean sum of squares														
		Plant height	Days to first flower	Height of first pod bearing node	Days to maturity	Primary branch/ plant	Secondary plant/ plant	Internode length	Cumulative length of petiole & rachis	Leaf length	Leaf width	Pods/ plant	Pod length	Seeds/ pod	100 seed weight	Yield/ plant
Replications	2	35.92	17.796	7.133	13.019	0.37	0.468	0.027	0.127	0.017	0.004	46.46	0.014	0.042	8.43	0.445
Varieties	2	131.366*	235.019**	13.532*	181.796**	0.479	10.795**	0.553**	1.856**	0.906**	0.308**	118.474*	0.86	0.860**	457.821**	40.753**
Treatments	5	265.294**	18.063	4.965	47.574**	0.749	24.627**	0.045	0.579**	0.032	0.01	101.159*	0.076*	0.076*	4.801	13.867**
Varieties × treatments	10	81.863*	40.452**	11.002**	64.752**	0.963*	20.572**	0.041	0.096	0.083**	0.018	155.312	0.04	0.04	8.107	10.501**
Error	34	35.171	11.581	26.362	7.705	0.402	1.836	0.033	0.07	0.017	0.007	32.773	0.026	0.026	9.722	1.232

*Significant at 5% level of probabaility ** Significant at 1% level of probabaility

The data on genotypic response at different mutagenic treatments for mean trait values are presented in Table 2. In PG114, mean values for characters such as plant height, days to maturity, internode length, cumulative length of petiole and rachis, leaf length, leaf width and pod length were decreased significantly from control for all mutagenic treatments. However, in PG186 mean of the characters decreased significantly for internode length, leaf length, leaf width, number of pods per plant and pod length. In PG3 mean for plant height, cumulative length of petiole and rachis, number

of pods per plant, number of seeds per pod and yield per plant were significantly reduced whereas number of secondary branches increased significantly in two treatments *i.e.* 300 Gy alone as well as in combination treatment with 0.5 EMS. Thus genotypic response was different at different mutagenic levels for various traits. Although none of the genotypes responded significantly better over control for yield per plant at any of the treatment level but one genotype PG114 showed significant increase in yield component trait pods per plant at 300 Gy + 0.5% EMS level.

Table 2: Mean for various traits in M_2 generation in three chickpea varieties at five mutagenic treatments

Treatment	Variance																	
	PG 114						PG 186						PG 3					
	Control	300 Gy	400 Gy	0.5 EMS	300Gy +0.5% EMS	400Gy +0.5% EMS	Control	300 Gy	400 Gy	0.5 EMS	300Gy +0.5% EMS	400Gy +0.5% EMS	Control	300 Gy	400 Gy	0.5 EMS	300Gy +0.5% EMS	400Gy +0.5% EMS
Plant height	54.13	39.50**	44.21**	41.00**	42.79**	40.71**	58.46	32.14**	57.58	45.81	48.70	43.02	47.33	44.36	44.10	35.42	39.62	43.75
Days to first flower	81.65	85.65**	83.70	83.60	83.50	82.50	73.25	76.00	79.35	76.75	78.6	77.45	80.30	81.50	81.35	78.50	79.25	81.60
Height of first pod bearing node	20.80	19.40	23.65*	18.80	19.30	20.40	19.73	20.37	21.75	24	23.44	23.33	21.67	23.23	20.89	20.34	18.98*	23.27
Days to maturity	149.67	138.67**	137.60**	139.60**	145.30*	150.30	140.30	147.00	152.00	143.67	147.30	144.60	136.00	135.67	139.00	140.00	143.00	143.67**
Number of primary branches	2.80	4.10	3.47	3.50	3.13.	4.23	2.50	3.45	4.13	3.37	4.20	3.76	4.30	3.45	4.13	3.37	4.20	3.77
Number of secondary branches	7.10	9.03*	7.93	11.30**	11.50**	8.87	6.13	4.67	12.4**	7.43	11.83**	12.10**	9.17	13.74**	10.43	6.69	13.36**	9.77
Internode length	2.10	1.88	1.97	1.67	1.75*	1.75*	2.16	1.92	2.23	2.11	2.08	2.04	2.25	2.24	2.07	2.16	2.28	2.18
Cumulative length of petioleand rachis	4.04	3.42**	3.75	3.62*	3.37**	3.76	4.30	3.42**	3.92	3.75*	4.06	4.12	4.64	3.96**	4.07*	4.04*	4.62	4.46
Leaf length	1.30	1.14	1.08*	1.05*	0.94*	0.98**	1.34	1.33	1.15	1.43	1.37	1.06*	1.55	1.55	1.52	1.24*	1.53	1.78
Leaf width	1.71	0.55**	0.57**	0.53**	0.52**	0.56**	0.71	0.67	0.59	0.73	0.65	0.55	0.79	0.83	0.76	0.76	0.93	0.91
Pods per plant	36.07	44.93	32.80	37.13	47.40**	28.90	34.62	27.40	41.33	29.83	33.67	31.80	45.66	28.46*	36.37	22.80**	31.60**	38.11
Pods length	1.98	1.60**	1.70**	1.60**	1.80	1.83	2.21	2.14	2.12	1.89	2.07	1.94	2.26	2.39	2.09*	2.15	2.09*	2.09*
Average number of seeds per pod	1.59	1.23*	1.60	1.57	1.30	1.23*	1.53	1.06*	0.80**	1.04**	0.87*	1.11*	1.80	1.03**	1.39	1.1**	0.89**	0.91**
100 seed weight	11.43	12.20	12.38	12.19	12.57	14.23	14.07	15.05	14.36	15.74	13.32	16.55	24.10	20.17	24.09	24.34	20.60	19.82
Yield per plant	5.80	6.67	4.63	6.60	4.89	5.86	7.90	5.10**	4.86**	5.36*	4.57**	7.97	14.03	6.67**	8.51**	6.36**	6.88**	8.85**

*Significant at 5% level of probabaility ** Significant at 1% level of probabaility

Table 3 depicts varietal response for variance of various traits at different treatment levels. In general, variance for yield and most of the yield related traits significantly increased in all five kinds of mutagenic treatments in all the three varieties. In PG114, gamma rays (300 Gy) alone induced more variability for flowering duration but in PG186 and PG 3 combination treatments induced more variability for this trait. However, high variance for yield component traits viz. number of primary branches, number of secondary branches, internodal length, and 100 seed weight was observed at 300 Gy + 0.5% EMS level and 400 Gy + 0.5% EMS level. This indicated that combination treatments were more effective in inducing variability for yield related traits in all the three genotypes in comparison to single physical or chemical mutagen treatment.

Table 2: Variance for various traits in M_2 generation in three chickpea varieties at gamma rays and EMS combination mutagenic treatments

Treatment	Variance																	
	PG 114						PG 186						PG 3					
	Control	300 Gy	400 Gy	0.5 EMS	300Gy +0.5% EMS	400Gy +0.5% EMS	Control	300 Gy	400 Gy	0.5 EMS	300Gy +0.5% EMS	400Gy +0.5% EMS	Control	300 Gy	400 Gy	0.5 EMS	300Gy +0.5% EMS	400Gy +0.5% EMS
Plant height	15.87	13.37	25.35	8.18	26.67	37.68*	6.14	19.16**	88.52**	40.25**	65.90**	66.24**	6.08	28.14**	25.94**	52.77**	73.03**	29.43**
Days to first flower	4.43	10.20*	12.24*	4.98	9.96*	9.88*	1.35	5.87**	12.80*	8.50**	14.82**	14.10**	3.80	11.32*	16.74**	9.42*	30.16**	19.27**
Height of first pod bearing node	3.85	5.59	2.65	7.27	10.35*	4.28	1.08	2.24*	3.57**	8.77**	13.11**	13.09**	0.86	2.85**	3.62**	2.77**	4.01**	8.20**
Days to maturity	0.37	0.99*	1.27**	1.09*	0.91*	1.87**	0.06	2.08**	0.72**	1.07**	0.91**	1.92**	0.02	0.51**	1.42**	1.01**	1.73**	3.87**
Number of primary branches	0.01	0.02*	0.03**	0.02*	0.05**	0.19**	0.01	0.07**	0.11**	0.11**	0.28**	0.34**	0.01	0.04**	0.08**	0.17**	0.47**	0.43**
Number of secondary branches	0.78	2.19*	2.35*	4.38**	6.05**	6.44**	0.48	0.81	5.38**	2.73**	9.22**	8.89**	1.56	4.82**	4.39*	2.02	14.59**	9.47**

Internode length	0.05	0.07	0.09	0.08	0.14*	0.15**	0.07	0.08	0.09*	0.09*	0.21*	0.29**	0.18	0.23	0.24	0.40	0.51*	0.56**
Cumulative length of petioleand rachis	0.09	0.09	0.21*	0.27**	0.31**	0.57**	0.01	0.18**	0.27**	0.29**	0.17**	0.35**	0.08	0.09	0.14	0.11	0.21*	0.16
Leaf length	0.01	0.02**	0.02**	0.03**	0.03**	0.04**	0.01	0.02**	0.06**	0.07**	0.14**	0.11**	0.02	0.03	0.10**	0.10**	0.14**	0.31**
Leaf width	0.01	0.02*	0.02*	0.02*	0.03**	0.05**	0.01	0.02**	0.01	0.03**	0.02**	0.04**	0.01	0.01	0.02	0.02	0.08**	0.07**
Pods per plant	79.11	313.37**	188.24*	276.57**	684.84**	296.97**	76.77	110.35	357.53**	163.76	305.01**	396.28**	81.73	80.42	113.01	43.26	210.97*	463.63**
Pods length	0.01	0.02*	0.01	0.02**	0.01	0.04**	0.01	0.03*	0.07**	0.06**	0.15**	0.15**	0.02	0.04*	0.05*	0.15**	0.13**	0.22**
Average number of seeds per pod	0.01	0.02**	0.05**	0.03**	0.06**	0.07**	0.01	0.01	0.02	0.02	0.01	0.05**	0.04	0.04	0.11*	0.10	0.07	0.09
100 seed weight	0.01	0.13**	0.35**	0.20**	1.09**	1.13**	0.05	0.50**	0.25**	1.11**	0.82**	1.62**	0.26	1.05**	1.22**	1.92**	1.31**	3.47**
Yield per plant	0.25	3.87**	0.82**	1.35**	1.55**	1.85**	0.22	1.28**	1.50**	1.78**	2.09**	6.61**	1.96	1.32	2.11	2.97	3.58	6.55**

*Significant at 5% level of probabaility ** Significant at 1% level of probabaility

Frequency and spectrum of mutants

A gradual decrease in germination with increase in the mutagen strength was observed in M_1 generation and it was also found that combination treatments caused more damage to seeds than individual mutagen treatment. Different types of morphological mutants were observed such as chlorophyll mutants, high anthocyanin pigmented type, narrow leaves with entire margin types, variegated leaf type, tall type and fasciated stem type.

In M_2 generation, different viable mutants were identified and they were categorized as stem mutants, leaf mutants, growth habit mutants, flowering mutants, maturity duration mutants and double mutants (mutant for two traits) in individual plant progeny rows (Table 4).

Table 4: Frequency and spectrum of different viable mutants in different mutagenic treatments in M_2 generation of three chickpea varieties

Treatment	Size of M_2 population	Total no. of viable mutants	Frequency of viable mutants	No. and frequency of different mutant types plants in each treatment					
				Stem mutant	Leaf mutant	Growth habit mutants	Flowering mutant	Maturity duration mutant	Double character mutants
PG114									
300Gy	411	2	0.49	-	-	2 (0.49)	-	-	-
400Gy	475	3	0.63	3 (0.63)	-	-	-	-	-
0.5%EMS	253	2	0.79	-	-	1 (0.4)	-	2 (0.79)	-
300Gy+0.5%EMS	458	4	0.87	-	2 (0.44)	-	-	-	2 (0.44)
400Gy+0.5%EMS	811	2	0.25	-	2 (0.25)	-	-	-	-
Total	2408	13	0.54	3 (0.12)	4 (0.16)	3 (0.13)	-	2 (0.08)	2 (0.08)
PG186									
300Gy	215	1	0.47	1 (0.47)	-	-	-	-	-
400Gy	266	2	0.75	-	-	-	-	-	2 (0.75)
0.5%EMS	243	3	1.24	-	2 (0.82)	-	-	-	1 (0.41)
300Gy+0.5%EMS	297	0	0	-	-	-	-	-	-
400Gy+0.5%EMS	290	3	1.04	-	-	3 (1.04)	-	-	-
Total	1310	9	0.69	1 (0.08)	2 (0.15)	3(0.23)		-	3 (0.23)
PG3									
300Gy	164	3	1.83	-	2 (1.22)	1 (0.61)	-	-	-
400Gy	197	5	2.54	-	2 (1.02)		2^+ (1.02)	-	3 (1.52)
0.5%EMS	237	2	0.84	-	-	2 (0.84)	-	-	1 (0.42)
300Gy+0.5%EMS	285	2	0.70	-	-	-	2^+ (0.70)	-	2 (0.70)
400Gy+0.5%EMS	271	0	0	-	-	-	-	-	-
Total	1156	12	1.04	-	4 (0.35)	3 (0.26)	4 (0.35)	-	6 (0.52)

Note: Value in parentheses indicate percent frequency $^+$ = value include double mutant type.

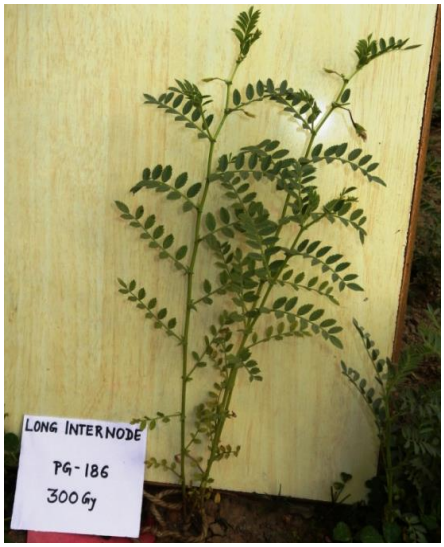
Variety PG114 had 0.12% stem type, 0.16% leaf type, 0.25 % growth type and 0.01% late maturity mutants. PG186 variety, showed 0.15% leaf mutants, 0.76% growth habit mutants and 0.15% flowering mutants. Highest leaf mutant frequency was observed in 0.5% EMS treatment of PG186. Similarly, PG3 showed mutants for growth habit (1.21%) and flowering duration (0.17%). Growth habit mutants were most frequent in all the three varieties. Gamma radiation treatments (300 Gy and 400 Gy) produced viable mutants most frequently and covered the whole spectrum of such mutants whereas 0.5% EMS and combined treatments of gamma ray and EMS (300 Gy + 0.5% EMS and 400 Gy + 0.5% EMS) produced viable mutants with more or less in equal frequency. Growth habit mutants were mostly due to recessive mutations as reported by Sandhu *et al.* (1990) [21].

Mutant type identified for quantitative traits in M_2 generation

A fasciated stem mutant was identified in population of 400 Gy treated PG114 plot (Fig. A). Fasciated mutants grew up to 41 to 45 cm in length and yielded in the range of 4.9 to 8.6 grams per plant. Fasciated stem mutant types were also isolated and the genetics of fasciation was reported by Gaur and Gour (1999) [7] and Srinivasan *et al.* (2008) [25]. Long internode type mutant was found in 300 Gy treatment of PG186 which had longer internode length of 2.8 cm as compared to normal plants with 2.0-2.2 cm length (Fig. B).

Two narrow leaved mutants with 1.1 cm leaf length and 0.5 cm leaf width as compared to control (with average length 1.3 cm and width 0.7 cm) appeared in combination treatment of 300 Gy + 0.5% EMS treatment of PG114 (Fig. C). However, Khan *et al.* (2011) [11] obtained narrow leaf mutant in two chickpea varieties using chemical mutagens only. **N**arrow leaf mutant was also reported by Van Rheenen (1993) [28] and Wani (2009) [30]. Barshile *et al.* (2009) [2] also isolated leaf shape mutants (round, compact and narrow) in M_2 generation by treating seeds by EMS and gamma rays. Two leaf mutants were found in 0.5% EMS treated progeny row of PG186 (Fig. D). More *et al.* (2011) [18] also obtained leaf mutants but with large leaves in two chickpea cultivars using chemical mutagens.

a. Fascinated stem mutant



b. Long internode type

c. Narrow leaf mutant

d. Leaf mutant

e. Compact type mutant

f. Tall + early flowering type

A bushy mutant of PG114 was found at 300 Gy treatment level with 8 primary and 17 secondary branches. Another bushy mutant was found in 0.5% EMS treatment of PG114 with 18 secondary branches as compared to 7-11 in normal plants. One bushy plant was also found in 400 Gy treatment of PG3 with 18 secondary branches. Wani (2009) [30] also reported bushy type mutants from 400 Gy gamma rays and 300 Gy + 0.3% EMS treatments in chickpea, whereas Khan *et al.* (2011) [11] obtained bushy mutant in two chickpea varieties using chemical mutagens alone. Sandhu *et al.* (2010) [22] also studied similar mutants.

One compact erect and early mutant was isolated from 400 Gy + 0.5% EMS treatment of PG186 variety which was also higher yielder with 8.2 grams seed per plant over control with 7.9 grams/plant yield (Fig. E). Tall early flowering type plants were found in 400 Gy treatment of PG186 with 55-57 cm height and took 60-65 days to flowering (Fig. F). Although Van Rheenen *et al. (*1993) [28] also observed early-flowering types by treating seeds with gamma rays but with small and large leaf sizes and other variations in M_2 generation of ICCV 2 and ICCV6 varieties. Gaur *et al.* (2007) [8] also isolated a mutant for compact growth through treatment of seeds with 0.6% EMS in cultivar JG 315. Kharkwal (2000) [15] and Barshile *et al.* (2009) [2] also isolated tall type mutant plant following EMS treatment in chickpea. Sagel *et al.* (2009) [20] also isolated similar mutants suitable to machinery harvest type with different gamma radiation dose rates. Barshile *et al.* (2015) [1] also identified early and tall mutants in M_2 generation by treating seeds with EMS and gamma rays in cv. Vishwas where tallest line also showed increase in number of pods and number of seeds.

In all the three varieties, EMS alone gave comparatively higher frequency of mutants than gamma rays alone and combined treatments. However, frequency of viable mutants

was higher in gamma rays treated plants which increased with increase in dose of gamma rays. Variety wise, viable mutants were more frequent in PG3 variety followed by PG186 and PG114. In PG114, leaf mutants were found in maximum frequency followed by growth habit mutants, stem mutants and double mutants. However in PG186 and PG3, double mutants were most frequent. Different viable mutants identified in individual plant progeny rows in M_2 generation like mutants with high number of primary branches, secondary branches and double mutants *i.e.* tall erect and compact plant types suitable for mechanical harvesting and ideal plant type with early flowering can be used as genetic stocks for useful traits and can be utilized in future crop improvement programs.

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