Theoretical and Applied Genetics 44, 266–269 (1974)

Effect of X-irradiation on the Size of the Mutated Sector in Diploid and Tetraploid Rice

S. R. SREE RANGASAMY and K. SREE RAMULU

Department of Biology, Tamilnadu Agricultural University, Coimbatore (India)

Summary. An investigation was carried out of the effect of X-irradiation on the size of the mutated sector in three diploids (A.S.D.8, T.N. 1 and G.E.B. 24) and the autotetraploid of G.E.B 24 of *Oryza sativa* L., by analyzing the M_2 segregation ratios of chlorophyll mutants. A total of 612 segregating M_1 panicle progenies in the diploids and 284 panicle progenies in the tetraploid was studied in the M_2 generation for the ratio of normal to chlorophyll mutant seedlings. Of the 573 segregating M_1 panicles analysed by chi square test in the diploids, 206 showed good fit with a 3:1 ratio, while the rest of the progenies (367) deviated significantly in both directions from the expected 3:1 segregation. This was interpreted as the result of M_1 panicles developing from both single cell and multicellular initials following X-irradiation, the former segregating for 3:1, the latter significantly deviating from 3:1. An assessment of fertility (as judged from the number of M_2 seedlings per mutated M_1 panicle), related to the distribution of chi square deviations from the 3:1 ratio in the diploids, indicated that the M_1 panicle progenies deriving from single cell initials had low fertility but those originating from multicellular initials ranged from low to high fertility. A comparison of the distribution pattern of the M_2 segregation ratios of chlorophyll mutant phenotypes in the diploid and autotetraploid G.E.B. 24 indicated that, in the tetraploid, a larger sector and only one, or fewer cell initials than in the diploid species, are involved.

A knowledge of the size distribution of mutated sectors is of fundamental importance for the development of optimal selection procedures in mutation breeding of crops. The extent of chimaerism in a mutated M_1 panicle is likely to depend upon whether the bud meristem of that panicle was uni-or multi-cellular at the time of treatment. The size of the sector and the number of cells are also known to vary in pre- and post-formed spikes. The induction of mutations in rice has attracted wide interest in the last two decades due to the great success of the 'mutation method' in other crop species. Radiation induced mutations in rice were first reported as early as 1934 by Ichijima, who used X-rays and U.V., but the practical use of 'mutation process' in rice improvement was demonstrated by several workers only after 1953, as reported by Gustafsson and Gadd (1966). The initiation by IAEA in 1965 of a co-ordinated programme in rice stimulated studies on the induction of mutations. The present study is taken up with three diploid varieties and one autotetraploid of *Oryza sativa* L., to examine chimaera formation in the M_1 panicles after X-irradiation of dry seeds by analysing the M_2 segregation ratios of chlorophyll mutant phenotypes. The results presented in the following on the distribution of M_2 segregation ratios are confined to pre-formed M_1 panicles.

Material and Methods

Three diploid varieties (T.N.1, A.S.D. 8, G.E.B 24) and an autotetraploid of G.E.B. 24 of *indica* rice were used in the study. The autotetraploid of G.E.B. 24 was induced by treating the seeds with colchicine and is being maintained for fifteen years at this institute. The seeds collected from selfed panicles of the diploids and autotetraploid were used for X-ray treatment. Dry, and well filled seeds of uniform size with a moisture content of 11.5% were irradiated with X-rays. A Philips X-ray machine operated at 50 kV, 2 mA, without filtration, delivering at an exposure rate of 500 R/Sec., was used for X-irradiation. The seeds were spread in a single layer in petridishes with their embryos facing upwards during irradiation. Immediately after X-ray treatment, the seeds were sown in nursery beds and later on the seedlings were transplanted to the field. At flowering time, the first two panicles formed in each of the M_1 plants were self-fertilized and used to raise the M_2 population as panicle progenies. Chlorophyll mutations were scored in the M_2 when the seedlings were 8–12 days old in the field. The number of chlorophyll mutants (*albina, xantha* and *chlorina*) to the number of normal looking seedlings was determined for each of the segregating M_1 panicle progenies. The segregation ratio of normal to mutant seedlings was worked out in individual segregating M_1 panicles and was tested for goodness of fit with a 3:1 ratio by the chi square method. A total of 612 segregating M_1 panicle progenies in the diploids and 284 panicle progenies in the autotetraploid were analysed from the different treatments.

Results

Table 1 gives data on the percentage distribution of M_1 panicles showing M_2 segregation ratios in the diploids (A.S.D. 8, T.N. 1, G.E.B. 24) and the autotetraploid of G.E.B. 24, following treatments with various doses of X-rays. It is clear that there was a high percentage of M_1 segregating panicles showing a deficit of recessives in the diploids. However, in the

Table 1. *Percentage distribution of mutated M_1 panicles under each of the different M_2 segregation ratio-classes following treatments with different doses of X-rays in the diploids and autotetraploid*

X-ray dose	M_2 segregation ratio (%)						Total no. of M_1 mutated panicles analysed
	1–5	6–10	11–15	16–20	21–25	26 ≥	
A.S.D.8							
30 kR	31	37	11	11	5	5	62
40 kR	52	15	12	8	3	10	40
50 kR	51	12	16	7	7	7	57
Total	43	23	13	9	5	7	159
T.N. 1							
30 kR	74	10	2	6	4	4	49
40 kR	87	8	2	1	1	1	104
50 kR	60	21	6	7	—	6	115
Total	73	14	4	5	1	3	268
G.E.B. 24–2x							
30 kR	46	14	14	8	8	10	78
40 kR	55	15	15	4	4	7	54
50 kR	75	8	9	2	4	2	53
Total	57	13	13	4	6	7	185
All diploids total	61	17	9	5	3	5	612
G.E.B. 24–4x							
30 kR	5	3	10	5	6	71	87
40 kR	13	26	20	15	4	22	92
50 kR	21	12	8	10	13	36	105
Total	13	14	12	10	9	42	284

tetraploid, the percentage of M_1 panicles showing a deficit of recessives was low.

Table 2. *Frequency distribution of M_1 panicles showing M_2 segregation ratios (%) as related to number of M_2 seedlings per M_1 panicle in diploids (Data from A.S.D. 8, T.N. 1 and G.E.B. 24 are pooled)*

No. of M_2 seedlings per M_1 panicle	M_2 segregation ratio (%)						Total
	1–5	6–10	11–15	16–20	21–25	26≥	
10–20	2	45	22	20	15	32	136
21–30	65	14	9	3	2	4	97
31–40	13	15	7	2	1	3	41
41–50	33	6	4	4	3	4	54
51–60	40	11	5	1	—	5	62
61–70	44	9	4	2	2	1	62
71–80	42	14	2	3	—	—	61
81 ≥	41	9	1	2	3	4	60
Total	280	123	54	37	26	53	573

Table 2 gives data on the frequency distribution of M_1 panicles showing M_2 segregation ratios as related to M_2 fertility in terms of number of M_2 seedlings per an M_1 panicle in the diploids. It will be seen from these results that the M_1 panicles showing a high deficit of recessives are distributed among all the fertility-classes. Of the 573 segregating M_1 panicles analysed by chi square test in the diploids, 206 showed good fit with a 3:1 ratio with chi square values ranging from 0.00 to 3.841 ($n = 1$ and $P = 0.05$), while there was a significant deviation from the expected ratio of 3:1 in 367 M_1 panicles with chi square values ranging from 4.01 to 10.01 and above (Table 3). These results further show that the M_1 panicles showing a good fit with 3:1 had a low number of M_2 seedlings per M_1 panicle, while those showing ratios significantly deviating from 3:1 were distributed among all the fertility-classes.

The data relating to the diploid G.E.B. 24 and its autotetraploid are given in Table 4 for the frequency distribution of M_1 panicles showing M_2 segregation ratios as related to M_2 fertility, and in Table 5 on the frequency distribution of M_1 panicles showing chi square deviation from the 3:1 ratio related to M_2 fertility. These results show that, in the diploid G.E.B. 24, the segregating M_1 panicles showing a good fit for the 3:1 ratio represented 28 per cent compared with 80 percent in the autotetraploid. It is also evident from the data that, in the diploid G.E.B. 24, those panicles which showed good fit with a 3:1 ratio had low fertility, whereas those showing a high deficit of recessives were distributed among all fertility-classes. In the case of the autotetraploid, those M_1 panicles which showed a good fit with 3:1 had only 10–20 M_2 seedlings per M_1 panicle and those with a deficit of recessives showed 10–40 M_2 seedlings per M_1 panicle.

Discussion

The results on the distribution of M_2 segregation ratios of chlorophyll mutant phenotypes in the three diploid varieties of *Oryza sativa* L. showed that there was a high percentage of mutated M_1 panicles showing a deficit in recessives following treatments with various doses of X-rays (Table 1). Previous studies

Table 3. *Frequency distribution of M_1 panicles showing chi square deviation from 3:1 ratio as related to number of M_2 seedlings per M_1 panicle in diploids (pooled data from A.S.D.8, T.N. 1 and G.E. B.24)*

No. of seedlings per M_1 panicle	Chi square distribution												
	0.00–0.50	0.51–1.00	1.01–1.50	1.51–2.00	2.01–2.50	2.51–3.00	3.01–3.50	3.51–3.84	4.01–6.00	6.01–8.00	8.01–10.00	10.01 ≥	Total
10–20	17	34	30	8	11	5	12	6	13	—	—	—	136
21–30	4	4	3	10	—	—	2	4	30	38	1	1	97
31–40	1	11	2	3	1	2	4	—	7	4	4	2	41
41–50	2	6	1	1	—	—	—	—	12	5	6	21	54
51–60	—	—	—	—	—	—	—	2	8	—	—	52	62
61–70	2	1	1	—	1	—	—	—	—	1	—	56	62
71–80	—	—	—	—	1	1	3	—	3	—	3	50	61
81 ≥	3	—	—	5	1	1	—	—	—	2	2	46	60
Total	29	56	37	27	15	9	21	12	73	50	16	228	573

Table 4. *Frequency distribution of M_1 panicles showing M_2 segregation ratios (%) as related to number of M_2 seedlings per M_1 panicle in diploid G.E.B. 24 and its autotetraploid (Data relating to autotetraploid are given in parentheses)*

No. of M_2 seedlings per M_1 panicle	M_2 segregation ratio (%)						
	1–5	6–10	11–15	16–20	21–25	26 ≥	Total
10–20	— (—)	— (6)	4 (21)	6 (15)	15 (24)	14 (80)	39 (146)
21–30	19 (13)	10 (8)	8 (4)	2 (4)	5 (2)	2 (3)	46 (34)
31–40	47 (1)	6 (3)	— (4)	— (1)	— (—)	— (—)	53 (9)
41–60	21 (5)	7 (—)	1 (—)	— (—)	2 (—)	1 (—)	32 (5)
61–80	6 (—)	3 (—)	3 (—)	1 (—)	2 (—)	1 (—)	16 (—)
Total	93 (19)	26 (17)	16 (29)	9 (20)	24 (26)	18 (83)	186 (194)

Table 5. *Frequency distribution of M_1 panicles showing chi square deviation from 3:1 ratio as related to number of M_2 seedlings per M_1 panicle in diploid G.E.B. 24 and its autotetraploid (Data relating to autotetraploid are presented in parentheses)*

No. of M_2 seedlings per M_1 panicle	Chi Square distribution												
	0.00–0.50	0.51–1.00	1.01–1.50	1.51–2.00	2.01–2.50	2.51–3.00	3.01–3.50	3.51–3.84	3.85–6.00	6.01–8.00	8.01–10.00	10.01 ≥	Total
10–20	7 (22)	3 (11)	3 (35)	6 (35)	1 (9)	3 (3)	5 (20)	4 (—)	7 (11)	— (—)	— (—)	— (—)	39 (146)
21–30	2 (6)	— (1)	1 (1)	— (—)	— (—)	— (—)	— (—)	3 (2)	17 (19)	10 (3)	6 (2)	7 (—)	46 (34)
31–40	— (—)	— (—)	— (—)	3 (—)	— (—)	— (—)	2 (3)	— (—)	5 (4)	3 (2)	5 (—)	35 (—)	53 (9)
41–60	5 (5)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	2 (—)	2 (—)	2 (—)	1 (—)	20 (—)	32 (5)
61–80	2 (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	1 (—)	3 (—)	— (—)	10 (—)	16 (—)
Total	16 (33)	3 (12)	4 (36)	9 (35)	1 (9)	3 (3)	7 (23)	9 (2)	32 (34)	18 (5)	12 (2)	72 (—)	186 (194)

in rice (Nishjmura and Kurakami, 1952; Matsuo et al. 1958; Bekendam, 1961; Yamaguchi, 1962; Kawai and Sato, 1965), barley (Stadler, 1930; Moh and Smith, 1951; Caldecott and Smith, 1952; Gaul, 1960, 1961; Sarvella et al., 1962; Frydenberg et al., 1964; Frydenberg and Jacobsen, 1966) and wheat (D'Amato et al., 1962; D'Amato, 1965) have shown that the spikes of plants raised from seeds treated with mutagenic agents are usually chimerical for induced mutations. The chi square estimates worked out for each of the segregating M_1 panicles (Table 3) showed good fit with a 3:1 ratio in 206 M_1 panicles and a significant deviation from the expected ratio of 3:1 in 367 M_1 panicles. These results indicate that in diploid rice two kinds of initials are probably involved, one with a single cell and the other multicellular. The present findings are in agreement with the previous reports in rice that some panicles are descendants of a single initial cell, but others contain descendants of many initials (Nishjmura and Kurakami, 1952; Matsuo et al., 1958; Bekendam, 1961; Yamaguchi, 1962; Osone, 1963; Kawai and Sato, 1965). Previously, Yamaguchi (1962) also applied the chi square method on the expected 25 per cent segregation (3:1 ratio) to estimate the number of initials and reported that individual tillers arise from single as well as from multicellular initials. The number of cell initials contributing to the sporophytic tissue in barley spikes was determined by Stadler (1930), Moh and Smith (1951), Caldecott and Smith (1952), Gaul (1960, 1961), Sarvella et al. (1962), Frydenberg et al. (1964), Frydenberg and Jacobsen (1966); it was reported that some spikes originate from a single cell, while others develop from two or more cell initials in the dormant seed. Similarly in wheat, the number of initials involved may vary from one to two or more (D'Amato et al., 1962; D'Amato, 1965). Jacobsen (1966) concluded that in barley seeds there are nine meristems which can produce nine mutually exclusive sectors in the plant. Each embryo in barley was also estimated to contain three, five or six partially or fully developed tillers or spikes (Jacobsen, 1966).

Fertility in the irradiated M_1 plants was affected to a different extent depending on dose, and the segregating M_1 panicle progenies showed variation in fertility as adjudged from the number of M_2 seedlings per mutated M_1 panicle (Table 2 and 3). Most of the M_1 panicles that showed a good fit with a 3:1 ratio also showed a low number of seedlings per panicle. This may be due to the mutagenic treatments drastically affecting the cell initials at higher doses, leading to cell killing and reduction to one. The low fertility in mutated M_1 panicles, however, may be caused by the drastic action of mutagenic treatments on cell initials resulting in high gametic and zygotic sterility (Gaul, 1961; Moh and Smith, 1951; Yamaguchi, 1962). The mutated M_1 panicles showing ratios significantly deviating from 3:1 are, however, distributed through all the fertility-classes. These results show that the mutated M_1 panicles deriving from single cell initials had low fertility, while those panicles originating from multicellular initials possessed low as well as high fertility.

A comparison of the distribution pattern of M_2 segregation ratios of chlorophyll mutant phenotypes in the diploid and autotetraploid G.E.B. 24 indicated that in the tetraploid the percentage of M_1 panicles showing a deficit in recessives was lower than in the diploid (Table 4). The results on the chi square estimates indicated that about 80 per cent of the segregating M_1 panicles showed a good fit with the 3:1 ratio in the tetraploid compared with 28 per cent in the diploid G.E.B. 24 (Table 5). These results suggest that the size of the sector and the number of cell initials contributing to the sporophytic tissue in the tetraploid panicles differ from those in the diploid species. It is likely from the data presented that in the tetraploid a larger sector and one or fewer cell initials are involved compared with diploid rice. The low number of M_2 seedlings per M_1 panicle in the tetraploid is, however, due to its low fertility compared with the diploid.

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Received July 9, 1973

Communicated by Å. Gustafsson

Prof. S. R. Sree Rangasamy
Dr. K. Sree Ramulu *
Department of Biology
Tamilnadu Agricultural University
Coimbatore (India)

* present address:
Laboratorio Applicazioni Agricoltura
Comitato Nazionale per l'Energia Nucleare Centro di Studi Nucleari della casaccia casella postale 2400
00100 Roma A. D. (Italy)