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THE RELATIVE MUTAGENIC EFFECTS OF IONIZING RADIATIONS AND ALKYLATING CHEMICALS IN RICE

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The rice plant which is a secondary polyploid with a partly disomic and partly polysomic genetic constitution having a strict self pollinating system is ideally suited for improvement through mutation breeding. Mutation research in rice from the point of view of its improvement dates back to 1934. Reports of early period relate to sparsely ionizing radiations such as X rays and *gamma* rays. The potentialities of fast neutrons have not been explored. Information on the usefulness of chemical mutagens is meagre. A critical comparative evaluation of mutagenic effectiveness and efficiency of radiations and chemicals has not yet been attempted. The present investigation has, therefore, been undertaken to study the relative mutagenic effects of three ionizing radiations and five alkylating chemicals in rice.

Materials and Methods

The biological material consisted of seeds of the short duration tall *Indica* rice variety Co.29. The radiations employed were X-rays, *gamma* rays and fast neutrons and the chemicals were monofunctional aikyiating agents such as Diethyl sulphate (DES), Ethyl methane sulphonate (EMS), Methy! methane sulphonate (MMS), Nitroso methyl urea (NMH) and Methyl nitro nitroso guanidine (MNNG).

Dry seeds stabilised for moisture content at 12% were irradiated. Seeds were soaked in water for 16 hours prior to treatment with chemical mutagens. The pre-soaked seeds were treated by keeping immersed in the solution for 8 hours (4 hrs for DES) with intermittent shaking. The temperature of the solution was maintained at 73 $\pm$ 22°F throughout the period of treatment. The DES solution was changed once in every 40 minutes with fresh solution to maintain the activity of the mutagen at a high level throughout the period of treatment.

Treated seeds were sown in three replications in a field nursery. Thirty days old seedlings were transplanted in the mainfield. Observations on germination, survival, seedling height, seed fertility and chlorophy! deficient chimeras were made in the M_1 generation. The apical and first axillary panicles of about 100 plants in each dose selected at random were selfed and harvested separately.

The M_2 generation was grown as M_1 ear progenies. The progenies segregating for chlorophyll mutants were scored to calculate mutation frequency per 100 M_1 ears. The number of mutants and normal seedlings in all progenies were counted to estimate the mutants frequency per 100 M_2 plants. In the segregating

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progenies, the mutants and normal seedlings were counted to calculate the segregation percentage of mutants, i.e. the percentage of mutants in segregating progenies. The differenttypes of mutants were scored separately for calculating the spectrum (relative % of different types) of mutants. Mutagenic effectiveness and efficiency were estimated adopting the formulae proposed by Konzak *et al.* (1965).

Results and Discussion

Effects of mutagens in the M_1 generation

Data relating to the effects of mutagens in the M_1 generation are presented in Table-1

Germination of seeds was not affected by radiations even at high doses. But survival was reduced at higher doses of X-rays and *gamma* rays. Mikaelsen (1968) while reporting similar results in barley has stated that initial germination of seeds is mainly through cell elongation and this stage is not inhibited by radiation. Chemical mutagens on the other hand inhibited germination. The percentage of reduction increased with increase in dose of mutagen. Several reports indicate that chemical mutagens such as DES, EMS and NMH inhibit germination of rice seeds considerably. Reduction in survival occurred in treatment with all mutagens except fast neutrons. Maximum reduction was recorded in treatment with NMH. The nature of lethal effects induced by radiations thus differed from those induced by chemical mutagens. Radiation induced lethality was manifested subsequent to germination through a prolonged lethal action, whereas chemical induced lethality was expressed mostly through inhibition of germination.

Radiations as well as chemical mutagens inhibited seedling growth. *gamma* rays and NMH were more effective in this respect. The magnitude of height reduction increased with increase in dose of mutagens. Growth inhibition following mutagen treatment has been attributed to factors such as auxin destruction, inhibition of auxin synthesis, production of growth retarding substances, changes in the activity of enzymes, delay in the onset of first mitosis and inhibition of DNA synthesis.

Seed fertility of M_1 plants decreased with increase in dose of radiations. Fast neutrons per unit dose had reduced fertility more drastically than X-rays and *gamma* rays. Low fertility following neutron irradiation was reported by Siddiq (1967). Sterility was comparatively low in treatments inolving chemical mutagens. Radiation induced sterility might be due to detectable chromosome aberrations, whereas chemical induced sterility might be due to cryptic deficiences and specific gene mutations. Sterility induced by radiations is mostiy haplontic whereas EMS induced Steriiity is diplontic (Gaul *et al.* 1966).

High frequeneies of chlorophyll deficient chimeras were found in treatments with NMH and EMS. The frequency of plants with chlorophyll deficient sectors increased with increase in doses of mutagens. At equimolar concentration, NMH

Table—1

Effects of mutagens in the M_1 generation

Mutagen and dose	Values as % of control				M_1 damage			Chlorophyll (%)
	Germination (7 th day)	Survival (30 th day)	Seedling height (30th day)	Seed fertility	Lethality (L)	Injury (I)	Sterility	
1	2	3	4	5	6	7	8	9
1) X-rays.								
10 krad	100	100	96	66	0	5	34	—
20 ,,	100	97	92	46	3	8	54	—
30 ,,	100	98	87	36	2	13	64	—
40 ,,	98	74	73	30	26	27	70	1.4
2) Gamma rays								
10 Krad	100	100	95	73	0	5	27	—
20 ,,	100	100	91	43	0	9	57	—
30 ,,	100	97	79	19	3	21	81	0.5
40 ,,	100	63	51	19	37	49	81	1.2
3) Fast neutrons								
705 rad	100	100	95	75	0	5	25	—
1170 ,,	100	98	92	60	2	8	40	0.4
1570 ,,	100	98	87	47	2	13	53	—
1880 ,,	100	97	83	44	1	17	56	0.7
4) DAS								
11.4 mM	100	93	96	90	7	4	10	—
22.8 ,,	93	87	96	89	13	4	11	—
34.2 ,,	78	79	97	88	21	3	12	—
45.6 ,,	75	70	96	88	30	4	12	—
57.0 ,,	76	71	95	87	29	5	13	—
5) EMS								
77 mM	100	97	91	90	3	9	10	0.6
154 ,,	100	88	85	87	12	15	13	2.6
240,,	88	88	84	71	12	16	29	2,6
336 ,,	90	85	84	69	15	16	31	2.7
384 ,.	86	83	81	66	17	19	34	3.5
6) MMS								
5.9 mM	100	97	91	93	3	9	7	—
11.8 ,,	90	82	88	88	18	12	12	—
17.7 ,,	86	73	85	91	27	15	9	—
23.6 ,,	85	72	84	87	28	16	13	0.8
29.5 ,,	84	70	80	87	30	20	13	—

1	2	3	4	5	6	7	8	9
7) NMH								
1,94 mM	100	79	76	96	21	24	4	4.3
3.88 ,,	99	46	68	96	54	32	4	6.4
5.82 ,,	100	35	50	95	65	50	4	6.1
7.76 ,,	94	24	45	92	76	55	8	10,2
9,70 ,,	82	22	41	90	78	59	10	20.0
8) MNNG								
1.36 mM	100	98	93	99	2	7	1	—
2.72 ,,	100	98	91	99	2	9	1	—
5.44 ,,	100	79	86	98	21	14	2	—
8.36 ,,	95	48	83	92	52	17	1	0.4
10.20 ,,	83	35	79	98	65	21	2	1,2

Table—2

Mutagenic effects in the M_2 generation [Chlorophyll mutations]

Mutagen and dose	Mutation frequency per $100 M_1$ ears (M)	Mutation frequency per $100 M_1$ plants	Segregation χ	Mutagenic effectiveness Mx100 / te or k rad	Mutagenic efficiency Mx100 / L	Mutagenic efficiency Mx100 / I	Mutagenic efficiency Mx100 / S
1	2	3	4	5	6	7	8
1) X-rays							
10 krad	11.2	0.83	7.5	112	∞	224	33
20 ,,	13.3	1.52	9.7	67	443	166	25
30 ,,	150	3,16	16.9	50	750	115	23
40 ,,	14.8	3.44	18.6	37	57	55	21
2) Gamma rays							
10 krad	11.3	0.85	7.9	113	∞	226	42
20 ,,	8,0	1.19	14,0	40	∞	89	14
30 ,,	6.8	1.19	18.5	23	227	32	8
40 ,,	24.3	5.31	20.3	61	66	50	30
3) Fast neutrons							
705 rad	10.1	1.16	11.0	1433	co	202	40
1170 ,,	12.5	1.03	8.5	1068	625	156	31
1570 ,,	12.1	2.07	14.2	771	605	93	23
1880 ,,	15.8	2.52	14.8	835	1580	93	28

1	2	3	4	5	6	7	8
4) DES							
11.4 mM	3.6	0.09	—	8	51	90	36
22.8 ,,	3.1	0.11	—	3	24	78	28
34.2 ,.	2.0	0.10	—	1	10	67	17
45.6 ,,	4.2	0.13	—	2	14	105	35
57.0 ,.	8·9	0.40	—	4	31	178	68
5} EMS							
77 mM	9.0	0.38	4.2	2	300	100	90
154 ,,	10.3	2.02	11.1	2	169	135	156
240,,	22.3	2.48	12.9	1	186	139	77
336 ,,	27.3	2.57	10.8	1	182	171	88
384 ,,	26.5	3,14	14.3	1	156	140	78
6) MMS							
5.9 mM	3.9	0.26	—	8	130	43	56
11.8 ,,	4.0	0.53	—	4	22	33	33
17.7 ,,	4.5	0.37	—	3	17	30	50
23.6 ,,	6.5	0.51	—	3	23	41	50
29.5 ,,	8.6	0.51	—	4	29	43	66
7) NMH							
1.95mM	13.3	1.14	9.0	86	63	55	333
3.88 ,,	13.1	0.92	6.7	42	24	41	328
5.82 ,,	18.9	1.57	7.9	41	29	38	472
7.76 ,,	29.5	3.00	9.9	47	39	54	369
9.70 ,,	22.6	2.82	13.1	29	29	38	226
8) MNNG							
1.36mM	2.8	0.10	—	26	140	40	280
2.72 ,,	1,7	0.04	—	8	85	19	170
8.16 ,,	3.4	0.40	—	8	16	24	170
5.44 ,,	1.1	0.05	—	2	2	6	110
10.20,,	0.6	0.01	—	1	1	3	30

induced a higher frequency of chimeras than EMS, indicating that NMH is the most effective mutagen in this respect Radiations and other chemicals have induced such chimeras in very low frequencies only. The superiority of chemical mutagens over radiations in inducing chlorophyyll deficient sectors was emphasised by several workers in a number of plant species. Chlorophyll deficient sectors have been reported to arise as a result of chromosomal aberrations, plastid mutation and destruction of chloroplast structure or DNA, Eriksson and Lindgren (1 970) stated that the dose dependance of sector frequency had made possible the use of thie criterion as an estimate of mutagenic effects in the M_1 generation.

Table—3

Spectrum of chlorophyll mutants in the M_2 generation

Mutagen	Total No of mutants	Spectrum (Relative percentage)							
		Albi-na	Xantha	Viri-dis	Chlo-rina	Albo-viri-dis	Stri-ata	Tig-rina	oth-ers
1) X-rays	629	39	4	31	12	8	2	—	4
2) Gamma rays	308	43	4	14	25	—	14	—	...
3) Fast neutrons	547	43	5	27	12	5	4	1	3
4) DES	108	24	12	50	5	—	9	—	—
5) EMS	1328	24	10	39	12	7	2	1	5
6) MMS	309	33	2	21	25	10	1	—	8
7) NMH	750	18	8	40	15	9	3	1	6
3) MNNG	77	4	1	69	19	3	1	—	3

Mutagenic effects in the M_2 generation

The data relating to mutagenic effects in the M_2 generation such as mutation frequency and segregaticn percentage are presented in Table-2·

Mutation frequencies were estimated as the number of mutations per 100 M_1 ears and number of mutants per 100 M_2 plants. The frequencies estimated on M_1 ear basis and M_2 plant basis showed similar trends indicating that the M_1 ear method is as efficient as the M_2 plant method. The frequencies of mutations induced by the radiations were similar in spite of the fact that the doses of fast neutrons employed were much lower than those of X-ray and *gamma* rays. Swaminathan *et al.* (1970) have reported that neutrons induce a higher frequency of chlorophyll mutations at low doses. Among the chemical mutagens employed, EMS was the most potent because it induced the highest frequency of mutations. Konzak *et al.* (1965) and Gaul *et al.* (1966) have also reported that EMS is the best among the chemical mutagens. NMH also induced high frequencies of mutations and ranked second to EMS. On the other hand, very low mutagenic effects were observed for DES, MES and MNNG.

The mean segregation percentage of mutants increased with increase in doses of radiations and chemical mutagens (Table-2). The dose dependance of M_2 segregation percentage was reported by Kawai and Sato (1966) in rice and Aastveit (1968) in barley. The increase in segregation percentage at higher doses of mutagens was explained by Aastveit (1968) as due to an increase in the size of the mutated sector resulting from lethality of some of the initial cells of the primordium. Mean segregation percentages were higher after treatment with radiations than with chemical mutagens indicating severe eliminations of initial cells in the panicle primordia following irradiation. The high segregation percentage increasesthe proportion of mutants and facilitates their selection in segregating populations.

The spectrum of chlorophyll mutants presented in Table-3 indicate differences following treatment with radiations and chemicals, Albina was the most frequent type in radiation treatments. In treatment with chemical mutagens there was a decrease in albina followed by an increase in viridis. Such differences in the spectrum of mutants between radiations and chemical mutagens were reported by Kawai 1966.

Mutagenic effectiveness and effciency

Konzak *et al.* (1965) proposed the terms "effectiveness" as a measure of gene mutations in relation to dose and "efficiency" as an estimate of the mutation rate in relation to other induced biological effects such as lethality, injury and sterility. The effectiveness and efficiency of mutagens in inducing chlorophyll mutations were estimated and presented in Table-2. Radiations in general were more effective than chemical mutagens. The most effective among radiations and chemical mutagens were fast neutrons and NMH respectively. The high effectiveness of fast neutrons was also reported by Swaminathan (1969) and that of NMH by Siddinq (1967). Effectiveness decreased with increase in doses of mutagens. This inverse relationship between mutagenic effectiveness and mutagen dose was reported by several investigators and could be explained as due to the failure of mutation frequency to increase proportionately with increase in mutagen doses.

Mutagenic efficiency was higher for radiations when estimated on the basisof lethality and injury, whereas based on sterility, chemical mutagens were more efficient. The most efficient radiation was fast neutrons. The higher efficiency of fast neutrons was reported by Rana and Swaminathan (1967) in wheat. EMS was the most efficient chemical mutagen based on lethality and injury whereas based on sterility NMH was the most efficient. The high mutagenic efficiency of EMS was repeatedly confirmed by several investigators in rice. barley, maize and other plants. This was probably because of its low phyto-toxicity. Mutagenic efficiency also decreased with increase in doses of mutagen. According to Konzak *et al,* (1965), the higher efficiency of low doses of mutagens was because of the increasing lethality, injury and sterility with increase in mutagen doses at rates faster than those for mutations.

The usefulness of any mutagen depends on its mutagenic effectiveness as well as efficiency. The most effective mutagen need not be the most efficient one (Konzak *et al.* (1965). In the present study, the most effective as well as efficient radiation was fast neutrons. Among chemical mutagens, the most effective was NMH whereas the most efficient was EMS based on lethality and injury and NMH based on sterility.

Summary

Studies were undertaken on the relative mutagenic effects of three ionizing radiations (X-rays, *gamma* rays, fast neutrons) and five alkylating chemicals (DES, EMS, MMS, NMH, MNNG) in rice. Observations on seed germination, seedling survival, seedling height, seed fertility and chlorophyll deficient chimeras were made in the M_1 generation. The M_2 generation was grown as M_1 ear progenies. Chlorophyll mutation frequency, segregation percentage and mutant spectrum were estimated. The mutagenic effectiveness and efficiency were also worked out.

Germination of seeds was not affected by radiations even at high doses. Chemical mutagens inhibited germination. Radiation induced lethality was manifested subsequent to germination, whereas lethality induced by chemical mutagens was expressed mostly through inhibition of germination. Seedling height was reduced fay mutagen treatments • *Gamma* rays and NMH were more effective in this respect Radiations induced high degreesof sterility than chemical mutagens. Fast neutrons induced more sterility per unit dose than sparsely ionizing radiations. Chlorophyll deficient sectors were more frequent in M_1 plants after treatment with NMH and EMS. NMH, however, was more effective than EMS in this respect.

Mutation frequency estimated on M_1ear basis was as efficient as that on M_2 plant basis because the study was confined to pre-formed ears. *Gamma* rays fast neutrons, EMS and NMH had induced high frequencies of chlorophyll mutations. Per unit dose, fast neutrons was more effective than *gamma rays* and at equimolar concentrations NMH was more effective than EMS. The mean segregation percentages were higher after treatment with radiation than with chemical mutagens. The spectra of mutants differed between radiations and chemical mutagens. Albina was the most frequent type following irradiation. In chemical mutagen treatments, the frequency of albina was low and that of viridis was high.

Radiations were more effective than chemical mutagens. Mutagenic effectiveness decreased with increase in doses of mutagens. This was due to the failure of mutation frequency to increase proportinately with increase in mutagen doses. Mutagenic efficiency was higher for radiations when estimated on the basis of lethality and injury whereas, based on sterility, chemical mutagens were more efficient. Mutagenic efficiency also decreased with increase in doses of mutagens. The most effective as well as efficient radiation was fast neutrons Among chemical mutagens the most effective was NMH whereas the most efficient was EMS.

സംഗ്രഹം

ഹ്രസ്വകാല മൂപ്പുള്ള ഇൻഡിക്കായിനത്തിൽപ്പെട്ട സി. a. 29 നെല്ലിൽ റേഡിയേഷനും രാസമ്യൂട്ടേജനുകളും ഉപയോഗിച്ച് ആകസ്മിക പാരമ്പര്യവ്യതിയാനം കൈവരുത്തുന്നതു സംബന്ധിച്ച പരീക്ഷണങ്ങളിൽ കൂടുതൽ വ്യതിയാനങ്ങൾ വരുത്തുന്നതിൽ ഫാസ്റ്റ് ന്യൂട്രോണുകൾ താരതമ്യേന കൂടുതൽ ഫലപ്രദമാണെന്ന് കാണുകയുണ്ടായി.

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