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INDUCED MUTATIONS IN CHICKPEA (*CICER ARIETINUM* L.) I. COMPARATIVE MUTAGENIC EFFECTIVENESS AND EFFICIENCY OF PHYSICAL & CHEMICAL MUTAGENS

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ABSTRACT

Mutagenic effectiveness usually means the rate of mutation as related to dose. Mutagenic efficiency refers to the mutation rate in relation to damage. Studies on comparative mutagenic effectiveness and efficiency of two physical (gamma rays and fast neutrons) and two chemical mutagens (NMU and EMS) on two *desi* (G 130 & H 214), one *kabuli* (C 104) and one green seeded (L 345) chickpea (*Cicer arietinum* L.) have been reported. The treatments included three doses each of gamma rays (400, 500 and 600 Gy) and fast neutrons (5, 10 and 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 and 0.2% 8h). Results indicated that chemical mutagens, particularly NMU are not only more effective but also efficient than physical mutagens in inducing mutations in chickpea. Mutagenic effectiveness and efficiency showed differential behaviour depending upon mutagen and varietal type. Chemical mutagens were more efficient than physical in inducing cholorophyll as well as viable and total number of mutations. Among the mutagens NMU was the most potent, while in the physical, gamma rays were more effective. Out of four mutagens, NMU was the most effective and efficient in inducing a high frequency and wide spectrum of chlorophyll mutations in the M_2 followed by fast neutrons. While gamma rays showed least effectiveness, EMS was least efficient mutagens. Major differences in the mutagenic response of the four cultivars were observed. The varieties of *desi* type were more resistant towards mutagenic treatment than *kabuli* and green seeded type.

Key words: Chickpea, mutagenic effectiveness and efficiency, chlorophyll mutations, frequency and spectrum

In order to induce variability and utilize useful mutations for efficient plant breeding, the systematic and comparative study of induced mutagenic effectiveness and efficiency in a variety of crop plants and cultivars is essential. Although extensive studies on induced mutations in cereal crops have been undertaken in the past [1-4], limited reports are available on pulse crops such as chickpea and pigeonpea [5-7].

However, no systematic reports on comparative effectiveness and efficiency of various physical and chemical mutagens are available for chickpea, which is the most important food legume crop in India.

While ionizing radiations still remain the potent and convenient tools inducing genetic variability, a number of chemicals have been found to be equally and even many times more effective and efficient mutagens [1-4, 8-10]. Rapoport [8] classified some of the nitroso compounds (viz., NMU) as supermutagen in view of their superiority over Ethylmethane sulphonate (EMS), a potent alkylating agent. Hence, prior knowledge of mutagenic effectiveness and efficiency of most frequently used chemical mutagens in relation to ionizing radiations in a number of varieties is essential to identify the range of useful mutagens and doses/concentrations for meaningful programme for genetic improvement of a crop through mutation breeding.

Extensive studies on mutagenic effectiveness and efficiency of several chemical mutagens [8-10], gamma rays, EMS, NMU and NMG on *durum* wheat [1-2], NMU, NG and NEU on Sorghum [3] gamma rays, X-rays, NMU and DES on peas [11-15] and lentils [16-17] and have been carried out. However, such information on comparative response of different types of chickpeas viz. *desi, kabuli* and green seeded culinary types to various physical and chemical mutagens is not reported in the literature. The present investigation was undertaken to understand the response of different types of chickpea varieties to more than one type and treatment of mutagens, both physical and chemicals.

MATERIALS AND METHODS

The material used for this comparative study of the effectiveness and efficiency comprised of four varieties of chickpea (*Cicer arietinum* L.), two *desi* (G 130 and H 214), one *kabuli* (C 104) and one green seeded type (L 345). 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) treatments were given. Two concentrations and two durations of the 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, I.A.R.I., New Delhi. Fast neutron treatments were given at B.A.R.C., Trombay, Mumbai. NMU and EMS of Pfaltz and Bauer Inc. USA were used for prepairing aqueous solutions of chemical mutagens at 5.2 pH. Treatments with chemical mutagens were given with intermittent shaking at $20 \pm 2^{o}C$. 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. Chlorophyll and viable mutations were scored in the seed beds in the field. Both mutagenic effectiveness and efficiency were determined using the following formulae [9]:

Mutagenic effectiveness = Mf/tc or Mf/Gy

Mutagenic efficiency = Mf/L or Mf/S where,

Mf = Percentage of families segregating for chlorophyll mutations

t = Period of treatment with chemical mutagen

C = Concentration of chemical mutagen in terms of percentage

Gy = Gray of physical mutagen

L = Percentage of lethality in M_1

S = Percentage of sterility in M_1

The frequencies of chlorophyll mutations were recorded using the following three methods:

a) mutations per cent M_1 plants

b) mutations per cent M_1 families

c) mutations per 1000 M_2 plants

RESULTS AND DISCUSSION

MUTAGENIC EFFECTIVENESS

The data presented in Table 1 indicate that the effectiveness of various mutagens and the response of varieties was varying. In *desi* varieties, 0.02% (8h) NMU was most effective in var. G 130, whereas in var. H 214 0.01% (20h) NMU was most effective. The least effective treatments in *desi* varieties appeared to be gamma rays. Between physical mutagens, neutrons were more effective than gamma rays.

In the *kabuli* and green seeded varieties, mutagenic effectiveness was different in two varieties used. In green seeded var. L 345, 0.02% (8h) NMU was most effective. As compared to NMU, other mutagens showed less effectiveness. EMS proved to be the least effective mutagen in these types also.

Effectiveness of treatments (treatments pooled over varieties) indicated (Fig. 1a) that 0.02% (8h) NMU was most effective and gamma rays showed minimum effectiveness compared to all other treatments. Physical mutagens showed inverse

Table 1. Mutagenic effectiveness and efficiency of physical and chemical mutagens in chickpea varieties

Treatment	% Lethality	% Mutated families	Mutagenic effectiveness	Mutagenic efficiency	% Lethality	% Mutated families	Mutagenic effectiveness	Mutagenic efficiency
	desi var. *G 130*				*desi* var. *H 214*			
Gamma rays								
400 Gy	22.1	32.0	0.80	1.45	16.0	32.0	0.80	2.00
500 Gy	8.1	42.0	0.84	5.19	24.0	18.0	0.36	0.75
600 Gy	19.4	18.0	0.30	0.93	17.4	16.0	0.27	0.92
Average	**16.5**	**30.7**	**0.65**	**2.52**	**19.1**	**22.0**	**0.47**	**1.22**
Neutrons								
5 Gy	5.8	6.0	12.00	1.03	9.2	18.0	36.00	1.96
10 Gy	14.5	20.0	20.00	1.38	29.7	16.0	16.00	0.54
15 Gy	4.0	14.0	9.33	3.50	5.6	22.0	14.67	3.93
Average	**8.1**	**13.3**	**13.77**	**1.97**	**14.8**	**18.7**	**22.22**	**2.14**
NMU								
0.01%(20h)	14.6	50.0	250.00	3.43	8.3	54.0	270.00	6.51
0.02% (8h)	17.2	62.0	387.50	3.61	13.9	38.0	237.50	2.73
Average	**15.9**	**56.0**	**318.75**	**3.52**	**11.1**	**46.0**	**253.75**	**4.62**
EMS								
0.1% (20h)	27.4	16.0	8.00	0.58	47.2	20.0	10.00	0.42
0.2% (8h)	39.4	12.0	7.50	0.31	46.1	36.0	22.50	0.78
Average	**33.4**	**14.0**	**7.75**	**0.44**	**46.6**	**28.0**	**16.25**	**0.60**
	kabuli var. *C 104*				green seeded var. *L 345*			
Gamma rays								
400 Gy	75.0	0.0	0.0	0.0	12.4	38.0	0.95	3.07
500 Gy	90.0	0.0	0.0	0.0	13.5	46.0	0.92	3.41
600 Gy	100.0	0.0	0.0	0.0	42.1	34.0	0.57	0.81
Average	**88.3**	**0.0**	**0.0**	**0.0**	**22.7**	**39.0**	**0.81**	**2.43**
Neutrons								
5 Gy	54.6	4.4	8.88	0.08	12.2	16.0	32.00	1.31
10 Gy	21.6	4.4	4.49	0.21	1.9	16.0	16.00	8.42
15 Gy	24.5	6.7	4.45	0.27	2.5	20.0	13.33	8.00
Average	**33.6**	**5.2**	**5.94**	**0.18**	**5.5**	**17.3**	**20.44**	**5.91**
NMU								
0.01% (20h)	22.6	13.3	66.65	0.59	12.7	60.0	300.00	4.72
0.02% (8h)	13.1	4.4	27.55	0.34	8.5	58.0	362.50	6.82
Average	**17.8**	**8.8**	**47.20**	**0.46**	**10.6**	**59.0**	**331.25**	**5.77**
EMS 0.2% (8h)	66.1	5.0	3.13	0.08	43.1	20.0	12.50	0.46

dose relationship, whereas the chemical mutagens showed positive and direct dose dependence. Overall effectiveness for mutagens (mutagens pooled over varieties) indicated (Fig. 1b) that NMU was most effective mutagen followed by fast neutrons and EMS. These results are in close confirmity with the findings of other workers reported in the literature [1-4, 8, 14-16].

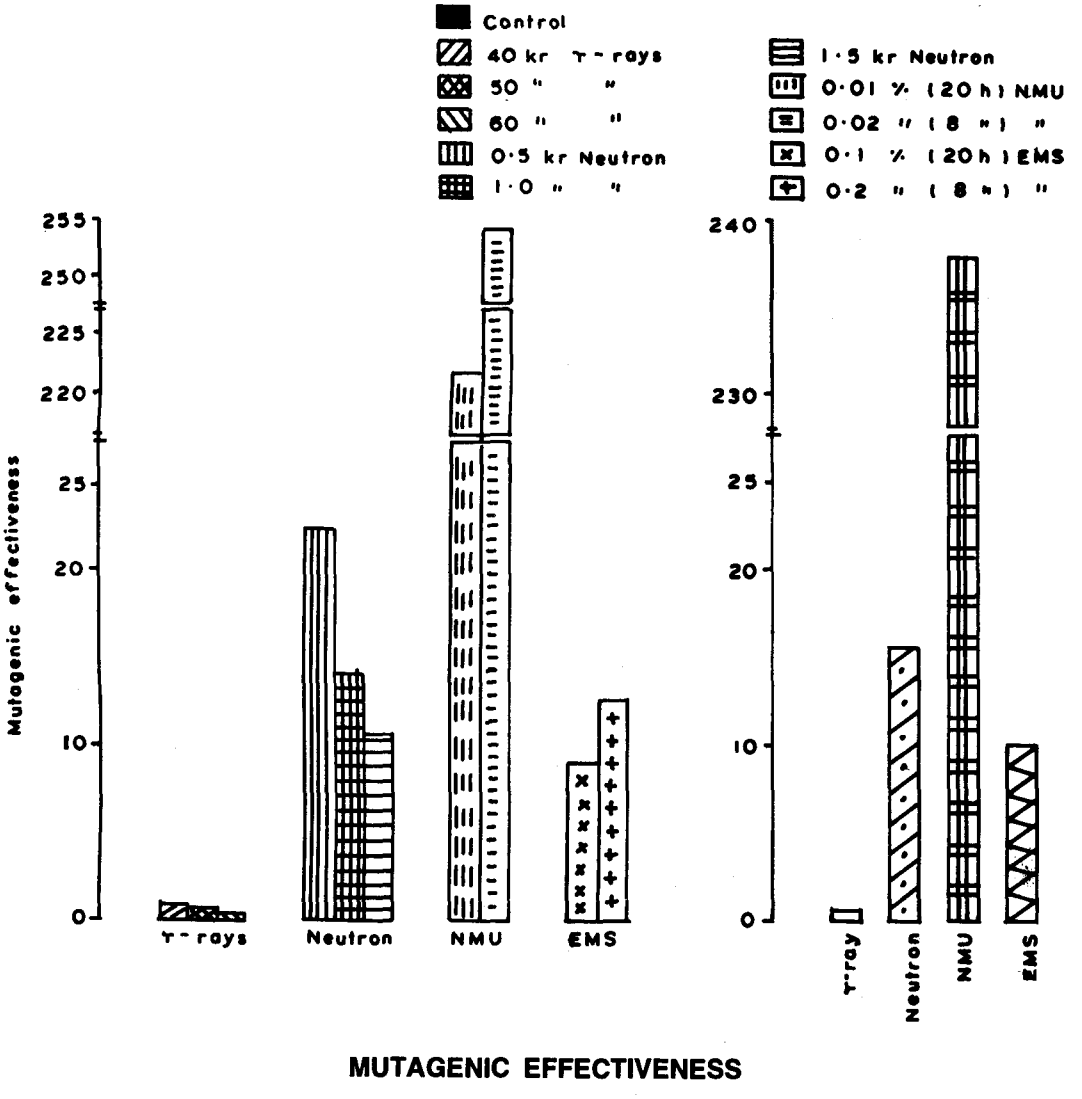

Fig. 1. (a) Treatments pooled over varieties; (b) Mutagens pooled over varieties

The degree of effectiveness of various mutagens and the response of varieties in the present study was observed to be varying. Physical mutagens showed inverse relationship for dose dependency whereas chemical mutagens showed positive dose dependency for effectiveness. Higher effectiveness of NMU over gamma rays and other mutagens has been demonstrated by several workers in *Triticum durum* [1 and 2] and *Sorghum* [3]. The average mutagenic effectiveness (Table 2) was higher at higher dose and shorter duration of NMU [0.02% (8h)] than at the lower dose but longer duration [0.01% (20h)]. Similarly in case of neutrons, EMS and gamma rays treatments, the effectiveness in general, decreased with the increase in dose or concentration. Similarly general decrease in effectiveness with increasing dose of gamma rays, DES and EMS has been reported in Foxtail millet [4].

Table 2. Average mutagenic effectiveness and efficiency of physical and chemical mutagens

Treatment basis†				Mutagen basis‡			
Mutagen	Treatment	Effectiveness	Efficiency	Mutagen	Effectiveness	Mutagen	Efficiency
Gamma rays	400 Gy	0.85	2.17	NMU	237.72	NMU	3.59
	500 Gy	0.70	3.11				
	600 Gy	0.38	0.88				
Neutrons	5 Gy	22.22	1.09	Neutrons	15.59	Neutron	2.59
	10 Gy	14.12	2.63				
	15 Gy	10.44	3.92				
NMU	0.01% (20h)	221.66	3.81	EMS	10.20	Gamma-rays	2.05
	0.02% (8h)	253.79	3.37				
EMS	0.1% (20h)	9.00	0.50	Gamma-rays	0.64	EMS	0.45
	0.2% (8h)	11.40	0.40				

†(Treatment pooled over varieties);
‡(Mutagens pooled over varieties and treatments)

MUTAGENIC EFFICIENCY

The results indicated (Table 1) that the degree of efficiency of various mutagens is varying. In *desi* varieties, 0.01% (20h) NMU showed highest efficiency in var. H 214, whereas in var. G 130 gamma rays 500 Gy was found the most efficient. Between the physical mutagens, neutrons appeared less efficient than gamma rays. The

difference between the efficiency of chemical mutagens, NMU and EMS was very distinct. In *Kabuli* var. C 104, NMU 0.01% (20h) was most efficient. On the other hand, in green seeded var. L 345, fast neutron 10 Gy was most efficient. EMS was found to be least efficient mutagen in all the four chickpea varieties.

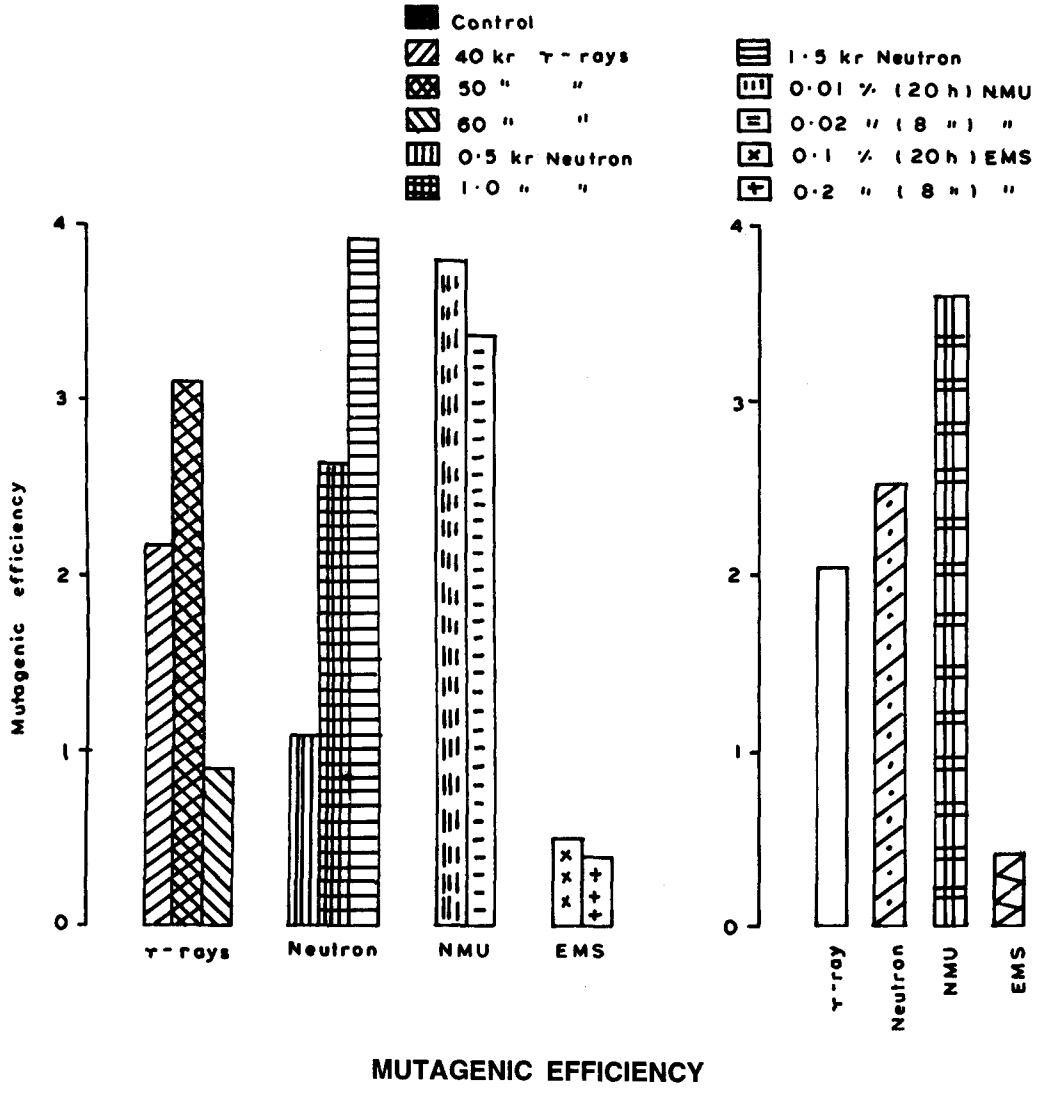

Fig. 2. (a) Treatments pooled over varieties; (b) Mutagens pooled over varieties

Efficiency of mutagenic treatments (treatments pooled over varieties) indicated (Fig 2a) that neutrons 15 Gy was most efficient followed by NMU. Physical mutagens, in general showed positive dose relationship with mutation rate whereas in chemical mutagens it was negative for concentration but positive for duration of treatments. Overall efficiency for mutagens (mutagens pooled over varieties) indicated (Fig 2b) that NMU was most efficient followed by neutrons and gamma rays and EMS was least efficient in inducing mutations in all the four chickpea varieties studied. This is in agreement with various earlier reports on cereals [1-4] and pulse crops [8, 14-16].

The usefulness of a mutagen depends both on its mutagenic effectiveness and efficiency, efficient mutagenesis being the production of maximum desirable changes accompanied by the least possible undesirable changes. Mutagenic effectiveness is a measure of the frequency of mutations induced by unit dose of mutagen. Mutagenic efficiency is indicative of the proportion of mutations as against associated undesirable biological effects such as gross chromosomal aberrations, lethality and sterility, induced by the mutagen in question [9 and 10].

In terms of mutagenic efficiency also NMU was found to be most efficient compared to EMS, gamma rays and neutrons. NMU was found to be more efficient than gamma rays, EMS and NG in case of *Triticum durum* when used at low concentrations [1]. In the present study also mutagenic efficiency was higher at lower doses of NMU and EMS. The higher efficiency of lower concentration of a mutagenic agent is due to the fact that the biological damage (seedling injury, lethality and sterility) increases with the increase in dose at a faster rate than the mutations (Konzak *et al* 1965). The efficiency of physical mutagens showed positive relationship for dose dependency, whereas in case of the chemical mutagens it was negative. Decrease in efficiency of gamma rays, EMS and DES with the increase in doses has also been observed in Foxtail millet [4].

Although both the mutagenic effectiveness and efficiency generally decreased with the increasing dose/concentration of mutagens with few exceptions, nevertheless, the highest total mutation rates were in general obtained with higher doses/ concentrations both in case of physical as well as chemical mutagens. It would thus be seen that higher mutagenic effectiveness and efficiency does not reflect the *per se* mutation frequency and they cannot be used as an index for maximization of mutation rates. In effect, these factors, modify the effectiveness (mutations per unit dose) and efficiency (rate of mutations to damage or unwanted effects such as chromosomal aberrations) of mutagens in cells of higher plants.

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