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Induced Chlorophyll Mutations in Black Gram

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Abstract: An investigation entitled "Induced chlorophyll mutations in black gram" was conducted during kharif (rainy season) 2004 at the field experimentation centre of the Department of Genetics and Plant Breeding, Allahabad Agricultural Institute - Deemed University, Allahabad. Seeds of IPU-982 of Black gram were exposed to gamma rays and then treated with sodium azide to obtain the spectrum and frequency of chlorophyll mutations in M_2 generation Combination treatment of gamma rays with sodium azide enhanced the frequency of chlorophyll mutations in M_2 generation. The treatments of sodium azide were found to be more efficient than gamma rays in inducing chlorophyll mutations when applied singly. A progressive increase in mutation frequency of chlorophyll mutations was observed with increasing doses. Six different types of chlorophyll mutants, namely, albino, xantha, dark xantha, chlorina, viridis and striata were induced. Out of these mutants, xantha and dark xantha were most frequent while striata was least frequent. The highest frequency of chlorophyll mutations (8.87) was reported in the combination of 60kR+0.03%SA. There was a dose dependent increase in the spectrum and frequency of chlorophyll mutations whether mutagens were employed singly or in combination.

Key words: Gamma rays, sodium azide, mutation, black gram

INTRODUCTION

A large number of desirable varieties have been developed through mutation breeding in field and horticulture crops. But the application and success of mutation breeding in improvement of grain legume crops is relatively limited except perhaps soybean and groundnut. Chlorophyll mutations offer one of the most reliable indices for the assessment of genetic effects of mutagenic treatments. Genotypic differences in response to induction of chlorophyll mutations can be observed as frequency of induced chlorophyll mutations in M_2 generation. Azide induces high frequencies of chlorophyll and morphological mutations with negligible frequency of chromosomal aberrations when used in acidic conditions. As expected, about 250-300 loci might be involved for breakdown of the chlorophyll apparatus in barley (Swami Nathan 1957). So also Von Wettstein (1980) in barley and Haque and Godward (1986) in Lectuceae reported involvement of considerable number of genes at different stages of plastid development as revealed from the plastid ultra structure of leaves. Hence, the probability of occurrence of such category of mutation is obvious in all mutagen treatments.

MATERIALS AND METHODS

The cultivar IPU-982 of Black gram was employed as experimental material during the present study. Seeds of this variety were irradiated with 10, 20, 40 and 60 kR doses of gamma radiation at ^{60}Co gamma cell and for chemical treatment seed samples were presoaked in the distilled water for 14 hours at room temperature and treated with sodium azide at 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06% and 0.07% concentrations. Irradiated seeds along with sodium azide treated seeds and control (parental variety), were grown in randomized block design to study the M_1 generation during kharif (rainy season) 2003-2004.

The M_2 population was screened for frequency and spectrum of chlorophyll mutations per 1000 M_2 plants. Lethal chlorophyll mutations were scored within 10 to 25 days of sowing whereas viable chlorophyll mutations were scored throughout the life period of plants. The spectrum of chlorophyll mutations was studied and the mutants were classified as per the scheme of Gustafson (1940) with modifications.

- Albino-white, lethal, no chlorophyll or carotenoids are formed.
- Xantha yellow to yellowish white, lethal, carotenoids present but chlorophyll absent.
- Dark xantha – dark yellow, lethal
- Chlorina – uniform green colour with white on tips, viable.
- Viridis – uniform light yellow green colour of leaves, viable.
- Striata – longitudinal strips of different colours, viable.

RESULTS AND DISCUSSION

Chlorophyll mutations provide one of the most dependable indices for the evaluation of genetic effects of mutagenic treatments and have been reported in

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Table 1: Effect of different combinations of gamma rays and sodium azide on frequency and spectrum of chlorophyll mutations and other macro mutations in M_2 generation of black gram.

Treatments,	Number of M_2 plants,	Chlorophyll mutants,		Spectrum/Frequency of chlorophyll mutants/1000M_2 plants					
		Number,	Frequency (per 1000M_2 plants)	Albino	Xantha	Darkxantha,	Chlorina	Viridis	Striata
0kR+0.00%SA	1354	00	0.00	-	-	-	-	-	-
0kR+0.01%SA	1317	7	5.31	2.28	-	1.51	1.51	-	-
0kR+0.02%SA	1414	8	5.65	-	1.41	-	2.12	2.12	-
0kR+0.03%SA	1345	9	6.69	2.23	-	1.48	-	-	2.97
0kR+0.04%SA	1256	11	8.81	1.60	2.40	1.60	-	2.40	1.60
0kR+0.05%SA	1242	5	4.05	-	-	1.61	-	2.41	-
0kR+0.06%SA	1211	0	0	-	-	-	-	-	-
0kR+0.07%SA	1201	5	4.16	1.66	-	1.66	-	0.83	-
10kR+0.00%SA	1315	7	5.32	2.28	1.52	-	1.52	-	-
10kR+0.01%SA	1302	3	2.30	-	0.76	-	0.76	0.76	-
10kR+0.02%SA	1248	9	7.16	0.79	0.79	-	1.59	-	-
10kR+0.03%SA	1205	8	6.64	2.48	2.48	-	-	1.65	-
10kR+0.04%SA	1200	4	3.33	0.83	0.83	-	-	1.66	-
10kR+0.05%SA	1191	5	4.19	1.67	-	0.83	-	-	-
10kR+0.06%SA	1176	3	2.55	-	0.85	-	-	-	-
10kR+0.07%SA	1152	8	6.94	-	1.73	-	2.60	2.60	-
20kR+0.00%SA	1234	9	7.29	3.24	2.43	-		-	1.62
20kR+0.01%SA	1215	5	4.11	1.64	-	-	0.82	1.64	-
20kR+0.02%SA	1137	3	2.64	-	1.76	0.87	-	-	-
20kR+0.03%SA	1126	6	5.32	2.66	0.88	-	1.77	-	-
20kR+0.04%SA	1111	4	3.60	-	-	0.90		1.80	0.90
20kR+0.05%SA	1096	8	7.29	2.73	-	1.82	2.73	-	-
20kR+0.06%SA	1048	7	6.68	-	-	-	-	-	-
20kR+0.07%SA	1024	3	2.92	-	1.95	-	-	0.97	-
40kR+0.00%SA	1246	5	4.01	-	-	0.80	1.60	1.60	-
40kR+0.01%SA	1213	8	6.59	2.47	1.64	-	2.47	-	-
40kR+0.02%SA	1175	9	7.66	3.40	-	1.70	2.55	-	-
40kR+0.03%SA	1134	9	7.93	-	-	0.88	-	2.64	1.76
40kR+0.04%SA	1127	6	5.32	3.22	0.88	0.88	-	0.88	-
40kR+0.05%SA	1046	3	2.86	-	0.95	-	0.45	0.95	-
40kR+0.06%SA	1028	3	2.91	0.97	-	0.97	0.97	-	-
40kR+0.07%SA	946	5	5.28	-	2.11	-		1.05	1.05
60kR+0.00%SA	1085	4	3.68	-	-	0.92	1.84	0.92	-
60kR+0.01%SA	985	5	5.07	2.03	-	2.03	1.01	-	-
60kR+0.02%SA	923	4	4.33	-	1.08	-		1.08	1.08
60kR+0.03%SA	901	8	8.87	2.22	4.43	-	2.22	-	-
60kR+0.04%SA	846	0	0.00	-	-	-	-	-	-
60kR+0.05%SA	826	4	4.84	1.21	-	3.63	1.21	-	-
60kR+0.06%SA	764	5	6.54	2.61	1.31		-	-	2.61
60kR+0.07%SA	613	3	4.89	1.63	-	1.63	-	1.63	-

Various pulse crops by several workers including Gautam *et al.* (1992). The data was recorded on the frequency of chlorophyll mutations per 1000 M_2 plants (Table 1) Chlorophyll mutations were found in almost all the mutagenic treatments. High frequency of chlorophyll mutations were found in the combination treatment of gamma rays and sodium azide and in the doses of sodium azide alone. The highest frequency of chlorophyll mutations (8.87) was reported in the combination of 60kR+0.03%SA while the lowest (2.30) frequency of chlorophyll mutations was found with the treatment combination of 10kR+0.01%SA. Sodium azide was found to be more effective for inducing chlorophyll mutations in comparison to gamma rays and their combinations.

Spectrum of chlorophyll mutations in segregating M_2 generation (Table 1) indicates presence of broad chlorophyll mutant spectrum comprising 5 types (maximum) was induced by 0.04%SA followed by 40kR+0.04%SA treatment which induced four types of chlorophyll mutants. Xantha mutant recorded highest frequency (4.43) in 60kR+0.03%SA treatment. Highest frequency of chlorina mutants was found with 20kR+0.05%SA and dark xantha in case of 60kR+0.05%SA (3.63) treatment. The higher frequency of viridis was observed in the treatment 40kR+0.03%SA (2.64), while albino mutants were found in 40kR+0.02%SA (3.40) and striata mutant was found under 0.03%SA treatment.

The combination treatments of gamma rays with sodium azide enhanced the frequency of chlorophyll mutations, which is supported by previous results found in various crops, such as mungbean Gautam and Mittal, 1998 in black gram. Mutation frequency in sodium azide with gamma ray treatments was higher than the sum of the two single treatments. Six different types of chlorophyll mutants produced in the present study are in agreement with the findings of several workers in the past. Ignacimuthu and Babu, 1988 reported albino, viridis, chlorina, xantha mutants in three species of *Vigna*. Out of six mutants induced in the present study, dark xantha and xantha mutants were most frequent while striata type was the least frequent. Earlier studies have revealed as many as 6 types of chlorophyll mutations Gustafson 1940.

There was a dose dependent increase in the spectrum and frequency of chlorophyll mutations in M_2. Similar results were obtained in *Vigna* species by Ignacimuthu and Babu, 1988 they reported that the spectrum and frequency of chlorophyll mutations increased with dosage, which is similar to the present study. A progressive increase in mutation frequency of chlorophyll mutations was observed with increasing doses. Manju *et al* (1983) observed that chlorophyll mutation frequency in M_2 seedlings showed dose dependence in horse gram. In the present study, interaction of sodium azide with gamma rays increased the chlorophyll mutation frequency, which is in agreement with the findings of Cheng and Gao (1988). Viable chlorophyll mutations, i.e., chlorina and viridis were produced more at lower doses/concentrations of mutagen whereas lethal mutants, namely, albino, xantha and dark xantha were observed more frequently at relatively higher doses/concentrations of the mutagens

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