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Chlorophyll Mutations in Mungbean (*Vigna radiata* (L.) Wilczek)

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Summary. Chlorophyll mutations in two varieties ('ML-5' and 'K-851') of mungbean (*Vigna radiata* (L.) Wilczek) were studied severally and in combination following treatment with gamma rays and EMS. Frequencies, spectra and inheritance patterns are reported and discussed. Genotypic differences were observed with regard to frequency and spectrum. Combined treatments produced higher mutation frequencies than individual treatments. Albina and xantha appeared more frequently, relative to other chlorophyll mutations. Inheritance of albina, chlorina, xantha and virescens types in different generations revealed that each of them was monogenically controlled and was recessive in nature.

Key words: *Vigna radiata* – Chlorophyll mutations – EMS – Gamma radiation

Introduction

Induced mutations have now been recognized as an important tool for crop improvement, and have sufficient scope in pulses. In order to induce useful mutations in mungbean (*Vigna radiata* (L.) Wilczek), two varieties ('ML-5' and 'K-851') were subjected to treatments with gamma rays and EMS, severally and in combination. Results on frequencies, spectra and inheritance patterns of induced chlorophyll mutations are communicated in this paper.

Materials and Methods

Seeds of two varieties, namely 'ML-5' and 'K-851', were subjected to the following treatments:

(i) Gamma rays: Seeds subjected to 20 kR, 30 kR, 40 kR and 50 kR doses of gamma irradiation were secured from the Division of Genetics, I.A.R.I., New Delhi, India which has a source of ^{60}Co.

(ii) Ethyl methane sulphonate (EMS): Seeds were first soaked in water for 2 h and then transferred to an aqueous solution of EMS (0.2% and 0.4%) for 6 h and 12 h. The solutions were changed every three hours. Following the treatments, seeds were thoroughly washed with water and dried with blotting paper. EMS of Eastman Kodak Chemicals, USA was used for preparing acqueous solutions of the chemical mutagen.

(iii) Combined treatment: Chemical treatment (0.2% EMS for 6 h) was given to seeds irradiated at doses, 20 kR, 30 kR, 40 kR and 50 kR.

In each treatment, 300 seeds were treated and grown in a randomized block design with three replications. Seeds from each plant in M_1 were harvested separately and sown in M_2 on a plant to progeny basis. Frequency, spectrum and segregation of different chlorophyll mutants were scored in M_2. A few plants were taken randomly from lines segregating for chlorophyll mutants in M_2 and seeds of these plants were sown for M_3 to confirm segregation of these chlorophyll mutants. M_4 was similarly raised from segregating M_3 lines.

Results

Chlorophyll mutations were classified following Gustafsson (1940). The following types were identified in the M_2 generation.

(i) albina: white, lethal (6–7 days), no chlorophyll or carotenoids are formed.

(ii) xantha: yellow to yellowish white, lethal (12–15 days), carotenoids present, but chlorophylls absent.

(iii) chlorina: yellowish green, lethal (10–12 days)

(iv) xanthovirdis: green with yellow apex, viable.

(v) alboviridis: green with white apex, viable.

(vi) virescens: light green, gradually changing to normal green, viable.

(vii) maculata: yellowish green spots on the leaves, viable.

Mutation frequencies were calculated per 100 M_1 plants and are represented in Figure 1. It can be seen that albina and xantha types are more common relative to others found in the M_2 generation. Different spectra of chlorophyll mutations were obtained in two variet-

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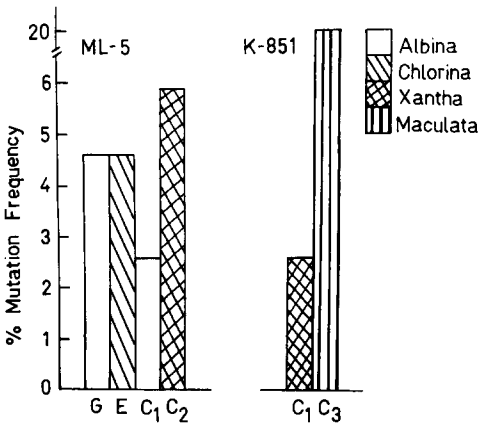

Fig. 1. Frequency of different chlorophyll mutations in the M_2 generation: G gamma rays (40 kR); E EMS (0.2%, 12 h); C_1 gamma rays (20 kR)+EMS (0.2%, 6 h); C_2 gamma rays (30 kR)+EMS (0.2%, 6 h); C_3 gamma rays (40 kR)+EMS (0.2%, 6 h)

ies. No chlorophyll mutations were seen in variety 'K-851' treated with gamma rays and EMS, while they were observed in variety 'ML-5' treated with high doses of gamma rays (40 kR) and EMS (0.2% 12 h). Combined treatments produced higher mutation frequencies in comparison to individual treatments. Further, with increasing dosages of gamma rays in combined treatment, mutation frequency increased (Fig. 1). Chlorophyll mutations like those of alboviridis and virescens, recorded in the M_2 generation, were excluded from analysis, since they did not prove to be true chlorophyll mutations in subsequent generations. Similarly, xanthoviridis, recorded in M_2, is included in xantha, since it segregated for xantha in subsequent generations.

Segregation of such different chlorophyll mutants such as albina, chlorina, xantha and virescens were studied in M_2 and in subsequent generations up to M_4,

Table 1. Segregation of various mungbean chlorophyll mutants of different lines in the M_2, M_3 and M_4 generations

Generation	Segregating line	Albina			Chlorina			Xantha			Maculata/Virescens		
		Normal	Mutant	P (3:1)	Normal	Mutant	P (3:1)	Normal	Mutant	P (3:1)	Normal	Mutant	P (3:1)
M_2	1	28	6	0.3 – 0.5	25	4	0.1 – 0.2	33	6	0.1 – 0.2	26	1	0.01 – 0.02
	2	28	12	0.3 – 0.5				28	4	0.1 – 0.2			
M_3	1	21	11	0.2 – 0.3	31	6	0.2 – 0.3	47	11	0.2 – 0.3	35	9	0.3 – 0.5
	2							36	7	0.1 – 0.2	43	13	0.7 – 0.8
	3							32	10	0.8 – 0.9			
	4							30	9	0.7 – 0.8			
M_4	1	54	16	0.5 – 0.7	20	3	0.1 – 0.2	9	4	0.5 – 0.7	16	3	0.3 – 0.5
	2	48	21	0.2 – 0.3	104	25	0.1 – 0.2	35	7	0.2 – 0.3	21	3	0.1 – 0.2
	3	8	4	0.5 – 0.7	45	10	0.2 – 0.3	29	7	0.3 – 0.5	9	1	0.2 – 0.3
	4	34	10	0.7 – 0.8				20	4	0.3 – 0.5	31	5	0.1 – 0.2
	5	5	1	0.5 – 0.7				8	2	0.7 – 0.8	45	11	0.3 – 0.5
	6							27	8	0.7 – 0.8	10	1	0.2 – 0.3
	7										8	2	0.7 – 0.8

Table 2. Frequencies of non-segregating and segregating rows derived as single plant progenies of segregating rows in the preceding generation

Generation	Albina			Chlorina			Xantha			Virescens		
	Non-seg.	Seg.	P (1:2)	Non-seg.	Seg.	P (1:2)	Non-seg.	Seg.	P (1:2)	Non-seg.	Seg.	P (1:2)
M_3	5	0	0.001 – 0.01	4	1	0.02 – 0.05	2	3	0.70 – 0.80	3	2	0.20 – 0.30
	4	1	0.02 – 0.05				4	1	0.02 – 0.05	5	0	0.001 – 0.01
M_4	2	5	0.70 – 0.80	4	3	0.10 – 0.20	1	2	1.0	4	2	0.05 – 0.10
							3	2	0.20 – 0.30	2	5	0.70 – 0.80
							5	2	0.02 – 0.05			

Non-seg. = Non-segregating, Seg. = Segregating

with the help of heterozygous plants obtained in a segregating line. The results are shown in Tables 1 and 2. The ratio fitted well for 3 : 1 for all types from different generations except that for maculata in the M_2 generation, but its subsequent generations segregated for virescens in the same fashion as found in other types. Probability for a good fit to 3 : 1 ratio ranged from 0.1 to 0.90 in all cases except one (Table 1). The ratio of non-segregating rows to segregating rows for different chlorophyll mutations in M_3 and M_4 are shown in Table 2. It can be seen that the number of rows segregated are less than expectation in most cases and χ^2 is also significant in about half the cases.

Discussion

Chlorophyll mutations provide one of the most dependable indices for the evaluation of genetic effects of mutagenic treatments and have been studied in various crops by several workers. In the present study, genotypic differences in response were observed in the form of frequencies of induced chlorophyll mutations in the M_2 generation.

This has been reported earlier in mungbean (Rathnaswamy et al. 1978), wheat (Goud 1967), sorghum (Goud et al. 1970) and several other crops. No difference in mutation frequencies was observed in variety 'ML-5' in treatments with gamma rays (40 kR) and EMS (0.2% 12 h) while in previous reports on several crops, some treatments with chemical mutagens were found to be more effective than those with physical mutagens. Combined treatments produced higher mutation frequencies in comparison to individual treatments, which support previous results found in such various crops as *Vicia faba* (Sjodin 1962; Hussain and Abdalla 1974), barley (Doll and Sandfoer 1969; Sharma 1970), and (Ramaswamy and Sree Rangaswamy 1972), Setaria (Gupta and Yashvir 1975) and mungbean (Grover and Tejpaul 1979; Krishnaswamy and Rathinam, 1980).

Chlorophyll mutations are recessive as revealed by segregation patterns in the M_2 generation. The expressivity of chlorophyll mutations varies with environment: xanthoviridis and maculata observed in M_2 generation behaved as xantha and virescens respectively in subsequent generations. Segregation of albina, chlorina, xantha and virescens types in different generations indicated that these are monogenically controlled. However, xantha and variegata (=maculata) were earlier reported as monogenic recessive, while chlorina was reported as digenic recessive (Santos 1969). Although the χ^2 value for the segregation ratio of different chlorophyll mutants in the M_2 generation fitted well for 3 : 1 for all cases except one, the probability of result is low in some of the cases. One of the possible explanations for the low number of mutant seedlings can be the chimeric nature of M_1 plants (Blixt 1960; Santos 1969). Blixt (1960) also stated that both kinds of cells (AA, Aa) occur in the same M_1 plant, which led to deviation in an expected ratio in its progeny by increasing the number of normal plants and decreasing the number of recessive homozygotes. Lower branches are known to produce more chlorophyll mutations in *Vicia sativa* and *Pisum sativum* (Debelyi et al. 1974), but Zannone (1968) did not find any preferential localization of chimeric mutations. This behaviour, however, is seen in subsequent generations also, which suggests that haplontic selection due to deficiency may be responsible as deficiencies may influence transmission rates and hence deviation in segregation ratio. The number of rows segregating for chlorophyll mutations were found to be less than expectation, which suggests an lethality effect on heterozygotes.

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