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Mutagenic Effectiveness and Efficiency of EMS, DES and Gamma-rays in Rice

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Summary. Data on chlorophyll mutation frequency after treatment with EMS, DES and gamma-rays and sequential administration of gamma-rays and the two alkylating agents in three varieties of rice have been used to work out quantitatively the effectiveness and efficiency of each mutagen and combination treatment. For effectiveness, the order is EMS > DES and for efficiency it is EMS > DES > gamma-rays. In some sequential treatments (Gamma-rays + DES in 'IR8' and 'Basmati'; DES + gamma-rays in 'IR8' and 'Jhona'; Gamma-rays + EMS in 'IR8' and 'Basmati'; and EMS + gamma-rays in 'IR8', 'Jhona' and 'Basmati') mutation frequency is more than additive (synergistic) but these treatments are decisively less efficient because of their relatively high injurious effects in the M_1 generation. EMS induces more albinas than gamma-rays do. The mutational spectrum patterns induced by gamma-rays and DES are alike. In general, combination treatments tend to increase the frequency of albinas over other types of chlorophyll mutants.

Key words: Rice - Chlorophyll Mutants - Gamma-Rays - EMS - DES - Mutation Spectrum

Introduction

It is well known that the most effective treatment may not be the most efficient. Efficient treatments are essential for the economic use of mutagens as tools for inducing variability. Thus, for induced mutagensis, the use of data on the relative effectiveness and efficiency of mutagens has been considered vital.

Kawai (1969), while attempting to review the effectiveness of various mutagens in rice, was handicapped by the lack of data on dose, treatment conditions and other biological effects in some cases. Moreover, he made no quantitative estimation of effectiveness and efficiency of the mutagens. Lack of conclusive results in this field prompted the present work. This paper records a quantitative estimation of effectiveness and efficiency of various doses of ethyl methanesulphonate (EMS), diethyl sulphate (DES), gamma-rays and sequential administration of physical and chemical mutagens in three rice varieties.

Materials and Methods

The material comprised of selfed seeds of rice varieties 'IR8', 'Jhona 349' and 'Basmati 370'.

Husked seeds of these varieties, with moisture content stabilized at 13 per cent, were irradiated with 20, 30, 40 and 50 Kr gamma-rays at a dose rate of 1250 rads/minute with a Co^{60} source, at the Indian Agricultural Research Institute, New Delhi.

For the chemical treatments, seeds were presoaked for 12 hours in distilled water and treated with 0.5, 1.0, and 1.5 per cent aqueous solutions of EMS and DES for 6 hours and 2 hours, respectively, at 30 ± 2°C. DES solution was changed after every half hour. After treatment the seeds were thoroughly washed.

For sequential administration of mutagens, 20 Kr gamma-rays and 1.0 per cent EMS or DES for 6 hours and 2 hours, respectively, were used. In this treatment the procedure adopted was similar to that for the individual treatments, but when the chemical treatment was administered before radiation, the seeds were allowed to dry to 13 per cent moisture at room temperature before being irradiated.

During the M_2 generation, panicles from main tillers of plants for each treatment were harvested separately, sown in separate rows in nursery beds and analysed for chlorophyll mutations according to the classification of Gustafsson (1940). The data were taken both on M_1 spike basis and M_2 seedlings basis. Formulae given by Konzak et al. (1965) were used for calculating efficiency and effectiveness of the mutagens.

Results

Chlorophyll mutation frequency on M_1 spike basis and M_2 seedling basis is represented in Figs. 1 and 2, respectively, and the spectrum of chlorophyll mutations induced by various treatments in Fig. 3.

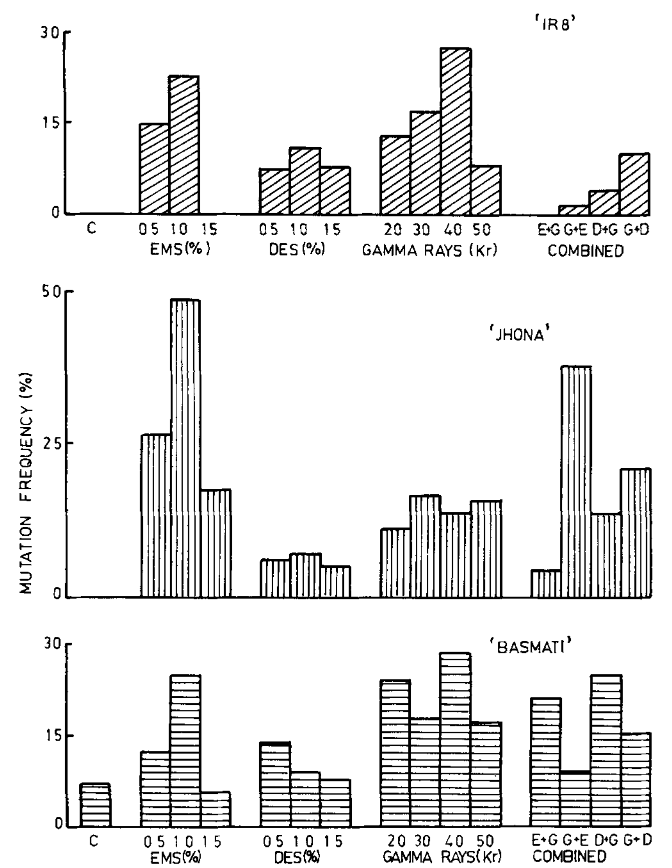

Fig.1. Chlorophyll mutation frequency (M_1 spike basis in rice varieties induced by EMS, DES and gamma-rays (c = control)

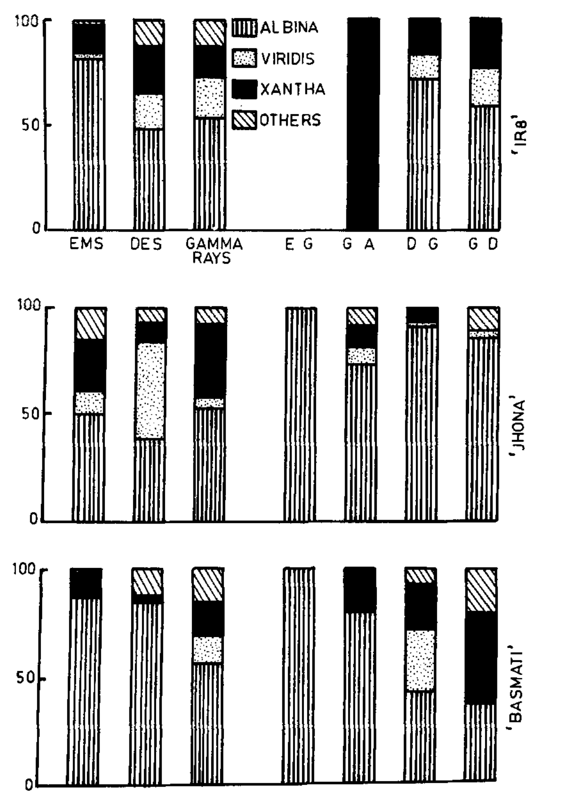

Fig.3. Spectrum of chlorophyll mutations induced by physical and chemical mutagens in rice varieties (data pooled for different doses)

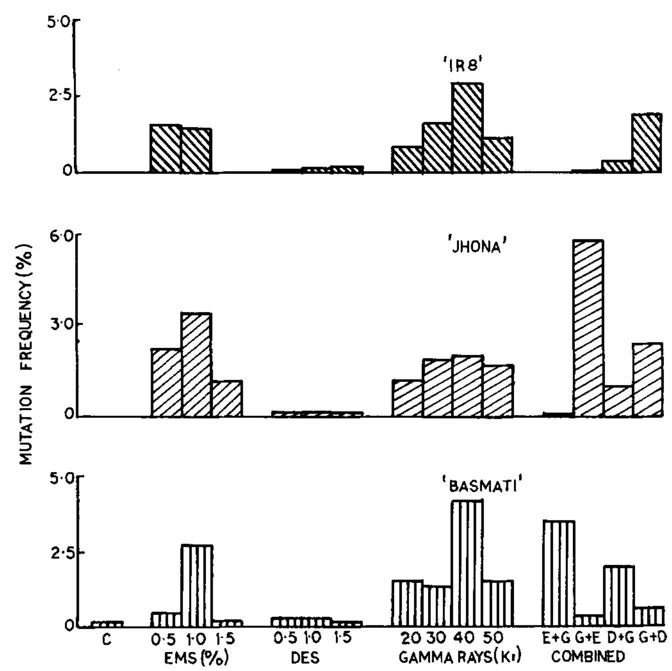

Fig.2. Chlorophyll mutation frequency (M_2 seedling basis) in rice varieties induced by EMS, DES and gamma-rays (c = control)

About 97 per cent of chlorophyll mutations detected in the M_2 populations were lethal; the rest were subvital and were able to assimilate to form ears and ripe kernels, although ripening was much retarded and poor.

Spontaneous chlorophyll mutation frequency on M_2 seedling basis was 0, 0 and 0.09% (Fig.2), and on M_1 spike basis was 0, 0 and 7.14% (Fig.1), for 'IR8', 'Jhona' and 'Basmati', respectively. On the other hand, average chlorophyll mutation frequency (on the basis of pooled values for all treatments given to a variety) was enhanced to 0.75, 1.26 and 0.93% on M_2 seedling basis and 10.08, 17.58 and 15.67% on M_1 spike basis in the three varieties, 'IR8', 'Jhona' and 'Basmati', respectively. While gamma-rays and EMS induced more chlorophyll mutations in 'IR8' and 'Jhona', respectively, both the mutagens were equally potent in 'Basmati' (Figs.1, 2). In general, the frequency of chlorophyll mutations was dose-de-

Table 1. Estimates of relative effectiveness and efficiency of chemical mutagens in rice

			Mutagenic effectiveness				Mutagenic efficiency				
			Msp*				Msp			Msd	
Variety	Mutagen	Dose(%)	t^C	$X^C(1-e^{-kt})$	$X^C(e^{-kt})$	$txC(e^{-kt})$	I	L	S	L	S
'IR8'	EMS	0.5	0.049	2.00	0.057	0.34	-	0.44	1.3	0.046	0.139
		1.0	0.039	1.60	0.046	0.27	1.71	0.35	0.5	0.023	0.035
		1.5	-	-	-	-	-	-	-	-	-
	DES	0.5	0.094	0.06	0.048	0.19	0.68	2.17	1.6	0.021	0.016
		1.0	0.072	0.05	0.037	0.15	3.23	0.55	4.1	0.068	0.050
		1.5	0.035	0.02	0.018	0.17	8.00	0.30	16.0	0.064	0.334
'Jhona'	EMS	0.5	0.091	3.76	0.107	0.64	2.52	1.30	1.0	0.106	0.079
		1.0	0.083	3.43	0.098	0.58	1.20	0.78	1.0	0.055	0.069
		1.5	0.019	0.80	0.023	0.14	0.36	0.18	0.3	0.012	0.020
	DES	0.5	0.079	0.05	0.040	0.16	30.00	-	0.9	-	0.015
		1.0	0.046	0.03	0.023	0.09	-	0.62	1.8	0.010	0.029
		1.5	0.022	0.01	0.011	0.04	1.92	0.27	0.9	0.005	0.017
'Basmati'	EMS	0.5	0.042	1.74	0.050	0.30	-	1.04	0.6	0.037	0.056
		1.0	0.043	1.79	0.051	0.00	1.85	1.04	0.9	0.044	0.096
		1.5	0.007	0.28	0.008	0.06	1.17	0.06	0.4	0.002	0.012
	DES	0.5	0.182	0.12	0.092	0.37	-	9.27	4.2	0.128	0.058
		1.0	0.060	0.04	0.030	0.12	-	0.90	45.5	0.020	0.990
		1.5	0.035	0.02	0.018	0.07	-	0.80	1.5	0.012	0.023

* Msp-Mutation frequency on spike basis; Msd - Mutation frequency on seedling basis; t^c is the product of time and C, the initial concentration; $X^c(e^{-kt})$ is the product of time and net available conc. (not hydrolyzed); $X^c(1-e^{-kt})$ is the total mM conc. of mutagen hydrolyzed during the treatment period; I is the seedling injury (seedling height reduction); L is the M_1 seedling lethality and S is the reduction in M_1 seed fertility

pendent except in the treatments where seedling lethality in M_1 exceeded 90 %. Unlike EMS and gamma-rays, various doses of DES induced insignificant changes in mutation frequency.

The mutagenic effectiveness of each mutagen decreased with increasing mutagen dose, and maximum effectiveness was shown by the lowest mutagen doses used (Tables 1, 2). Because of a difference in the dose units between physical and chemical mutagens, their mutagenic effectiveness can not be compared. Different doses of EMS (0.5 % in 'IR8' and 'Jhona'; 1.0 % in 'Basmati'), DES (0.5, 1.0 and 1.5 % in 'Jhona', 'Basmati' and 'IR8', respectively) and gamma-rays (20 Kr in 'Basmati' and 20-30 Kr in 'IR8' and 'Basmati') represent the most efficient doses for the three varieties (Tables 1, 2).

Only some of the sequential treatments (i.e. Gamma-rays + DES in 'IR8' and 'Basmati', DES + gamma-rays in 'IR8' and 'Jhona'; Gamma-rays + EMS in 'IR8' and 'Basmati', and, EMS + gamma-rys in 'IR8', 'Jhona' and 'Basmati'), showed synergistic effects (Fig.2) as far as the induced chlorophyll mutation frequency on M_2 seedling basis was concerned. Most of the sequential treatments were not as effective and efficient as individual treatments (Tables 1, 2, 3).

Since different doses of a mutagen produced almost identical spectra of chlorophyll mutations, values for different doses of the same mutagen were pooled and a break-up is shown in Fig.3. Chlorophyll aberrant types other than albina, viridis and xantha were induced only at very low frequency, so they have been pooled and labelled as 'others' in Fig.3. Gamma-rays induced more than 50 % albinas in each of the three varieties, with viridis and xantha types occurring in about equal proportions. Effects of EMS and DES varied with genotype. Only in one case (DES on 'Jhona') was frequency of viridis higher than that of albina; in all other cases, the frequency of albinas was much higher than the sum of all other classes. The proportion of albinas was more than 80 % in EMS on 'IR8' and 'Basmati' and DES on 'Basmati'. In general, sequential treatments showed a tendency to produce a higher proportion of the albina type.

Table 2. Estimates of relative effectiveness and efficiency of various doses of gamma-rays in rice

Variety	Dosage (kr)	Mutagenic effectiveness (msp/dose in kr)	Mutagenic efficiency				
			Msp	Msp	Msp	Msd	
			I	L	S	L	S
'IR8'	20	0.65	2.7	-	0.27	-	0.019
	30	0.57	1.0	0.68	0.22	0.067	0.022
	40	0.69	0.9	0.54	0.34	0.059	0.038
	50	0.17	0.3	0.11	0.01	0.015	0.015
'Jhona'	20	0.55	1.1	-	0.57	-	0.019
	30	0.55	0.9	2.47	0.21	0.286	0.024
	40	0.34	0.4	0.25	0.16	0.036	0.023
	50	0.31	0.4	0.22	0.20	0.023	0.021
'Basmati'	20	1.19	-	76.67	0.42	4.940	0.026
	30	0.58	1.5	0.97	0.34	0.072	0.025
	40	0.71	0.8	0.34	0.31	0.030	0.045
	50	0.34	0.5	0.18	0.21	0.016	0.018

* For explanation of abbreviations see footnote of Table 1

Table 3. Estimates of relative efficiency of combined physical and chemical mutagen treatments on rice

Variety	Mutagen and dosage		Mutagenic efficiency				
			Msp*			Msd	
			I	L	S	L	S
'IR8'	1 % EMS + 20 KrGR	(A)	-	-	-	-	-
	20 KrGR + 1 % EMS	(B)	-	0.017	-	-	-
	1 % DES + 20 KrGR	(C)	0.23	0.102	0.088	0.010	0.009
	20 KrGR + 1 % DES	(D)	0.61	0.472	0.248	0.089	0.047
'Jhona'	1 % EMS + 20 KrGR	(A)	0.43	0.67	0.313	0.001	0.003
	20 KrGR + 1 % EMS	(B)	0.90	0.429	0.468	0.006	0.071
	1 % DES + 20 KrGR	(C)	0.94	0.472	0.227	0.033	0.016
	20 KrGR + 1 % DES	(D)	1.34	0.959	0.390	0.108	0.044
'Basmati'	1 % EMS + 20 KrGR	(A)	-	0.211	0.302	0.035	0.052
	20 KrGR + 1 % EMS	(B)	0.45	0.097	0.207	0.002	0.005
	1 % DES + 20 KrGR	(C)	5.25	1.108	0.389	0.088	0.031
	20 KrGR + 1 % DES	(D)	2.73	1.987	0.314	0.079	0.012

* For explanation of abbreviations see footnote of Table 1

Discussion

The chlorophyll mutation frequency of 27.5 % induced by 40 Kr gamma-rays in 'IR8' (on M_1 spike basis) is higher than the highest recorded by Kawai (1969) in his review on effectiveness of mutagens. Similarly, the mutation frequency of 4.2 % by 40 Kr gamma-rays (on M_2 seedling basis) in 'Basmati' compares favourably with the highest frequency (viz. 4.1 %) recorded by him.

A drop in frequency of chlorophyll mutations in treatments involving very high doses of mutagens (the ones which induce over 90 % seedling lethality in M_1 generation) could either be due to death of the mutated plants leading to their consequent elimination or due to a selection within the plant, i.e. the diplontic selection (Bekendam 1961; Gaul 1961, 1964; Yamaguchi 1962). The latter is a possibility because two or more primordial cells are involved in the ontogeny of a spike in cereals (Mackey 1954). In such treatments we observed a very high seedling lethality in the M_1 generation and that corroborates the contention of Gaul (1961) that whole plants may be dying due to extensive damage and only the relatively resistant ones survive.

Chlorophyll mutation rate in M_2 generation has been customarily used as a test of the effectiveness of a particular mutagen. The unmistakable fall in chlorophyll mutation frequency at high doses thus necessitates judicious utilization of this test at high doses of mutagens. We suggest that chlorophyll mutation frequency should not be taken as an index of effectiveness and efficiency beyond LD 75 doses.

EMS was both the most effective and the most efficient mutagen in the present material; gamma-rays were less efficient than DES. EMS has been regarded as superior to gamma-rays and fast neutrons in inducing useful mutations for practical plant breeding in rice (Mikaelsen et al. 1971).

By using dehusked seeds, brief pre-soaking periods, long mutagen treatment with low concentrations and very brief post-washing, neutrons were found to be more effective and efficient than EMS by Siddiq and Swaminathan (1968). Results from the present study reveal the possibility of attaining high efficiency with EMS even when husked seeds are used. This can be achieved by using long pre-soaking periods but a brief treatment duration. Similar observations have been made by Osone and Mikaelsen (1971) in a Hungarian rice variety.

Some of the sequential treatments produce a chlorophyll mutation frequency higher than the expected values obtained by adding the frequencies induced by single mutagen treatments. Such synergestic effects in combinational treatments have been reported earlier for rice (Rao and Gopal Ayengar 1964) and barley (Sharma 1970; Valeva 1965), the possible reasons being enumerated by Sharma (1970). The reasons for the low mutation frequency in some sequential treatments, where M_1 damage was considerable, are the same as forwarded for high doses of single mutagen treatments. Efficiency of almost all the sequential treatments was low (Table 3). Similar observations have been made by Doll and Sandfaer (1969). This low efficiency is due to extensive injury caused by the sequential administration of mutagens under the present treatment conditions.

The high frequency of albina types in EMS treated populations as compared with gamma-ray treated ones is interesting, since it is contrary to the earlier reports for barley (Nilan, 1967) and rice (Rao and Gopal Ayengar 1964). These workers have shown that physical mutagens induce higher frequencies of albina types than chemical mutagens do. With the present state of knowledge, no reason for such differential responses can be given.

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