INDUCTION OF MUTATIONS IN CULTIVATED RICE BY CHEMICAL AND PHYSICAL AGENTS

II. The Effect of M_1 Damage and M_2 Mutations[(1)]

SHIU-CHU WOO, CHI-MING NG and ING-JIUNN TUNG[(2)]

Abstract

Japonica variety Chianung 242 was treated with gamma irradiation (20–50 kr) and diethyl sulfate (pH 3, 7, 11) to study the sterility of M_1 plants and the morphological mutations of the M_2. Fertility of M_1 plants was affected by radiation treatments. It decreased with the increase of dose. Plant with poor fertility provided higher mutation frequency. Chlorophyll mutations were found at M_2 seedling stage. It did not associate with morphological mutations. Mutations induced by diethyl sulfate were less than those induced by gamma irradiations. The number of chlorophyll mutations decreased with pH level, however, the frequency of morphological mutations increased. No correlation was found between these two forms of mutations.

Introduction

Cultivated Taiwan rice has few inferior agronomic characters. It is therefore used as the experimental material to treat with mutagens in order to study the mutation mechanism of this organism and to discover the desirable mutations which may be used to improve the deficiency of the present variety. The chemical and physical treatments at seedling stage are discussed in our previous paper (Woo *et al.*, 1971). Chemical isopropyl methanesulfonate does not induce significant seedling height injury. However, diethyl sulfate (dES) causes severe damage when mutagen solutions were buffered at pH 3 and 7. Those buffered at pH 11, on the contrary, stimulate seedling growth. Gamma irradiation also gives significant seedling height injury, but seed germination is little affected. This paper reports the continuous result of our previous experiment initiated in 1970.

---

(1) This research was financially supported supported by the National Research Council of the Republic of China and the International Atomic Energy Agency, Vienna. Paper No. 114 of Scientific Journal Series, Inst. Bot., Academia Sinica.

(2) Associate Research Fellow and Assistants of the Institute of Botany, Academia Sinica.

Materials and Methods

Variety Chianung 242 (CN242, *Oryza sativa*, 2n=24, *japonica* type) was used as the experimental material. Treatments applied to the variety were indicated in our previous paper, experiments 4 and 5. M_2 seeds from the treatments were grown in flats to study chlorophyll mutation. Seedlings were transferred to nursery field after 35–40 days on seed beds. Sterility was studied during maturity. They were classified into four categories i. e., fertile (over 80%), semi-fertile (50–80%), semi-sterile (30–50%) and highly sterile (less than 30%). M_2 seeds harvested from each M_1 plant were randomly sampled and grown for 20 M_2 progenies. Morphological mutation frequency was based upon M_1 plant basis.

Results

The variation of M_1 fertility from treatments of gamma irradiation is given in Table 1. It is apparently shown that the levels of fertility varied with different doses of treatments. The fertility generally decreased with the increase of dose. At low doses of 20 and 25 kr treatments, the predominant M_1 plants were semi-fertile, i. e., 53.46 and 40.91% respectively. However, at 30 kr treatment the predominant group was semi-sterile. At higher doses of 40 and 50 kr, most of the M_1 plants were highly sterile.

Table 1. *Degrees of fertility in M_1 generation, gamma irradiation treatments.*

Dose (kr)	highly sterile		semi-sterile		semi-fertile		fertile		Total No. plants	Average fertility %
	No. pls.	% mut.	No. pls.	% mut.	No. pls.	% mut.	No. pls.	% mut.		
20	33	12.69	81	31.15	139	53.46	7	2.69	260	64.85
25	71	21.52	100	30.30	135	40.91	24	7.27	330	61.61
30	43	22.16	83	42.78	61	31.44	7	3.63	194	56.80
40	43	53.75	22	27.50	13	16.25	2	2.50	80	45.38
50	12	66.67	3	16.67	3	16.67	0	—	18	41.67
control	—	—	—	—	—	—	100	0	100	85.90

Of the result shown in Table 2, the average chlorophyll mutations from a single treatment on M_1 plant basis varied from 8.3–45.5%. The 50 kr treatment having the smallest number of 17 plants gave the highest mutation rate, while those of other treatments were independent with the doses. Mutations on M_2 seedling basis also showed the same trend with those calculated at M_1 plant basis. It differed from 0.43–6.57%. The 50 kr treatment gave the highest average mutation rate. Mutations from other treatments varied and were also independent with the doses. A high correlation coeffieient, r = 0.94 was found between the mutation rates calculated from M_1 plant basis and those at per 100 M_2 seedlings, indicating a consistent pattern of chlorophyll mutations induced from the mutated M_1 plants.

Of the spectrum of chlorophyll mutations found, albina, xantha and chlorina mutations were discovered. The albino was most frequent. Xantha seedlings happened at certain plant progenies. Chlorina mutation was least frequent of the three.

Table 2. *Chlorophyll mutations induced by gamma irradiation*

Doses kr	M_1 plant basis					100 M_2 seedling basis
	fertility %	%, No. pls.	Albina %	Xantha %	Chlorina %	%, No. pls.
20	> 80	12.5 (8)	22.2	66.6	11.1	1.13 (880)
20	50-80	8.5 (82)	31.5	31.5	36.8	0.53 (10835)
20	30-50	14.3 (42)	49.0	16.9	33.9	13.5 (3927)
20	< 30	30.0 (20)	66.6	26.6	6.6	1.36 (1100)
	Ave.*	13.2				0.80
25	> 80	11.5 (26)	0	100	0	0.18 (7098)
25	50-80	8.9 (78)	60.7	21.5	17.6	0.47 (10741)
25	30-50	8.5 (71)	18.9	56.7	24.3	0.48 (4603)
25	< 30	7.3 (41)	66.6	0	33.3	0.20 (1271)
	Ave.	8.3				0.43
30	> 80	1.7 (6)	100	0	0	0.14 (696)
30	50-80	16.7 (30)	95.4	0	4.5	1.16 (3792)
30	30-50	25.0 (36)	26.0	65.7	8.2	2.30 (3172)
30	< 30	19.1 (21)	56.2	9.3	34.2	2.61 (1228)
	Ave.	20.4				1.68
40	50-80	14.3 (14)	0	44.4	55.5	2.09 (2149)
40	30-50	20.0 (20)	30.0	50.0	20.0	0.84 (1190)
40	< 30	7.3 (41)	9.5	47.6	42.8	1.20 (1749)
	Ave.	12.00				1.49
50	50-80	66.7 (3)	69.7	0	30.2	8.74 (492)
50	30-50	66.7 (3)	95.6	4.3	0	12.50 (184)
50	< 30	9.1 (11)	0	66.6	33.3	0.80 (374)
	Ave.	45.5				6.57
Control	85.9	0 (50)	0	0	0	0 (4521)

r=0.94

$$* \text{ Average } \% = \frac{\text{Total numbers of mutated plants}}{\text{Total numbers of } M_1\ (M_2) \text{ plants}}$$

Of the result given in Table 3, the 20 kr treatment gave the lowest frequency of morphological mutations, average 29.7%. Mutations from other treatments varied from 47.4–57.5%, independent with the dose. M_1 fertility trended to associate with average number of mutations in M_2 generation. They differed from 32.5—63.8% as the fertility decreased from fertile to highly sterile. However, this tending did not always exist in 20 kr treatment. In this treatment fertile M_1 plants gave higher rate of mutations than those with lower fertility.

Table 3. *Number of morphological mutations induced by gamma radiations.*

Dose (kr)	Generations	M_1 fertility				Total (average %)
		fertile	semi-fer.	semi-ster.	H. ster.	
20	M_1 plants	8	80	42	18	148
	mutants (M_2)	3	22	13	6	44 (29.7)
	%	37.5	27.5	30.9	33.3	
25	M_1 plants	26	80	73	40	219
	mutants (M_2)	8	32	50	36	126 (57.5)
	%	30.8	40.0	68.5	90.0	
30	M_1 plants	6	30	42	19	97
	mutants (M_2)	2	14	21	9	41 (47.4)
	%	33.3	30.0	50.0	47.4	
40	M_1 plants	3	14	19	35	74
	mutants (M_3)	1	1	11	20	39 (52.7)
	%	33.3	7.1	57.9	57.1	
50	M_1 plants	—	3	4	7	14
	mutants (M_2)	—	—	2	5	7 (50.0)
	%			50.0	71.4	
Control		50				0
	mutants (M_2)	0				
total M_1 plants		43	207	180	119	
average mutants (M_2)		14	69	97	76	
%		32.5	33.3	53.8	63.8	

Of the result given in Table 4, M_2 seedlings from the pH 3 treatments have the highest mutation frequency, average 12.95%, and those from pH 11 the lowest, 3.9%. Within a single pH level, mutations increased with doses at pH 3. However, this tendency did not exist in pH 7 and 11. Mutations per 100 M_2 seedlings also showed their highest frequencies of 1.79 and 1.41% from treatments of 120 and 96 hours respectively at pH 3. Mutations from other treatments varied from 0.02–0.73%. A very high correlation coefficient, $r=0.92$ was also found between mutations calculated at M_1 plant basis and per 100 M_2 seedlings, indicating that all chlorophyll mutations were induced to the same pattern. That is mutated M_1 plants having a number of chlorophyll mutations rather than a large number of M_1 plants carrying a few mutation

Table 4. *Chlorophyll mutations induced by dES treatments in M_2 seedling stage*

treatment No.	pH	hours	M_1 plant basis				Per 100 M_2 seedlings
			%, No. pls.	Albina %	Xantha %	Chlorina %	%, No. pls.
1	3	120	40.0 (20)	41 4	46.3	12.1	1.79 (2290)
2	3	96	20.4 (98)	54.9	21.8	25.2	1.41 (10079)
3	3	72	16.2 (99)	48.2	20.0	31.7	0.56 (15159)
4	3	48	5.0 (100)	72.7	18.1	9.0	0.09 (12220)
5	3	24	5.0 (100)	0	57.8	42.1	0.37 (17080)
	Ave.*		12.95				0.60
6	7	120	15.0 (100)	87.5	7.1	4.3	0.36 (15393)
7	7	96	5.2 (96)	84.2	15.7	0	0.04 (46772)
8	7	72	10.0 (100)	59.5	19.0	21.4	0.34 (12240)
9	7	48	3.0 (100)	100.0	0	0	0.02 (19809)
10	7	24	9.8 (92)	31.0	41.3	27.5	0.73 (3976)
	Ave.		8.61				0.15
11	11	120	4.2 (96)	6.2	0	93.7	0.24 (6768)
12	11	96	2.1 (95)	60.0	0	40.0	0.04 (11495)
13	11	72	7.1 (99)	20.5	53.8	25.6	0.33 (11851)
14	11	48	3.1 (99)	76.9	7.6	15.3	0.18 (7260)
15	11	24	3.1 (98)	64.2	14.2	21.4	0.02 (9277)
	Ave.		3.90				0.18
control	6.2		0 (50)				0 (5847)

r=0.92

$$* \text{ Average } \% = \frac{\text{Total numbers of mutated plants}}{\text{Total numbers of } M_1 \text{ plants}} \times 100$$

seedlings or a mixture of both patterns.

Of the result given in Table 5, the treatment of pH 3 gave the lowest mutation frequency, average 8.49% and those of pH 11 provided a mutation of 19.58%, which was the highest of the three. Within a single pH level, the 120 hours treatment at pH 3 gave 40.9% mutation. On the other hand, at pH 7 and 11, the highest mutation frequencies, 25.3 and 27.6% respectively were induced by 72 hours treatments.

Discussion

Since most mutations are genetically recessive, they do not, in general, appear until M_2 or subsequent generations. Mutation frequency is generally low, thus, it needs a large population to discover a small numbers of mutations. Because of the existing of the these two barriers, the discovery of mutations is labor and time consuming. Therefore, for the purpose of reco-

Table 5. *Morphological mutations induced by dES at 3 pH levels*

Treatment No.	pH	hrs.	M_1		Mutations %
			No. plants	with mutated M_2 progenies	
1	3	120	22	9	40.9
2	3	96	92	7	7.6
3	3	72	100	10	10.0
4	3	48	98	6	6.1
5	3	24	100	3	3.0
Ave.					8.49
6	7	120	92	0	0
7	7	96	79	18	22.8
8	7	72	91	23	25.3
9	7	48	93	9	9.7
10	7	24	72	2	2.8
Ave.					12.18
11	11	120	77	13	16.9
12	11	96	95	22	23.2
13	11	72	58	16	27.6
14	11	48	81	11	13.6
15	11	24	67	12	17.9
Ave.					19.58
control	6.2	0	50	0	0

vering these two weaknesses, any characters at M_1 plant or M_2 seedling, which may be associated with mutations in later generations will be worthwile investigated. This aims for the purpose of making M_1 selections and of eliminating M_2 population size as well as of increasing mutation frequency.

In M_1 generation, sterility is usually shown during maturity. It is caused by treatments, indicating that the mutagen has affected the plant material. Mutagen is presumed to react with the germplasm as equal chance as to react with those of somatic cells. Mesken and Van der Veen (1968) reported that in *Arabidopsis* the M_1 fertility is correlated with M_2 fertility, suggesting that the fertility is inherited. It can be used as a selection marker in M_1 generation. Higher M_1 fertility gave better M_2 mutation frequency. It may be due to more progenies derived from highly fertile M_1 plants. Therefore, fertile M_1 plants furnish better chance for mutation discovery. However, in our experiment of rice, fertility decreases with dose increase (Table 1); the morphological mutations in M_2 generation increase with dose (Table 3). Other words, M_1 plants with poor fertility provide better mutation frequency in the M_2 generation. This may suit our assumption that those genes controlling fertility and those conditioning morphological mutations are equally affected by the mutagen. Therefore, those doses caused lower fertility would give higher mutation frequency although the survivals from the treatment of higher dose are fewer than those from lower dose.

Chlorophyll mutations can be observed in M_2 seedlings before transplantation. Doll and Sandfaer (1969) found that the frequency of barley morphological mutations was proportional to the chlorophyll mutations induced by ethyl methanesulfonate (EMS), dES, and gamma irradiation. If this correlation also exists in rice, the mutation frequency can be greatly increased by eliminating a large numbers of M_2 seedlings which carry no chlorophyll mutations. However, to our surprise, the chlorophyll and morphological mutations induced by gamma irradiation are independent with the doses. The two forms of mutations are not associated with each other (Table 2 and 3). This may suggest that chlorophyll and morphological mutations are controlled by genes with various locations, since the chlorophyll mutations are known to be controlled by a numbers of genes located on several chromosomes (Desai, 1969) as well as conditioned by genes in cytoplasmic organelles.

Mutation frequency induced by dES is generally lower than those by gamma irradiation. This is different with EMS used by Marki and Morea (1970) in flax and Rao (1969) in barley. They reported a higher frequency of chlorophyll mutations in the progeny of EMS treated plants than those treated with gamma irradiation. Levels of pH affect chlorophyll and morphological mutations. However, the frequencies are reversed (Tables 4 and 5). The chlorophyll mutation decreases with pH level, and on the other hand, the frequency of morphological mutations increases with pH. No correlation was found between these two forms of mutations. This may be because the genes controlling chlorophyll formation react differently from those conditioning morphological characters with the chemical mutagen, dES.

Mutagen buffered at high pH level probably would give less physiological damage to genes located at cytoplasmic organelles. Therefore, more M_1 plants having mutation can survive. This agrees with our results of seedling test. It indicates that seedling heignt injury is much less at high pH level rather than at low pH.

The spectrum of chlorophyll mutations can not be concluded at this study. In general the albina mutation is most common and the chlorina the least. This may differ with the nature of mutagens and the species of the cultivars as well (Rao, 1969).

水稻化學及物理誘變之研究

II. M_1代之損傷和M_2代誘變率關係之研究

吳旭初 吳智明 董英俊

用 gamma 線，20–50 kr 及化學品 diethyl sulfate (pH 3, 7, 11)，處理臺灣蓬萊稻嘉農 242 號，以研究處理第一代 (M_1) 之稔實性和第二代 (M_2) 形態誘變率之關係。

處理第一代之稔實性，受放射線之影響，隨放射量之增加而遞減。稔實性較低之 M_1 植株，其 M_2 代之誘變率較高。葉綠素誘變體出現於秧苗期，但和形態誘變率無相關。

用 diethyl sulfate 劑之誘變率較 gamma 線爲低，該劑配合低 pH 緩衝劑使用時，葉綠素誘變率較高 pH 時爲高，但形態誘變率却相反。這兩類誘變率無相關。

Literature Cited

DESAI, P. A. 1969. Induced mutations in *Triticum durum*. Indian J. Genet. Plant Breeding **29**: 115-122.

DOLL, H., and J. SANDFAER 1969. Mutagenic effect of gamma-rays, diethyl sulfate, ethyl methanesulfonate, and various combinations of gamma-rays and the chemicals. Induced Mutations in Plants (IAEA-SM-121/5) p. 195/205.

MARKI, A., and M. BIANU MOREA 1970. Gamma-rays and EMS-induced mutations of flax. Genetika **6**(1): 24-28.

MESKEN, M., and J. H. VAN DER VEEN 1968. The problem of induced sterility. A comparison between EMS and X-rays in *Arabidopsis thalians*. Euphytica **17**: 363-370.

RAO, M. V. P. 1969. Alteration in the rate and spectrum of chlorophyll mutations induced by ethyl methanesulfphonate in wheat. Gurr. Sci. (India) **38**: 22-23.

WOO, SHIU-CHU, CHI-MING MG, and EE-MOOI TYE 1971. Induction of mutations in cultivated rice by chemical and physical agents. 1. The effects of isopropyl methanesulfonate, diethyl sulfate, and gamma-radiation on seed germination. Bot. Bull. Acad, Sinica **12**: 10-23.