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Frequency and spectrum of chlorophyll mutants induced in rice by chemical mutagens

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Summary. Dry seeds of rice varieties 'T(N)1', 'IR 8' and 'Sona', with stabilised moisture content and presoaked in distilled water, were treated with chemical mutagens MMS, dMS, dEMS and dES with the purpose of evaluating chlorophyll mutation frequency and spectrum. In the M_2 generation, mutants occurred in 24 lines of 'T(N)1; 94 lines of 'IR 8' but only in six of 'Sona'. They include albino, viridis, xantha and other categories of which viridis was predominant. dES was found to be most effective of all mutagens used in all the three varieties and varietal differences were observed.

Key words: Chemical mutagen – Chlorophyll mutants – Mutagenicity – Varietal difference

Introduction

Mutations for chlorophyll deficiency, leaf, panicle and spikelet character were obtained by Ramiah and Parthasarathy (1938). Chang and Hsieh (1957) and Hsieh (1959) found that over 10% of the R_1 plants of a variety showed cream, albino and variegated mutants and these were suggested to be plastid mutations. Shastry and Ramiah (1961) observed that the mutation rate in ^{35}S treatments of the variety 'NP 130' exhibited a steady increase with increase in activity of isotope when compared to X-rays and thermal neutron treatments. The increased chlorophyll mutation rate (57.2%) at the dose of 9 μc of ^{35}S per seed was suggested as a positive approach to the maximization of mutation effect. Xantha mutants were more frequently encountered following treatment with ^{35}S while albinos were more frequent following X-irradiation.

Kawai (1963) obtained chlorophyll lethals by treatment with radiophosphorus. According to Nilan (1967) the spectrum of chlorophyll mutations induced is specific to the type of mutagen used in rice and barley. Basu and Basu (1969) reported a predominance of albino mutations followed by others, by treating the seed of rice varieties 'Rupsail', 'Latisail' and 'Patnai' with ^{32}P, ^{35}S and X-rays. Reddy et al. (1973) recorded that both hydrazine (HZ) and hydroxylamine (HA) induced M_2 chlorophyll mutants in a local variety of rice 'Tellakattera' but the spectra were significantly different. Nanda and Misra (1975) suggested that for screening chloroplast mutants it was desirable to grow the same M_2 in both dry and wet seasons in order to eliminate the effect of environmental factors. Bhan and Kaul (1976) studied the frequency and spectrum of chlorophyll mutants in rice varieties 'IR 8', 'Jhona 349' and 'Basmati 370' in relation to the genotype and the nature of the mutagen.

In the present paper results are reported of a study of chlorophyll mutation frequency and spectrum in 'T(N)1', 'IR 8' and 'Sona' varieties after treatments with chemical mutagens methylmethane sulfonate (MMS), dimethylsulphate (dMS), dichloroethylmethane sulfonate (dEMS) and diethylsulphate (dES).

Materials and methods

Five hundred normal seeds from each of the three varieties cited with 14% moisture content were presoaked for 12 h in distilled water under aseptic conditions. Treatments were subsequently carried out with chemical mutagens at concentrations of 0.03, 0.05 and 0.1% by volume, except in the case of dEMS in which 0.05, 0.1 and 0.2 were used. In view of the short half-life of dES, the solution was replenished every 30 min. The seeds were also continuously shaken in the mutagen solutions throughout the 12 h period of treatment. As a post-treatment care the seeds were washed in running water for 30 min, allowed recovery in fresh water for 3 h and sown directly in seed beds. Comparable controls were maintained throughout the period of the experiment. Twenty panicles per dose were collected at random with which raise the M_2 populations.

Results and discussion

The highest frequency of chlorophyll mutants occurred in a total of 94 M_1 panicles of 'IR 8' representing all the mutagen treatments. A large number of them turned

Table 1. Frequency of chlorophyll mutants in 'T(N)1'. 'IR 8' and 'Sona' in M_2

Mutagen	No. of M_2 spikes studies	'T(N)1' M_2 spikes segregating		No. of M_2 spikes studied	'IR 8' M_2 spikes segregating		No. of M_2 spikes studied	'Sona' M_2 spikes segregating	
		No.	%		No.	%		No.	%
Control	20	–	–	20	–	–	20	–	–
MMS	60	2	3.33	60	20	33.33	60	–	–
dMS	60	4	6.66	60	18	30.00	60	–	–
dEMS	60	–	–	60	6	10.00	60	2	3.33
dES	120	18	15.00	120	50	41.66	120	4	3.33

Table 2. Frequency of chlorophyll mutations (perf 100 M_2 seedlings) in three varieties of rice

Mutagen	Dose	'T(N)1'			'IR 8'			'Sona'		
		Seedlings analysed	Mutant seedlings	%	Seedlings analysed	Mutant seedlings	%	Seedlings analysed	Mutant seedlings	%
Control		1072	–	–	1655	–	–	1876	–	–
MMS	0.03	1148	2	0.17	1318	26	1.97	1692	–	–
	0.05	1028	–	–	1554	28	1.80	1976	–	–
	0.1	1074	–	–	1140	28	2.45	–	–	–
dMS	0.03	1178	–	–	1139	–	–	2059	–	–
	0.05	1124	2	0.17	1450	16	1.10	2064	–	–
	0.1	1078	2	0.18	1528	14	0.91	2024	–	–
dEMS	0.05	1152	–	–	1322	14	1.05	1908	–	–
	0.1	982	–	–	1526	2	0.13	1924	2	0.10
	0.2	1316	–	–	1931	–	–	2041	–	–
dES	0.03	1954	–	–	2452	44	1.79	2536	–	–
	0.05	2384	28	1.17	2846	60	2.10	3832	–	–
	0.1	2018	22	1.09	3098	30	0.96	3210	4	0.12

out to be the viridis type and only in two lines were completely albino and striped categories found. In 'T(N)1', a total of 24 lines segregated only for chlorophyll mutants. Although many of them were of the viridis type, again, as in 'IR 8', two new mutant types, namely xantha and alboviridis, were located. In 'Sona', the mutants were limited to only six panicles, representing two different treatments of the mutagens dEMS and dES (Table 1). The data reveals that in 'T(N)1', 15% of the M_1 spikes treated with dES segregated for chlorophyll mutants. In 'IR 8', on the other hand, all the treatments used were effective in inducing mutations in a fraction of M_1 spikes. A maximum of 41.66% were found in dES followed by 33.33, 30.00 and 10.00 in MMS, dMS and dEMS treatments respectively.

When the dose-wise analysis was made on the basis of seedlings, 'T(N)1' recorded a maximum of 28 (1.17%) in dES 0.05 followed by 22 (1.09%), 2 (0.18%), 2 (0.17%) and 2 (0.17%) in dES 0.1, 0.1 and 0.05 of dMS and MMS 0.03 respectively (Table 2). In 'IR 8', mutants were observed in all the doses except in dMS 0.03 and dEMS 0.2. Higher responses were noticed in MMS when compared to dMS and dEMS. dES was equally effective as MMS treatment in this case. No dose dependent relationship could be established with reference to any chemical in 'IR 8' but the average frequency of induced chlorophyll mutations was higher. The lowest response was recorded in 'Sona', in which 0.10% and 0.12% were observed in dEMS and dES treatments respectively.

Viridis type of chlorophyll mutants were induced by all the mutagens used whereas the albino type occurred with MMS and dES treatments. Alboviridis was observed with MMS treatment only. Xantha type was limited to dEMS and dES treatments.

In general, dES treatment was the most effective mutagenic agent for all the varieties of the present study. The chemicals with ethyl groups had uniform effects in both 'T(N)1' and 'IR 8', but MMS treatments were less effective in 'T(N)1' than in 'IR 8'. In this respect varietal differences were also evident as indicated by the overall frequency and spectrum of the chlorophyll mutations induced.

Similar varietal differences in the yield of chlorophyll mutations induced by EMS and NMU were reported by Nanda and Misra (1975). In the present investigation, viridis mutants occurred at a maximum frequency followed by albino, xantha and other types. Earlier workers reported the higher frequency of albino mutants (Swaminathan et al. 1970 in 'IR 8' and 'Tainan 3'; Nanda and Misra 1975 in 'Saturn', 'Ch. 45' and 'Ratna'; Bhan and Kaul 1976 in 'IR 8', 'Jhona 349' and 'Basmati 370'). However, Bhan and Kaul (1976) reported a higher frequency of viridis than albina in dES treatment of 'Jhona' but in all other treatments the frequency of albina was reported to be higher. The study of Reddy and Reddy (1971) suggests that alkylating agents such as diethyl sulphate induced a maximum frequency of chlorophyll mutants in the secondary tillers as compared to primary tiller types.

The chimeric nature of the M_1 plants and of the individual panicles are considered among the causes accounting for the shortage of mutant class of seedlings in M_2. In the present study panicles segregating for chlorophyll mutants were planted separately. The shortage observed for the mutant class in many cases probably indicate that even the single tiller of a M_1 plant is of a chimeral constitution originating from more than one cell of the primordium.

Earlier studies on rice by Bekendam (1961) and Shastry and Ramiah (1961) on irradiated rice varieties led to a similar conclusion. Additional cytological evidence was presented by Reddi and Reddi (1975) concerning the chimeral nature of the ring of six forming plant in which some florets showed normal 12 bivalents whereas others showed translocation complex.

The data presented endoreses the views of Gregory (1960) that the chief limiting factor in mutation production and mutant recovery depends upon the genetic constitution of the experimental organism and not the type of mutagen used, at least for alkylating agents. Even with well controlled environmental conditions one should not hope to predict, let alone direct, the spectrum of mutations likely to be introduced by merely using a specific class of mutagen.

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