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Studies on Induced Chlorophyll Mutations in *Cajanus cajan* (L.) Mill. Sp.*

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In the study of induced chlorophll mutations with two varieties of redgram (*Cajanus cajan*), the frequencies increased with increasing doses of mutagens up to middle doses and thereafter decreased. Multiple chlorophyll mutants comprising two types were more frequent than single and three types. The pooled segregation ratios did not show any dose dependency. None among the segregating progenies showed the theoretical expected proportions of 25 per cent of recessive mutants. The spectrum of chloropyll mutants consisted of *albina*, *xantha chlorina* and *viridis*. *Chlorina* and *viridis* were the predominant types in gamma rays DES treatments respectively.

The use of induced mutations has now come of age as one of the methods of plant breeding. In *Cajanus cajan* attempts on artificial induction of mutations have been made only recently. An attempt has been made in this paper to study the induced chlorophyll mutations in this important grain legume.

MATERIAL AND METHODS

Two redgram varieties SA.1 and Co.2 formed the material for the study. Gamma irradiation was carried out using a 60 CO Gamma Cell with the doses of 1, 5, 10, 15, 20, 25, 30 and 40 krad. Treatments with chemical mutagen (Diethyl sulphate) were performed by keeping the pre-water soaked seeds immersed for 6 hours in the concentrations of 3, 6, 9, 12 and 15 mM of DES with intermittent shaking. The DES solutions were changed for every half an hour with a freshly prepared one. Treatments were at room temperature, 23 ± 1°C.

After mutagenic treatments, M_1 generation was raised. The M_2 generation was raised as individual M_1 plant and M_1 branch progeny bases. The M_2 seedlings were observed from the day of germination up to 15th day to spot out chlorophyll mutants which were classified according to the system proposed by Gustafsson (1940) and Blixt (1961). The frequency of mutation per 100 M_1 plant and 100 M_1 branch were calculated from the data. The total number of M_2 mutants and normal seedlings were counted from all the families (both segregating and non-segregating) to calculate the frequency of mutation per 100 M_2 seedlings. The different types of seedling muta-

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tions for chlorophyll deficiency were scored separately for calculation of the spectrum of mutants.

RESULTS AND DISCUSSION

a) *Mutation frequency:* The chlorophyll mutation frequencies estimated as number of mutations per 100 M_2 plants, 100 M_1 branches and 100 M_2 seedlings are presented in Table 1. The trend in mutation frequency on M_1 plant and M_1 branch bases was more or less similar and they did not show any relationship with increase in doses of both mutagens in both varieties. The frequencies of mutations are usually estimated on M_1 plant M_1 spike and M_2 plant bases. Among the three, the third method proposed by Gaul (1960) is preferred as it is proportional to the initial mutation rate and is rather independent of variation in progeny and size of mutated sector. Mohan Rao (1972) also expressed the same view from his study with barley. In the present investigation, in SA.1, the frequency of mutation on M_2 seedling basis increased upto 15 krad of gamma rays and upto 9 mM of DES beyond which there was a decrease. In Co.2, the frequency on M_2 seedling basis increased upto 10 krad of gamma rays and 6 mM of DES after which, there was a decrease. Similar results were reported by Ramasamy (1973) in blackgram and Vindhiyavarman *et al.* (1980) in *Vigna marine*. The reduction in frequency at higher doses has been attributed to the rigour of both diplontic and haplontic selections in the biological material (Swaminathan, 1961) and elimination of mutations.

b) *Frequency of single and multiple mutations:* The data on relative percentage of M_1 plant progenies segregating for single and multiple mutant types are presented in Table 1. In both the varieties, after gamma rays and DES treatments, multiple chlorophyll mutations comprising two types were more frequent than single and three types. This agreed with the results reported by Muthiah (1975) in sesame. In general, the occurrence of multiple mutations did not bear any consistent relationship with doses of mutagens in both the varieties.

c) *Pooled segregation ratio:* The pooled segregation ratio of chlorophyll mutants in the M_2 generation estimated on M_1 branch basis was higher than those of M_1 plant basis following gamma rays and DES treatments in both the varieties (Table 2). The ratios did not show any dose dependency on both M_1 plant and M_1 branch progeny bases. Such an inconsistent relationship with doses was reported after gamma rays and EMS treatments by Sundaram (1974) in redgram. All the segregating M_1 plant and M_1 branch progenies showed less than 25 per cent of recessive mutants in treatments with both mutagens in both the varieties. The reason for not realising the expected segregation ratio of 25 per cent might probably be due to the induced genic changes with a low transmission frequency closely associated with certain minute chromosomal aberration (deletions) which alter the gene transmission as reported by Moh and Alan (1968) with studies on *Phaseolus vulgaris*. The small mutated sector involving more number of initial cells and lethality of mutated cells may be the other cause for the realisation of a lower segregation ratio.

d) *Mutation spectrum:* The spectra of mutants observed in the segregating progenies were estimated and their relative percentages are given in Table 3. In both the varieties, the spectra of mutants comprised of only four types namely, *albina, xantha, chlorina* and *viridis*. Alikhan and Veerasamy (1974) also reported the occurrence of four types only in the same crop. In both the varieties, *chlorina* was the most frequent type following treatments with gamma irradiation, whereas *viridis* followed by *xantha* occurred in a high proportion after DES treatments. Alikhan and Veerasamy (1974) reported the frequent occurrence of *chlorina* types in gamma rays treated population of redgram. Gustafsson (1963) and Nilan (1972) reported that alkylating agents induced high proportion of *viridis* and low proportion of *albina*. Westergaard (1960) found that the alkylating chemicals induced the less drastic mutatons such as *viridis* instead of extreme mutations such as *albina* in higher proportions than those induced by radiations possibly because of their apparently less drastic effect on chromosomes. A maximum of three types of mutants were recorded in the individual doses of gamma rays or DES in both the varieties. The occurrence of mutant types, however, was not proportional with the doses of mutagens. Similer inconsistent result with doses of mutagens was reported by Vindhiyavarman *et al.* (1980) in *Vigna marina*.

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Table 1 Mutation frequency and relative % of multiple mutations

Mutagen and dose	Chlorophyll mutation frequency						Relative % of M_1 plant progenies seregating for mutations of					
	Per 100 M_1 plnats		Per 100 M_1 branches		Per 100 M_2 seedlings		One type		Two type		Three type	
	SA. 1	CO. 2	SA. 1	CO. 2	SA. 1	CO. 2	SA. 1	CO. 2	SA. 1	CO. 2	SA. 1	CO. 2
I *Gamma rays* (krad)												
1	28.9	24.7	9.4	10.2	1.8	1.5	11.5	16.7	88.5	83.3	—	—
5	28.4	28.9	19.8	19.1	2.4	2.8	14.3	—	85.7	88.5	—	—
10	17.1	17.8	16.1	16.4	3.0	4.1	16.7	—	66.6	100.0	16.7	—
15	18.0	24.6	17.6	23.6	3.7	3.8	—	23.5	100.0	76.5	—	—
20	16.7	15.0	15.7	13.8	2.2	2.3	—	16.7	100.0	66.6	—	—
25	14.8	14.3	11.1	11.5	1.2	1.0	—	20.0	75.0	80.0	25.0	—
30	18.2	22.2	12.5	17.3	1.1	1.4	—	—	100.0	100.0	—	—
40	25.0	20.0	10.5	16.7	0.7	0.9	—	—	100.0	100.0	—	—
II *DES* (*mM*)												
3	25.0	26.4	25.5	12.5	1.2	1.6	18.2	17.4	81.8	82.6	—	—
6	24.0	29.2	16.5	24.7	2.2	3.5	—	14.3	100.0	76.2	—	9.5
9	45.2	22.2	26.9	20.6	2.9	2.6	10.5	—	73.7	100.0	15.8	—
12	36.8	28.1	21.6	20.0	1.6	1.1	14.3	11.1	85.7	88.9	—	—
15	—	25.0	—	20.7	—	0.9	—	33.3	—	63.7	—	—

Table 2 Segregation percentages of chlorophyll mutants in M_2 generation

Mutagen and dose	Total No. of M_1 plants seregating		Pooled segregation ratio (%)		Total No. of M_1 branches seregating		Pooled seregation ratio (%)	
	SA. 1	CO. 2	SA. 1	CO. 2	SA. 1	CO.2	SA. 1	CO. 2
I. Gamma rays (krad)								
1	26	24	13.7	11.7	28	26	15.1	13.1
5	28	26	11.8	12.5	34	50	13.4	14.3
10	12	13	12.1	10.6	30	30	14.3	13.5
15	11	17	10.2	11.9	29	42	14.9	13.5
20	7	6	11.8	12.5	18	15	13.1	14.8
25	4	5	10.0	12.5	7	10	13.9	14.5
30	2	4	10.0	10.0	3	9	14.2	13.6
40	2	2	12.5	12.5	2	4	15.0	15.0
II. DES (mM)								
3	22	23	10.2	11.0	53	29	13.0	13.4
6	12	21	10.4	11.8	20	44	14.5	14.4
9	19	12	13.8	11.3	28	29	16.0	13.7
12	14	9	9.6	12.5	21	16	12.7	13.8
15	—	3	—	12.5	—	6	—	14.2

Table 3. Spectrum of chlorophyll mutants in M_2 generation

Mutagen and dose	Total No. of mutants in M_2		Spectrum (Relative percentage) of chlorophyll mutants							
			Albina		*Xantha*		*Chlorina*		*Viridis*	
	SA. 1	CO. 2	SA. 1	CO. 2	SA. 1	CO. 2	SA. 1	CO. 2	SA. 1	CO. 2
I *Gamma rays* (krad)										
1	217	159	2.3	—	40.1	—	57.6	58.5	—	41.5
5	232	295	—	2.0	32.8	33.2	67.2	64.8	—	—
10	264	384	6.1	—	—	—	58.7	58.6	35.2	41.4
15	304	321	—	—	—	24.6	50.3	21.5	49.7	53.9
20	110	108	—	5.7	—	31.3	22.7	63.2	77.3	—
25	41	38	7.3	—	—	65.8	26.8	—	65.9	34.2
30	19	25	—	—	26.3	—	73.7	40.0	—	60.0
40	4	7	—	14.3	25.0	—	—	85.7	75.0	—
II *DES* (mM)										
3	110	157	—	—	59.1	56.1	—	—	40.9	43.9
6	154	247	—	1.6	—	36.8	64.3	—	35.7	61.5
9	151	145	3.3	—	29.8	—	—	64.1	66.9	35.9
12	51	33	—	—	39.2	27.3	—	—	60.8	72.7
15	—	8	—	—	—	50.0	—	50.0	—	—