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Mutagenic Effects on Chlorophyll Mutation Frequencies and Spectrum in Rice

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Seeds of the rice variety Co 37 were subjected to gamma rays and EMS alone and in combination with two treatments. The effects of frequency and spectrum were studied. The frequency of mutations estimated as number of mutants per 100 M_1 spikes was as efficient as the number of mutants per 100 M_2 plants. Gamma rays proved more potent in inducing chlorophyll mutations than EMS. The combination of treatments induced a higher frequency of chlorophyll mutations than individual treatments showing antagonistic effect on M_1 spike and additive effect on M_2 seedling basis. Regarding the spectrum of chlorophyll mutations gamma rays induced *Albina*, *Xantha* and *Striata* types, whereas EMS and combination treatments produced *Virescent* in addition to three types obtained through gamma rays. *Albina* and *Xantha* occurred more frequently than other types in all the treatments but the combination treatments produced more *Albina* than individual treatments.

The enhancement of mutation frequency and the alteration of the mutation spectrum in a predictable manner are the two important goals of mutation research. In the past, varied approaches have been tried by many workers to achieve these goals. The mutagenic efficency of combination treatments involving different mutagens in rice, has been indicated earlier in a few reports. Keeping this in view, a study on inducing chlorophyll mutations with treatments of gamma rays and EMS and results obtained are presented below.

MATERIAL AND METHODS

The material comprised selfed seeds of rice (*Oryza sativa* L.) variety Co 37 Husked seeds with a moisture content of 11% were irradiated with 10, 20, 30 40 and 50 krad of gamma rays with a Co^{60} source at a dose rate of 2500 rads/min.

Seeds pre-soaked in distilled water for 24 hours were treated with aqueous solutions of EMS (10mM to 50mM) for six hours at room temperature 23± 1°C with intermittent shaking. Immediately after the completion of treatment the treated seeds were thoroughly washed in running tap water for half an hour.

For combination treatments, the dry seeds were exposed to 40 krad of gamma rays and then presoaked for 24 hours in distilled water. The presoaked seeds were kept immersed for 6 hours in EMS simultaneously with the individual EMS treatments under similar conditions and were sown as such.

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Twenty two day old seedlings were transplanted in the main field at a spacing of 20x10 cm. In the M_1 generation the first formed three panicles at flowering were selfed by bagging. At maturity, the first three panicles were harvested, dried and stored separately in paper bags. The seeds of each panicle of individual plants were sown in separate beds for M_2 generation. All the seeds were sown simultaneously under field conditions thus providing almost uniform conditions to the whole nursery.

The primary panicle of each of the surviving M_1 plants was used for working out the chlorophyll mutation frequency in M_2. The ear progenies segregating for chlorophyll mutants were scored first to calculate mutation frequency per 100 M_1 plants and per 100 M_1 spikes. The total number of mutants and normal seedlings were also counted to estimate mutation frequency per 100 M_2 plants. The chlorophyll mutants were classified according to the system suggested by Gustafsson (1940). The data on uumber of M_1 spikes and M_2 seedlings tested are furnished in the Table 1. The overall statistical significance of the deviation between the observed and expected values in each series of combination treatments was tested by Chi-square test. The interaction effects of combined treatments on M_1 spikes and M_2 seedling basis were calculated according to the farmula.

$$K = \frac{(a+b)}{(a)+(b)}$$ (Sharma, 1970), where,

K = interaction coefficient
(a) and (b) = mutation frequencies of individual mutagens
(a+b) = mutation frequency of combination treatment

If k=1 additive; K>1, Synergistic; K<1, antagonistic.

RESULTS AND DISCUSSION

a Chlorophyll mutations frequency:

The frequency of chlorophyll mutations on M_1 plant basis was higher than those on M_1 spike and M_2 seedling bases for both individual and combination treatments (Table 2). Both in single and combination treatments, the chlorophyll mutation frequencies increased with increasing doses in all the three bases except 50 krad of gamma rays and 40 krad+ 50mM of combination treatment. Higher exposures of 40 krad +50 mM gave lower mutagenic efficiency on all the three bases, since these treatments showed greater injury. This is in agreement with observations of Khalatkar and Bhatia (1975) in barley. In M_1 plant, M_1 spike and M_2 seedlings, the maximum frequency was obtained at the dose levels of 40 krad, 50mM and combination treatment of 40 krad+ 40 mM. With the increase in doses of EMS, mutation frequencies on all the three bases increased, but there was no such relationship between the frequency of mutations and doses of gamma rays. Of the mutagens used in the study, gamma rays produced higher frequencies of chlorophyll mutation than EMS.

The combination treatments produced higher frequency of chlorophyll mutations than individual treatments of gamma rays and EMS. This is in conformity with the results of Chakrabarti (1975) in rice. The chlorophyll mutation frequency for combination treatments ranged from 24.5 to 32.5, 22.0 to 27.3 and 3.0 to 4.0 on M_1 plant, M_1 spike and M_2 seedling bases respectively. The chi-square value showed that the deviations from the expected values were in the negative direction. These deviations were statistically significant ($X^2=18.78$, $0.01 > p > 0.001$) regarding chlorophyll mutation frequency on M_1 spike basis (table 3). This showed that the mutagens combined each others action antagonistically. This fact was also confirmed by interaction coefficients which ranged from 0.61 to 0.74.

In M_2 seedling basis, the 'p' value showed the deviation to be not significant (X^2 - 0.29, $p > 0.99$). This implies that the mutagens combined additively in their effect (table 3). This fact was also borne out by interaction coefficients in most of the combination treatments except 40 krad +40 mM where synergistic effect was indicated. Most of the combination teatments showed additive effect on chlorophyll mutation frequency on M_2 seedling basis. The overall consideration of the effects of combination treatments showed that the two mutagens were combined to act additively in their effect. The additive effect of two mutagens in combination treatment may be due to the independent action of the two mutagens in inducing mutations, probably by different mechanisms.

b. Spectrum of chlorophyll mutants.

The spectrum of mutants comprising *albina*, *xantha*, *striata* and *virescent* is prsented in table 2. Among these, *albina* and *xantha* were more frequent than others. *Striata* was very rare. All these four types were observed in EMS and combination treatments, whereas gamma rays gave only three types viz., *albina*, *xantha* and *striata*. The relative percentage of *albina* mutants increased with increasing doses of gamma rays, EMS alone and their combination treatments. But there was consistent relationship observed between other types of chlorophyll mutants and the dose of mutagens employed.

Both in single and combination treátments, *albina* was the most frequent type than the other kinds of mutants, followed by *xantha*. The relative percentage of *albina* mutants was higher in combination treatments than in single treatments whereas the relative percentage of *xantha* mutants was higher in individual treatments than in combination treatments. Such differences in the spectrum of chlorophyll mutants were reported by Kawai (1966) and Gopinathan Nair (1971) in rice.

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Table 1 Number of M_1 Spikes and M_2 Seedlings tested.

Mutagen (Dose/conc).	Number of M_1 spikes tested	Number of M_2 seedlings tested
Gamma rays (krad)		
Control	876	45552
10	831	43212
20	678	35256
30	642	33384
40	442	25984
50	210	10920
EMS (mM)		
Control	870	45240
10	821	42692
20	803	41756
30	788	40976
40	778	40456
50	641	33432
Gamma rays+EMS		
Control	876	45452
40+10	432	22464
40+20	416	21632
40+30	371	19292
40+40	362	18824
40+50	272	14144

Table 2. Freqnency and spectrum of chlorophyll mntations in the M_2 generation.

Mutagen (Dose/conc)	Mutation frequency per 100			Total number of mutants	Spectrum (relative percentage) of chlorophyll mutants			
	M_1 plants	M_1 spikes	M_2 seedlings		Albina	Xantha	Striata	Viresant
Gamma rays (krad)								
Control	—	—	—					
10	22.3	18.8	1.1	483	51.0	38.7	10.3	—
20	22.9	18.9	1 5	512	53.1	46.9	—	—
30	25.1	19 3	1 7	568	55.4	37.5	7.1	—
40	25.3	19.3	2.0	520	69.4	27.6	3.1	—
50	25.0	18.6	1.5	212	80 3	19.7	—	—
EMS (mM)								
Control	—	—	—					
10	18.4	16 0	0.9	419	46.7	22.2	2 2	28.9
20	18.9	16.1	1.0	435	52.2	19.6	—	28.3
30	19.6	16.2	1.1	452	41.9	32.6	7.0	18.6
40	21.4	17.1	1.2	492	60.8	27.5	—	11.8
50	22.2	18.6	1.5	516	72.3	13.8	2.1	11.7
Gamma rays + EMS								
Control	—	—	—					
40+10	24.5	22.0	3.0	682	70.5	15.4	2.3	11.7
40+20	27.0	23.3	3.1	692	73.4	11.1	—	15.5
40+30	28.5	25.6	3.5	683	71.5	23.8	0.9	3.7
40+40	32.5	27.3	4 0	754	80.5	11.5	—	8.0
40+50	32.3	27.2	3.9	552	82.8	16.4	0.9	—

Table 3 Effect of combined Treatment on Chlorophyll Mutation Frequency in the M_2 Generation

	No. of mutants per 100 M_1 spikes				
	O	E	O—E	$\frac{(O-E^2)}{E}$	K
Gamma rays+EMS (mM)					
Control					
40+10	21.99	35.87	—13.88	5.37	0.61
40+20	23.22	35.97	—12.75	4.52	0.64
40+30	25.61	36.15	—10.54	3.07	0.71
40+40	27.34	37.00	—9.66	2.52	0.74
40+50	27.20	38.47	—11.27	3.30	0.71

X^2 Value for diviation 18.73 0.01 P> 0.001

	No. of mutants per 100 M_2 seedings				
	O	E	O—E	$\frac{(O-E^2)}{E}$	K
Gamma rays+EMS (mM)					
Control					
40+10	3.04	2.99	0.05	—	1.02
40+20	3.19	3.05	0.74	0.01	1.05
40+30	3.54	3.11	0.43	0.06	1.13
40+40	4.01	3.23	0.78	0.19	1.24
40+50	3.90	3.55	0.36	0.03	0.29

X^2 Value for diviation 0.29 P> 0.99