EFFECT OF X-RAYS AND THERMAL NEUTRONS ON CHROMOSOMES OF RICE

Mutations in Rice Induced by X-rays IV

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Many observations have been reported regarding the effects of X-rays and other radiations on rice plants (Komuro, 1931; Imai, 1935; Nishimura, 1950, 1952; Nagamatsu, 1950; Chang, 1955, etc.). Recently, the effect of thermal neutrons produced by nuclear reactors has also been observed (Matsuo, 1955; Caldecott, 1952 and 1954, in barley; Ygyu *et al.*, in tomato). From the results of observations of chromosomal aberrations and damage of seedling, Caldecott *et al.* (1952) assumed that thermal neutrons might give a more serious effect than X-rays. Matsuo (1955) made a comparative study of biological effects of X-rays and thermal neutrons in rice, by estimating the frequencies of segmental interchanges of chromosomes from fertility variation. The present author (Hsieh, 1957) has formerly observed ring chromosomes in pollen mother cells of semi-sterile rice plants which had been induced by X-rays, and have isolated reciprocal translocations. In this work, however, he could not work out the relation between frequency of segmental interchanges and the dosage of different kinds of radiations. Intervarietal difference in radio-sensitivity was also a problem to be studied.

With the view to acquire more informations on these problems, many rice plants treated with X-rays and thermal neutrons were observed with respect to chromosomes in pollen mother cells. The results are reported in this paper. The writer expresses his hearty thanks to Dr. Hikoich Oka of National Institute of Genetics, Japan, for a reading of the manuscript.

Materials and Methods

Seeds of five Japonica varieties, Taichung No. 65, Taichung No. 155, Kaushung No. 27, Chianan No. 8, and Chianunyu No. 242, were treated with X-rays and thermal neutrons in the Brookhaven National Laboratory, New York, U. S. A. in May 1959. Regarding these treatments, the author is obliged to the authorities of the above given institution and of JCRR for their kind arrangement and cooperation, and also to Mr. C. H. Hwang of this Institute who prepared the seeds for treatment and transferred the materials to the author.

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The dosages of X-rays applied were 20,000 r and 25,000 r. The thermal neutron irradiation was made in the thermal column of a nuclear reactor, exposing the seed to neutrons at the flux value of 3.60×10^{13} nth/cm^2 for 15 hours, and 4.90×10^{13} nth/cm^2 for 20 hours. The materials thus irradiated were sent back to our laboratory in June, 1959. Plants were then raised from seeds and were grown in an experimental field, and their ratoons were kept for two years in the same field until our cytological observations were completed.

For cytological observations, two young panicles from each plant were fixed with Farmer's fluid for 24 hours, and were preserved in 70% alcohol. Staining was made by the aceto-carmine smear method. 20–30 PMCs of each panicle were observed.

Results and Discussion

In plants treated with X-rays as well as with thermal neutrons, the major chromosomal change found in pollen mother cells was ring chromosomes. Their frequencies in different varieties are given in Table 1. As shown in the table, the frequency of segmental interchanges caused by X-rays ranged from 29% to 50%. That caused by thermal neutrons ranged from 33% to 59%. Dosage of radiations needed for producing 1% of chromosomal interchanges was calculated to be approximately 600 r with X-rays, that was 0.10×10^{13} nth/cm^2 with thermal neutrons. The biological equivalence value for chromosomal interchanges then, was estimated to be $1\,r=1.6\times10^9$ nth/cm^2 of thermal neutron. The value calculated by Matsuo (1958) based on 5% chlorophyll mutation was $1\,r=1.35$ 10^9 nth/cm^2 and that based on fertility was $1\,r=0.85\times10^9$ nth/cm^2. As expected, plants irradiated at higher dosage showed higher frequency. These percentages seem to be of the same order as that found in barley by Caldecott (1953), 31% after 24,000 r of X-rays.

The number of ring chromosomes per cell ranged from zero to three, as shown in Table 2. A ring of four was sometimes broken to form a chain-shaped quadrivalent (Fig. 1 C, H) or of other shapes (Fig. 1 D), but such was also counted as a ring.

The ring of six which showed interchanges in different arms of two different chromosomes was occasionally found with low frequency (Fig. 1, G), and were not included in Table 1.

Intervarital difference in the frequencies of segmental interchanges, and in fertility, were tested by analysis of variance, after arc-sine transformation of the data. The results are given in Table 4. The data in the table suggest that the frequency of chromosome aberrations may differ according to varieties, especially in plants treated with X-rays.

Table 1. Effects of X-rays and thermal neutrons on dormant seeds of rice as measured by frequency of chromosome aberrations in microsporocytes.

Varieties	Dose of radiation	Frequency of Quardrivalent*		Total No. of cells observed
		Total	%	
	X-ray			
Taichung No. 65	20,000 r	250	29.10	859
	25,000 r	237	41.00	578
	Control		0	332
Taichung No. 155	20,000 r	224	35.10	638
	25,000 r	340	47.09	722
	Control		0	85
Kaushung No. 27	20,000 r	245	31.17	786
	25,000 r	296	33.04	896
	Control		0	150
Chianan No. 8	20,000 r	163	29.26	557
	25,000 r	243	32.40	750
	Control		0	172
Chianunyu No. 242	20,000 r	217	45.20	480
	25,000 r	479	50.10	956
	Control		0	170
	Thermal neutron ($\times 10^{13}$nth/cm^2)			
Taichung No. 65	3.6	226	37.29	606
	4.9	370	59.01	627
Taichung No. 155	3.6	294	44.74	657
	4.9	1,048	54.30	1,930
Kaushung No. 27	3.6	582	32.76	1,776
	4.9	416	41.03	1,014
Chianan No. 8	3.6	416	30.81	1,350
	4.9	608	40.47	1,502
Total		6,654	42.81	16,684**

* One, two or three rings of four were seen in a cell as mentioned in the text.
** Not including controls.

Though X-rays and thermal neutrons differ in physical nature, the present study indicates that their effects on chromosomes of rice are quite similar. Both bring about segmental interchange and univalents in pollen mother cells of the first generation plant. A segmental interchange is considered to be due to two simultaneous breaks of chromosomes, which may or may not be caused by a single ionizing particle. Univalents were found in addition to quardrivalent and they may be due to early separation of homologous chromosomes once paired in the pachytene stage. It may be inferred that minor structural changes of chromosomes caused by radiation hinder the formation of chiasmata. Various chromosomal changes may be produced in irradiated cells, but it is possible those changes are rearranged in the course of development, if the plant survives from the damage and grows. A part of the aberrations produced in embryos of seeds may be eliminated before meiosis. Gametic selection may also eliminate aberration. The behavior of radiation induced chromosomal changes in a plant as well as in a population of plants is a problem in which the writer is interested after carrying out this observation.

Table 2. Varietal difference in frequency of quardrivalent chromosomes. (Data due to different doses are pooled).

Variety	X-ray				No. of cells observed	Thermal neutron				No. of cells observed
	0	1_{IV}	2_{IV}	3_{IV}		0	1_{IV}	2_{IV}	3_{IV}	
Taichung No. 65	961 (66.9)	465 (32.4)	11 (7.65)		1,437	662 (53.69)	546 (44.28)	25 (2.03)		1,233
Taichung No. 155	810 (59.6)	535 (39.3)	15 (11.08)		1,360	1,343 (51.9)	1,153 (44.57)	89 (3.44)	2 (0.08)	2,587
Kaushung No. 27	1,152 (68.5)	519 (30.9)	8 (4.76)	3 (1.78)	1,682	1,859 (66.6)	864 (30.97)	67 (2.40)		2,790
Chianan No. 8	923 (70.6)	362 (27.7)	22 (16.8)		1,307	1,737 (60.9)	1,032 (36.19)	77 (2.69)	6 (0.21)	2,852
Chianunyu No. 242	834 (58.1)	524 (36.5)	62 (4.31)	16 (1.11)	1,436	—	—	—	—	
Total	4,680	2,405	118	19	7,222	5,601	3,595	258	8	9,461

* Figures in parenthesis are percentages.

Table 3. Frequencies of cells with different numbers of ring chromosomes.

Kinds of radiation	Doses	Type of interchange								Total No. of cells observed
		0		1_{IV}		2_{IV}		3_{IV}		
		No.	%	No.	%	No.	%	No.	%	
X-ray	20,000 r	2,272	68.43	998	30.06	49	1.48	1	0.03	3,320
	25,000 r	2,411	61.78	1,406	36.03	68	1.74	17	0.44	3,902
Total		4,683	64.84	2,404	33.23	117	1.62	18	0.25	7,222
Thermal neutrons $(x10^{13})/cm^2$	3.6	2,962	67.49	1,341	30.55	86	1.95			4,389
	4.9	2,819	55.57	2,074	40.88	172	3.39	8	0.16	5,073
Total		5,781	61.09	3,415	36.09	258	2.73	8	0.09	9,462
Control		909	100.00	0	0	0	0	0	0	909

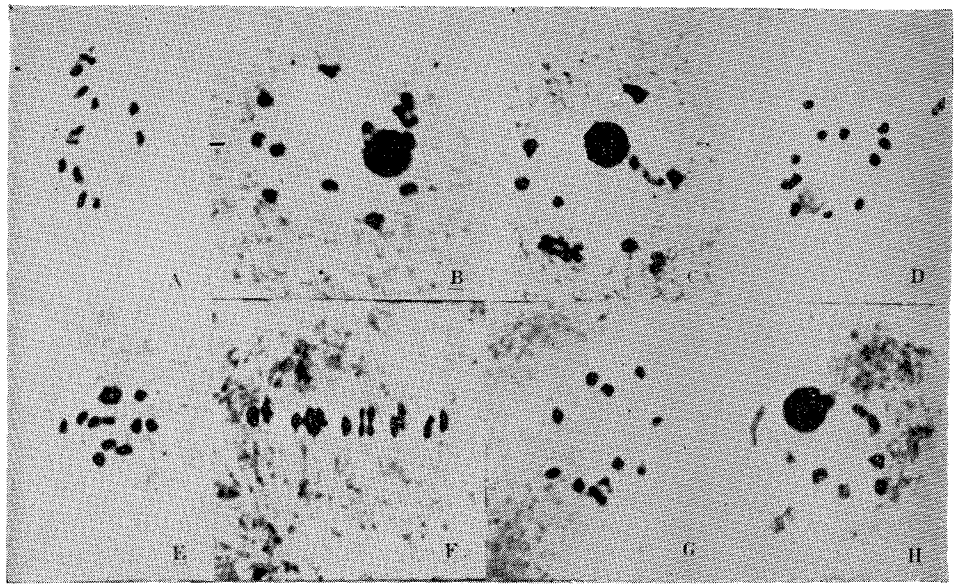

Fig. 1. Quardrivalent chromosomes due to induced segmental interchange in pollen mother cells of rice.

(A) 12_{II}; Metaphase-I
(B) $1(\cdot)4+10_{II}$; Diakinesis
(C) $1(\cdot)4+1$ chain$+8_{II}$; Diakinesis
(D) $1_{IV}+10_{II}$; Diakinesis
(E) $1(\cdot)4+10_{II}$; Metaphase-I
(F) $1(\cdot)4+10_{II}$; Metaphase-I (side view)
(G) $1(\cdot)6+9_{II}$; Metaphase-I
(H) $1_{IV}+2$ chains$+6_{II}$; Diakinesis.

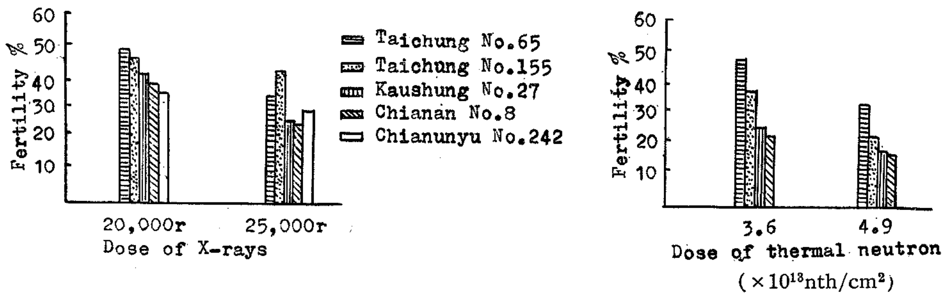

Fig. 2. Effects of different radiations on the percentage of seed setting.

Table 4. F values for difference due to treatments and varieties.

(a) Quardrivalents.

Source of variation	X-ray			Thermal neutron		
	D. f	Mean square	F	D. f	Mean square	F
Treatments	1	40.000	13.4*	1	103.248	13.4*
Varieties	4	34.261	11.5*	3	34.863	4.5
Error	4	2.983		3	7.683	
Total	9			7		

(b) Fertility

Source of variation	X-ray			Thermal neutron		
	D. f	Mean square	F	D. f	Mean square	F
Treatments	1	92.416	19.1*	1	80.01	45.7**
Varieties	4	18.886	3.9	3	54.51	31.1**
Error	4	1.322		3	8.04	
Total	9					

* Significant at 5% level.
** Significant at 1% level.

Summary

Seeds of five "Ponlai" varieties (Japonica type) were sent to the Brookhaven National Laboratory, U. S. A, for irradiation with X-ray and thermal neutrons. The relation of doses as well as kinds of radiation to the rate of chromosomal changes, and varietal differences in radiation sensitivity were studied cytologically with the first generation plants.

Ring chromosomes and univalents were found at both diakinesis and first metaphase of pollen mother cells. The frequency of cells with a ring chromosomes was proportional to the dosage of radiation. The effect of thermal

neutron appeared to be more serious than that of X-rays. Dosage of radiation producing 1% chromosomal interchanges was approximately 600 r with X-rays, and was 0.10×10^{13} nth/cm^2 with thermal neutrons. The biological equivalence value for chromosomal changes was then, calculated to be 1 r of X-ray $= 1.6 \times 10^9$ nth/cm^2 of thermal neutrons. Varietal differences in radiation response were proved with X-rayed plants, but were not significant with thermal neutron.

X線及熱核子對水稻染色體之影響

水稻經X線處理後所發生的突然變異之第四報

謝　順　景

將五個蓬萊種水稻種子寄往美國 Brookhaven National Laboratory 處理不同劑量的X線及熱核子。處理後的種子寄回臺灣省農業試驗所種植，以觀察不同放射線之量及質對處理當代花粉母細胞染色體之影響。放射線處理後代所出現的主要變異爲染色體之相互轉座，其出現頻度以X線處理者介於29～50%，以熱核子處理者則介於33～59%。如所期處理量愈高則染色體變異之頻度亦愈高。據變量分析結果認爲放射線對不同品種間染色體之影響不同，尤其以X線處理者爲然。產生1%的染色體轉座所需的X線量計算爲 600 r，熱核子爲 0.10×10^{13} nth/cm^2。熱核子之生物的影響 (Biological effect) 計算結果，X線1 r 相當於熱核子之 1.6×10^9 nth/cm^2。(摘要)

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