CYTOLOGICAL AND GENETIC CHANGES INDUCED BY X-RAYS AND THERMAL NEUTRONS IN RICE[1]

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Introduction

A number of ionizing radiations have been used by genetists to induce mutations in plants, among which X-ray was the first one to have been employed. Since the introduction of nuclear reactor, thermal neutron has also been utilized to induce mutations in plants.

In barley, Caldecott *et al.* (1952, 1954) have already shown that X-rays and thermal neutrons have different biological effects. Besides affecting the genetic materials, X-rays have physiological effect on extranuclear materials. Comparisons of biological effects between X-rays and thermal neutrons on rice have been made by Matsuo *et al.* (1958). However, their studies were mainly on seedling height, seedling mutations, and fertility. The present experiment was designed to obtain more informations about the effects of X-rays and thermal neutrons on two rice varieties. In addition to seedling height and seedling mutations, data for chromosomal interchanges and pollen sterility were also collected and presented in this paper.

Materials and Methods

Two rice varieties, Taichung No. 65 and Chianan No. 8, were used in this experiment. Before irradiation, dry seeds of the two varieties were stored in a room to stabilize the uniform moisture content at 60 per cent relative humidity.

The X-ray irradiations of the seeds were made at the Brookhaven National Laboratory, U. S. A., with a G. E. Maxitron operated at 250 KVP and 30 ma., giving a dose rate at approximately 1,000 r per minute. The thermal neutrons were generated in the thermal neutron column of the nuclear reactor at the Brookhaven National Laboratory. Its dosages were determined by exposing gold foils with the seeds and relating the extent of induced radioactivity in the foils to the flux of thermal neutron in the column.

Treated seeds were presoaked in water for 24 hours and then sown and grown in pots. Number of seedlings survived and seedling height were determined at the time of transplanting (18 days after sowing).

(1) This investigation is supported in part by a grant from the Society of the Sigma Xi, U.S.A.

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At microsporogenesis, one spike was picked from each plant surviving each treatment and fixed in acetic alcohol. Frequencies of chromosomal interchanges at diakinesis and first meiotic metaphase were determined. Aceto-carmine smear technique was employed throughout the study.

Pollen abortion was checked and counted from random samples of spikelets picked from 40 to 50 plants of each treatment before anthesis.

At maturity two spikes were taken from each plant of the treated generation. Twenty to fourty seeds of each spike were sown and grown for studying the mutations in seedlings.

Results

Number of seedlings survived and seedling height: Data for number of seedlings survived and seedling height are presented in Table 1. For X-ray

Table 1. Effects of X-rays and thermal neutrons on dry seeds of rice as measured by the number of seedlings survived and seedling height

Variety	Treatment	Dose	No. of seeds treated	Seedlings survived		Av. seedling height (cm)	C. V. (seedling height)
				No.	% of control		
Taichung No. 65	Control	0	160	144	100.0	23.6	16.4
	X-rays	20,000 r	300	266	98 5	20.6	23.5
	X-rays	25,000 r	300	171	63.3	11.3	43.6
	Thermal neutrons	2.65×10^{13}nth/cm^2	300	276	102.2	20.0	20.4
	Thermal neutrons	3.99×10^{13}nth/cm^2	300	283	104.8	17.7	25.7
Chianan No. 8	Control	0	100	99	100.0	20.2	18.7
	X-rays	20,000 r	300	267	90.0	17.4	22.7
	X-rays	25,000 r	300	231	77.8	13.2	42.8
	Thermal neutrons	2.65×10^{13}nth/cm^2	300	260	87.6	18.2	22.3
	Thermal neutrons	3.99×10^{13}nth/cm^2	300	270	90.9	17.0	26.3

irradiated materials, the number of seedlings survived was considerably decreased when compared with that of the unirradiated ones. Greater reduction in the number of seedlings survived at high dose than at low one was observed in both varieties tested. However, this is not the case for the thermal neutron irradiated materials. The survival of seedlings grown from seeds treated with thermal neutrons was as good as the controls. Similar results have been reported by Caldecott *et al.* (1952) in barley. They found that even under field conditions, the survivals from seeds treated with the most doses of thermal neutrons were about as good as the unirradiated ones.

Both X-ray and thermal neutron irradiations inhibited seedling height to a considerable degree. Seedlings grown from seeds treated with 25,000 r X-rays were about half the length of the controls. The coefficients of variability for seedling height presented in the last column in Table 1 indicate that seedlings grown from seeds irradiated with either X-rays or thermal neutrons were more variable than those of the unirradiated ones. Ranges of seedling height became greater at high dose of either X-rays or thermal neutrons than at low dose. The coefficients of variability also show that seeds irradiated with thermal neutrons were more uniformly affected than those irradiated with X-rays in seedling height. Thus, the variability of seedling height increases in the following ascending order: controls, thermal neutron irradiated ones, X-ray irradiated ones.

Data for number of seedlings survived and seedling height shown that X-ray and thermal neutron irradiations manifest differently to the rice seeds.

In order to find out whether there existed varietal difference as to the response to X-ray and thermal neutron irradiations, analysis of variance was made for seedling height. The F value for varieties is 5.24 which is not significant. This may indicate that dry seeds of the two varieties treated are similar in response to X-rays and thermal neutrons as far as seedling height is concerned.

Chromosomal Interchanges in the R_1 Microsporocytes: Chromosomal abnormalities were checked in the pollen mother cells. The chromosomal abnormalities mostly observed at diakinesis and first meiotic metaphase were those of interchanges of nonhomologous chromosomes either in ring or chain configurations. Since such chromosomal configurations were not consistent in cells of a single spike, more than 20 cells were checked before a definite conclusion was made for each spike. The fluctuation of such chromosomal configurations might be related to the length of the interchange segments which affect the number and position of chiasmata and consequently the chromosomal configurations at diakinesis and first metaphase.

Table 2 presents the frequencies of different types of chromosomal interchanges determined from the R_1 microsporocytes. The most frequent type of chromosomal interchanges observed was a ring- or chain-of-four. The next to the most frequent types were two rings- or chain-of-four and a ring- or chain-of-six. Ring- or chain-of-eight was very rare and only observed in the microsporocytes of the variety Taichung No. 65.

In barley, Caldecott *et al.* (1952) found that the only types of chromosomal anomalies observed at meiosis in plants grown from the X-ray and thermal neutron irradiated seeds were reciprocal interchanges. No clear case of "gross" chromosomal deficiency was observed. In this study, however, two monosomic

spikes were found in the variety Taichung No. 65 after X-ray irradiation (Table 2). Besides monosomics, one spike analyzed had two univalents and eleven bivalents at diakinesis and first meiotic metaphase (Table 2). The cause of failure in pairing of the chromosomes was not determined. The per cent of chromosomal interchanges of each treatment is presented in the last column in Table 2. When the data for types of chromosomal interchanges were trans-

Table 2. Frequencies of chromosomal interchanges in the R_1 microsporocytes from dry seeds irradiated with X-rays and thermal neutrons

Variety	Treatment	Dose	No. of spikes analyzed	No. of spikes showing ring- or chain-of-					Total per* cent of inter-changes
				4	4+4	6	8	Others	
Taichung No. 65	Control	0	73	0	0	0	0	0	0.00
	X-ray	20,000 r	122	20	4	2	0	1(a)	26.20
	X-ray	25,000 r	66	15	4	2	1	1(a)	45.45
	Thermal neutron	2.65×10^{13}nth/cm^2	88	16	1	2	0	0	25.00
	Thermal neutron	3.99×10^{13}nth/cm^2	90	23	1	3	1	0	37.78
Chianan No. 8	Control	0	41	0	0	0	0	0	0.00
	X-ray	20,000 r	113	19	1	1	0	0	20.36
	X-ray	25,000 r	90	20	0	1	0	0	24.44
	Thermal neutron	2.65×10^{13}nth/cm^2	84	12	1	1	0	0	19.05
	Thermal neutron	3.99×10^{13}nth/cm^2	91	16	3	1	0	1(b)	26.37

* When interchange types were transformed into per cent, ring-of-six was counted as 2 interchanges, etc.

(a) Monosomics

(b) 2 univalents+11 bivalents

formed into per cent, ring- or chain-of-six was counted as 2 interchanges, ring- or chain-of-eight as 3 interchanges, etc. The per cent of interchanges is significantly greater at high dose than at low dose in both X-ray and thermal neutron irradiated materials. With the same treatment and dose, greater per cent of interchanges was found in the variety Taichung No. 65 than in the variety Chianan No. 8 (Fig. 1).

It is interesting to find out whether the two varieties would produce the same relative frequencies of the different types of chromosomal interchanges after their dry seeds were irradiated with X-rays and thermal neutrons. By adding together each of the interchange types of each variety (Table 3), a Chi-square analysis using a 2×5 contigency table was employed to determine the likelihood that the two varieties tested would yield the same relative frequencies of different types of interchanges. The Chi-square value obtained is 2.96 which

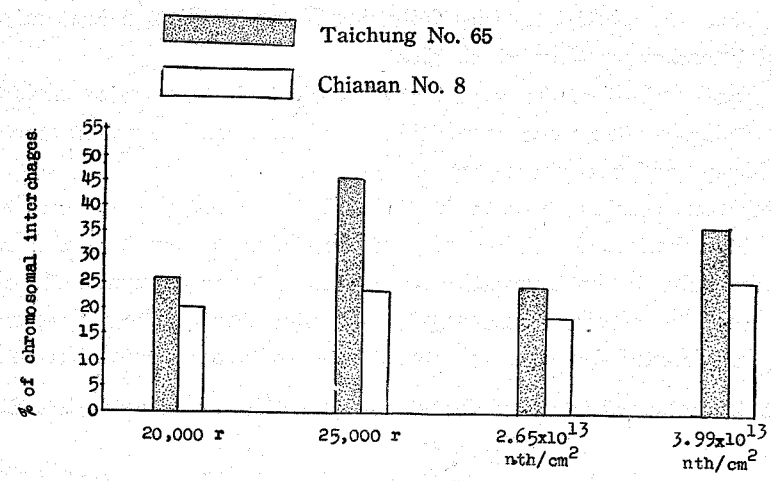

Fig. 1. Per cent of chromosomal interchanges in PMC from seeds irradiated with X-rays and thermal neutrons.

is not statistically significant, indicating that the two varieties produced similar relative frequencies of interchanges after irradiations.

Table 3. Summary of chromosomal interchange types in the R_1 microsporocytes from plants grown from dry seeds irradiated with X-rays and thermal neutrons

Variety	No. of spikes showing ring- or chain-of				
	0	4	4+4	6	8
Taichung No. 65 (all doses and treatments)	269	74	10	9	2
Chianan No. 8 (all doses and treatments)	301	67	5	4	0

Similar analysis was conducted to determine the resemblance of the two types of radiations in producing the relative frequencies of different classes of interchanges in each variety. The Chi-square values thus obtained are not significant for either variety.

It has been calculated from the data for chromosomal interchanges that 1 r of X-rays is approximately equal to 1.4×10^9 nth/cm^2 of thermal neutrons. The result is very close to 1 r equivalent to 1.35 nth/cm^2 calculated by Matsuo *et al.* (1958) who based upon the dose necessary for the induction in rice of the frequency of chlorophyll deficient mutation of five per cent.

Pollen Sterility: Cytogentists are aware of the fact that many chromosomal abnormalities may affect the fertility of organisms. Thus, many heterozygous translocation plants are semisterile. The sterility resulting from irradiations has been attributed to the chromosomal abnormalities by many investigators.

In rice plants, the partial sterility following X-ray irradiation is mostly due to reciprocal interchanges (Oka *et al*, 1953).

The results obtained by Beard *et al* (1958) in barley, maize, mustard, and safflower indicate that close correlation exists between the pollen sterility and doses of X-rays and thermal neutrons.

As expected, results presented in Table 2 show that the per cent of chromosomal interchanges is higher at high dose than at low dose of X-rays and thermal neutrons in both varieties tested. In order to determine the relations between the chromosomal interchanges and pollen steriliy, the per cent of pollen abortion was counted for each treatment. The results are summarized in Table 4.

Table 4. Pollen abortion of the plants grown from the seeds irradiated with X-rays and thermal neutrons

Variety	Treatment	Dose	No. of samples checked	Total pollen	Aborted pollen	Per cent of abortion
Taichung No. 65	Control	0	45	11,599	1,011	8.7
	X-ray	20,000 r	43	11,139	2,916	26.2
	X-ray	25,000 r	49	14,235	7,713	54.1
	Thermal neutron	2.65×10^{13}nth/cm^2	40	13,640	8,664	63.5
	Thermal neutron	3.99×10^{13}nth/cm^2	47	13,362	8,854	66.2
Chianan No. 8	Centrol	0	42	9,781	1,102	11.3
	X-ray	20,000 r	45	12,430	4,053	32.6
	X-ray	25,000 r	42	11,543	6,081	52.7
	Thermal neutron	2.65×10^{13}nth/cm^2	43	14,052	9,630	48.0
	Thermal neutron	3.99×10^{13}nth/cm^2	43	10,834	4,943	46.4

For X-ray irradiated materials, significant differences in pollen abortion between low and high doses were found in both varieties tested. Such a case is being observed in the per cent of chromosomal interchanges. However, for thermal neutron irradiated materials no significant differences in pollen sterility were found between plants grown from seeds irradiated with low and high doses. Since the pollen samples were taken from the regenerated plants (the original R_1 plants were severely damaged by *Chilo* sp. before anthesis), the results of pollen abortion obtained might not truly represent the effect of thermal neutrons. For this reason no general conclusion can be made in this study concerning the relationship between chromosomal interchanges and pollen abortion.

Seedling Mutations: Data for seedling mutations are given in Table 5. The seedling mutations include albino, yellow green, light green, yellow, striped, and variegated types, among which albino is the most frequent one. Since

the original R_1 plants were damaged by *Chilo* sp., the spikes used for seedling mutation study were picked from the regenerated plants. Thus, only relatively small number of spikes were tested for each treatment. Errors involved in the data for seedling mutaions may have been great.

Table 5. Frequencies of chlorophyll deficient mutants induced by X-rays and thermal neutrons

Variety	Treatment	Dose	No. of spikes tested	No. of spikes showing mutations	Per cent of mutation
Taichung No. 65	Control	0	140	0	0
	X-ray	20,000 r	158	6	3.8
	X-ray	25,000 r	94	3	3.2
	Thermal neutron	2.65×10^{13}nth/cm^2	129	3	2.3
	Thermal neutron	3.99×10^{13}nth/cm^2	140	9	6.4
		Average			4.03
Chianan No. 8	Control	0	145	0	0
	X-ray	20,000 r	148	7	4.7
	X-ray	25,000 r	146	13	8.9
	Thermal neutron	2.65×10^{13}nth/cm^2	131	15	11.5
	Thermal neutron	3.99×10^{13}nth/cm^2	120	6	5.0
		Average			7.52

Data given in Table 5 show that frequencies of chlorophyll deficient mutations induced by X-rays and thermal neutrons were higher in the variety Chianan No. 8 than in the variety Taichung No. 65. It should be recalled that the reverse is the case for the chromosomal interchanges (Table 2 and Fig. 1).

In testing the chlorophyll deficient mutations, it was observed that air temperature had affected greatly on the expression of such mutants. This is especially true with those of variegated types. Genetists are often handicapped with the interference to obtain a precise segregating ratio of such mutants. In order to clarify the temperaure effect on the segregating ratio of such mutants, 4 variegated R_3 lines were taken for this study. The R_3 seeds from the heterozygous plants of each line were divided into 3 parts. They were germinated and grown under 3 different conditions, namely: (1) under natural condition (20°–36°C), (2) under constant low temperature (15°–20°C), and (3) under constant high temperature (25°–30°C), and their segregating ratios were determined. The results are given in Table 6.

Table 6. Segregation of variegated seedlings grown under different conditions

Line No.	Natural condition (20°-36°C)				Constant high temp. (25°-30°C)				Constant low temp. (15°-20°C)			
	Total seedlings	No. of mutant seedlings	X^2	P	Total seedlings	No. of mutant seedlings	X^2	P	Total seedlings	No. of mutant seedlings	X^2	P
1	1,167	252	6.9	0.01	789	193	0.061	0.9-0.8	746	187	0.006	0.98-0.95
2	1,090	202	17.7	0.01	841	199	0.71	0.5-0.3	675	175	0.284	0.70-0.50
24	379	87	0.864	0.5-0.3	314	63	4.26	0.05-0.02	189	39	2.05	0.20-0.10
25	342	45	10.00	0.01	134	23	4.25	0.05-0.02	123	31	0.01	0.95-0.90

The Segregating ratios of all the 4 lines tested under the constant low temperature fit very closely to the 3:1 ratio while under the constant high temperature only 2 out of the 4 lines fit to the 3:1 ratio, and those under natural condition, only one of the lines fits to the monogenic ratio. By the analysis of the variance, it was found that significant difference for the conditions was demonstrated, indicating that in testing chlorophyll deficient mutants temperature takes great importance in the expression of their phenotypes especially those of variegated types.

Discussion

In barley, Caldecott *et al.* (1952, 1954) demonstrated that X-rays have a pronounced effect on extra-chromosomal elements of the cells, while the effect of thermal neutrons is largely chromosomal. Their interpretation is mainly based on the data for seedling height and number or seedlings survived.

Data for the number of seedlings survived and seedling height obtained in this experiment indicate that rice seeds treated with thermal neutrons were much more uniformly affected than seeds irradiated with X-rays. This finding supports the conclusion made by Caldecott *et al.* in barley.

Intervarietal differences in radio-sensitivity has been observed in some species. In this study intervarietal differences in response to X-rays and thermal neutrons were found to have expressed in chromosomal interchanges and seedling mutations but not in seedling height. It is interesting to note that the variety whose chromosomes are relatively resistant to radiations, *i.e.* chromosomes less subjected to chromosomal interchanges yielded more chlorophyll deficient mutations and vice versa. This might be explained by the difference in elimination of the aberrant chromosomes between varieties during ontogenesis or due to difference in genes sensitive to radiations. In any case, for the practical purpose of plant breeders, selection of variety for the induction of mutations is important.

In genetic studies, it is a recognized fact that recessive mutants often appear in relatively low proportions. This is especially true for chlorophyll deficient mutants. The results obtained in this experiment show that the proportion of chlorophyll variegated mutants in the segregating populations was influenced by temperatures. Thus, in testing chlorophyll deficient mutants, control of temperature may be necessary.

Summary

Dry seeds of two rice varieties were treated with both X-rays and thermal neutrons in two doses each. Comparisons of the effects were made between the two types of irradiations as well as between the two varieties as measured by seedling height, number of seedlings survived, chromosomal interchanges, pollen

abortion, and seedling mutations. From these studies, the following conclusions have been reached:

1. Seeds of both varieties treated with thermal neutrons were much more uniformly affected than seeds irradiated with X-rays as shown by the seedling height and number of seedlings survived.

2. Intervarietal differences in response to both irradiations were observed. Seeds of the variety Taichung No. 65 after being irradiated with X-rays and thermal neutrons produced more chromosomal interchanges than that of the variety Chianan No. 8. The reverse was the case for the seedling mutations.

3. It is calculated from the data for chromosomal interchanges that 1 r of X-rays was approximately equal to 1.4×10^9 nth/cm^2 of thermal neutrons.

4. The relationship between the per cent of chromosomal interchanges and the per cent of pollen abortion was rather obscure.

5. In testing the segregating ratio of the variegated type of chlorophyll deficient mutants, it was observed that temperature had an important effect on the expression of such mutants in the segregating populations.

經X光線與中子處理後所引起水稻之細胞遺傳的變異

趙傳纓　柴淑文

二水稻品種，臺中65號與嘉南8號之種子，各以二强度之X光線與中子處理。由苗之成活數，苗高，染色體轉接，花粉不孕率，以及苗葉綠素突變等比較二品種間與各處理間之差異，所得結果如下：

（1） 就苗高及苗之成活數而言，經中子處理的比X光線處理的要齊整。

（2） 二品種對於X光線與中子之反應顯有不同。臺中65號所發生之染色體轉接率比嘉南8號的高，而苗葉綠素突變率則反之。

（3） 如以染色體轉接率計算，X光線之1r的作用，約相等於中子之 1.4×10^9 nth/cm^2。

（4） 測定斑型苗突變型之分離比時，發現溫度對於突變性狀之顯現與否頗有影響。

(摘要)

Literature Cited

BEARE, B. H., F. A. HASKINS, and C. O. GARDNER. Comparison of effects of X-rays and thermal neutrons on dormant seeds of barley, maize, mustard, and safflower. Genetics 43: 728-736, 1958.

CALDECOTT, R. S. and L. SMITH. A study of X-ray induced chromosomal aberrations in barley. Cytologia 17: 224-242, 1952.

CALDECOTT, R. S., E. F. FROLIK, and ROSALIND MORRIS. A comparison of the effects of X-rays and thermal neutrons on dormant seeds of barley. Proc. Nat. Acad. Sci. 38: 804-809, 1952.

CALDECOTT, R. S. *et al.* Cytogenetic effects of X-ray and thermal neutron irradiation on seeds of barley. Genetics 39: 240-?59, 1954.

MATSUO, TAKANE, *et al.* A comparison of biological effects between thermal neutrons and X-rays on rice seeds. Jap. Journ. Breeding 8: 35-45, 1958.

OKA, H., T. T. CHANG, and M. S. HONG. Reciprocal translocations in rice—A preliminary note. Jap. Journ. Genetics 28: 87-91, 1953.