Studies on the Radiation Breeding in the genus *Mentha*

(XV) A Comparison of Biological Effects between γ-rays and X-rays on Mint Seeds

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Introduction

In attempts to utilize radiation-induced mutation for crop breeding, it is of interest to find an efficient mutagenic agent among various kinds of radiation and to search for an effective physiological condition of the material to be treated.

For their physical characteristics γ-rays are penetrating readily in biological material and exert a different action within a cell than the X-rays. Because the amount of the physiological damage depends upon environmental condition, the possibility to modify the X-ray effects is of interest. From practical point of view, however, it may be noted that γ-rays leading always to a uniform biological action is more effective than X-radiation for the production of various mutations.

This experiment was carried out to obtain the fundamental data of the effects of X-rays and γ-rays on mint.

Material and Method

Using dry seeds of a five species the radiosensitivity of mint seeds was compared by measuring the seedling height at 50 days after X-rays or γ-rays irradiation. The materials used in this experiment are shown in Table 1.

Table 1. Genome constitutions and chromosome numbers of the species used

Species	No. of chromosome ($2n$)	Genome
M. rotundifolia (L.) Huds	24	RR
M. spicata L.	48	RRSS
M. arvensis L.	72	R^aR^aSSJJ
M. arvensis L. var. *piperascens*	96	$R^aR^aSSJJAA$
M. piperita L.	144	$RRRRSSS^cS^cJJAA$

The X-rays were produced at 200 kV and 19 mA through 1.0 mm Al filter. The γ-rays were irradiated by ^{60}Co source in the γ-room of University of Kyoto. That is, a sample of two petri dishes, with 200 seeds in each, was irradiated at a dose rate of 110 R/min with ^{60}Co gamma rays of 5 KR, 10 KR, 20 KR and 40 KR.

Result

The growth inhibition of each radiation dose is shown as ratio of height of seedling produced from the irradiated seeds to that of unirradiated ones (Fig. 1).

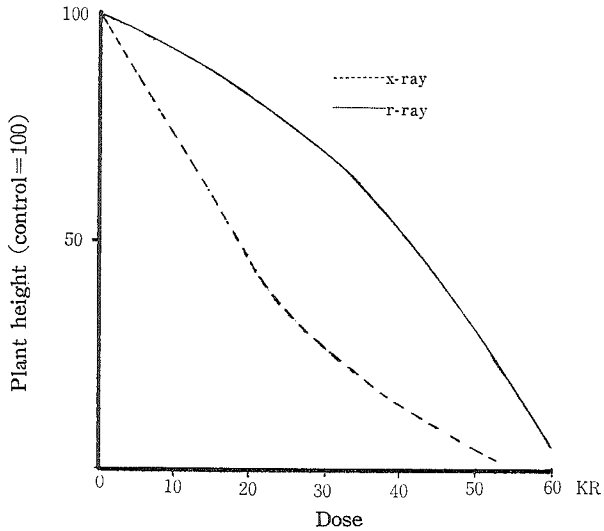

Fig. 1. Effect of dry on seedling height produced by X-rays and γ-rays
(species : *M. arvensis* L. $2n=72$)

With the purpose to find whether there was intervarietal difference of radiosensitivity in *Mentha*, dry seeds of the five species were irradiated with X-rays and γ-rays. The sensitivity was determined by scoring the number of seedlings surviving at 50 days after treatment and showing the survival as the ratio of the number of seedling resulting from the irradiated seeds to that from unirradiated one. There is a variation in the sensitivity to X-rays or γ-rays of mint species.

Dry seeds treated with X-rays and γ-rays wers cultivated in field of Laboratory of plant breeding. The dosages of radiation used and the number of grains sown are shown in Table 2. Seedling height measured at 30 days after transplantation (at 50

Table 2. The effect of radiation on some characters of mint in treated generation

Species	Radiation	Dosage (KR)	No. of sown seeds	Survivals (%)	Seedling height (cm)
M. rotundifolia (L.) Huds	None		500	100	14.3
	X-ray	5	500	96	10.2
		10	500	90	9.3
		40	500	95	7.1
	γ-ray	5	500	86	8.9
		10	500	72	6.3
		40	500	62	5.9
M. spicata L.	None		500	100	15.9
	X-ray	5	500	83	13.2
		10	500	80	10.2
		40	500	72	8.9
	γ-ray	5	500	63	11.2
		10	500	59	7.3
		40	500	48	6.4
M. piperita L.	None		500	100	18.2
	X-ray	5	500	82	12.1
		10	500	70	9.2
		40	500	66	8.4
	γ-ray	5	500	58	10.2
		10	500	42	7.1
		40	500	39	6.3

days after irradiation) is inhibited with increasing dose in X-rays and γ-rays treatment. The plant height produced from γ-rays treated seeds 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 (Fig. 2). Number of mature plants given as a ratio

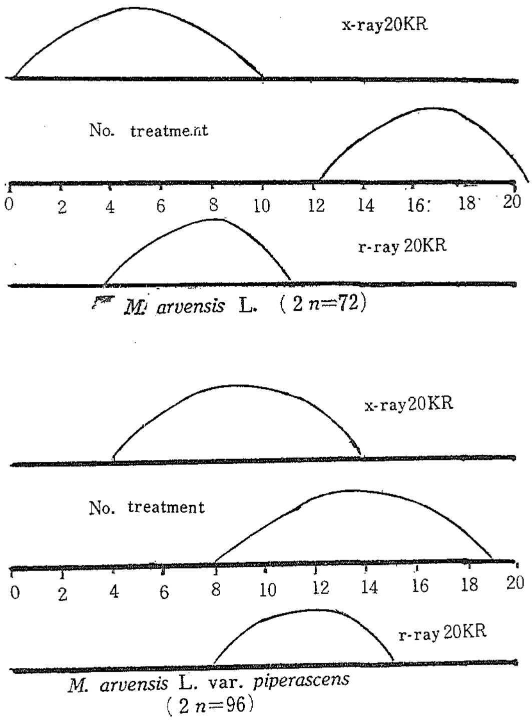

Fig. 2. Frequency distribution of seedling heights resulting from X-rays and γ-rays irradiation

of survivals from irradiated seeds to those from unirradiated ones decreased with increasing dosage and the survivals from seeds receiving a dose of 40 KR γ-rays 1 KR numbered only 10～25% of unirradiated ones.

The relation between the fertility and the dosage was linear in both types of radiation. Linear equations of regression listed below were obtained by least square method as regards the relation between the reduction in seed-setting and the radiation dosage:

$\widehat{Y} = 99.6 - 1.4216X$ in the case of *M. rotundifolia* treated with X-rays

$\widehat{Y} = 94.2 - 10.56X$ in the case of *M. spicata* exposed with γ-rays, and

$\widehat{Y} = 97.3 - 18.4X$ in the case of *M. piperita* exposed with γ-rays.

Although range of γ-rays dosage was dissimilar, a significant difference of liner coefficient of regression equation was found between *M. rotundifolia* and *M. piperita*. As regards the fertility, therefore, the latter species seems to be more sensitive to γ-rays than the former.

If the reduction in fertility induced by irradiation is due to chromosome aberration as supposed by many workers, a distribution of this fertility must show a discontinuous curve taking some constant values. In order to ascertain this problem, the distribution of various grades of fertility produced from irradiated material was analyzed for *M. rotundifolia.*

The frequency of mutation was determined by inspecting the appearance of chlorophyll deficiency in a sown species. The relation between the frequency of seedling mutation and the dosage of each radiation estimated by least square method is demonstrated in Fig. 3. In the irradiation by γ-rays of *M. piperita,* a linear equation

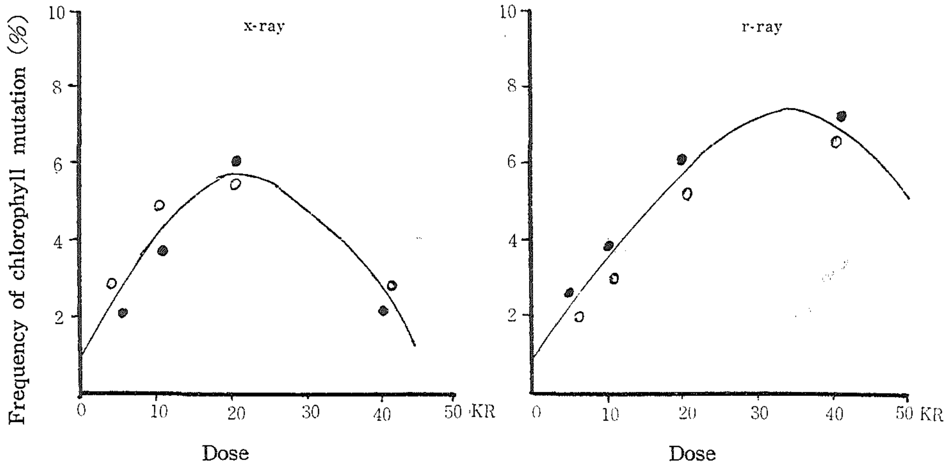

Fig. 3. A relation between frequency of chlorophyll mutation in X_2 generation and dosage of radiation

of regression, $\widehat{Y} = 0.512 + 1.946X$, was obtained, but in *M. spicata* quadratic equations of regression were fitted for the observed values in the case of X-rays and γ-rays, the former $\widehat{Y} = 0.426 + 0.219X - 0.0063X^2$ and the latter $\widehat{Y} = 0.429 + 2.910X - 0.195X^2$. As shown in Table 3, both quadratic coefficients of regression equations

Table 3. Regression analysis of the effects of dose on frequency of chlorophyll deficiency mutation in mint

Radiation	Sources of variation	D.F.	S.S.	M.S.	F-value
X-rays	Linear regression	1	5.921	6.001	103.21※※
	Excess due to quadratic	1	11.363	11.183	168.36※※
	Residual	2	0.216	0.057	
γ-rays	Linear regression	1	22.671	21.177	276.51※※
	Excess due to quadratic	1	4.653	4.803	61.98※
	Residual	2	0.156	0.069	

deviate significantly from zero. Therefore, at lower doses the frequency of mutation is proportional to the dosage of γ-rays, but at higher doses in both radiations the efficiency of mutation per unit dose decreases as compared with that at lower doses, especially in X-rays.

Maximum frequency of chlorophyll deficiency mutation induced by each radiation was estimated, using these equations of regression in *M. rotundifolia.* As a result of calculations it came to the value of 4.8% and 6.7% for X-rays and γ-rays respectively. Consequently we could induce more mutants by using γ-rays than X-rays.

Sowing for X_2 generation was performed in two replications due to shortage of space in greenhouse. But it was perceived that there was a remarkable deviation between the frequencies of mutation obtained in the first time and those in the second. Therefore, the chlorophyll deficiency mutants were classified according to their types and the differences between the frequencies of each type obtained in different sowing times were examined in Table 4. By statistical analysis it was demonstrated that a

Table 4. A deviation of the frequency of chlorophyll deficiency mutation due to a difference of the sowing time in X_2 generation

Sowing time	I	II	Total
Total no. of chlorophyll mutants	129	91	220
albina	56	44	100
viridis	32	29	61
xantha	16	6	22
striata	10	8	18
others	15	4	19
No. of analyzed strains	1962	1635	3597

low frequency of the seedling mutation in the second sowing time was mostly due to a reduction xantha type mutants.

Although it has been thought that embryo of dry seed is composed of many cells and each inflorescence is produced separately from a single cell, it is of interest to find from practical point of view or morphogenesis how many inflorescence are independent of each other. All inflorescence of a X_1 plant obtained from the highest doses of X-rays or γ-rays were divided according to an order of tillering and were examined on the induction of chlorophyll mutation in the X_2 generation. A difference between the sterility and mutation equivalents vanishes rapidly when the frequency of mutation produced by X-radiation reaches a saturation point (Fig. 4). Therefore,

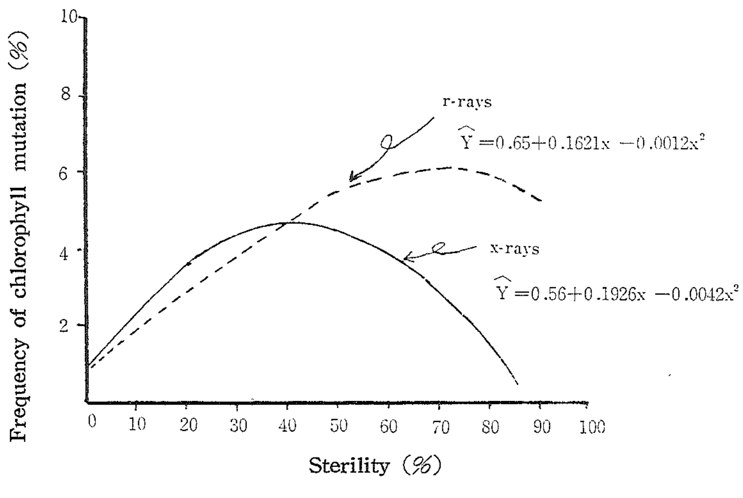

Fig. 4. Regression of sterility in X_1 generation on frequency of chlorophyll mutation in X_2 generation

as X-rays and γ-rays are used to obtain the frequency of mutation of a certain value, γ-rays induce more sterility than X-rays provided the used dosage of X-rays is relatively low.

Discussion

Consequently, it has been thought that the biological action of γ-rays is attributed to proton and alpha radiation, depending upon the chemical composition of treated material. Because all of these radiations originate internally and cause localized destructive action, it is suggestable that the biological effect induced by γ-rays is different from that by X-radiation administered externally.

Besides the physiological condition of irradiated material, radiosensitivity is greatly influenced according to genetical constitution within a species. Otherwise, STUBBE (1935) reported that the mutability induced by X-radiation was influenced by a presence of the plasmagenes of *Epilobium luteum* on the intranuclear genes of *E. hirsutum*. It is important from breeder's practical stand point to find the intervarietal differences in cultivated crop with regard to the lethal effect of ionizing radiation and whether this is due to extranuclear particles or chromosomal genes must be ascertained in future.

In barley, at higher doses there has been a drop in the frequency of mutation produced per unit dose, more effectively in the case of X-rays than γ-rays, and the cause of this phenomenon has been thought to be attriliutable to an intrasomatic selection, taking place during ontogenesis from the irradiated seed to the mature plant (EHRENBERG., 1953). EHRENBERG and NYBOM assumed that such intrasomatic selection influenced by some factors modifying the X-rays sensitivity was primarily due to the varying sensitivity of cells or seeds and the most sensitive one was eliminated by the radiation action to cell constituents outside chromosome. In this experiment carried out with mint seeds it was also found the elimination proccesses occurred less effectively in the case of γ-rays than X-rays. Because the investigation on a relation between the order of tiller and the induction of mutation demonstrated that the inflorescence of secondary tillers induced the very same mutants as resulting from the inflorescence of their primary tiller, from which were grown the secondary tillers, it is assumed that the intrasomatic selection leading to the elimination of mostly affected cells takes place at early stage during ontogenesis.

At any rate, because the saturated frequency of mutation after irradiation with γ-rays is higher than when X-rays are used, it can be concluded for the practical use of breeder that the more mutants are rather obtained in the case of treatment with γ-rays than X-rays, especially when irradiation is executed in high doses.

It was found that the frequency of chlorophyll mutation was influenced by the time of sowing of X_2 seeds, especially in xantha type. HOLM (1954) obtained some information on the dependence of the manifestation of chlorophyll deficiency in barley on the environmental condition. Furthermore, KAPLAN observed in barley the deviation of the frequency of mutation resulting from a partition of sowing time in X_2 generation. And in his investigation, the manifestation of albino-type mutants was independent of varying conditions, whereas that of other chlorophyll deficiency and morphological mutants were influenced greatly. Thus, in a comparison of the efficiency of mutation induction among various mutagenic agents a simultaneous sowing of X_2 strains is necessary. Moreover, from breeder's point of view, it is desirable to carry out the screening of mutants produced with treatment on the spot, expressing fully the mutated gene itself.

Summary

1. Using dry seeds of a species the radio-sensitivity of mint seeds was compared by measuring the seedling height at 50 days after X-rays or γ-rays irradiation.
2. The plant height produced from γ-rays seeds 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.
3. The frequency of mutation was determined by inspecting the appearance of chlorophyll deficiency in a sown strain.
4. The induce more mutants by using γ-rays than X-rays.
5. A remarkable deviation between the frequencies of mutation obtained in the first time and those in the second.
6. As X-rays and γ-rays are used to obtain the frequency of mutation of a certain value, γ-rays induce more sterility than X-rays provided the used dosage of X-rays is relatively low.
7. In this experiment carried out with mint seeds it was also found the elimination processes occurred less effectively in the case of γ-rays than X-rays.
8. The frequency of chlorophyll mutation was influenced by the time of sowing of X_2 seeds, especially in xantha type.

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ハッカ属植物の放射線育種に関する基礎研究
（第15報） γ線およびX線の種子照射がハッカの当代および後代に及ぼす影響の比較

小 野 清 六

ハッカ属植物の染色体数の異なる種（$2n=24$, 48, 72, 96および144）を材料として，乾燥種子にγ線（^{60}Co）とX線（200kV, 19mA）を照射，その後に現われる作用を観察し，両放射線を比較した．その結果，幼植物の草丈の変異の幅は，X線よりもγ線の方が狭い．また，稔性は線量の増加によって直線的に減少することが両放射線で観察され，***M. piperita*** は他の種より不稔に関しγ線に対する感受性が高いことが認められた．つぎに，葉緑素変異個体の出現数から求められた突然変異率は，高い線量でかえって減少し，とくにX線で著しかった．そのため，高い線量照射ではγ線を用いた時の方が生存率当りの突然変異率が高く，γ線とX線照射によって生ずる葉緑素突然変異体の種類に関し，有意な差が見出されなかった．

以上から，ある一定の突然変異率を得るためにγ線とX線とを用いると，前者の方が，不稔を多くおこし易いことが認められた．