MUTATIONS IN RICE INDUCED BY X-RAYS

(A PRELIMINARY REPORT)

by

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The early studies of X-ray treatment in rice were mostly aimed to induce genic changes, while structural changes of the chromosomes were rarely reported, though Ramiah and Parthasarathy (1935) found chromosome ring in X-rayed rice.

Since Yamashita (1949) advocated that reciprocal translocations induced by X-rays were usable for linkage analysis, Nishimura (1950), Oka (1953) and Chang (1955) began to study this problem and some reciprocal translocation stocks were obtained.

For the purpose of isolating reciprocal translocation lines for linkage group analysis, writers initiated an experiment to treat rice with X-rays in 1947 and again in 1954 and have obtained mutants such as semi-sterile rice, dwarf plants, chlorophyll deficiencies and others. The general characteristics and inheritance of these mutants, and the effect of different dosages of X-rays on the quantitative characters will be reported in this paper.

Materials and the conditions of X-ray treatments are shown in table 1.

Table 1. Conditions of X-ray treatment

	1st treatment	2nd treatment
Variety	Taichung no. 65, Pei-ko-fa-lo	do
Date	Feb. 14, 1948	Feb. 17, 1954
Place	Plant Breed. lab. of Taiwan University	Taiwan University Hospital
Seeds	Germinating seeds	do
Current	4 m.a.	5 m.a.
Voltage	80 k.v.	100 k.v.
Filter	no	¼Aluminum filter (Beryllium window) open (no cone)
Distance	30 cm.	
Duration of treatment	50, 60, 70, 80, 90, 100, 120 min.	19 min. 15 sec., 26 min. 56 sec.,30 min. 5 sec., 34 min. 27 sec.
Dosage		5,000r, 7,000r, 8,000r, 10,000r.
Temperature	Indoor temp.	do

Growth conditions, percentage of the lethal seedlings, plant height and number of tillers were observed in the 1st generation (X1). Panicles of X1 generation were harvested individually as the different X2 lines. Special care was paid to the different kinds of mutants.

1. Effect of different dosages of X-rays upon the frequencies of lethal seedlings, mutants and quantitative characters

The percentage of lethal seedlings in X1 generation was ranged from 22 to 100% and increased as the duration of X-ray treatment was prolonged. This is shown in table 2. The seedling growth of treated plants was slower than that of untreated ones.

Table 2. Percentage of lethal seedlings in X1 generation

Variety \ Duration of X-ray treatment (min.) / Percentage of lethal seedlings	80	90	100	110	120	Control
Taichung no. 65	22	92	92	84	98	0
Pei-ko-fa-lo	60	90	92	86	100	0

In some cases, seedlings with twisted leaves were observed but recovered to normal condition before long. As mentioned above, dwarf, semi-sterile, variegated, albino and other mutants were found in X1 generation. Their frequencies are given in table 3.

Table 3. Frequency of occurrence of mutants in X1 generation

	No. of fertile plant	No. of semi-sterile plant	No. of dwarf plant	No. of other plant	Total no. of plant observed
No. of plant observed	854	134	2	18	1,008
Percentage of mutant	85.2	13.2	0.2	1.4	100

Different degrees of chlorophyll deficiencies were found in X1 generation of Taichung no. 65 in lst treatment. These abnormal chlorophyll deficiencies were classified into three classes; namely, cream, variegated and albino plants. The cream plants showed light yellowish white coloration and without chlorophyll content. Albino plants were colorless, being wholly lack chlorophyll and died when the plant heights reached 5–10 cm. Variegated plants showed to have 1 to 3 colorless strips along the vein of leaves and also in leaf sheathes. Such variegated seedlings were viable except a few ones which dies at the early growing stage. Sometimes twisted leaves were found among the variegated plants. In some cases, the white strips turned green at the late growing stage. These chlorophyll deficiencies induced by X-rays might be due to the plastid mutations as explained by Imai (1935).

The rate of occurrence of chlorophyll deficiencies in the seedlings of X1 generation of X-rayed rice is given in table 4. The table shows that there were no clearcut relations between the rate of chlorophyll deficiency occurrence and the duration of

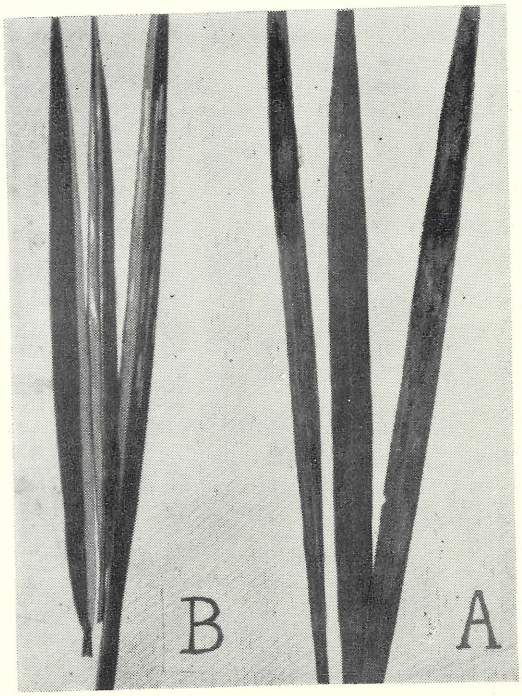


Fig. 1. Chlorophyll deficiency in X-rayed rice
A. Normal leaf
B. Variegated leaf

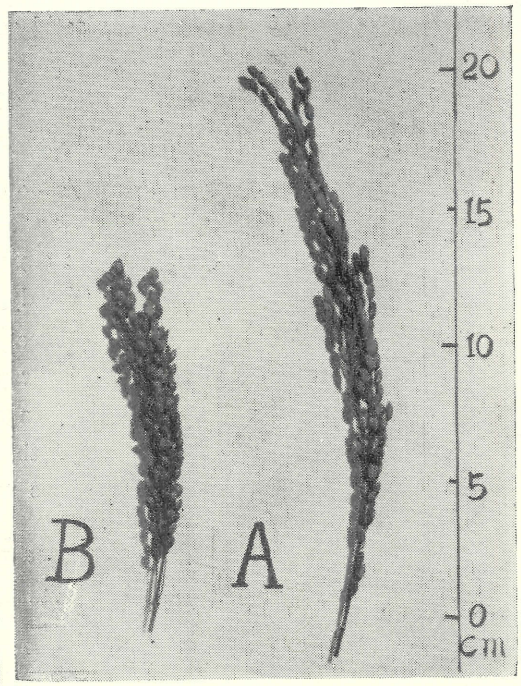


Fig. 2. Panicles of rice
A. Normal panicle of Taichung no. 65.
B. Panicle of dwarf rice induced by X-rays

Table 4. Rate of occurrence of chlorophyll deficiencies of X-rayed strains in X1 generation

Duration of X-ray treatment \ leaf color / No. of seedlings and percentage	Green	Cream	Variegated	Albino	Total no. of plant
50 min.	1492 (85.1)	87 (4.9)	48 (8.4)	27 (1.6)	1654 (100)
60	503 (87.9)	5 (0.9)	58 (10.1)	6 (1.1)	572 (100)
70	110 (91.6)	0	10 (8.4)	0	120 (100)
80	251 (86.8)	26 (8.9)	9 (3.1)	3 (1.2)	289 (100)
90	108 (99.1)	0	1 (0.9)	0	109 (100)
Total	2464 (89.8)	118 (4.1)	126 (4.6)	36 (1.5)	2744 (100)
Control	458 (98.3)	0	8 (1.7)	0	466 (100)

X-ray treatments. Therefore, this plastid mutations seemed to be relatively easy to occur regardless of dosages of X-ray applied.

Seedling heights (measured on March 22, i.e. 35 days after sowing), plant heights (measured on July 7, i.e. 110 days after sowing), and the number of tillers were observed in the field while the length and weight of panicles were calculated in the laboratory after the harvest. These results are given in table 5. It will be seen in table 5, that the seedling heights were supressed differently by different dosages of X-rays. There was a tendency that, the degree of X-ray supression to seedlings was increased by the increase of X-ray dosages. The rate of seedling growth was varied differently according to the dosages of X-rays. The range of variation was wide in 10,000r treatment, being less in 7,000r treatment. The table also indicates that the effects of different dosages of X-rays on the plant heights, number of tillers, number of panicles, length and weight of panicles were not very distinct.

Table 5. Effect of different dosages of X-rays on the quantitative characters in rice

Treatments	Average of each character						
	Seedling height (cm)	Plant height (cm)	No. of tillers	No. of panicles	Length of panicles (cm)	weight of panicles (gr)	Total no. of plant calculated
Taicung no. 65							
7,000r	16.2	113.5	11.9	8.6	23.2	3.8	180
8,000r	14.4	113.3	10.6	8.6	23.2	3.9	200
10,000r	14.6	112.4	10.5	7.8	23.7	3.2	60
Control	16.8	114.3	11.7	8.4	23.7	3.5	50
Pei-ko-fa-lo							
7,000r	14.0	137.0	10.8	7.9	23.4	3.5	130
8,000r	17.8	133.0	9.2	7.4	23.3	3.4	170
10,000r	17.7	140.0	10.2	7.6	23.7	3.6	60
Control	18.2	140.0	10.5	7.6	23.7	3.6	50

2. Variation of fertility and its inheritance

As mentiond above, there occurred semi-sterile plants in X1 generation. The rate of occurrence of semi-sterile plants was different according to different dosage of X-ray treatments. The fertility was different in different plants and even different in the different panicles within a plant (table 6). It will be seen in table 6 and fig. 3, that the fertility was reduced when seeds were treated with strong dosage of X-rays. If aiming to obtain semi-sterile rice plants, at least 5,000r of X-ray is necessary for germinating seeds and more than 10,000r will get better results.

Table 6. Effect of different dosages of X-rays on the fertility in X1 generation

Treatments \ Fertility in % / Freq. of single panicle in %	10	20	30	40	50	60	70	80	90	100	Total (%)	Total no. of panicles calculated
Taichung no. 65												
5,000r	3.9	4.2	5.1	7.0	8.3	8.7	11.7	13.7	20.4	17.0	100	2,162
7,000r	9.1	4.6	5.8	4.9	7.9	7.6	8.6	10.2	18.6	22.5	100	1,586
10,000r	8.3	5.8	8.3	2.0	9.9	8.8	9.7	7.2	10.2	19.9	100	433
Control	0	0	0	0	1.4	2.6	5.4	25.2	32.4	32.8	100	425
Pei-ko-fa-lo												
7,000r	12.8	10.1	10.5	9.7	9.5	8.8	12.5	9.9	9.8	6.5	100	891
8,000r	12.1	9.2	8.3	9.8	8.6	9.7	10.1	11.8	12.7	7.6	100	1,402
10,000r	16.5	9.7	7.8	10.7	9.6	7.7	9.4	10.1	12.3	6.1	100	934
Control	3.7	3.0	7.4	11.1	6.7	8.1	15.5	12.6	16.3	15.6	100	135

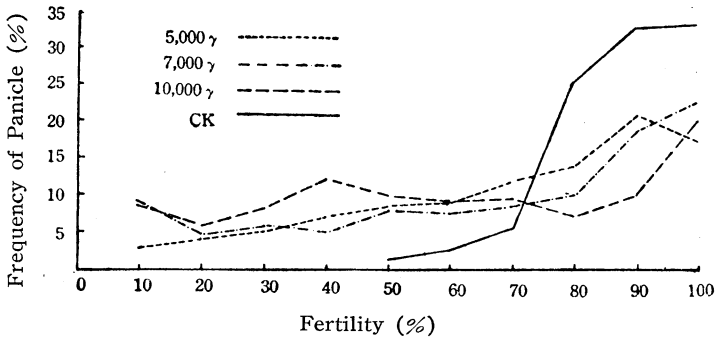


Fig. 3. Effect of different dosages of X-rays on the fertility in X1 generation (Taichung no. 65)

As mentioned above, the different panicles of semi-sterile plants of X1 generation were harvested seperately, and their fertility seemed to show both continuous and discontinuous variations. Some lines of those showing discontinuous variation in fertility, segregated the fertile and semi-sterile plants in approximately 1 to 1 ratio in the X2 generation. In the X3 and later generations the fertile plants were found to breed true, but the semi-sterile plants continues to segregate in the same manner as in the X2 generation (table 7). Such a behavior of sterility to be of the same type as reported by Oka (1953), Chang (1955) and others [6,8]. Based on the result of cytological observation, 10 to 90% of ring, chain and other types of quardrivalent chromosomes (Unpublished) found in the semi-sterile plants of the said lines by ordinary aceto-carmine smear method, the semi-sterility was proved to be attributed to the segmental interchange.

Table 7. Segregation of fertile and semi-sterile plants in semi-sterile rice

Strains	Fertility in Semi-sterile plant(%)	Fertility of self-pollinated plants (%)										Total no. of plant
		10	20	30	40	50	60	70	80	90	100	
B S1-3-1	35.3				10 (14)	4		2	5 (16)	8	1	30
B S2-3-1	33.9	1		2 (16)	7	5	1		1	14 (19)	4	35
B S3-3-1	28.8		1	2	7 (17)	2	5	5 (7)	2			24
B S5-1-1	23.0			1	8 (13)	4		2	5 (14)	7		27
B S6-3-4	33.0	1		(14)	8	5		3	2 (12)	6	1	26
B S10-2-7	33.8			2	6 (12)	4		4	4 (13)	3	2	25
B S11-1-20	33.8				2 (4)	2			2	2 (5)	1	9
B S12-1-1	21.8			4	(5)	1				2	3 (5)	10
B S13-2-1	26.5			2	6 (9)		1	1	3 (11)	4	3	20
B S14-1-1	29.5			7	6 (14)	1			3	8 (12)	1	26
B S16-1-1	29.4			1	4 (15)	8	2		1	5 (12)	6	27
B S17-3-2	27.3			6 (13)	7				1	7 (14)	6	27
B S18-2-18	24.0			1	7 (16)	8			3	7 (12)	2	28
B S19-2-2	26.0			4		3 (13)	6	6	3 (15)	6		28

3. Occurrence of dwarf plant and its inheritance

As indicated before, two dwarf plants were obtained from 80-minute treatment of Taichung no. 65. Panicles of these plants were short, grains were small in size but fertility was normal. No segregation was taken place in the subsequent generations after selfing, and all characters shown to breed true. Table 8 shows differences of general characterisics between Taichung no. 65 and dwarf rice.

Table 8. Comparison of general characteristics between Taichung no. 65 and dwarf rice

Items	Original Taichung no. 65	Induced dwarf mutant
Plant height (cm)	100.3	75.5
No. of tillers	8.5	6.7
Length of boot leaf (cm)	36.5	27.8
Width of boot leaf (cm)	1.4	1.8
Length of panicle (cm)	20.6	15.5
Weight of panicle (g)	3.4	2.8
Average density of panicle	7.2	11.2
Weight of 1,000 grains (g)	27.9	20.9
Length of hulled rice (mm)	5.2	4.2
Width of hulled rice (mm)	3.1	2.8
Thickness of hulled rice (mm)	2.1	2.0
Percentage of fertile grain	92.3	83.3

In order to know genetic behavior of dwarf rice, it was crossed to original variety Taichung no. 65. F1 plants were normal but normal and dwarf plants were segregated into 3 to 1 ratio in the F2 generation (table 9). This shows that dwarfism is controlled by single gene and that the dwarf gene is recessive. This indicates that dwarf rice might be resulted from genic change as it could be expressed as A→a. Whether there were small chromosome deficiencies which were hard to recognize under microscope, was a problem of further studies.

Table 9. Segregation ratio of F2 in the "hybrid between Taichung no. 65 and dwarf rice

	Normal plant	Dwarf plant	Total
Expected (3:1)	56.25	18.75	75
Observed	54	21	75

$X^2=0.36$ d.f.=1 $0.5<P<0.7$

It is concluded, according to the observation, that those semi-sterile lines, which segregated fertile and semi-sterile plants in approximately 1 to 1 ratio and showed the occurrence of ring chromosomes, might be reciprocal translocation heterozygotes. If plants of these semi-sterile strains were crossed to the original Taichung no. 65, both fertile and semi-sterile plants should be expected as the same manner as the selfing of semi-sterile plants. If the original variety were crossed to fertile plants, either fertile or sterile plants could be produced. Whether these lines fitted this genetical behavior, further cytological observations and test cross are necessary for comfirmation. As reciprocal translocation lines are useful materials for linkage analysis and for other genetic studies, the writers are now on the way to isolate more strains of this kind.

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RÉSUMÉ

The aims of these experiments are to obtain mutations by X-ray irradiation and to take advantages of the treatment thus induced for linkage analysis. Germinating seeds of two varieties, Taichung no. 65 (a representative of Ponlai variety or Japanica type in Taiwan) and Pei-ko-fa-lo (a native variety of lst crop nature) were used. Dose

of X-rays: lst treatment; 4 m.a. 80 k.v. 30 cm, 50–120 minutes. 2nd treatment; 5 m.a. 100 k.v., 19–35 mimutes, 5,000r, 7,000r, 8,000r and 10,000r. The percentage of lethal seedlings of the X1 generation was ranged from 22 to 100% being related the dulation of irradiation. The fertility of X1 generation tended to decrease markedly with the duration of irradiation.

The frequency of chlorophyll deficiencies was quite high in the treated Taichung no. 65. in the X1 generation. The effect of different dosages of X-ray treatment on various quantitative characters was not very distinct.

Dwarf rice as tall as 3/4 of the original variety was obtained among the X2 generation of Taichung no. 65 from 80-minute treatment. It was crossed to original variety. The F2 generation fitted 3 to 1 segregation ratio. Part of the segregating progenies of semi-sterile rice showed continuous variation, and the mechanism involed is rather complex and remained to be elucidated. Part of semi-sterile strains showed discontinuous variation in fertility, which segregated fertile and semi-sterile plants in approximately 1 to 1 ratio and showed the occurrence of ring chromosomes considered to be attributed to the segmental interchange.

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水稻經 X 線處理後所發生之突然變異

（第 一 報）

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摘　　要

爲誘發染色體構造的變化，以獲得多數不同之相互轉座系統，進一步作稻之連鎖分析爲目的，乃舉行本試驗。試驗材料爲臺中 65 號（蓬萊種）及白殼花螺（在來種）之發芽種子。X線處理分爲兩次：第一次之處理條件；電壓 80 kv，管電流 4 ma. 距離 30 cm 時間 80–120 分，第二次處理條件；電壓 100 kv，管電流 5 ma. 時間10–35 分，放射線量爲5,000r, 7,000r, 8,000r, 10,000r 等四種。

處理當代幼苗之致死率爲 22–100%，隨X線量照射時間而增，處理當代（80 分處理區）不稔個體之發生頻度爲 13.2% 矮稻之發生率爲 0.2%。

在處理第一代臺中65號之幼苗發現淡黃色苗(Cream)，條紋苗(Variageted)，及白苗 (Albino) 等葉綠素缺乏之系統。淡黃色苗及白苗生長後不久即告死亡，但條紋苗可繼續生長，此種變異體可能爲色素體之變異 (Plastid mutation) 所致，不同X線量對於第一代生長初期之苗高有抑制影響外，對收穫前的各量性狀之影響不顯明。

在X線處理後代中發現矮稻，該稻較原來品種株高低矮，穗短而小，粒小而密，結實爲正常。矮稻出現後不再有分離現象，故此種突變體爲已固定者。爲明瞭矮稻發生之機構及其遺傳行爲，曾將之與原來品種雜交，在 F2 分離出正常稻與矮稻，其比例符合於理論值 3:1 之比率，由此而觀，矮稻可能由於因子之劣變所致。

處理後代所生的半不稔稻自交後分離爲結實稻及半不稔稻，其比例爲 1:1。結實率高者不再有分離現象，但半不稔稻復以 1:1 之比例分離出結實稻與半不稔稻兩種，以後之分離情形相同。此種結實率表示不連續變異之系統以細胞遺傳之觀察結果，已知由於染色體構造變化所致。對於結實率表示連續變異者，所包括的機構較複雜，詳細情形尙待研究。