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Research Article

Spectrum and Frequency of Induced Chlorophyll Mutation in Chickpea (*Cicer arietinum* L.) in M_2 Generation.

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Abstract The chlorophyll mutation frequency in M_2 generation is the most effective and dependable index for evaluating the genetic effects of mutagenic treatments. In general, all mutagenic treatments induced fairly high frequency of chlorophyll mutations. It is also obvious from these results that chlorophyll mutation rate increased with increasing dose of radiation (gamma-rays) exception in few cases, whereas medium dose of chemicals was found to be the most efficient for inducing chlorophyll mutations. The present investigation is found to have obtained higher frequency of chlorophyll mutation with medium or low doses mutagens. It seems that the strong mutagens reach their saturation point even at lower doses in the highly mutable genotypes, and further increase in dose does not add to mutation frequency, with increase in dose beyond a point, the strong mutagens become more toxic than the higher dose of relatively weaker mutagens. It was assumed that specific chemical reactions would take place between a mutagen and a gene mutations in a definite manner if the right chemical applied. Several morphological characters through different mutagens *i.e.* xantha > albino > viridis, are founded respectively.

Key words: Chickpea, chlorophyll, frequency and spectrum

Introduction

Mutation has been a dominant tool to produce new additional heritable variation which may be utilized in traditional recombination breeding programme. Mutation breeding programme needs to be sufficiently standardized for improvement of polygenic traits to gain general confidence and wide acceptance of its usefulness among the breeders so that experiments could be planned with reasonable expectations of success. The induced mutation in fundamental studies, induced mutagenesis can be used to create additional variability for quantitative traits. The success of a mutation breeding programme depends not only on the quality of induced mutation, but also on the screening techniques to identify these mutations, which occur with a very low frequency among a large number of others of little breeding value. An attempt has been made here to develop standard screening techniques for micro mutations affecting the polygenic system. In general, selection for quantitative traits, such as yield, should be preferably carried out in early generations because most of the desired combinations of favorable alleles are likely to be lost in advanced generations due to intensive or even no selection for other traits. It became a common practice to advance only normal looking M_3 plants to M_2 generation and apply the first cycle of selection not earlier than M_3 This results in increased volume of non-mutated material and delay in isolation of promising variants. This was most probably because the material already selected once in M_2 was confirmed with higher probability in subsequent generations. Even if the material selected in M_2 generations has higher probability of getting fixed as promising strains, there is no evidence to suggest that the frequency of promising mutations per se over the entire population is higher in M_2 than M_3. Therefore, an attempt has been made to explore the possibility of selecting for polygenic variability in an early generations (M_2) following treatments with three mutagens.

Materials and Methods

The experimental materials for this study consisted of 3 varieties of chickpea having good agronomic base and belonging to diverse group viz.,V_1 Desi (BGM-524),V_2 Kabuli (BG-1053) and V_3 green seeded (KSB-220) were treated with different doses of gamma rays Ethyl Methane Sulphonate (EMS) and their combination (EMS + Gamma rays, details are presented in below

Mutagens

The physical chemical and combined mutagens were employed to induce mutations. The brief description about the source mode of action and method of application is given below. Details of mutagens and their doses for each three varieties used in present investigation detailed showed in table-1

Physical Mutagen

The gamma rays obtained from a 2000 curie CO60 gamma cell installed in the Division of NRL, IARI New Delhi with a dose rate of 2500 radius per minutes was used as physical mutagen. Gamma rays are electromagnetic type of radiations induce mutations through ionization when a biological material is irradiated a gamma rays photon hits an orbital electron of the atom. The electron gets excited and in turn ejected tremendous energy and is capable of causing further ionization along its path. Well dried uniform seeds of each variety size about 10 per cent moisture content were filled in a seed envelope and exposed to gamma rays irradiations in the gamma cell. Different doses of gamma rays were used for irradiation of seeds i.e.10, 20, 30, 40, 50 and 60 kR accordingly. Ethyl methane sulphonate (EMS) a potent alkylating agent obtained from Eastman Kodak Chemicals USA was used as chemical mutagen. It is a most widely used chemical mutagen and is also a powerful carcinogen. It induces through alkylation of DNA.

Well dried selected seeds of uniform size were presoaked in water for 6 hours and then treated with freshly prepared aqueous solutions of EMS at 25+ 20 C. To have maximum absorption of solution, the seeds contained in the conical flask were repeatedly subjected to shaking. Treatments with five different concentrations i.e. 0.10, 0.15, 0.20, 0.25 and 0.30 percent for 4 to 6 hr each were given with intermittent shaking. As soon as treatment is over, seeds were washed in running tap water for 5-10 minutes to remove traces of chemical from the seed surface. Thereafter by spreading the seeds on blotting paper excessive water was removed. The seeds were sown in the field immediately after treatment whereas seeds soaked in tops water for 6 hours were used as control. Seeds irradiated at different doses viz. 20, 30, 40, 50, and 60kR were treated with 0.10, 0.15, 0.20, 0.25 and 0.30% EMS aqueous solution of ethyl methane sulphonate for 6 hrs by the method described above.

The investigation was carried out from Experimental Farm at Monad University and Janta Vaidic College, Baraut, Baghpat on M_1 and M_2 generations. Both generations were grown in well prepared land and data taken very precautionary. The experimental material in M_1 and, M_2 generations was handled as described below:

M_1 Generation

The M_1 generation was raised with control and treated seeds at distance of 45 cm between rows and 25 cm between seeds with in row. The recommended agronomic, cultivar and plant protection practices were followed to raise a good crop. The characters were recorded M_1 generation in during the entire crop season.

M_2 Generation

The individual M_1 plant progenies were sown in the field in separate M_2 progeny and data were recorded following characters.

Chlorophyll Mutation

All the treated as well as control progenies were screened for the frequency and spectrum of chlorophyll mutations from emergence till the age of 4 weeks after so wing. Mutation frequency was estimated on population basis as well as percentage of segregating M_1 plant progenies in each treatment. The spectrum by chlorophyll mutation was studied and the mutants were classified as per the scheme of Lamprecht (Blixt, 1972) with modification.

Using the data obtained on chlorophyll mutations both mutagenic effectiveness is a measure of the frequency of mutation induced by a unit dose on mutagen, while mutagenic efficiency gives an idea of the proportion of the mutations in relation to the total biological damage measured through lethality and sterility etc. The mutagenic effectiveness and efficiency were determined as per the formulae suggested by Konzak et al. (1965) as follows.

$$\text{Mutagenic effectiveness} = \frac{\text{Mutation rate (\% } M_2 \text{ seedlings)}}{\text{(Gamma rays) Dose in Gy}}$$

$$\text{Mutagenic effectiveness (Chemical treatment)} = \frac{\text{Mutation rate (\% } M_2 \text{ seedlings)}}{\text{Concentration} \quad \text{time}}$$

$$\text{Mutagenic efficiency} = \frac{\text{Mutation rate (\% } M_2 \text{ seedlings)}}{\text{\% lethality or sterility}}$$

Throughout the entire growth period all M_2 progenies were examined several times to detect viable mutations affecting various morphological attributes. The frequency of morphological mutations was also calculated as earlier for the chlorophyll mutations.

Mutation Frequency

The frequencies of chlorophyll and morphological mutations were estimated as follows:

M_2 family basis (percentage of segregating M_2 progenies)

$$\text{Mutation frequency (\%)} \quad \frac{\text{No. of mutated progenies}}{\text{Total } M_2 \text{ progenies}} \quad 100$$

M_2 Population basis (percentage of mutated M_2 plants or mutants

$$\text{Mutation frequency (\%)} \quad \frac{\text{No. of Mutants}}{\text{Total } M_2 \text{ progenies}} \quad 100$$

Result and Discussion

The frequency of chlorophyll mutation recorded on the basis of M_1 plant (M_2 progenies) with some suitable modifications are showed following. The chlorophyll mutations were indentified in M_2 generation in Table 2 given below.

1. Spectrum and frequency of chlorophyll and viable mutations
2. Estimates of the totals mutations rate

Albina : The colour of young seedlings was white on germination and they did not survival after a week from germination. No chlorophyll or carotenoids was found absent.

Xantha : The colour of young seedling was yellow, high pale yellow or orange but they did not survive beyond two week. The carotenoids were present but chlorophyll was absent.

Chlorina: The colour seedling were greenish yellow/yellowish green and started dying within 10-15 days after germination, few survived a little longer without seed setting.

Viridis: Light green, fully viable and more or less normally productive.

Chlorophyll mutation: The variety BGM-524 of chickpea was noticeably different in chlorophyll mutation under different treatments showed in table 4.2. Chlorophyll mutation based on different seedlings were classified on the lines suggested by Gustafson (1940). Mutation rates under various treatments has been represented the under following heads:

Chlorophyll Mutation Frequency

Table 2 shows that highest (1.40%) frequency of chlorophyll mutation was observed under (0.30%) EMS followed by (1.20%) under 20Kr+0.10% EMS treatments while the lowest (0.20%) being under 0.15% EMS.

Frequency of Viable Mutation

The spectrum and frequency of induced chlorophyll viable mutation are presented in Table 3. The maximum leaf morphological mutation were shown in 0.15% EMS (2.00%) and lowest (0.20%) in 50 kR treatments of gamma rays. The maximum (0.80%) plant type mutation were shown in 30kR+0.15% EMS treatments and the lowest (0.20%) under 30kR dose of gamma-rays and 0.20% EMS. The maximum (0.80%) frequency was observed under 20 kR+0.10% EMS and the lowest (0.20%) under 30kR, 60kR of gamma rays and 0.10% and 0.20% EMS dose for pod type mutation. The highest 0.60%frequency and percentage of seed type mutation was observed under 0.15% EMS and 50kR +0.25%, while the lowest 0.20%was observed under 20 kR dose of gamma rays, 0.20%EMS,20kR+0.10%EMS and 40kR+0.20%EMS.

Total Mutation Rate

The total (chlorophyll + viable) mutation under various treatments are presented in table 2. The mutation rate most of the treatments are deviated between (1.4-3.0%) except for the treatments 40kR (1.20%) having 4 plants. The total mutation rate in treatments of gamma rays was observed less comparison in comparison to the treatments of EMS with different concentration on the basis of Table 2. It is clear from the combined treatments of gamma rays with ethyl methane sulphonate had more mutation rate in BGM-524 genotypes.

Table 1: Details of mutagens and their doses for each three varieties

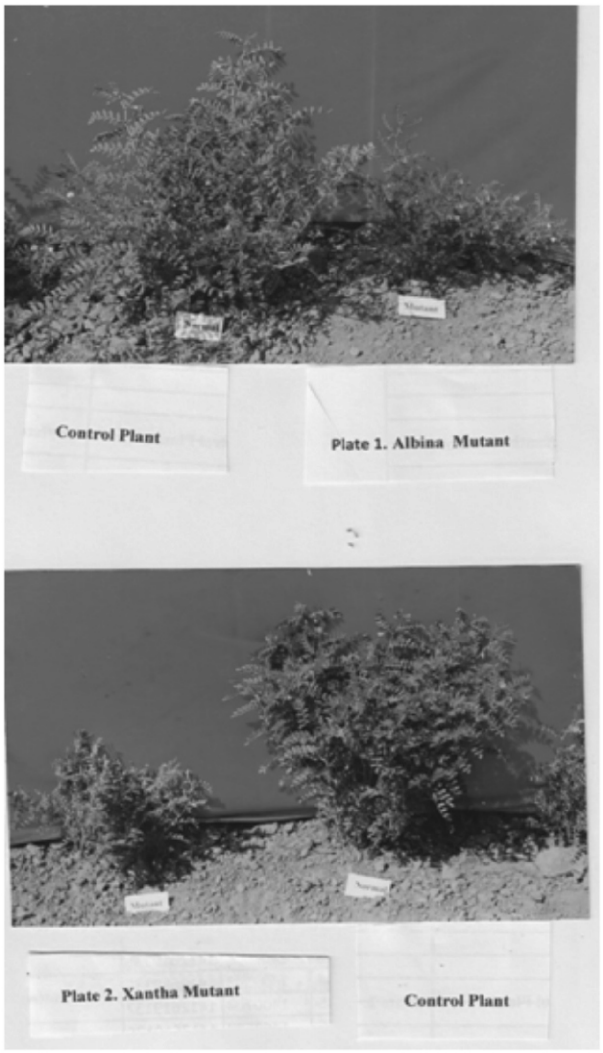
Mutagen	Dose	No. of seeds treated	Treatment condition
Control	0.00	100×3	Dry
Gamma rays	20kR	100×3	Dry
	30kR	100×3	Dry
	40kR	100×3	Dry
	50kR	100×3	Dry
	60kR	100×3	Dry
EMS	010%	100×3	Soaking (400ml)
	015%	100×3	
	020%	100×3	
	025%	100×3	
	030%	100×3	
Combined treatment	20kR+0.10	100×3	Dry + Soaking (400ml)
	30kR+0.15	100×3	
	40kR+0.15	100×3	
	50kR+0.15	100×3	
	60kR+0.15	100×3	

Total treatment = 16×3= 48

Total experimental seeds = 48×100=4800

Table 2: Spectrum and frequency of induced chlorophyII mutation for BGM-524 in M_2 generation

Treatments Total mutant in M_2	Types of mutations										
		Chlorina		Xantha		Albina		Viridis		Plant (overall)	
	No. of M_2	No.	%	No.	%	No.	%	No.	%	No.	%
Control	500	-	-	-	-	-	-	-	-	-	-
20 kR	500	-	-	-	-	2	0.40	-	-	2	0.40
30 kR	500	1	0.20	1	0.20	-	-	2	0.40	4	0.80
40 kR	500	2	0.40	-	0.40	1	0.20	-	-	3	0.60
50 kR	500	1	0.20	2	0.20	-	-	2	0.40	5	0.10
60 kR	500	1	0.20	1	-	2	0.40	-	-	4	0.80
0.10% EMS	500	1	0.20	-	-	3	0.60	2	0.40	6	0.12
0.15% EMS	500	-	-	-	-	1	0.20	-	-	1	0.20
0.20% EMS	500	2	0.40	-	-	-	-	1	0.20	3	0.60
0.25% EMS	500	1	0.20	-	0.40	1	0.20	2	0.40	4	0.80
0.30% EMS	500	-	-	2	0.80	1	0.20	4	0.80	7	1.40
20 kR+0.10% EMS	500	1	0.20	4	0.40	1	0.20	-	-	6	1.20
30 kR+0.15% EMS	500	-	-	2	0.20	-	-	1	0.20	3	0.60
40 kR+0.20% EMS	500	1	0.20	1	-	-	-	-	-	2	0.40
50 kR+0.25% EMS	500	2	0.40	-	-	2	0.40	-	-	4	0.80
60 kR+0.30% EMS	500	1	0.20	-	-	1	0.20	2	0.40	5	1.00
Total	500	14		13		15		17		59	-



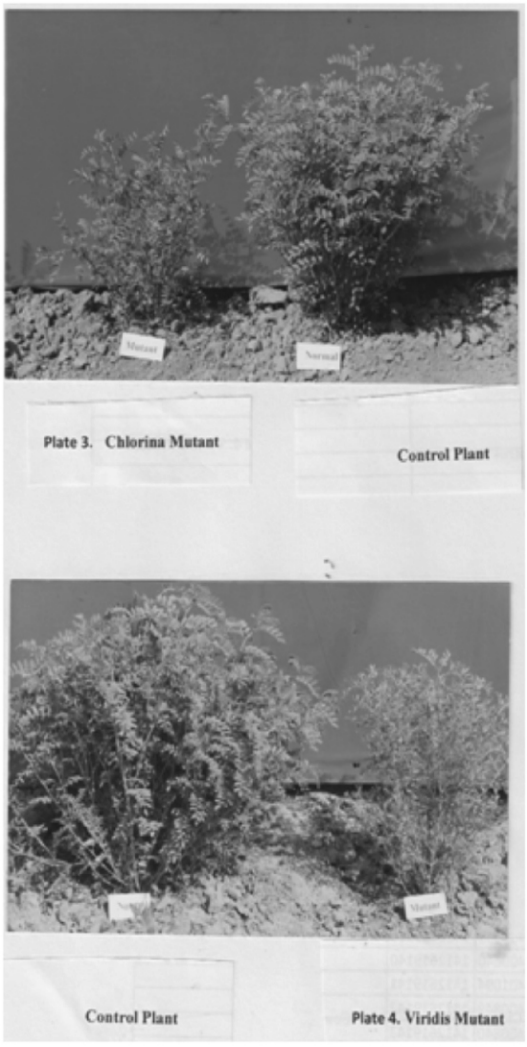


Table 3. Range population mean, CV, heritability and genetic advance for days to 50% flowering in M_2 generation for V_1V_2 and V_3

Statistical Parameter		Control	Gamma rays (kR)					Ethyl Methance Sulphonate (EMS,%)					Combination (Gama rays kR+EMS,%)				
			20	30	40	50	60	1.10	0.15	0.20	0.25	0.30	20+0.10	30+0.15	40+0.20	50+0.25	60+0.30
Range																	
	V_1	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
		19.0	21.3	29.0	27.5	56.6	27.7	27.9	24.4	27.7	19.1	27.3	27.7	27.0	21.7	30.0	21.3
	V_2	16.0	11.0	11.0	11.0	6.3	11.1	11.0	11.2	11.1	7.4	17.3	11.1	11.3	8.7	6.8	11.3
		26.0	31.0	37.0	39.5	27.2	40.3	31.0	29.3	27.7	33.3	32.4	33.1	19.3	19.3	29.3	29.9
	V_3	11.0	7.0	7.4	11.0	3.4	7.0	9.3	6.3	8.9	6.4	1.0	1.5	7.5	11.4	4.4	7.1
		19.7	21.3	27.0	22.8	29.3	17.5	36.3	19.5	27.9	27.7	10.3	27.7	27.0	30.0	22.4	21.2
Mean																	
	V_1	13.39	13.16	14.66	14.48	14.13	18.34	15.80	17.63	17.57	9.87	14.30	15.92	15.70	13.64	18.42	15.81
	V_2	21.00	18.38	23.23	25.82	14.33	25.30	23.03	21.50	19.31	17.46	24.57	19.93	24.06	13.84	18.66	22.71
	V_3	14.20	13.26	14.11	14.47	13.57	12.50	18.21	19.91	18.60	16.39	5.55	14.31	14.88	17.44	14.15	14.05

CV																	
	V_1	17.64	21.73	30.01	40.74	56.05	29.60	32.59	28.07	41.52	53.59	49.03	43.34	28.47	21.48	31.48	22.13
	V_2	24.14	29.43	26.90	24.40	36.15	24.74	25.23	23.67	27.19	30.18	14.57	27.25	21.03	16.44	36.55	23.21
	V_3	19.15	23.22	25.42	19.58	45.48	20.80	48.88	24.17	29.73	30.50	45.05	46.12	28.96	30.10	30.17	22.92
Heritability																	
	V_1	17.64	21.73	30.01	40.74	56.05	29.60	32.59	28.07	41.52	53.59	49.03	43.34	28.47	21.48	31.48	22.13
	V_2	24.14	29.43	26.90	24.40	36.15	24.74	25.23	23.67	27.19	30.18	14.57	27.25	21.03	16.44	36.55	23.21
	V_3	19.15	23.22	25.42	19.58	45.48	20.80	48.88	24.17	29.73	30.50	45.05	46.12	28.96	30.10	30.17	22.92
Genetic advance																	
	V_1	17.64	21.73	30.01	40.74	56.05	29.60	32.59	28.07	41.52	53.59	49.03	43.34	28.47	21.48	31.48	22.13
	V_2	24.14	29.43	26.90	24.40	36.15	24.74	25.23	23.67	27.19	30.18	14.57	27.25	21.03	16.44	36.55	23.21
	V_3	19.15	23.22	25.42	19.58	45.48	20.80	48.88	24.17	29.73	30.50	45.05	46.12	28.96	30.10	30.17	22.92
V_1= BGM-524 (desi) V_2=BG-1053 kabuli) V_3=KSB-220(green)																	

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