Indian J. Genet., 59(4): 451-464 (1999)

INDUCED MUTATIONS IN CHICKPEA (*CICER ARIETINUM* L.) III. FREQUENCY AND SPECTRUM OF VIABLE MUTATIONS

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(Received: December 29, 1997; accepted: September 30, 1999)

ABSTRACT

A comparative study of frequency and spectrum of chlorophyll mutations induced by two physical (gamma rays and fast neutrons) and two chemical mutagens (NMU and EMS) in relation to the effects in M_1 plants and induction of mutations in M_2 was made in four chickpea (*Cicer arietinum* L.) varieties, two *desi* (G 130 and H 214) one *kabuli* (C 104) and one green seeded (L 345). The treatments included three doses each of gamma rays (400, 500 & 600 Gy) and fast neutrons (5, 10 & 15 Gy) and two concentrations with two different durations of two chemical mutagens, NMU [0.01% (20h), and 0.02% (8h)] and EMS [0.1% (20h) & 0.2% (8h)]. Chemical mutagens were more efficient than physical in inducing viable and total number of mutations. Among the chemical mutagens NMU was the most potent, while among the physical mutagens, gamma rays were more effective. Green seeded var. L 345 was most responsive for mutations while the *kabuli* var. C 104 was least suitable. Major differences in the mutagenic response of the four cultivars were observed. The desi varietiies were more resistant towards mutagenic treatment than *kabuli* and green seeded type.

Key Words : Chickpea, *Cicer arietinum*, viable mutations, frequency and spectrum, mutagens, radiation

Chickpea is most important pulse crop of the Indian subcontinent accounting for more than 50% in area and production of all the pulse crops. In order to induce variability and utilize useful mutations for efficient plant breeding, the systematic and comparative study of induced chlorophyll and viable mutation frequency in M_2 generation is the most dependable index for evaluating the effectiveness of mutagenic treatments. Although studies on induced mutations in some legumes [1-14] have been undertaken in the past, limited reports are available on crops such as chickpea [1-5].

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 identify the mutagen and treatment causing maximum viable mutations in M_2 generation.

MATERIALS AND METHODS

The material for this 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 neutrons 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 ^{0}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 row. The treated as well as control poulations after germination were carefully screened for viable mutations throughout the life period of the plants in the seed beds in the field.

RESULTS AND DISCUSSION

The mutations affecting gross morphological changes in branching, stem structure, growth habit, leaf, flower, pod and seed size, maturity and plant type etc. were scored as viable mutations. There were differences in the mutation spectrum both between varieties and mutagenic treatments. However, many similarieties were also noticed between the *desi* and *kabuli* varieties in respect of the spectrum and frequency of a particular mutation. The frequencies of viable mutations were computed as in the case of chlorophyll mutations [4].

Frequency of viable mutations: It can be seen (Table 1) that the total mutation requency increased with the increase in dose, except in case of neutron 15 Gy and EMS 0.2%(8h) which showed a sudden decrease in mutation rate. Differences were observed in the mutation rates obtained with different varieties as well as mutagens applied. Among the mutagens the highest frequency of total viable mutations on the basis of M_1 population (Fig. 1-a) showed that NMU 0.02%(8h) was most efficient (51.8%), followed by EMS 0.1% (20h) (44.0%). Physical mutagens gave similar results

Table 1. Frequency of induced viable mutations in the M_2 generation in chickpea

Mutagen	Treatment Dose/duration	Varieties: *desi* G 130 M_2 pl.	*desi* G 130 % Mut.	*desi* H 214 M_2 pl.	*desi* H 214 % Mut.	*kabuli* C 104 M_2 pl.	*kabuli* C 104 % Mut.	green-seeded L 345 M_2 pl.	green-seeded L 345 % Mut.	Total	Population size M_1 family	Population size M_2 plants	Frequence of Mut./100 M_1 family	Frequence of Mut./1000 M_2 plants
Gamma rays	400 Gy	2020	0.08	1834	0.20	-	-	2490	0.09	0.38	150	6344	16.0	3.8
	500 Gy	2014	0.32	1019	0.08	-	-	2218	0.17	0.51	150	5251	20.0	5.7
	600 Gy	1948	0.23	850	0.21	-	-	1926	0.19	0.64	150	4724	20.0	6.3
	Overall	**5982**	**0.20**	**3703**	**0.16**	-	-	**6634**	**0.15**	**0.51**	**450**	**16319**	**18.7**	**5.1**
Neutrons	5 Gy	2466	0.26	2596	0.07	652	0.07	1890	0.03	0.42	195	7604	16.4	4.2
	10 Gy	2090	0.01	2082	0.21	780	0.04	2020	0.23	0.50	195	6972	17.9	5.0
	15 Gy	1654	0.07	2520	0.04	306	0.03	2190	0.09	0.24	175	6670	9.2	2.4
	Overall	**6210**	**0.12**	**7198**	**0.11**	**1738**	**0.05**	**6100**	**0.11**	**0.39**	**565**	**21246**	**14.2**	**3.9**
NMU	0.01%(20h)	2150	0.40	1900	0.11	866	0.19	2354	0.40	1.10	195	7270	41.0	11.0
	0.02%(8h)	2604	0.30	1836	0.32	412	0.08	2406	0.70	1.39	195	7258	51.8	13.9
	Overall	**4754**	**0.34**	**3736**	**0.21**	**1278**	**0.14**	**4760**	**0.55**	**1.24**	**390**	**14528**	**46.4**	**12.5**
EMS	0.1%(20h)	1990	0.73	338	0.69	-	-	-	-	1.42	75	2328	44.0	14.2
	0.2%(8h)	1940	0.19	306	0.77	132	0.35	224	0.35	1.65	120	2602	35.8	16.5
	Overall	**3930**	**0.45**	**644**	**0.73**	**132**	**0.18**	**224**	**0.18**	**1.54**	**195**	**4930**	**39.0**	**15.4**
	Total	**20876**	**0.23**	**15281**	**0.20**	**3148**	**0.07**	**17718**	**0.24**	**0.74**	**1600**	**57023**	**26.5**	**7.4**

- Population not available

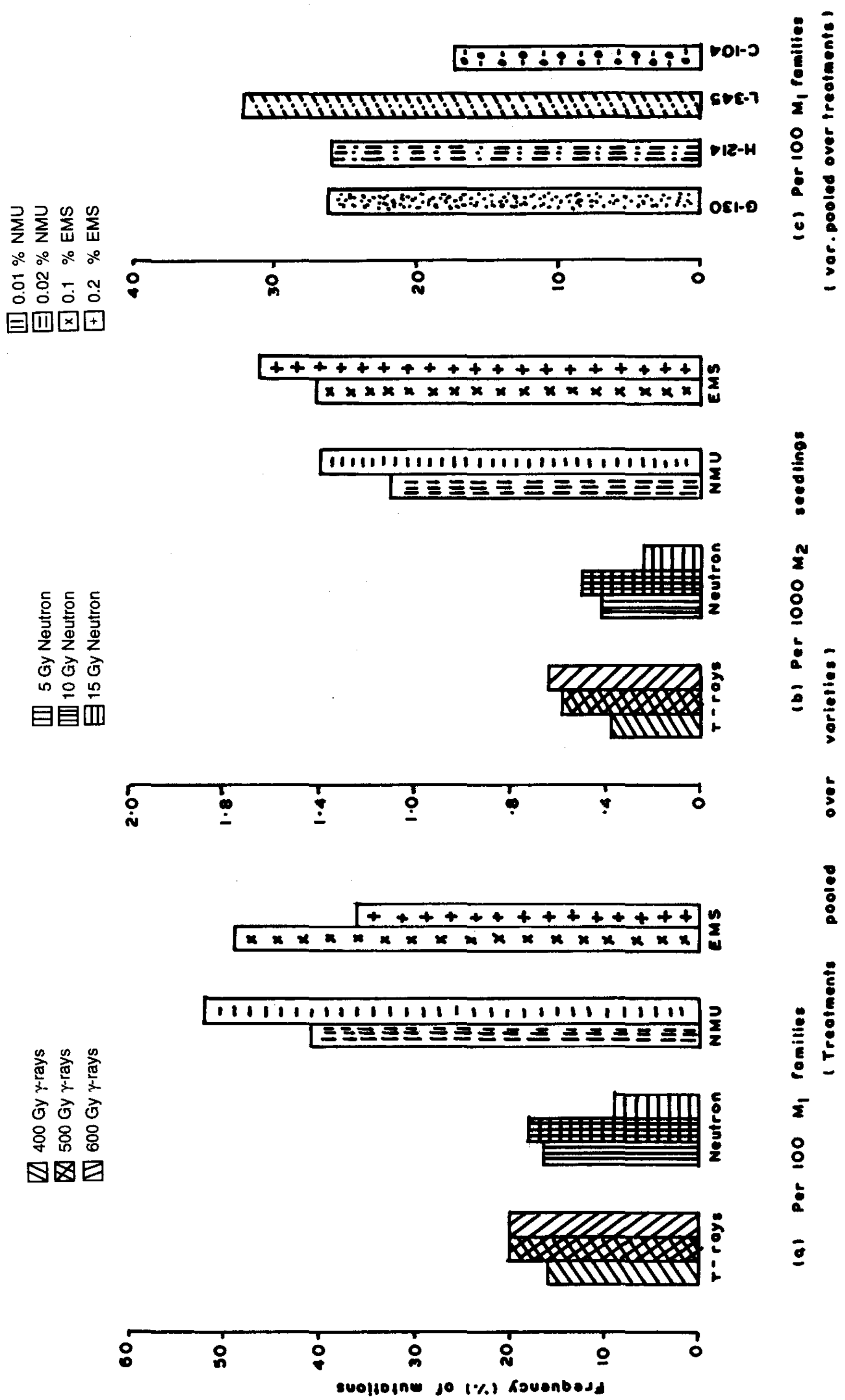

Fig . 1(a,b,c). Frequency of viable mutations in M2 generation on M_1 and M_2 population basis and their highest mutation rate in 600 Gy gamma rays was about 50% of the mutation rate recorded with the chemical mutagens. Neutrons 15 Gy was found to be the least effective treatment. On the basis of M_2 population (Fig. 1-b), highest frequency of viable mutations was observed in EMS treatment 0.2%(8h). The frequency of mutations in physical mutagens was very low as compared to chemical and neutrons gave lowest frequency among all mutagens. Among the varieties, total viable mutation frequency on the basis of M_1 population (Fig. 1-c) indicated the maximum rate in green seeded var. L 345 (32.2%), followed by *desi* var. G 130 (26.2%). The lowest number of mutants was observed in *kabuli* var. C 104 (17.3%).

Average mutation rate showed that the chemical mutagens gave 2 to 5 times more mutations than the radiations while in chemical mutagens NMU gave higher mutation rate than EMS, in radiations gamma rays gave higher mutation rate than neutrons.

a) **Viable mutations** : The chemical mutagens have also been found to be more effective compared to the physical mutagens in inducing morphological mutations (Table 1). NMU, which gave the highest frequency of M_2 families segrgating for chlorophyll mutations, has also yielded the largest number of M_1 families segregating for morphological mutations (Fig. 1-a). Thus we may conclude that NMU is highly useful mutagen when one is looking for major gene changes which can be readily scored in a segregating population. However, it may be noted that EMS, another alkylating agent is also highly efficient in inducing viable mutations particularly on M_2 population basis (Fig. 1-b). The neutron treatments in contrast has been found to be least effective in inducing mutants of this kind. It is known from earlier studies that densely ionising particles like neutrons are more efficient in inducing chromosome breaks than point mutations. The present findings would appear to support this conclusion. Another interesting finding is that in contrast to the high sensitivity of *desi* varieties as a group for chlorophyll mutations, for viable mutations the culinary varieties are relatively more sensitive (Fig. 1-c). Higher frequency of chlorophyll and viable mutations following treatments with chemical mutagens than radiations as observed in this study, have also been reported in peas [6-9] and lentils [10-13].

Results on the basis of frequency of viable morphological mutations per 100 M_1 families also indicated maximum mutability of green seeded var. L 345 (32.2%) followed by *desi* var. G 130 (26.2%) whereas again *kabuli* var. C 104 had lowest mutation rate (17.3%). The data obtained suggested that induced mutability is also governed by the genetic architecture of the material used.

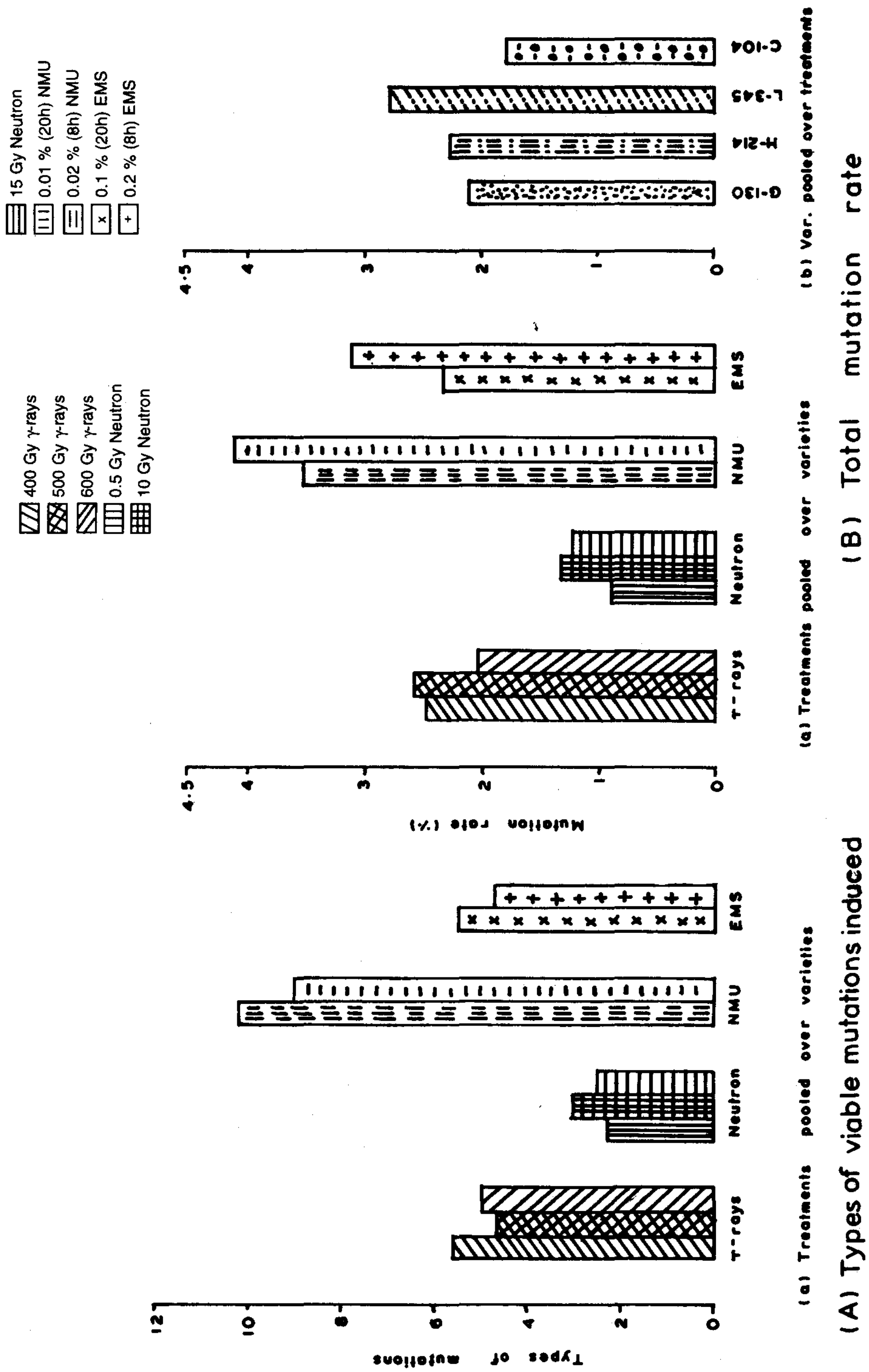

Fig . 2(a,b). Frequency of viable mutations in M_2 generation

The sharp differences in the response of the different varieties in terms of induction of mutations by the different mutagens raises some important issues. The choice of variety for mutation experiment is determined largely by the agronomic characteristics of the different varieties. It is important for this reason to find why different varieties differ so much in their mutability and whether experimental conditions can be manipulated to enhance mutation rates in varieties which normally appear to be resistant to genetic changes. The *kabuli* var. C 104 in the course of present study has been found to be relatively resistant to mutational changes in general.

It is well recognised of course that induced mutability at *locus* or group of *loci* is affected by the general genetic background of the plant. That the varieties of spring barley of non-hybrid ones were less mutable than the hybrid ones was noted by Gustafsson [6] as far back as 1947. Later on, the genotypic control of mutation process has been reported by several workers [20-22].

The wider spectrum of induced mutations by NMU holds good for all the varieties. In addition to the mutagen, the genetic background of the chickpea variety has a significant role in determining its mutability. This is clear from the observation presented in Table 2, Fig. 2-B, that even a highly potent mutagen like NMU is not equally effective in all the varieties. Gregory [21] also stated that the chief factor that influences the induction and recovery of mutations is the genetic constitution of the experimental organism and not the mutagen used. Similar evidence that genetic differences as small as single gene difference even could bring about significant changes not only in the frequency but also in the spectrum of recoverable mutations have been provided by many workers [16-22].

The mutation spectrum induced in the more radio and Chemo resistant *desi* varieties was quite broad and the mutations affecting almost all the parts of the plant and characteristics were isolated (Table 3). On the other hand, in the most radio and chemo-sensitive *kabuli* var. C 104 which was also least mutable, the mutation spectrum was also narrowest (Table 4). These observations also agree with the conclusion that the closer the varieties are in their genotypes, the greater is the similarity in their spectra and frequency of mutations. Thus the "Varietal mutability" in the frequency and spectrum of induced mutations is largely affected by the genotype of the variety under definite mutagen treatment and treatment conditions.

It was also interesting to note that the spectrum of the mutations was dependent upon the nature of the mutagen employed. For example, most of the foliage mutations in all the varieties were induced by NMU alone. The fasciata, simple, curly and long leaf and upright mutations were induced by NMU and EMS treatments

Table 2. Spectrum and frequency of induced viable mutations in the M_2 generation in chickpea

Mutagen	Treatment Dose/duration	Mutation (%) Foliage	Growth habit	Seed char.	Total	Population size M_1 family	M_2 plants	Frequency (%) Mut./100 M_1 family	Mut./1000 M_2 plants
				desi variety G 130					
Gamma rays	400 Gy	0.00	0.14	0.09	0.25	50	2020	10	2.5
	500 Gy	0.79	0.00	0.05	0.84	50	2014	34	8.4
	600 Gy	0.10	0.15	0.31	0.56	50	1948	22	5.6
	Overall	0.44	**0.10**	**0.15**	**0.55**	**150**	**5982**	**22**	**5.5**
Neutrons	5 Gy	0.81	0.00	0.00	0.81	50	2466	40	8.1
	10 Gy	0.00	0.05	0.00	0.05	50	2090	2	0.5
	15 Gy	0.06	0.12	0.12	0.30	50	1654	10	3.0
	Overall	**0.29**	**0.06**	**0.04**	**0.39**	**150**	**6210**	**17**	**3.8**
NMU	0.01%(20h)	0.80	0.42	0.14	1.35	50	2150	58	13.5
	0.02%(8h)	0.35	0.42	0.04	0.81	50	2604	42	8.1
	Overall	**0.57**	**0.42**	**0.09**	**1.08**	**100**	**4754**	**50**	**10.8**
EMS	0.1%(20h)	0.30	0.45	0.10	0.85	50	1990	34	8.5
	0.2%(8h)	0.00	0.00	0.00	0.26	50	1940	10	2.6
	Overall	**0.15**	**0.22**	**0.05**	**0.58**	**100**	**3930**	**22**	**5.5**
	Total	**0.32**	**0.17**	**0.08**	**0.61**	**500**	**20876**	**26**	**6.1**
				desi variety H 214					
Gamma rays	400 Gy	0.32	0.21	0.16	0.71	50	1834	26	7.1
	500 Gy	0.39	0.00	0.00	0.39	50	1019	8	3.9
	600 Gy	0.82	0.11	0.23	1.18	50	850	20	11.8
	Overall	**0.51**	**0.11**	**0.13**	**0.76**	**150**	**3703**	**18**	**0.8**
Neutrons	5 Gy	0.07	0.11	0.00	0.19	50	2596	10	1.9
	10 Gy	0.19	0.00	0.52	0.72	50	2082	30	7.2
	15 Gy	0.07	0.03	0.00	0.12	50	2520	6	1.2
	Overall	**0.11**	**0.47**	**0.17**	**0.34**	**150**	**7198**	**15**	**3.4**

NMU	0.01%(20h)	0.10	0.15	0.15	0.42	50	1900	16	4.2
	0.02% (8h)	0.98	0.27	0.00	1.25	50	1836	46	12.5
	Overall	**0.54**	**0.21**	**0.07**	**0.83**	**100**	**3736**	**31**	**8.3**
EMS	0.1% (20h)	4.43	0.00	0.29	4.73	25	338	64	47.3
	0.2% (8h)	5.55	0.32	0.65	6.54	25	306	80	65.4
	Overall	**4.99**	**0.16**	**0.47**	**5.63**	**50**	**644**	**72**	**56.3**
	Total	**1.29**	**0.12**	**0.20**	**1.52**	**450**	**15281**	**31**	**16.2**
				kabuli variety C 104					
Neutrons	5 Gy	0.31	0.00	0.46	0.77	45	652	11.1	7.7
	10 Gy	0.00	0.00	0.38	0.38	45	780	6.6	3.8
	15 Gy	0.00	0.65	0.00	0.65	45	306	8.0	6.5
	Overall	**0.10**	**0.22**	**0.28**	**0.60**	**135**	**1738**	**8.6**	**6.0**
NMU	0.01%(20h)	0.23	0.92	0.46	1.62	45	866	31.1	16.2
	0.02% (8h)	0.97	0.48	0.00	1.46	45	412	13.3	14.6
	Overall	**0.40**	**0.70**	**0.23**	**1.54**	**90**	**1276**	**22.2**	**15.4**
EMS	0.2% (8h)	0.00	6.82	0.00	6.82	20	132	45.0	68.2
	Total	**0.25**	**1.48**	**0.22**	**1.95**	**245**	**3146**	**19.2**	**19.5**
				green seeded variety L 345					
Gamma rays	400 Gy	0.12	0.04	0.08	0.24	50	2490	12.0	2.4
	500 Gy	0.13	0.13	0.13	0.41	50	2218	18.0	4.1
	600 Gy	0.30	0.09	0.05	0.47	50	1926	18.0	4.7
	Overall	**0.18**	**0.09**	**0.09**	**0.37**	**150**	**6634**	**16.0**	**3.7**
Neutrons	5 Gy	0.00	0.00	0.11	0.11	50	1890	4.0	1.1
	10 Gy	0.10	0.20	0.50	0.79	50	2020	32.0	7.9
	15 Gy	0.05	0.00	0.23	0.27	50	2190	12.0	2.7
	Overall	**0.05**	**0.07**	**0.28**	**0.39**	**150**	**6100**	**16.0**	**3.9**
NMU	0.01%(20h)	0.68	0.47	0.08	1.23	50	2354	58.0	12.3
	0.02% (8h)	0.25	1.50	0.37	2.12	50	2406	102.0	21.2
	Overall	**0.46**	**0.98**	**0.22**	**1.67**	**100**	**4760**	**80.0**	**16.7**
EMS	0.2% (8h)	0.45	3.12	0.45	4.02	25	224	36.0	40.2
	Total	**0.23**	**0.62**	**0.22**	**1.07**	**425**	**17718**	**32.4**	**10.7**

alone. On the other hand, the loabbed and stalked leaflet and open flower mutations were induced by gamma-rays only. On the whole, NMU and gamma-rays induced 15 and 8 different types of mutations respectively in *desi* and *kabuli* varieties. Thus NMU induced widest spectrum of mutations among all the four mutagens in all the four varieties. Occurrence of dwarf mutations in all the varieties under most of the treatments showed the presence and similar mutability of this gene in all the varieties thereby confirming the above discussed views. The differential spectrum of viable mutations, as observed in the present experiment, has been reported by several workers [10-14].

In addition to a large number of simple viable mutations, multiple mutations affecting two or more characters were found common in all the mutagenic treatments in the present study. However, their frequency varied with variety and mutagen. For example, the frequency of multimutants was lowest in *kabuli* var. C 104 and very high in *desi* var. G 130 and H 214. Similar differences were also found in treatments with different mutagens. NMU and EMS, in general, were more effective in inducing multiple mutations followed by gamma-rays and neutrons. Occurrence of multiple mutations has also been reported in groundnut by Gregory [21]. This phenomenon is attributed to either mutation of pleiotropic gene, or mutation of gene clusters or due to loss of chromosomal segments.

Data on the spectrum and relative frequency of viable mutations [Tables 1-4] revealed that both varied depending on variety and treatment. Of all the mutagens NMU indicated the widest spectrum of viable mutations (Fig. 2. A-a). EMS gave a much narrower spectrum than NMU. Comparison of varieties indicated that *desi* var. G 130 produced the widest spectrum of mutations. On the other hand, *kabuli* var. C 104 showed the narrowest spectrum. Frequency and spectrum of mutations on mutagen basis (Table 2 & 3) indicated that mutagens show relative specificity in case of induction of various types of mutations. NMU, for example, showed widest spectrum of mutations for leaf-morphology and plant type in all the four varieties. Neutrons showed maximum specificity for seed colour mutants and gamma-rays for plant type.

Mutations affecting leaf morphology, growth habit and plant type (Table 3) appeared more frequently in *desi* varieties under chemical mutagens than with radiations. Out of the total number of viable mutations scored, 45% belonged to leaf morphology, 32.8% to growth habit, 19.3% to seed colour mutations and rest 2.8% to all other types of mutations. Of all the mutations induced by different mutagens 42.7% were produced by NMU alone and the rest by gamma rays, neutrons and EMS in about equal numbers. Frequency of mutations on variety basis indicated

Table 3. Total (pooled) frequency and spectrum of different types of viable mutations induced in M_2 generation in chickpea

Mutagen/ Varieties	Population scored	Mutants scored									
		Foliage type		Growth Habit		Seed charact.		Others		Total	
		No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
		Mutagen basis (mutagens pooled over varieties)									
Gamma rays	16319	46	54.8	14	16.7	16	19.0	8	9.5	84	19.8
Neutrons	21246	32	38.5	13	15.7	35	42.2	3	3.6	83	19.6
NMU	14528	73	40.3	85	47.0	22	12.1	1	0.5	181	42.7
EMS	4930	40	52.6	27	35.5	9	11.8	0	0.0	76	17.9
Overall	**57023**	**191**	**46.5**	**139**	**28.7**	**82**	**21.3**	**12**	**3.4**	**424**	**25.0**
		Variety basis (varieties pooled over treatments)									
G 130 (*desi*)	20876	72	55.0	39	29.8	20	15.3	0	0.0	131	30.9
H 214 (*desi*)	15281	77	65.8	18	15.4	21	17.9	1	0.8	117	27.6
C 104 (*kabuli*)	3148	8	20.6	21	53.8	9	23.1	1	2.6	137	9.2
L 345 (green)	17718	34	24.8	61	44.5	32	23.3	10	7.3	39	32.3
Overall	**57023**	**191**	**47.1**	**139**	**33.0**	**82**	**18.8**	**12**	**1.1**	**424**	**22.6**
Total			**45.0**		**32.8**		**19.3**		**2.8**	**424**	

Table 4. Total mutation rate (chlorophyll + viable) in M_2 generation in chickpea

Mutagen	Treatment Dose/duration	Var. G 130		Var. H 214		Var. C 104		Var. L 345		Total	
		No. M_2 plants	Mutant %	No. M_2 plants	Mutant %	No. M_2 plants	Mutant %	No. M_2 plants	Mutant %	No. M_2 plants	Mutant %
Gamma rays	400 Gy	2020	2.38	1834	2.57	-	-	2490	2.53	6344	2.49
	500 Gy	2014	3.23	1019	1.67	-	-	2218	2.39	5251	2.57
	600 Gy	1948	1.39	850	2.47	-	-	1926	2.55	4724	2.06
	Overall	**5982**	**2.34**	**3703**	**2.24**	-	-	**6634**	**2.49**	**16319**	**2.38**
Neutrons	5 Gy	2466	0.94	2596	0.89	652	1.08	1890	0.80	7604	0.90
	10 Gy	2090	1.34	2082	1.40	780	0.65	2020	1.54	6972	1.34
	15 Gy	1654	1.09	2520	0.96	306	1.64	2190	1.74	6670	1.28
	Overall	**6210**	**1.13**	**7198**	**1.09**	**1738**	**1.13**	**6100**	**1.36**	**21246**	**1.18**
NMU	0.01%(20h)	2150	3.63	1900	3.43	866	2.43	2354	3.91	7270	3.53
	0.02% (8h)	2604	3.77	1836	3.54	412	1.95	2406	5.28	7258	4.11
	Overall	**4754**	**3.70**	**3736**	**3.49**	**1278**	**2.19**	**4760**	**4.60**	**14528**	**3.62**
EMS	0.1% (20h)	1990	1.61	338	6.51	-	-	-	-	2328	2.32
	0.2% (8h)	1940	0.93	306	10.13	132	7.58	224	9.83	2602	3.12
	Overall	**3930**	**1.27**	**644**	**8.32**	**132**	**7.58**	**224**	**9.83**	**4930**	**2.72**
	Total	**20876**	**2.09**	**15281**	**2.26**	**3148**	**1.78**	**17718**	**2.77**	**57023**	**2.32**

- Population not available

almost equal response of the three var. L 345, G 130 and H 214, while *kabuli* var. C 104 showed poorest response.

Total mutation rate : The frequency of total mutations (chlorophyll + viable) in different treatments of *desi* and culinary varieties (Table 4 & Fig. 2-B) indicated that the mutation rate in most of the treatments increased with the increase in the dose of the mutagen.

The highest frequency of all the mutations (Fig. 2. B-a) was recorded (treatments pooled over varieties) in NMU treatment 0.02%(8h) (4.11%) follwed by NMU 0.01%(20h) (3.53%). Thus, both the chemical mutagen tretments showed higher mutation rate than physical mutagens and between physical, gamma rays treatments gave higher rate than neutrons which gave lowest rate among all. Among mutagens, overall average of total mutation rate indicated highest mutation rate in NMU followed by EMS, gamma-rays and neutrons giving lowest rate of mutations. Total mutation rate on variety basis (Fig. 2. B-b) was highest in green seeded var. L 345 followed by *desi* var. H 214. *Kabuli* var. C 104 showed poorest mutation rate among all the four varieties.

The relative low mutation rate at high doses may be due to the high percentage of zygotic lethal mutants due to the poor germination in the M_2 generation. The dose dependent increase in the mutation frequency and a close relationship between the frequencies of chlorophyll and viable mutations as observed in the present studies is in confirmity with the observations in other crops [6-14].

Total mutation rate on mutagen basis (treatments pooled over varieties) indicated positive dose dependency in general and in chemical mutagens in particular. These observations lead to important conclusion that high mutation rates can be obtained with moderate doses and shorter duration of treatments and acute doses and prolonged direct treatments are not suitable for achieving high mutation rates. Similarly the varieties with moderate mutagenic resistance (ie. *desi* var. H 214 and green seeded var. L 345) [Fig. 2. B-b] can be expected to give higher rates of mutations than those highly sensitive (*kabuli* var. C 104) or resistant (*desi* var. G 130).

REFERENCES

1. M. C. Kharkwal, H.K. Jain and B. Sharma. 1988. Induced Mutations for Improvement of Chickpea, Lentil, Pea and Cowpea. *In*: Proc. FAO/IAEA Workshop on "Improvement of Grain Legume Production using Induced Mutations". 1-5, July, 1986. Pullman, U.S.A. IAEA Vienna, 1988. 89-109.
2. M. C. Kharkwal. 1998. Induced mutations for improvement of protein in chickpea (*Cicer arietinum* L.). Indian J. Genet. **58**: 61-68.

3. M. C. Kharkwal. 1998. Induced mutations in chickpea (*Cicer arietinum* L.) I. Comparative mutagenic effectiveness and efficiency of physical and chemical mutagens. Indian J. Genet., **58**: 159-167.

4. M. C. Kharkwal. 1998. Induced mutations in chickpea (*Cicer arietinum* L.) II. Frequency and spectrum of chlorophyll mutations. Indian J. Genet., **58**: 465-474.

5. L. S. Srivatava, H. Chand and S. Kumar. 1973. Dose response study on EMS and MMS treated gram. Sci. and Cult., **39**: 345-347.

6. L. M. Monti. 1968. Mutations in peas induced by diethyl sulphate and X-rays. Mutation Res., **5**: 187-91.

7. S. Blixt, L. Ehrenberg and O. Galin. 1963. Studies of induced mutations in peas. VII. Mutation spectrum and mutation rate of different mutagenic agents. Agri. Hort. Genet., **21**: 178-216.

8. S. Blixt. 1972. Mutation Genetics in *Pisum*. Agri. Hort. Genet., **30**: 1-293.

9. A. Fillipeti, C. F. Marzano, L. M. Monti and G. T. S. Mungozza. 1977. Studies on breeding commercial varieties of peas for industrial use by experimental mutagenesis. 1. Frequency and types of mutations induced with X-rays and diethyl sulphate. Genet. Agrar., **31**: 40-48.

10. S. K. Sharma and B. Sharma. 1981. Induced chlorophyll mutations in lentil. Indian J. Genet., **41**: 328-333.

11. P. Dixit and D. K. Dubey. 1986. Mutagenic efficiency of gamma rays, NMU and their combinations in lentil (*Lens culinaris* Medik) var. T.36. Indian J. Genet., **46**: 501-505.

12. A. Sarkar and B. Sharma. 1989. Frequency and spectrum of chlorophyll mutations in lentil (*Lens culinaris* Medik.). Thai J. Agric. Biol., **22**: 107-111.

13. Vandana, A. Tripathi and D. K. Dubey. 1994. Frequency and spectrum of mutations induced by ethyl methane sulphonate (EMS) and diethyl sulphate (DES) in lentil var. K-85. LENS., **21**: 16-19.

14. Subramanyan, D. 1980. Effect of gamma-irradiation in *Vigna*. Indian J. Genet., **40**: 187-94.

15. A. Gustaffson. 1940. The mutation system of the chlorophyll apparatus. Lund Univ. Arskr. **36**: 1-40.

16. A. Gustafsson. 1947. Mutations in agricultural plants. Hereditas., **33**: 1-100.

17. I. A. Rapoport. 1966. Supermutagens: Peculiarities and Mechanism of Action of Supermutagens. Publ. House, Nauka, Moscow, USSR, pp. 9-23.

18. P. K. Gupta and Yashvir, 1975. Induced mutations in foxtail millet (*Setaria italica* Beauv.). I. Chlorophyll mutations induced by gamma rays, EMS and DES. TAG., **45**: 242-49.

19. C. P. Konzak, R. A. Nilan, J. Wagner and R. J. Foster. 1965. "Efficient chemical mutagenesis", Radiat. Bot. (Suppl.), **5**: 49-70.

20. R. A. Nilan. 1967. "Nature of Induced Mutations in Higher Plants", Induced Mutations and their Utilization, Proc. Symp. Erwin-Bauer-Gedachtnis Vorlesungen IV. Gatersleben, 1966, Akademie-Verlag. Berlin, K1. Mediz., **2**: 5-20.

21. W. C. Gregory. 1968. A radiation breeding experiment with peanuts. Radiation. Bot., **8**: 81-147.

22. G. Varughese and M. S. Swaminathan. 1968. A comparison of the frequency and spectrum of mutations induced by gamma rays and EMS in wheat. Indian J. Genet., 28: 158.165.