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BREEDING EXPERIMENT ON MUTATION INDUCTION BY IRRADIATION

(2) Effects of X-ray and Thermal Neutron Irradiation on Dry Seeds of Chincse Cabbage and Radish.

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金東祐・金垠椿・趙美卿：放射線 處理에 依한 突然變異育種
(2) X線 및 熱中性子가 배추와 무우 氣乾種子에 미치는 影響

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ABSTRACT

Kim, Dawng Woo, Yang Choon Kim and Mi Kyung Cho (Horticultural Experiment Station, Seoul, Korea)—Breeding Experiment on Mutation Induction by Irradiation. (2) Effects of X-ray and Thermal Neutron Irradiation on Dry seeds of Chinese cabbage and Radish. Kor. Jour. Bot. Vol. V. No. 1. p.1~6. 1962 : With the aims to find the biological effects of X-ray and thermal neutron on dry seeds of Chinese cabbage and radish, and to examine the utility of artificial mutation in plant breeding, this experiment was carried out through the treated and next generation. The results obtained are summarized as follows:

1) Germination rate was rather irregular than decreasing as increasing dose of radiation and there were no differences between Kyong-Sam and Chyong-Bang of Chinese cabbage.
2) In R_1 generation, abnormal leaves from seedling of irradiated seeds were observed. These were more apparent in X-ray irradiation than in thermal neutron.
3) Seedling height was inhibited with increasing dose of X-ray and thermal neutrons. Growth inhibition was more remarkable in X-ray than in thermal neutron. Kyong-Sam demonstrated more sensitivity than Chyong-Bang in both X-ray and thermal neutron.
4) Seedling height produced from seeds subjected to thermal neutrons showed small variation around its mean value, while in X-irradiation there was a greater deviation from the mean value.
5) Fertility was decreased as increasing with dose, while the frequency of abortive pollen was increased. There were variability of the fertility and frequency of abortive pollen among plants or branches of a plant.
6) The mutants were obtained more in thermal neutron irradiation than in X-ray. The types of mutations obtained in Chinese radish of R_2 generation were abnormal leaf, densely glowing leaf, degeneration in growing point and dwarf. The maximum frequency of phenotypic mutations was abnormal leaf mutation.

INTRODUCTION

Since Muller(1927) and Stadler(1928) demonstrated the mutagenic properties of X-ray, it was well konwn that various mutagens including X-ray were able to induce artificial mutations. Their potential utility of radiation mutation in plant breeding has been discussed by numerous investigators. Here it is very important to find out the most efficient agent among various kinds of radiation and their biological effect and to examine the utility of radiation mutation in plant breeding.

Several experiments were made in order to find the biological effect of X-ray and thermal neutron on dry seeds of Chinese cabbage and radish, and to examine the utility of artificial mutations in plant breeding. This paper deals with radiosensitivity in the R_1 generation and the phenotypic variation in the R_2 generation.

MATERIALS AND METHODS

Dry seeds of Kyong-Sam and Chong-Bang in Chinese cabbage (Brassica pekinensis L.) and Seoul-Danchoo in Chinese radish (Raphanus sativus L.) were used for this serial experiments. These seeds were produced at Horticultural Experiment Station in 1959 and originated from the progeny of same line and thus would be expected to have a high degree of genetic uniformity. They were selected for uniformity of size and for mechanical injury, to minimize variation in germination and seedling development. In order to be in equilibrium of water contents, all dry seeds were kept in desiccator filled with $CaCl_2$ for six weeks before irradiation.

The X-radiation was filled through 1 mm of aluminum and the radiation facility was operated at 250kvp and 30ma giving approximately 900r per minute in distance of 31cm. Each sample of 50 seeds was subjected to dose of 5, 10, 15, 20, 30 and 40 kr respectively.

For treatment with thermal nertrons the seeds were placed in a single layer in lucite container and exposed in the thermal column of the nuclear reactor at Brookhaven National Laboratory, U.S.A. Sample were exposed to thermal neutrons at $7.00\times10^{8}Nth/cm^2/sec$ for 1, 2, 3, 5, 7 and 10 hours, respectively.

RESULT AND DISCUSSION

The radio-sensitivity of Chinese cabbage and Chinese radish was compared by measuring the seedling height at 25 days after planting. The growth inhibition of each radiation dose was shown as ratio of height of seedling produced from the irradiated seeds to that of unirradiated ones. Seedling height was inhibited with increasing dose in X-ray and thermal neutron. Growth inhibition was more remarkable in X-ray than in thermal neutrons (Tables 1 and 2, Figs. 1 and 2).

In comparison of varietal differences of radio-sensitivity with seedling height, Kyong-Sam demonstrated more sensitivity than Chong-Bang in both X-ray and thermal neutron, but it was not severe(Fig. 1). This was similiar to the result of earlier work (unpublished) made with same varieties seeds subjected to X-rays.

Frequency distribution of seedling height in Kyong-Sam variety of Chinese cabbage are shown in Fig. 3. Seedling height produced from seeds subjectd to thermal neutrons showed a small variation around its mean value, while in X-irradiation there was a greater deviation from the mean value on the growth inhibition. From this, it was found that an irregular distribution of seedling height in X-ray is more apparent than that in thermal neutron. These were reported by Ehrenberg and Nybom and Caldecott (1954, 1955) in barley and Yamaguchi(1958) in rice. Caldecott (1955) assumed that from the fact that thermal neutrons have a much higher specific ionization than X-ray, the difference may result from the spatial distribution on ion pairs in the irradiated material.

Germination rate (percentage of seedling at cotyledon stage) was rather irregular than decrease with increasing dose of X-ray and thermal neutron, and there were no differences between two varieties (Tables 1 and 2). It is assumed that irregularity and no decreasing appearance in germination rate of irradiated seeds of genera Brassica and Raphanus are tolerant to radiation. Fujii and Matsumura(1958) reported also that LD-50 in Brassica and Raphanus was the extent of 70kr. And according to the experimental report of Brookhaven National Laboratory, U.S.A., Brassica had been known to be more resistant to radiation in comparison with other plants.

Table 1. Comparison of effects of X-rays and thermal neutrons on dry seeds of Chincse cabbage.

Variety	Radiation	Dose	Germ. rate	Abnor. leaf	Seedling height	
					cm	%(of cont.)
Kyong-Sam	Cont. X-rays	0	66%	· %	8.89	100
		5 kr	70	·	8.83	99.32
		10	64	·	8.50	95.61
		15	57	3.6	8.39	94.38
		20	54	7.7	7.85	88.30
		30	80	33.3	7.19	80.88
		40	74	62.2	6.31	70.98
	t. n.	1 hr	72	2.9	8.54	96.06
		2	64	6.3	8.65	97.30
		3	80	8.1	7.99	89.88
		5	68	5.9	8.06	90 06
		7	76	11.8	7.61	85.60
		10	63	12.5	7.28	81.89
Chong-Bang	Cont. X-rays	0	68	·	8.68	100
		5 kr	80	5.0	8.58	98.84
		10	71	10.0	8.18	94.23
		15	82	5.0	8.27	95.27
		20	70	12.1	7.34	84.56
		30	76	24.3	7.29	83.98
		40	70	69.2	6.95	80.06
	t. n.	1 hr	80	9.8	8.72	100.46
		2	69	5.9	8.44	97.24
		3	80	5.0	7.37	84.94
		5	71	2.9	8.25	95.05
		7	90	23.8	8.51	98.04
		10	69	41.2	8.11	93.43

Table 2. Comparison of effect of X-rays and thermal neutrons on dry seeds of Chinese Radish.

Variety	Radiation	Dose	Germ. rate	Abnor. leaf	Seedling height	
					cm	%(of cont.)
Seoul-Danchoo	Cont. X-rays	0	88%	· %	11.19	100
		5 kr	82	2.5	10.15	90.71
		10	90	·	9.80	87.58
		15	94	6.4	10.18	90.97
		20	85	2.6	9.11	81.41
		30	84	80.9	10.00	89.37
		40	92	95.5	8.96	80.07
	t. n.	1 hr	88	4.8	11.80	105.45
		2	90	16.3	11.66	104.20
		3	92	13.0	11.44	102.23
		5	85	17.1	11.41	101.97
		7	88	25.0	10.64	95.08
		10	94	26.1	10.96	95.53

Fig. 1. Effects of seedling height of Chinese cabbage by X-rays and thermal neutrons irradiation.

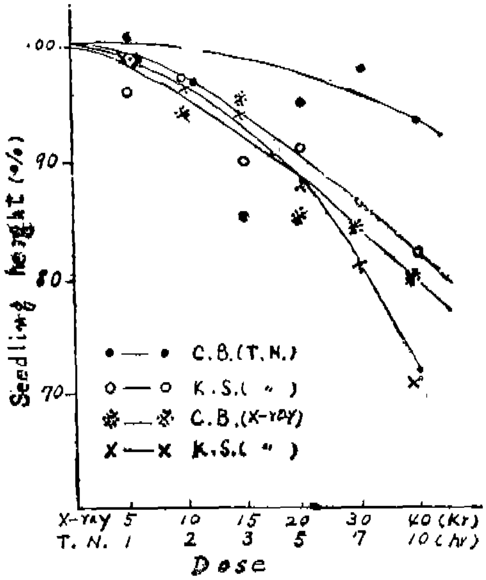


Fig. 2. Effects of seedling height of Chinese radish by X-rays and thermal neutrons irradiation.

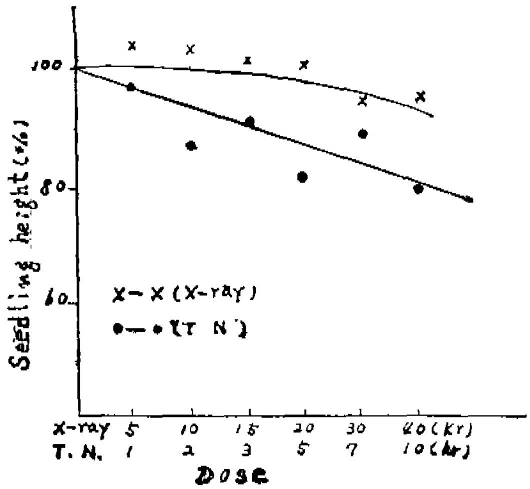


Fig. 3. Frequency distribution of seedling height resulting from X-rays and thermal neutrons irradiation in Chinese cabbge.

X-ray, 40 kr.
Av. Ht. 6.31 cm

No treat.
Av. Ht. 8.89 cm

Thermal neutrons, 10 hrs.
Av. Ht. 7.28 cm

Survival rate to maturity was not observed. Kim, Y.R. (1959) reported that approximately 50 per cent of seedlings from presoaked seeds irradiated to 5,000 r of X-ray was died after reaching the two or three leaves stage. In our present studies, however, no died seedling was observed at four or five true leaves stage in 40 days after planting even in highly dose of X-ray and thermal neutrons. Similiar result was obtained in earlier studies (unpublished) made with same varieties seeds subjected to dose of 30 kr with X-ray. This suggests that as numerous investigators reported, the seeds with a low water content are more tolerant to X-rayed seeds than with a high water content. Ehrenberg and Nybom reported that X-ray sensitivity in presoaked barley seeds was 5–7 times as compared to dry condition and thermal neutron sensitivity was 2–3 times.

Abnormal leaves from seedling of irradiated seeds were observed. This morphological effect was more remarkable in X-ray than in thermal neutron. These phenomena were reported by Gunkel and Sparrow (1954) and Fujii and Matsumura(1958). Kim, Y.R.(1959) reported that mottled and narrowing leaf were observed in Chinese cabbage. Fujii and Matsumura assumed that these effects might due to radiation damage to the cell itself and cell division.

In order to study of fertility, 10 plants of R_1 generation were selected randomly at all of the plots except 5 and 10kr plots X-rayed and 1 and 3 hours plots thermal neutron-exposed in early October, 1960. They were transplanted in the earthern pots and kept at green house without heating system to obtain a chilling requirement for winter. Fertility in Chinese cabbage was determined by ratio of total number of seeds by the artificial bud pollination method to total number of ovule. In Chinese radish it was determined by number of seeds per capsule. Abortive pollen was inspected by staining of aceto-carmine under microscope.

Fertility was decreased as increasing with dose, while the frequency of abortive pollen was increased. This was remarkably apparent in Kyong-Sam than in Chyong-Bang in X-ray. Many abortive pollens were not observed in spite of increasing of dose in thermal neutron of both Kyong-Sam and Chyong-Bang (Table 3). But contrary to fact that fertility should be variabled as with proportion of abortive, it was found that there was some extent of fertility variation. And differences of the fertility and frequency of abortive pollen among individuals and first flower branch of a plant were observed.

It was assumed that it was due to smaller number of plants used, pollination's error and environmental physiological differences among plants or branches. However, it was found that a negative correlation exists between fertility and frequency of abortive pollens.

Table. 3: Differences of fertility and abortive pollens frequency by increase of dose of X-rays and thermal neutron irradiation.

Radiation	Dose	Chinese Cabbage				Chinese Radish	
		Kyong-Sam		Chyong-Bang		Seoul-Danchoo	
		Abor. pollen	Fer.	Abor. pollen	Fer.	Abor. pollen	Fer.
		%	%	%	%	%	grain
Cont. x-ray	0	3.3	64.2	4.1	66.7	1.5	0.92
	10 kr	9.6	43.2	14.8	45.4	5.8	1.12
	20	10.3	31.1	4.2	64.4	12.2	1.85
	30	102.2	36.3	11.9	55.0	80.1	0.75
	40	97.6	16.0	51.0	60.7	57.2	0.49
t. n.	2 hrs	2.6	47.1	17.0	51.6	14.2	0.96
	5	6.6	30.8	6.0	53.2	29.5	1.28
	7	15.6	21.3	•	26.0	33.3	0.76
	10	8.8	28.3	11.0	45.7	31.2	1.28

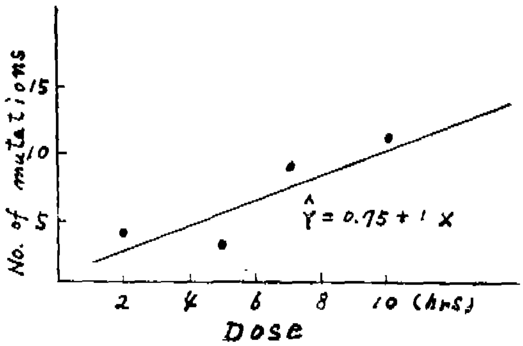
It was supposed that the severe decrease of fertility respectively in high dose might be due to poor fertilization by occurring of many abortive pollens and that abortive pollens might be attributable to structural change of chromosome in meiosis. Previous workers reported that the decrease of fertility by irradiation was due to chromosomal aberration (Kaplan, 1949; Nysiyama, 1959; Oka et al, 1953 and Matsuo et al, 1958).

The phenotypic variation frequency induced in the R_2 generation of Chinese radish were shown in Table 4. And in Fig. 4, mutation frequencies are plotted against dose of thermal neutrons. The mutation in Chinese cabbage were not observed because of damages cause by heavy rainfall in 1961 and various desease. Maximum frequency of mutation induced by each radiation was abnormal leaf mutations. It came to the value of 66.7% and 77.8% for X-rays and thermal neutrons respectively. The ratio to total mutations was I part in X-rays and 9 part in thermal neutrons. This suggests that we may obtain many a mutants by using thermal neutron than X-rays. Ehrenberg and Saeland (1954) reported that chlorophyll deficiency mutants induced rarely by X-radiation were obtained in abundance by thermal neutrons.

Table. 4: A frequency of mutation types induced in the R_2 generation of dry seeds of Chinese Radish by X-rays and thermal neutrons (%).

Radiation	No. of plants used	Mutations observed				Total	Ratio
		Abnor. leaf	Densely growing leaf	Degenerated in growing point	Dwarf		
X-rays	458	2(66.7)	•	1(33.3)	•	3(100)	10
t. n.	435	21(77.8)	3(11.0)	1 (3.8)	2(7.4)	27(100)	90
Total	895	23(76.6)	3(10.0)	2 (6.7)	2(6.7)	30(100)	100

Fig. 4. Regression of mutations on dose of thermal neutrons in Chinese radish.



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摘　要

X線 및 熱中性子를 배추 무우의 氣乾種子에 處理하여 이의 生物學的 影響을 調査하며 放射線 突然變異의 育種的 利用性을 考究하기 爲하여 行하였다.

(1) 品種間 및 處理間에있어서 線量의 增加에 依한 發芽率의 差는 없었고 오히려 不均一한 樣相을 보였다.

(2) 處理當代의 可視的 變異로서는 撚出異常葉의 個體가 發見되었고 X線 處理區에서 더 顯著히 나타났다.

(3) 草長은 線量을 增加함에 따라서 抑制現象이 있었으며 X線 處理區에서 顯著하였고 京都三號가 더 radio-sensitive 하였다.

(4) 草長의 分布는 熱中性子보다 X線 處理에서 變異가 더 컸다.

(5) 稔性은 X線의 線量을 增加함에 따라 減少되는 反面 不充實花粉은 增加되었고 熱中性子 處理에서는 處理間에 別로 差가 없었다.

(6) 무우의 處理次代에 나타난 突然變異는 異常葉 叢生葉 生長點退化 矮性 等이었고 異常葉의 出現率이 가장 많았으며 突然變異 出現率은 X線보다 熱中性子 處理에서 훨씬 많았다.

LITERATURE CITED

1. Caldecott, R. et al, 1954, Genetics 39 : 240-259.
2. ______ et al, 1955, Ann. of N.Y. Acad. of Sci. 59 : 514-535.
3. ______, 1955, Radiation Research, 3 : 316-330.
4. ______, 1954, Science, 120 : 809-810.
5. ______, 1955, Nature, 176 : 306-307.
6. Fujii, T. and Matsumura, S., 1958, Jap. Jour. Genet. 33 : 387-397.
7. Kim Y.R., 1959, Proc. Nuclear Sci. Korea, 228-239.
8. Matsuo, T. et al, 1958, Jap. Jour. Breeding. 8 : 37-45.
9. Nyshiyama, I. and Tsukuda, S., 1959, Jap. J. Genetic 34 : 363-370.
10. Yamaguchi H., 1958, Jap. J. Breeding 7 : 213-220.
11. Yamaguta, H. and Sakudo, K., 1960, Jap. J. Breeding 10 : 153-162.