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Induced mutations in chickpea (*Cicer arietinum* L.) V. Evaluation of micromutations

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Abstract

A wide range of induced polygenic variability in the form of micromutations was generated in M_2 and M_3 generations of chickpea. Treated M_2 population showed a much greater range of variability for all the characters than the controls. The enormous range of variability observed fully demonstrated the effectiveness of the mutagenic treatments in generating variability for different quantitative characters. High magnitude of increased ranges of variability towards positive side showed that some extremely useful variability has been induced following mutagenic treatments. Differential varietal response for quantitative characters indicated that even though the *kabuli* varieties are relatively more sensitive in respect of induced genetic damage, it is the *desi* varieties which gave a higher frequency of micromutations for the various quantitative characters. The study indicated that not all the mutagens are equally effective in generating variability for quantitative characters and showed a differential response to the different varieties. In general, chemical mutagens have been found to be relatively more efficient than physical in generating variability in M_2 and M_3 generation. The increased coefficient of variability (CV) in M_2 populations for all the characters suggested that a part of the varibility recorded was genotypic. In M_3 generation the CV was considerably lower than in M_2 for most of the charactres suggesting that selection technique employed in M_2 has been highly effective and played a key role in shifting useful variability in the positive direction to M_3 generation. The usefulness of induced variability was also evident from the higher estimates of heritability and genetic advance in M_2 and M_3 populations. The study also revealed that characters such as grain yield, number of pods and grains per plant, grain weight and biological yield showed a higher response to mutagenic treatments, indicating that remarkable opportunities exist for marked improvement of these polygenic characters in chickpea.

Key words: Chickpea, micromutations, polygenic variability, selection technique

Introduction

In order to induce variability and utilize useful mutations directly for efficient plant breeding, a systematic study of induced polygenic variability or micromutations based on a critical analysis of variability in M_2 and M_3 generations is not only necessary but also is the most dependable index. Although sporedic/limited reports are available on induced viable morphological mutations in chickpea [1-4], detailed studies on induced polygenic variability have hardly been reported.

The present investigation was undertaken to understand the response of different *desi* and *kabuli* chickpea genotypes to more than one type of mutagenic treatment with a view to inducing maximum micromutations through generation of polygenic variability in M_2 and M_3 generations and screening the same through various selection techniques.

Materials and methods

The material for the present study comprised of two *desi* (G 130 and H 214), one *kabuli* (C 104) and one green seeded type (L 345) chickpea genotypes. Five hundred dry seeds with a moisture content of 10-12% approx. were used for each treatment. Three doses each of two physical mutagens, gamma rays (400, 500 and 600 Gy) and fast neutron (5, 10 and 15 Gy) were given. Two concentrations and two durations of the two radiomimetic monofunctional alkylating agents *viz.*, N-nitroso-N-methyle urea (NMU) 0.01% (20h) and 0.02% (8h) and ethylmethane sulphonate (EMS) 0.1% (20h) and 0.2% (8h) were used. Gamma rays were secured from Gamma Cell-200 having a 2000 Curie ^{60}Co source available at Genetics Division, IARI, New Delhi. Fast neutron treatments were given at BARC, Trombay, Mumbai. NMU and EMS of Pfaltz and Bauer Inc. USA were used for preparing aqueous solutions of chemical mutagens at 5.2 pH. Treatments with chemical mutagens were given with intermittant shaking at 20 ± 2°C. Dry seeds were used as controls. The seeds treated with chemical mutagens were thoroughly washed in running water for 30 minutes to leach out the residual chemicals and then dried on blotting paper.

Treated and control seeds were sown at a spacing of 15 cm in rows of 5 m long and 0.45 m apart on

the same day in well prepared seed beds in the field. Each M_1 plant was harvested individually and M_2 progeny raised in separate rows. The treated as well as control poulations were carefully screened for polygenic variability/ micromutations by recording quantitative data on five random plants drawn from M_2 (unreplicated) and twenty random plants in M_3 (replicated) generation. The number of treatments and families and plants studied under each variety in M_2 and M_3 generations are given in Table 1.

for quantitative characters in mutagen treated populations. In the present study, the induced variability for the different quantitative characters has been analysed in a number of ways. Firstly, the plant to plant variation has been estimated in the control as well as in each of the treatment population. The population for the purpose of these observations consisted of five normal looking, random plants from each of the M_2 families. This part of the analysis of induced variability is presented in the form of range

Table 1. Number of plants, families, replications and varieties studied in different treatments for induced variability in quantitative characters in M_2 to M_3 generation

Treatment	No. seeds treated per var. for M_1 gen.		Number of plants (P) and families (F) per treatment in each variety									
			M_2 gen. (5 plants/Fam.)					M_3 gen. (10 plants × two replication/Fam.)				
			desi varieties		*kabuli* var.	green seeded	Total per treat in M_2	*desi* varieties		*kabuli var.*	green seeded	Total per treat in M_3
			G 130	H 214	C 104	L 345		G 130	H 214	C 104	L 345	
Control	500	P	150	150	150	150	600	100	100	100	100	400
		F	30	30	30	30	120	5	5	5	5	20
γ-rays 400 Gy	500	P	160	170	-	165	495	180	300	-	-	480
		F	32	34	-	33	99	9	15	-	-	24
500 Gy	500	P	160	165	-	165	490	160	320	-	-	480
		F	32	33	-	33	98	8	16	-	-	24
600 Gy	500	P	160	160	-	170	490	320	80	-	120	520
		F	32	32	-	34	98	16	4	-	6	26
Neutrons 5 Gy	500	P	160	160	165	160	645	60	-	400	440	900
		F	32	32	33	32	129	3	-	20	22	45
10 Gy	500	P	160	110	170	165	605	60	-	200	220	480
		F	32	22	34	33	121	3	-	10	11	24
15 Gy	500	P	165	105	80	150	500	100	-	80	160	340
		F	33	21	16	30	100	5	-	4	8	17
NMU 0.01%(20h)	500	P	175	145	165	170	655	100	100	200	80	480
		F	35	29	33	34	131	5	5	10	4	24
0.02%(8h)	500	P	160	145	140	160	605	40	140	80	140	400
		F	32	29	28	32	121	2	7	4	7	20
EMS 0.1%(20h)	500	P	170	40	-	-	210	180	-	-	-	180
		F	34	8	-	-	42	9	-	-	-	9
0.2% (8h)	500	P	170	55	50	60	335	220	120	140	-	480
		F	34	11	10	12	67	11	6	7	-	24
Total	500	P	1790	1405	920	1515	5630	1520	1160	1200	1260	5140
		F	358	281	184	303	1126	76	58	60	63	257

- = Population not available

Results and discussion

Evaluation of Micromutations in M_2 generation: In M_2 generation, the progenies of *desi* varieties G 130 annd H 214, *kabuli* var. C 104 and green seeded var L 345 were screened for micromutations. Data in respect of four quantitative characters *viz.*, grain yield per plant, number of pods per plant, number of grains per plant, and 100 grain weight were analysed on individual plant basis.

Micromutations can be detected in the form of increased population variance and shift in mean values and mean values for different characters (Table 2). The different M_2 families pertaining to each of the treatments have also been compared for their variability. Inter- and intra-family variance has been estimated for each of the different mutagenic treatments and control families.

These observations on inter and intra-family variances and the mean value have made it possible to estimate the heritability and genetic advance in treated populations. Finally, the induced variability in the form of the coefficient of variability (CV), has been

Table 2. Range, mean, S.E. and 't' value for treatment means (control *vs* treatment) for grain yield (g) per plant in M_2 generation

Treatment	Range	Mean S.E.	't' value	Range	Mean SE	't' value
	desi Var. G 130			*desi* Var. H 214		
Control	5.5-11.5	8.20 ± 0.09	-	4.5-14.0	9.45 ± 0.11	-
γ-rays 400 Gy	0.1-27.1	4.98 ± 0.38	8.131**	0.6-17.5	5.40 ± 0.26	14.516**
500 Gy	0.6-22.4	7.44 ± 0.37	1.954	0.9-22.9	5.55 ± 0.30	12.264**
600 Gy	0.9-30.5	7.35 ± 0.39	2.114*	0.2-18.2	3.50 ± 0.20	25.647**
Neutrons						
5 Gy	0.8-18.4	5.17 ± 0.24	11.434**	0.1-9.9	2.29 ± 0.14	42.874**
10 Gy	0.6-18.3	5.84 ± 0.30	7.398**	0.1-6.4	1.82 ± 0.14	44.882**
15 Gy	0.9-34.4	6.48 ± 0.37	4.514**	0.1-6.8	1.78 ± 0.14	43.580**
NMU 0.01% (20h)	0.4-19.1	5.30 ± 0.30	9.325**	0.1-17.6	3.42 ± 0.22	23.463**
0.02% (8h)	0.3-19.0	4.45 ± 0.24	14.368**	0.4-21.5	4.27 ± 0.28	17.383**
EMS 0.1% (20h)	0.2-28.3	6.50 ± 0.35	4.735**	0.1-10.7	2.56 ± 0.44	15.243**
0.2% (8h)	1.4-33.7	8.10 ± 0.40	0.244	0.2-25.2	4.97 ± 0.72	6.162**
	kabuli Var. C 104			green seeded var. L 345		
Control	11.0-17.7	14.74 ± 0.11	-	4.0-11.5	7.02 ± 0.09	-
γ-rays 400 Gy	-	-	-	0.1-11.1	3.40 ± 0.16	19.674**
500 Gy	-	-	-	0.7-12.5	4.14 ± 0.20	13.151**
600 Gy	-	-	-	0.5-20.4	6.30 ± 0.30	2.286*
neutrons						
5 Gy	0.3-22.7	7.96 ± 0.37	17.429**	0.6-30.3	8.30 ± 0.42	2.963**
10 Gy	0.8-19.6	7.11 ± 0.30	23.769**	0.4-20.4	7.21 ± 0.35	0.535
15 Gy	0.7-18.0	6.09 ± 0.45	18.723**	0.5-24.7	6.92 ± 0.35	0.279
NMU 0.01% (20h)	0.9-27.3	6.90 ± 0.36	20.686**	0.3-22.3	5.30 ± 0.28	5.677**
0.02% (8h)	0.5-17.5	4.94 ± 0.28	32.343**	0.4-21.4	6.05 ± 0.33	2.779**
EMS 0.2% (8h)	0.6-19.8	7.88 ± 0.69	9.828**	0.2-5.0	2.14 ± 0.17	24.158**

*, **significant at 5% and 1% respectively

Table 3. Observed CV range, CV, percentage of M_2 families above control CV and test of heterogeneity for grain yield (g) per plant in M_2 generation

Treatment	Observed CV range	CV	% M_2 Fam. above cont. CV	Observed CV range	CV	% M_2 Fam. above cont. CV
	desi Var. G 130			*desi* Var. H 214		
Control	7.5-15.9	13.92	-	4.2-13.5	13.49	-
γ-rays 400 Gy	10.4-141.2	97.49	97 *	17.5-109.9	62.62	100 *
500 Gy	20.3-110.1	63.94	100 *	21.5-121.1	69.34	100 *
600 Gy	17.0-92.6	67.30	100 *	11.0-112.7	74.66	97 *
neutrons						
5 Gy	16.7-99.6	60.46	100 *	17.0-114.1	72.31	100 *
10 Gy	11.1-111.3	66.11	97 *	26.4-115.9	78.22	100 *
15 Gy	24.7-133.7	73.01	100 *	32.6-101.8	81.58	100 *
NMU 0.01% (20h)	20.8-107.1	73.92	100 *	13.9-98.3	82.22	100 *
0.02% (8h)	19.6-121.0	68.78	100 *	23.2-165.7	78.55	100 *
EMS 0.1% (20h)	17.0-96.3	69.34	100 *	56.4-133.8	108.52	100 *
0.2% (8h)	20.9-114.5	63.85	100 *	15.9-143.9	107.09	100 *
	kabuli Var. C 104			green seeded Var. L 345		
Control	3.4-6.2	9.24	-	6.1-17.0	16.44	-
γ-rays 400 Gy	-	-	-	14.6-98.6	59.74	97 *
500 Gy	-	-	-	26.3-91.4	61.13	100 *
600 Gy	-	-	-	16.3-102.0	61.98	97 *
neutrons						
5 Gy	23.1-86.7	60.13	100 *	23.2-85.0	64.23	100 *
10 Gy	15.0-68.9	55.25	100 *	10.9-108.9	60.73	97 *
15 Gy	21.9-86.6	65.70	100 *	16.4-98.6	61.07	100 *
NMU 0.01% (20h)	26.3-120.1	67.46	100 *	22.2-152.7	70.79	100 *
0.02% (8h)	13.9-104.1	67.65	100 *	21.5-95.4	70.73	100 *
EMS 0.2% (8h)	39.8-81.4	61.85	100 *	28.4-140.6	64.81	100 *

- = Population not available; * = variances are heterogeneous

estimated for each of the families. The range of CV values for different M_2 and also the CV values for the entire M_2 population corresponding to each treatment has been given in Table 3.

Grain yield per plant: It is clear that with most of the mutagen treatments the M_2 population (Table 2) shows a larger range than the corresponding control population. Inspite of the greatly increased range, the mean value for this character has been significantly depressed in the M_2 population corresponding to most of the mutagenic treatments than the control population. This is true both in case of the two *desi* varieties as well as the *kabuli* and the green seeded variety. The negative shift of the mean has been reported earlier in barley [5] and corn [6]. Observations on the magnitude of induced variability for grain yield are given in Fig 1A. It is evident that the mutagenised population shows a much greater range of frequency distribution than in the control population. However, it is only a very small fraction of the M_2 families in which the increased variance may be associated with increase in the mean value of these characters. The frequency distribution curves have helped to locate these promising plants from the few exceptional M_2 families. The emphasis has been to clearly demarcate in each of the M_2 population those plants which correspond with the extreme right hand part of the frequency distribution curve. This has helped to locate more promising of the M_2 plants in respect of single plant yield and yield components. While increased ranges of very wide magnitude on the negative side is not uncommon, it is extremely rare to find the distribution curves showing such a wide-spread towards both the ends. This observation on M_2 population indicates that some very useful variability has been induced following mutagenic treatments.

A more meaningful comparison of the induced variability in the different M_2 families has been made in Table 3 where the range of CV values for this character have been given in the control and the different groups of M_2 families. The range of CV values in the treatment families in general is much greater than the control families. In most treatments all the families show a CV range greater than that of the control population. From a purely practical point of view the M_2 families which are of greater interest, are those where the CV has increased and at the same time the mean value for the character has either increased or not greatly depressed than the control. Families of this kind are associated more commonly with some of the mutagenic treatments as shown in Table 4.

This combination of high CV values and relatively high mean values for this character suggest that a large part of the induced variability has been in the positive directon. These treatments, therefore, deserve special attention for carrying forward to further generations.

Analysis of variability: The variability induced (Table 5) has been analysed statistically and partitioned into inter-family and intra-family variance components for this character in the different treatments. The analysis clearly shows that the variances greatly increased over control in each of the treatment at both the inter and intra-family levels. The largest increase in inter-family variance is associated with the treatments of gamma-rays in case of *desi* var. G 130 and neutrons in the *kabuli* var. C 104 and green seeded var. L 345

Table 4. Treatments with increased mean and CV for quantitative characters in M_2 generation

Character	*desi* Var. G 130	*desi* Var. H 214	*kabuli* Var. C 104	green seeded Var. L 345
Grain yield (g) per plant				
	EMS 0.2%(8h)	EMS 0.2%(8h)	EMS 0.2%(8h)	neutrons 5 and 10 Gy
	EMS 0.1%(20h)	γ-rays 500 Gy	neutrons 5 and 15 Gy	neutrons 15 Gy
	γ-rays 500 Gy	NMU 0.02%(8h)	NMU 0.01%(20h)	γ-rays 600 Gy
	γ-rays 600 Gy			
Number of pods per plant				
	γ-rays 600 Gy	EMS 0.2%(8h)	EMS 0.2%(8h)	neutrons 5,10 and 15 Gy
	γ-rays 500 Gy	γ-rays 500 Gy	neutrons 5 Gy	γ-rays 600 Gy
Number of grains per plant				
	EMS 0.2%(8h)	EMS 0.2%(8h)	EMS 0.2%(8h)	neutrons 5 and 10 Gy
	EMS 0.1%(20h)	γ-rays 500 Gy	neutrons 5,10 and 15 Gy	neutrons 15 Gy
	neutrons 15 Gy			NMU 0.02%(8h)
100 grain weight (g)				
	NMU 0.01%(20h)	γ-rays 500 Gy	neutrons 5 Gy	γ-rays 400 Gy
	neutrons 5 Gy	EMS 0.2%(8h)	neutrons 15 Gy	NMU 0.01%(20h)
	neutrons 15 Gy	neutrons 5 Gy	NMU 0.01%(20h)	γ-rays 500 Gy

Table 5. Inter and intra family variances, heritability (h^2) and expected genetic advance (EGA) for grain yield (g) per plant in M_2 generation

Treatment	Variance		h^2(%)	EGA(%)	Variance		h^2(%)	EGA(%)
	Inter family	Intra family			Inter family	Intra family		
	desi Var. G 130				*desi* Var. H 214			
Control	1.025	0.959			0.605	0.530		
γ-rays 400 Gy	63.197	14.026	41.2	71.08	18.462	9.734	15.2	16.85
500 Gy	36.733	19.234	15.3	17.40	16.804	14.372	0.3	0.36
600 Gy	67.706	14.049	43.3	51.56	11.199	5.773	15.8	20.86
neutrons								
5 Gy	21.650	6.933	29.7	31.72	4.550	2.313	16.1	20.52
10 Gy	27.070	11.990	20.0	23.29	3.795	1.607	21.3	29.67
15 Gy	34.963	19.356	13.8	17.75	5.507	1.305	39.1	56.74
NMU 0.01% (20 h)	33.302	10.995	28.8	37.55	25.185	3.784	53.0	77.49
0.02 % (8h)	16.028	7.803	17.4	21.12	19.163	9.342	17.3	23.89
EMS 0.1% (20h)	41.209	15.310	25.2	30.92	12.689	6.631	15.4	29.69
0.2% (8h)	41.565	23.221	13.6	15.31	63.565	20.434	19.6	56.54
	kabuli Var. C 104				green seeded Var. L 345			
Control	0.542	0.470			0.683	0.597		
γ-rays 400 Gy	-	-	-	-	7.554	3.310	20.3	21.47
500 Gy	-	-	-	-	8.919	5.818	9.6	10.39
600 Gy	-	-	-	-	15.636	13.730	2.7	2.86
neutrons								
5 Gy	37.358	19.451	15.5	16.46	49.913	23.285	18.6	21.08
10 Gy	36.039	10.462	32.8	32.07	31.685	16.203	16.0	17.20
15 Gy	30.642	12.631	22.1	25.78	36.617	13.355	25.8	27.89
NMU 0.01% (20h)	28.259	20.109	7.4	8.4	22.715	12.016	15.1	18.87
0.02% (8h)	28.837	6.914	38.8	46.46	32.422	14.538	19.7	24.46
EMS 0.2% (8h)	24.949	18.540	6.5	6.47	2.084	1.901	1.8	1.87

- = Population not available

and EMS 0.2%(8hr) treatment in *desi* var. H 214. On the other hand, intra-family variances are largest in chemical treatments in all the varieties except green seeded var. L 345 where neutrons 5 Gy generated highest variance.

Based on the above analysis the heritability estimates as well as the genetic advance has been computed for the different populations (Table 5). It is not surprising to find high heritability and genetic advance values in the case of treatment populations. In general, it is clear that the physical mutagens have generated more variability and the CV is higher in these treated populations with some exceptions.

Components of grain yield: A similar analysis has been made for number of pods, number of grains per plant and 100 grain weight (g) which are the main components of the grain yield per plant. In general, we find the same trend as for the grain yield per plant for the different yield components. However, the treatments mentioned in Table 4 deserve special attention for respective characters, as the mean value in these treatments either increased or did not drastically depress even with increase in CV values and, therefore, these treatments could be of greater practical utility in further generations.

Evaluation of micromutations in M_3 generation: The induced variability in the M_3 generation populations has been analysed in more or less the same manner as that in the M_2 generation. In all 28 treatments, 10 from *desi* var. G 130, 6 each from *desi* var. H 214, *kabuli* var. C 104 and green seeded L 345 and one each control were grown in a bireplicated randomised block design trial. The number of families varied from treatment to treatment. However, the number of plants per family was 20 in all cases. The number of treatments, families and plants studied under each variety are given in Table 1. It may be recalled that plants from only those selected M_2 families (only 5% of the plant population on the positive direction of the total frequency distribution curve) were carried forward to raise M_3 generation which showed a good combination of a relatively high mean value and a large CV. If the variability is genetic, one may reasonably expect that the high mean value will be maintained or further increased in the M_3 progenies and it should be possible to select individual plants showing good expression of various yield components or yield itself. Therefore, a comparative study was done to estimate the nature of induced variation with respect to its size, direction, heritable components and response to selection in M_3 generation and also to estimate correlations in M_3

Table 6. Means, percentage of M_3 families above control mean, CV range and GCV for grain yield (g) per plant in M_3 generation

Treatment	Mean (g)	% M_3 Fam. above cont. mean	CV range	GCV	Mean (g)	% M_3 Fam. above cont. mean	CV range	GCV
	desi Var. G 130					*desi* Var. H 214		
Control	28.86		9.7-23.5		25.53		13.3-26.0	
γ-rays 400 Gy	42.86**	100	31.4-46.0	24.50	38.56**	100	26.1-53.1	23.81
500 Gy	41.60**	100	29.7-49.0	32.25	37.06**	100	30.1- 43.4	51.64
600 Gy	43.81**	94	22.8-52.9	43.02	37.41**	100	25.0- 51.1	24.86
neutrons								
5 Gy	39.94**	100	30.9-38.2	7.12	-	-	-	-
10 Gy	33.54	67	33.3-49.6	51.34	-	-	-	-
15 Gy	39.12**	80	25.9-35.8	53.66	-	-	-	-
NMU 0.01% (20h)	37.48**	100	22.0-39.5	37.79	37.28**	80	23.5-46.4	99.92
0.02% (8h)	37.05**	100	26.9-36.8	7.39	36.39**	86	22.4-48.3	70.51
EMS 0.1% (20h)	40.11**	100	28.5-60.4	60.35	-	-	-	-
0.2% (8h)	39.09**	100	16.9-46.7	22.72	39.88**	100	31.8-44.5	53.61
	kabuli Var. C 104					green seeded Var. L 345		
Control	26.93		14.28-22.2		27.95		15.5-23.7	
γ-rays 600 Gy	-	-	-	-	37.07**	83	22.2-44.8	43.09
neutrons								
5 Gy	37.03**	95	18.4- 58.4	38.58	36.24**	95	23.3-53.3	29.97
10 Gy	36.98**	100	23.6-42.3	47.19	36.65**	91	26.5-58.3	40.28
15 Gy	40.89**	100	35.6-42.7	51.54	34.42**	87	29.3-59.7	32.43
NMU 0.01% (20 h)	36.73**	100	23.5-47.3	41.70	32.66	75	25.4-37.5	38.65
0.02% (8h)	33.56*	75	23.8-41.3	59.93	33.96*	100	31.3-48.6	26.54
EMS 0.2% (*8h)	38.81**	86	23.5-47.2	75.10	-	-	-	-

GM = 36.42; S. E. = 2.56; C.D. at 5% = 5.185; C.D. at 1% = 6.938; *Significant at 5% **Significant at 1%; - Population not available.

Table 7. Inter-family variances, overall variance, heritability (h^2), expected genetic advance (EGA) and test of heterogeneity for grain yield (g) per plant in M_3 generation

Treatment	Variance		h^2 (%)	EGA (%) of mean	Variance		h^2(%)	EGA (%) of mean
	Inter-family	Overall			Interfamily	Overall		
	desi Var. G 130					*desi* Var. H 214		
Control	379.15	44.92			31.32	39.00		
γ-rays 400 Gy	523.87	291.11**	26.65	26.05	493.14	221.00**	20.64	22.28 +
500 Gy	834.28	296.17**	27.52	34.86	884.93**	218.91**	70.65	89.41
600 Gy	1208.13*	306.09**	41.65	57.19	163.67	193.36**	0.0	0.0
neutrons								
5 Gy	347.20	187.69**	0.0	0.0	-	-	-	-
10 Gy	600.85**	197.75**	97.58	104.49	-	-	-	-
15 Gy	1030.75*	167.99**	74.70	95.55	-	-	-	-
NMU 0.01% (20h)	495.50	152.44*	68.11	64.25	2971.75**	301.30**	87.61	192.67 +
0.02% (8h)	220.00	135.51	0.0	0.0 +	1449.66**	189.54**	83.27	132.55 +
EMS 0.1% (20 h)	1326.62**	319.93**	79.14	110.59	-	-	-	-
0.2% (8h)	253.40	172.23**	0.0	0.0	1337.40**	268.94**	51.94	79.59
	kabuli Var. C 104					green seeded Var. L 345		
Control	233.77	41.50			92.20	36.82		
γ-rays 600 Gy	-	-	-	-	741.20	186.57**	51.45	63.67
neutrons								
5 Gy	834.74	218.64**	32.36	45.00 +	595.24	232.96**	24.72	30.70
10 Gy	912.00	191.78**	50.18	68.87 +	894.10	296.55**	32.23	47.11
15 Gy	1156.66	295.87**	62.38	83.85	524.00	238.03**	31.20	37.32
NMU 0.01 % (20 h)	657.78*	189.59**	55.44	63.96	375.00*	114.07	73.87	68.42
0.02% (8h)	885.00*	160.87*	84.25	113.32	342.83	201.75**	31.06	30.47 +
EMS 0.2% (8h)	2009.66**	255.94	73.22	132.38	-	-	-	-

S. E. = 320.63, 45.17; C.D. at 5% = 647.99, 91.29; C.D. at 1% = 866.98, 122.15; *Significant at 5%; **Significant at 1%; +Variances are heterogeneous; - Population not available

which might have been established due to breakage of linkage.

Grain yield per plant: Table 6 records observations on the M_3 populations for grain yield (g) per plant raised from selected M_2 progenies in relation to each of the different treatments. All the M_3 mean values in different treatments are higher than those in control population and several of them show significant increase in yield. This shows that the selection technique employed in M_2 population has been highly effective and the M_3 populations include a large proportion of induced variability in the positive direction. The new selection technique for efficient screening of useful induced variability is based primarily on the coefficient of variability (CV) and mean of the mutagenized populations and controls. This technique is based on the principle that from a purely practical breeding point of view, the M_2 families of greater interest are only those for which the CV has increased and, at the same time, the mean value for the character has also changed in the desired direction, or has not greatly altered over the control. The main point of this technique is to rigorously reject most of the induced variability and pinpoint those segregating families which exhibit a definite superiority over the controls, because it is only a very small fraction of the M_2 families in which the increased variance may be associated with an increase in the mean value of the same character. Thus, the mean and CV values of these families, compared to those of the controls, have been used as the basis for their identification. Identification and selection can be done at the interfamily and intrafamily levels. In this respect the M_3 families show a very different behaviour from the M_2 families which in general had a lower mean than the M_2 control population.

The second observation of interest is that the CV values in the M_3 families are lower than those in the M_2 families. This is only to be expected as the maximum segregation for induced varibility is expected in the M_2 generation.

The entire M_3 population in relation to each of the different varieties has been plotted along with the control for the frequency distribution of their plants as shown in Fig. 1B to 4B. The wide ranges of the curves for M_3 clearly indicate that the M_3 population have continued to show a great deal of variability indicating thereby that the possibilities of selecting superior genotypes in this M_3 population were by no means exhausted. The M_3 populations like M_2, were showing segregation for various quantitative characters. However, the pattern of segregation in the M_2 and M_3 populations has been found to be different. This is clear from the shape and spread of the frequency distribution curves. The M_2 populations (Fig 1A to 4A) showed widespread curves in both directions with a pronounced shift in the negative direction and with only a few individual plants distributed on the positive side. In M_3 populations (Fig 1B to 4B) the shift is largely in the positive direction. This obviously again reflects the effectiveness of the selection technique and selections made in the M_2 generation. The direction of the shift appears to be correlated with the past selection history of the varieties. The present observations, thus are in conformity with the hypothesis proposed by Brock [7, 8] and other workers [9-12].

The different M_3 populations have also been analysed for inter-family variance, overall variance and for heritability and genetic advance at treatment level (Table 7). It will be seen that the two variances are highly significant in many treatments, but their magitude is less than in the M_2 population. The heritability and genetic advance values are particularly very high in certain treatments than in others.

Analysis of variance for grain yield (g) per plant given in Table 8 shows that the inter-family and overall variances were significantly altered in treated populations in M_3 generation. Significant differences in the populations, treatments and varieties verses treatments in respect of overall mean and variance were observed for grain yield per plant. However, the varieties were unaffected at all levels. Similar increase in variance for several quantitative characters in mutagenised populations has been reported in rice [9-10], soybean [13] and wheat [14-16].

Table 8. Analysis of variance for grain yield (g) per plant in the M_3 generation

Source	D.F.	Inter-family MS	Overall MS
Replications	1	736684.16*	6767.27
Populations	31	196148.78*	13098.59**
Varieties	3	15063.66	24.20
Treatments	27	198692.04*	6548.00*
Var. vs. Treat.	1	670735.92*	229187.52**
Error	31	102804.33	2040.68

Components of grain yield in M_3 generation: A similar analysis has been made for other quantitative characters including number of pods, grains per plant, number of grains per pod, 100 grain weight, biological yield and harvest index per plant, which are the main components of the grain yield per plant. In general, we find the same trend as for the grain yield per plant for the different characters. In other words the mean values in M_3 populations have reached a relatively high level on the basis of M_2 selection and the amount of variability in the M_3 families has been relatively reduced.

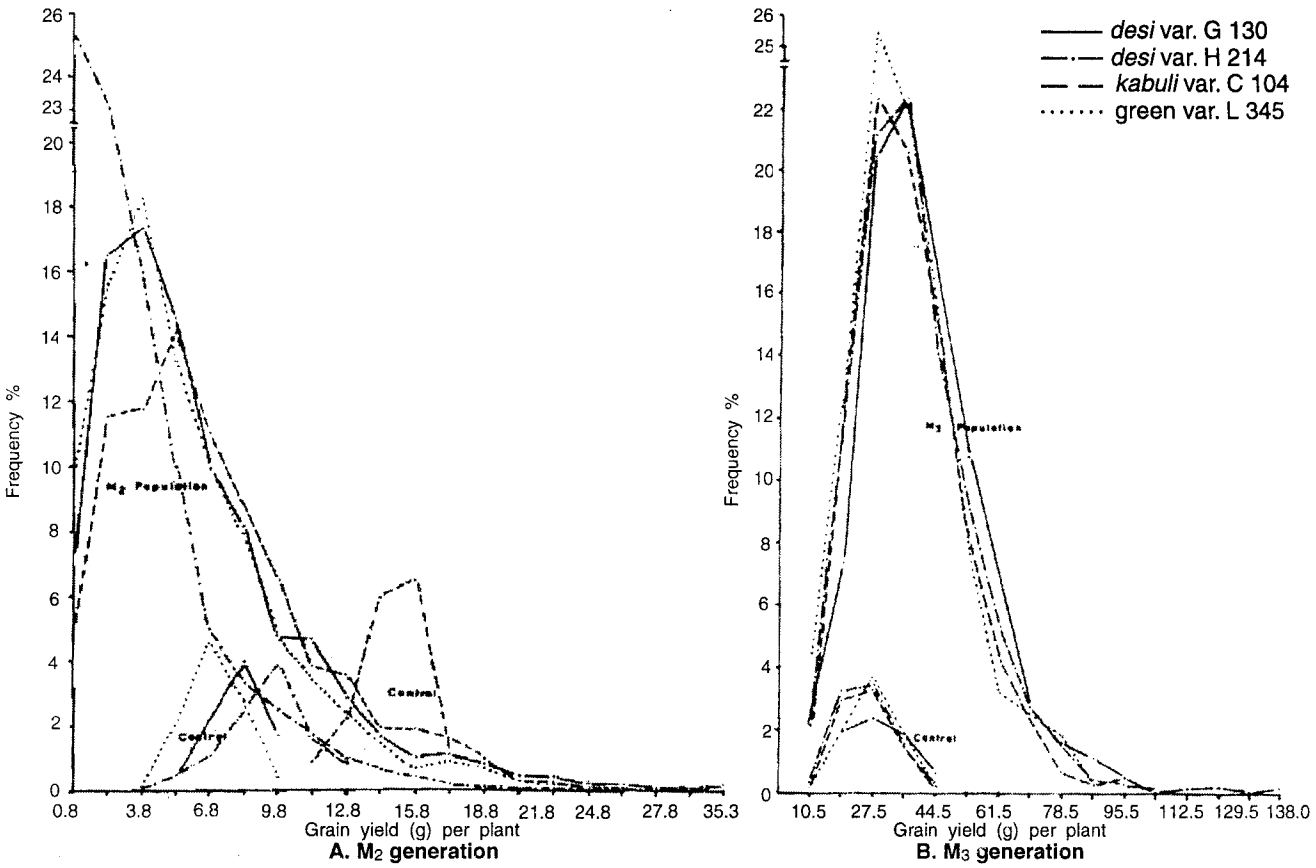

Fig. 1. Frequency distribution for grain yield (g) per plant in M_2 and M_3 generations

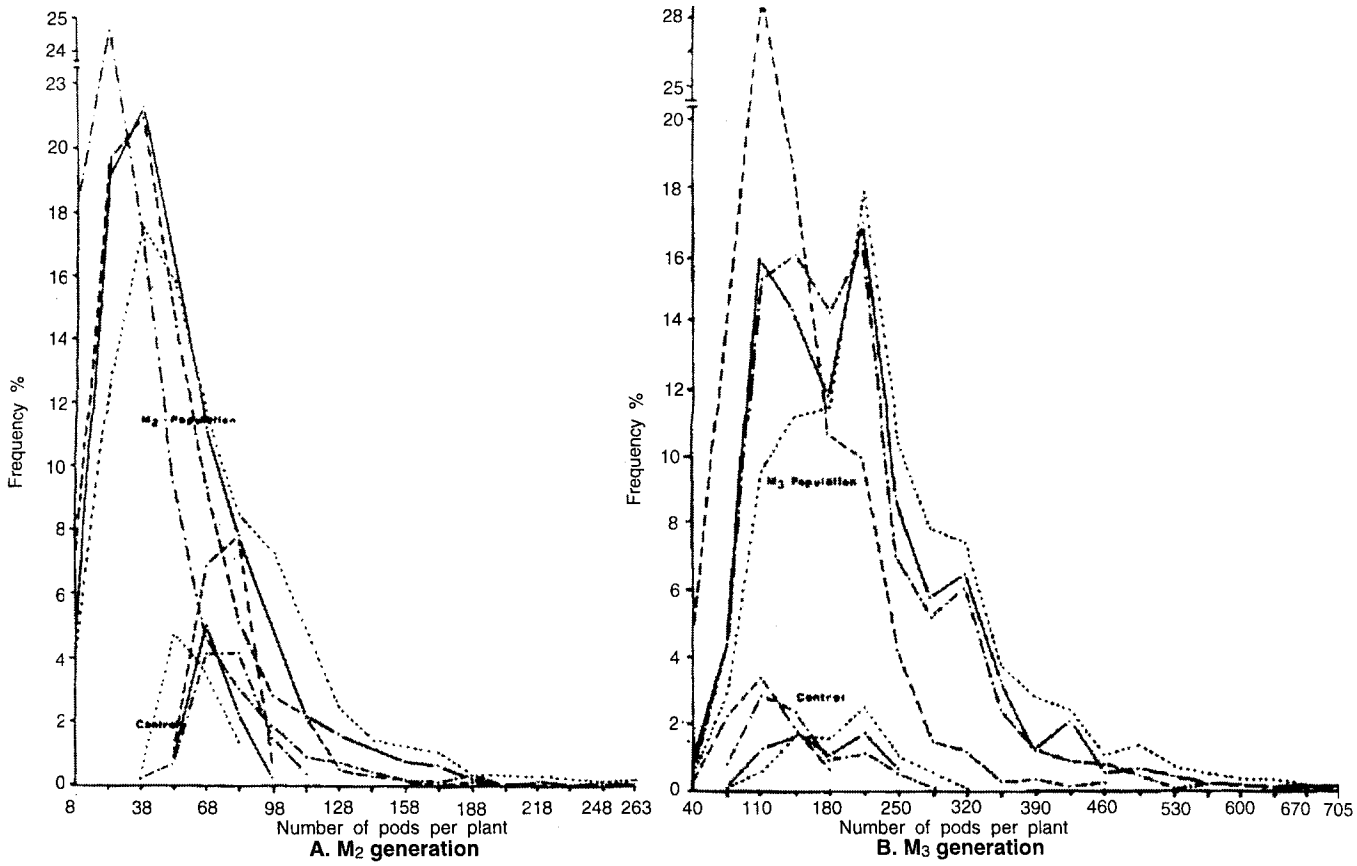

Fig. 2. Frequency distribution for number of pods per plant in M_2 and M_3 generations

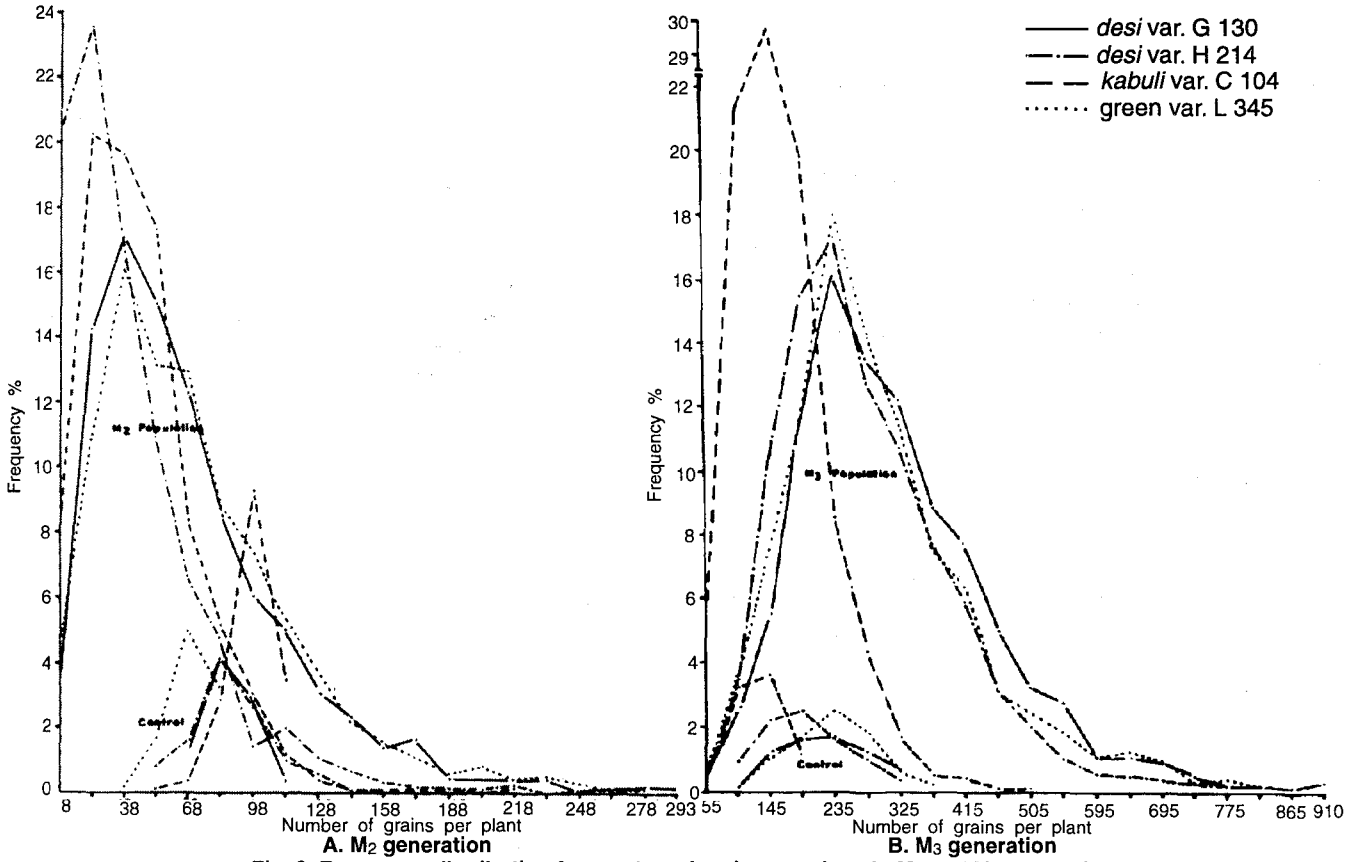

Fig. 3. Frequency distribution for number of grains per plant in M_2 and M_3 generations

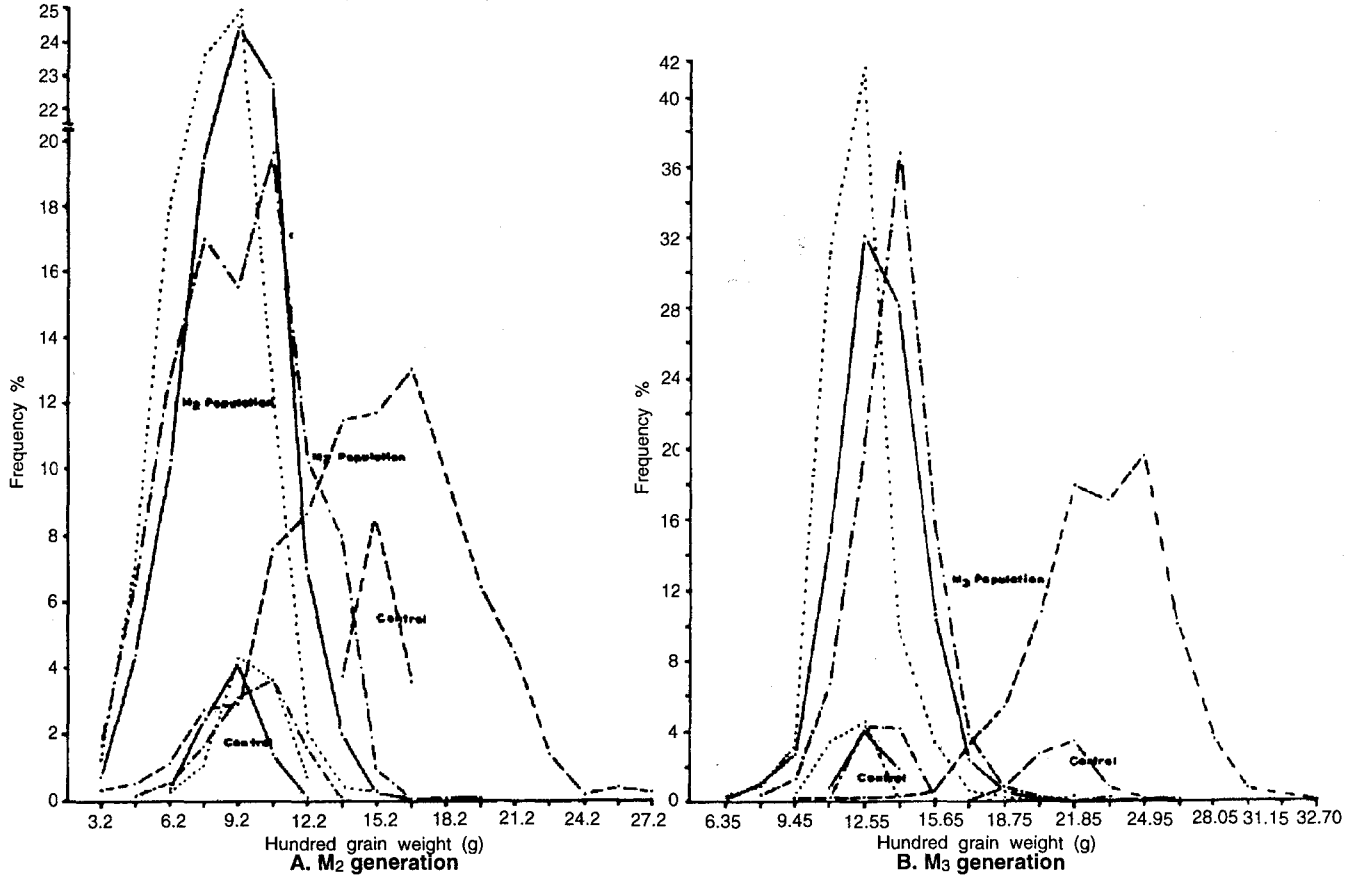

Fig. 4. Frequency distribution for hundred grain weight (g) in M_2 and M_3 generations

It is clear from the data that unlike the behaviour of mean in M_2 generation, in case of M_3 a great majority of the treatments showed large increase in mean over the respective controls in most of the characters. Few marginal exceptions to this trend were observed in case of number of grains per pod, hundred grain weight and harvest index. A very large amount of variability generated particularly in the positive direction in the mutagenised M_3 population can be clearly seen from frequency distribution curves in Fig. 1 to 4. It is also evident that while in *desi* varieties gamma-rays treatments have shown higher means than others, on the other hand, in *kabuli* variety, neutrons have shown better results.

The M_3 families with their relatively high mean values and considerable amount of inter-plant variability, high heritability and genetic advance offered a good opportunity for single plant selections based largely on important components of yield and on the basis of single plant yield. Based on a very rigorous selection of this kind, a large number of M_3 single plant progenies from the combined M_3 population of all the different treatments were selected for raising families in M_4 generation. The present study concludes that mutagen treatment of chickpea varieties generated a tremendous amount of variability. An effective selection technique based on CV and mean successfully shifted useful variability in the desirable direction to M_3 generation, a large portion of which was heritable. On the basis of symmetry of the variation, selection may be excercised towards positive or negative direction on such populations to select desirable genotypes for further evaluation in advanced generations.

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