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# Study of Chlorophyll Mutants in IR. 8 Rice

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The frequency of chlorophyll mutations as number of mutations per 100 $M_1$ spikes was as efficient as the number of mutants per 100 $M_2$ plants. The relationship between dose and frequency remained the same irrespective of the method of estimation of mutation frequencies. The frequencies decreased at high doses even when estimated on $M_2$ plant basis. The frequency of chlorophyll mutation in gamma irradiation increased upto the middle dose and thereafter followed an inconsistent trend whereas, in EMS treatments the frequency increased with increase in dosage. The rate of mutation was higher in primary spikes than the first and second formed spikes. Induced chlorophyll mutation spectrum differed between gamma rays and EMS. *Albina* was the most frequent type following irradiation while *xantha* was predominant with EMS treatments.

Various types of chlorophyll mutants were found to occur invariably in most of the studies on the artificial induction of mutations. Several investigators reported that the mutation frequency reached a maximum at moderate doses and decreased at high doses of gamma and x-rays. Linear relationship between mutation frequency and dose of X-rays and gamma rays was also reported. Chemical mutagens like ethyl methane sulphonate and nitroso methyl urea have also been found to be effective in producing high chlorophyll mutation frequencies and the differences in the spectrum of mutations induced by physical and chemical mutagens have been observed by many workers. In this paper, the frequency and spectrum of chlorophyll mutants which occurred in the $M_2$ generation in the study of induced mutagenesis in IR 8 rice are reported.

## MATERIAL AND METHODS

Unhulled seeds of IR 8 were treated with of gamma rays from 10 to 50 kR at 5 kR intervals ethyl methane sulphonate (EMS) viz., 5, 7.5, 10, 25, 50, 75. 100, 150 and 200 mM. Seeds were soaked in distilled water for 16 hours before EMS treatment. From each $M_1$ plant, panicles of the primary, first and second tiller were harvested separately.

The seeds of $M_1$ plants were sown in a field nursery. All the seeds from each ear were sown in separate beds. A total of 1013 $M_1$ spikes under gamma rays and 2361 spikes under EMS was sown. The nursery beds were examined

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at intervals of three to five days during the period of eight to 20 days following sowing for record of chlorophyll deficient seedlings. The ear-progenies segregating for chlorophyll mutants were scored first to calculate mutation frequency per 100 $M_1$ plants and per $M_1$ spikes. The total number of mutants and normal seedlings also counted to estimate the mutation frequency per 100 $M_2$ plants. The chlorophyll mutants were classified according to the system suggested by Gustafsson (1940) and expanded by Konzak *et al.* (1968). The different types were scored separately for calculating the spectrum of mutations.

## RESULTS AND DISCUSSION

(i) **Frequency:** The frequencies of chlorophyll mutations estimated as number of mutants per 100 $M_1$ plants, $M_1$ spikes and 100 $M_2$ plants are presented in Table I.

TABLE I. Frequency of chlorophyll mutations in the $M_2$ Generation

| Mutagen and dose | No. of $M_1$ plant progenies | | No. of $M_1$ spike progenies | | No. of $M_2$ | | Mutation frequency | | |
|---|---|---|---|---|---|---|---|---|---|
| | Scored | Segregating | Scored | Segregating | Seedlings scored | Chlorophyll mutants | Per 100 $M_1$ plants | Per 100 $M_1$ spikes | Per 100 $M_2$ seedlings |
| Control | 82 | — | 213 | — | 8,343 | — | — | — | — |
| (i) Gamma rays (KR) | | | | | | | | | |
| 10 | 78 | 24 | 216 | 48 | 10,117 | 226 | 30.77 | 22.22 | 2.23 |
| 15 | 75 | 24 | 195 | 51 | 6,646 | 218 | 32.00 | 26.15 | 3.28 |
| 20 | 62 | 22 | 151 | 49 | 3,103 | 171 | 35.48 | 32.45 | 5.51 |
| 25 | 41 | 6 | 77 | 13 | 576 | 26 | 14.63 | 16.88 | 4.51 |
| 30 | 47 | 13 | 104 | 21 | 1,664 | 52 | 27.66 | 20.19 | 3.13 |
| 35 | 40 | 12 | 104 | 26 | 2,020 | 104 | 30.00 | 25.00 | 5.15 |
| 40 | 32 | 9 | 88 | 17 | 1,063 | 42 | 28.13 | 19.32 | 3.95 |
| 45 | 22 | 6 | 49 | 12 | 578 | 27 | 27.27 | 24.49 | 4.67 |
| 50 | 11 | 1 | 29 | 3 | 298 | 5 | 9.09 | 10.34 | 1.68 |
| (ii) EMS (mM) | | | | | | | | | |
| Control | 88 | — | 257 | — | 29,236 | — | — | — | — |
| 5 | 82 | — | 246 | — | 25,507 | — | — | — | — |
| 7.5 | 86 | — | 257 | — | 26,230 | — | — | — | — |
| 10 | 90 | — | 266 | — | 30,342 | — | — | — | — |
| 25 | 85 | 2 | 255 | 6 | 26,772 | 26 | 2.35 | 2.35 | 0.027 |
| 50 | 92 | 4 | 272 | 10 | 32,449 | 64 | 4.36 | 3.68 | 0.197 |
| 75 | 87 | 6 | 259 | 14 | 30.083 | 110 | 6.90 | 5.41 | 0.366 |
| 100 | 93 | 10 | 275 | 30 | 32,094 | 252 | 10.75 | 10.91 | 0.785 |
| 150 | 95 | 15 | 278 | 37 | 30,786 | 359 | 15.79 | 13.32 | 1.166 |
| 200 | 87 | 18 | 259 | 51 | 24,318 | 593 | 20.69 | 19.69 | 2.439 |

It was seen that the frequency of chlorophyll mutation estimated on $M_1$ plant basis was high. The rate of mutation ranged from 9.09 to 35.48 on $M_1$ plant, 10.34 to 32.45 on $M_1$ spike and 1.68 to 5.51 on $M_2$ plant bases. The frequency of mutation showed an increase trend from 10 to 20 kR on all the three bases and thereafter no linear response with increasing dosages was evident. The highest frequency occurred at 20 kR and increased dosages caused reduction. At 50 kR, low values were observed indicating elimination of mutations. Osone (1960), Yamaguchi (1964) and Miah *et al*. (1970) have reported similar results in rice. The vigour of both diplontic and haplontic selection in the biological material (Swaminathan 1961) is attributed to be the reason. In the present study, the dose frequency relationship remained the same, irrespective of the method of estimation of frequencies thereby indicating that the mutation frequencies decreased at the highest dose even when estimated on $M_2$ plant basis.

In EMS treatment, chlorophyll mutants were observed at concentrations 25 mM and above. The mutation frequency ranged from 2.35 to 20.69 on $M_1$ plant, 2.35 to 19.69 on $M_1$ spike and 0.097 to 2.439 on $M_2$ plant bases. The frequency increased with increasing concentrations of EMS on all the three bases of estimation. When plotted against the doses, the rate of mutation showed linearity upto the highest dosage employed. This shows that the values recorded in the present investigation were not the highest possible. The frequencies recorded were lower in EMS than gamma irradiation.

The frequency of chlorophyll mutants expressed on $M_1$ spike basis, observed in three spikes in their developmental sequence is presented in Table II.

In treatments with gamma rays, chlorophyll mutants occurred in primary, first and second formed tillers. The rate of mutation was higher in primary spikes than the first and second formed spikes. Among the plants studied at 50 kR dosage, only one plant segregated and also all the three categories of spikes resulting in equal rate of mutation frequency. The percentage of mutants increased in the three spikes from 10 to 20 kR and an inconsistent trend was observed at higher dosages The occurrence of chlorophyll mutants was noticed in all the three spikes at various dosages of EMS. The rate of mutation was more in primary spikes than first and second formed spikes. Such dilution of mutation in late formed tillers in rice was reported by Chao and Chai (1961). The Osone (1963) and Siddiq and Swaminathan (1966). The dilution in mutation rate in the later formed tillers has been explained on the basis of 'Intra somatic selection' due to competition between healthy and affected cells and Gaul (1958) has termed this as 'Diplontic selection'.

ii) **Spectrum :** The types of mutations observed in the segregating $M_1$ spikes were estimated and their relative percentages are given in Table III.

In treatments with gamma rays, three types of chlorophyll mutants viz., *albina*, *xantha* and *striata* were observ-

TABLE II. Variation in chlorophyll mutation frequency in tillers ($M_1$ spike basis

| Mutagen and dose | Number of $M_1$ spikes | | $M_1$ spikes | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | | | Frequency | | | Percentage | | | |
| | Scored | Segregating | Primary | First | Second | Primary | First | Second | percentage |
| (i) Gamma rays (KR) | | | | | | | | | |
| 10 | 216 | 48 | 22 | 17 | 9 | 10.2 | 7.9 | 4.2 | 22.3 |
| 15 | 195 | 51 | 21 | 17 | 13 | 10.8 | 8.7 | 6.7 | 26.2 |
| 20 | 151 | 49 | 20 | 17 | 12 | 13.2 | 11.3 | 7.9 | 32.4 |
| 25 | 77 | 13 | 5 | 5 | 3 | 6.5 | 6.5 | 3.9 | 16.9 |
| 30 | 104 | 21 | 10 | 7 | 4 | 9.6 | 6.7 | 3.9 | 20.2 |
| 35 | 104 | 26 | 11 | 9 | 6 | 10.6 | 8.7 | 5.7 | 25.0 |
| 40 | 88 | 17 | 8 | 5 | 4 | 9.1 | 5.7 | 4.5 | 19.3 |
| 45 | 49 | 12 | 5 | 5 | 2 | 10.2 | 10.2 | 4.1 | 24.5 |
| 50 | 29 | 3 | 1 | 1 | 1 | 3.4 | 3.4 | 3.4 | 10.2 |
| (ii) EMS (mM) | | | | | | | | | |
| 25 | 255 | 6 | 2 | 2 | 2 | 0.78 | 0.78 | 0.78 | 2.34 |
| 50 | 272 | 10 | 4 | 3 | 3 | 1.48 | 1.10 | 1.10 | 3.68 |
| 75 | 259 | 14 | 6 | 4 | 4 | 2.31 | 1.54 | 1.54 | 5.39 |
| 100 | 275 | 30 | 10 | 10 | 10 | 3.64 | 3.64 | 3.64 | 10.92 |
| 150 | 278 | 37 | 15 | 13 | 9 | 5.40 | 4.68 | 3.23 | 13.31 |
| 200 | 259 | 51 | 18 | 18 | 15 | 6.95 | 6.95 | 5.79 | 19.69 |

ed. The widest spectrum was noted at 25, 35 and 40 kR doses and only *albinas* were seen at 50 kR. *Albinas* were found to occur more than the other kinds of mutants. The spectrum of mutants observed show no linear relationship with dosage.

Four types of chlorophyll mutants viz.. *albina*, *zantha*, *virescent* and *striata* were observed with EMS treatments. The widest spectrum was found at 150 and 200 mM doses. The spectrum of mutation increased with increasing dosage. Among the types, *xantha* was predominant; the order of occurrence following *Xantha* being *albina*, *virescent* and *striata*. *Xantha* was seen under all treatments where as *striata* was confined to the higher doses of 150 and 200 mM of EMS. Such differences in the spectrum of chlorophyll mutants between ratations and chemical mutagens were reported by Bekendam (1961), Chao and Chai (1961), Basu and Basu (1969) and Gopinathan Nair (1971) in rice.

TABLE III. Relative percentages of different types (Spectrum) of Chlorophyll Mutants in the $M_2$ Generation

| Mutagen and dose | Total number of mutants in $M_2$ | Relative percentage of chlorophyll mutants | | | |
|---|---|---|---|---|---|
| | | Albina | Xantha | Striate | Virescent |
| (i) Gamma rays (kR) | | | | | |
| 10 | 226 | 61.94 | 38.06 | — | — |
| 15 | 218 | 74.77 | 25.23 | — | — |
| 20 | 171 | 59.06 | 40.94 | — | — |
| 25 | 26 | 61.54 | 23.08 | 15.38 | — |
| 30 | 52 | 94.23 | 5.77 | — | — |
| 35 | 104 | 54.81 | 44.23 | 0.96 | — |
| 40 | 42 | 76.19 | 16.67 | 7.14 | — |
| 45 | 27 | 55.56 | 44.44 | — | — |
| 50 | 5 | 100.00 | — | — | — |
| (ii) EMS (mM) | | | | | |
| 25 | 26 | — | 38.46 | — | 61.54 |
| 50 | 64 | 62.50 | 37.50 | — | — |
| 75 | 110 | 44.55 | 55.45 | — | — |
| 100 | 252 | 21.83 | 52.78 | — | 26.39 |
| 150 | 359 | 29.53 | 40.67 | 12.26 | 17.54 |
| 200 | 593 | 41.32 | 46.20 | 2.19 | 10.29 |

iii) **Frequency of single and multiple chlorophyll mutations:** The data on $M_1$ spike progenies segregating for one and two types and mean number of chlorophyll mutations per segregating $M_1$ spike are furnished in Table IV.

More than one type of mutations induced in a spike primordium can ba identified if they have different phenotypes. In the present study, multiple chlorophyll mutations comprising two types were found to be induced in most of the dosages of gamma rays and EMS. In gamma irradiation, an increase in the frequency of two types of mutations was observed from 10 to 20 kR. The frequency of occurrence of two types of mutants was higher in gamma rays than EMS and in the latter, the frequency increased with increasing concetrations. A dose dependent increase in multiple chlorophyll mutations in treatments with EMS was

TABLE IV. Frequency and Percentage of $M_1$ spike progenies segregating for Single and Multiple Chlorophyll Mutations

| Mutagen and dose | Number of spike progenies segregating | Chlorophyll Mutations: Total number | Spike progenies segregating for mutations of: Mean number per spike | One type (Frequency) | two types (Frequency) | one type (Relative percentage) | two types (Relative percentage) |
|---|---|---|---|---|---|---|---|
| (i) Gamma rays (kR) | | | | | | | |
| 10 | 48 | 50 | 1.04 | 46 | 2 | 95.8 | 4.2 |
| 15 | 51 | 56 | 1.09 | 46 | 5 | 90.2 | 9.6 |
| 20 | 49 | 54 | 1.10 | 44 | 5 | 89.8 | 10.2 |
| 25 | 13 | 6 | 1.23 | 10 | 3 | 76.9 | 23.1 |
| 30 | 21 | 22 | 1.05 | 20 | 1 | 95.2 | 4.8 |
| 35 | 26 | 27 | 1.04 | 25 | 1 | 96.2 | 3.8 |
| 40 | 17 | 18 | 1.06 | 16 | 1 | 94.1 | 5.9 |
| 45 | 12 | 13 | 1.08 | 11 | 1 | 91.7 | 8.3 |
| 50 | 3 | 3 | 1.00 | 3 | — | 100.0 | — |
| (ii) EMS (mM) | | | | | | | |
| 25 | 6 | 6 | 1.00 | 6 | — | 100.0 | — |
| 50 | 10 | 11 | 1.10 | 9 | 1 | 90.0 | 10.0 |
| 75 | 14 | 14 | 1.00 | 14 | — | 100.0 | — |
| 100 | 30 | 32 | 1.07 | 28 | 2 | 93.3 | 6.7 |
| 150 | 37 | 39 | 1.05 | 35 | 2 | 94.6 | 5.4 |
| 200 | 51 | 54 | 1.06 | 48 | 3 | 94.1 | 5.9 |

reported by Siddiq (1967) and Gopinathan Nair (1971).

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