

A BIOMETRICAL-GENETIC STUDY OF IRRADIATED  
POPULATIONS IN RICE; GENETIC VARIANCES  
DUE TO DIFFERENT DOSES OF X-RAYS<sup>(1)</sup>

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Since the pioneer work of Gustafsson (1953) as well as Gregory (1956), utilization of induced mutations has become an important method in plant breeding. However, it seems that most breeders in this field have been interested in picking up "good mutants" on the basis of their visual judgement. In contrast, through repeated experiences in hybridization work, breeders know that in so far as they deal with yield of crop plants, it is difficult to detect good individual plants in a segregating population; various biometrical-genetic investigations have been reported to find better selection techniques. The same problems as in hybridization may be involved in radiation-breeding. Under the leadership of Dr. H. W. Li of the Institute of Botany, Academia Sinica, the writers have been in touch with radiation-breeding of rice since 1957, while their experiences have led them to consider biometrical studies of irradiated populations.

An important problem in the practice of radiation-breeding is how to determine a proper dosage of radiation. It may be assumed that the proper dosage is the one which makes the heritability value for yield or certain desired characters highest when selection is made. In order to look into this problem, seeds of a representative "Japonica" rice variety of Taiwan, Taichung no. 65, were X-rayed at different doses, and the second and third generations were investigated regarding several agronomic characters. The results proved that the genetic variance due to irradiation did not increase in proportion to the dosage of X-rays, so far as the  $X_3$  lines are concerned, for which selection had to be made.

**Materials and Methods**

A bulk of seeds of Taichung No. 65 was divided into five batches, and four

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of them were X-rayed at a dosage of 16 Kr, 20 Kr, 25 Kr and 30 Kr, respectively, leaving the remaining one as the control. This treatment was made in the Radiology Department, University Hospital, National Taiwan University, by courtesy of the authorities, to whom the writers are greatly indebted.

The X-rayed plants were grown in the experimental field in Taiwan Provincial College of Agriculture. Seeds of the  $X_1$  plants (grown in second-crop season, 1958) were harvested on an individual plant basis. The resultant  $X_2$  lines were grown according to a complete block design with two replications (first-crop season, 1959). Each line in a replication consisted of five plants. From each of them, two plants were harvested for seeds, dividing an  $X_2$  line into four  $X_3$  lines. However, in treating the data for  $X_3$  (the design being the same as in  $X_2$ ; second-crop season, 1959) the four lines or plots from an  $X_2$  line were regarded merely to be replications, so that it may be said that  $X_3$  lines each representing a  $X_2$  line were investigated with four replications. No artificial selection was made except that  $X_2$  plants showing sterility or extreme dwarfness were discarded.

Records were taken on an individual plant basis for heading date, plant height, panicle length and other agronomic characters.

#### Expectation of Genetic Variances in Irradiated Populations

Let us assume that a gene  $A$  mutates to  $a$  at the rate  $p$  when an  $AA$  plant is irradiated. The  $X_1$  population then consists of  $1-p$   $AA$  and  $p$   $Aa$  plants. When  $X_2$  lines are grown, those segregating into  $\frac{1}{4}$   $AA$ ,  $\frac{1}{2}$   $Aa$  and  $\frac{1}{4}$   $aa$  will occur with the frequency  $p$ . Denoting the effects of the three genotypes,  $AA$ ,  $Aa$  and  $aa$ , on the magnitude of a given character by 0,  $\bar{h}$  and  $d$ , respectively, the  $X_2$  variances among and within lines are written as follows:

$$V_{\bar{x}_2} = \frac{1}{16} p(1-p) d^2 + \frac{1}{4} p(1-p) \bar{h}^2 + \frac{1}{4} p(1-p) dh \text{ (among lines),}$$

$$\bar{V}_{x_2} = \frac{3}{16} p d^2 + \frac{1}{4} p h^2 - \frac{1}{4} p dh \text{ (within line).}$$

Since the mutation rate  $p$  is a small value,  $p^2$  may be assumed to be zero. If the increment  $d$  occurs in plus and minus direction with similar frequencies as suggested by Oka *et al* (1958), this assumption will not bring about a serious bias even if many genic changes are taken into account. When many genes are considered,  $\frac{1}{2} \Sigma p d^2$  may be denoted by  $D$ , and  $\Sigma p h^2$  by  $H$ , assuming that the  $Aa$  plants produced in  $X_1$  can contribute these variances to the population  $H$  in  $X_1$ , and  $D$  after repeated self-pollinations). The term  $dh$  may not always be plus in sign. Neglecting the term  $\Sigma p dh$  for the sake of simplicity, we

obtain the following approximations to the expected radiation-induced variances in  $X_2$  lines.

$$V_{\bar{x}_2} = \frac{1}{8} D + \frac{1}{4} H,$$

$$\bar{V}_{x_2} = \frac{3}{8} D + \frac{1}{4} H.$$

When  $X_2$  is grown in bulk, its variance is written as

$$V_{x_2} = \frac{1}{2} D + \frac{1}{2} H.$$

In the same manner, formulas for  $X_3$  lines are found as follows:

$$V_{\bar{x}_3} = \frac{9}{16} D + \frac{1}{8} H,$$

$$\bar{V}_{x_3} = \frac{3}{16} D + \frac{1}{8} H.$$

It is interesting to note that the fixable genetic variance  $D$  is distributed in the variances among and within  $X_2$  lines in a 1:3 ratio, while in a 3:1 ratio in  $X_3$ .

#### Data and Results of Computations

The results of variance analysis of the data for  $X_2$  and  $X_3$  plants are given in Tables 1 and 2, respectively. Examining the figures in the tables, it is recognized that the variances in  $X_2$  and  $X_3$  are not comparable with each other; for instance, in  $X_2$ , the 30 Kr plot had the largest variance, while in  $X_3$ , the 20 Kr plot had it. This might be due to the effect of selection as discussed later. This indicates that the radiation-induced variances in  $X_2$  and in  $X_3$  should be estimated separately. It seems difficult to estimate the relative magnitude of  $D$  and  $H$  components from this type of data.

Table 1. Results of variance analysis of the data for  $X_2$ .

Plot	Variation due to	d.f.	Mean square	
			Plant height	Panicle length
Control	Among lines	155	36.624	2.866
	Within line	624	18.553	2.052
16 Kr	Among lines	77	40.244	2.683
	Within line	310	21.968	1.663
20 Kr	Among lines	77	53.786	3.311
	Within line	284	21.683	2.585
25 Kr	Among lines	153	59.068	3.905
	Within line	579	19.296	2.003
30 Kr	Among lines	147	79.578	6.039
	Within line	560	25.875	2.319

Table 2. Results of variance analysis of the data for  $X_3$ .

Plot	Variation due to	d.f.	Mean square		
			Heading date	Plant height	Panicle length
Control	Among lines	150	5.959	53.185	3.986
	Within line	614	2.431	10.536	1.848
16 Kr	Among lines	148	9.270	60.203	3.376
	Within line	602	2.863	12.384	1.638
20 Kr	Among lines	146	20.495	83.425	5.381
	Within line	594	3.727	17.367	2.401
25 Kr	Among lines	150	11.341	64.547	4.566
	Within line	610	3.023	14.718	2.426
30 Kr	Among lines	146	11.980	55.188	4.248
	Within line	594	3.589	15.879	2.363

Formulas to estimate the induced variances in  $X_2$  and  $X_3$  were then constructed on the basis of those presented in the previous section. They are given in Table 3. In those formulas, the induced genetic component in the variance among line means was denoted by  $\sigma^2_{x_2}$  (in  $X_2$ ) or  $\sigma^2_{x_3}$  (in  $X_3$ ), and it was conventionally assumed that the variance within  $X_2$  lines contains  $2\sigma^2_{x_2}$ , while that within  $X_3$  lines contains  $\frac{1}{2}\sigma^2_{x_3}$ . It should be kept in mind that, as pointed out in the previous section,  $\sigma^2_{x_3}$  in  $X_3$  (represented by  $\frac{9}{16}D + \frac{1}{8}H$ ) would be much larger than  $\sigma^2_{x_2}$  in  $X_2$  ( $\frac{1}{8}D + \frac{1}{4}H$ ), if the values of D and H in both generations were comparable.

Table 3. Formulas used for computation.

Plot	Variation due to	Expectation of variance components
Control	Among lines	$\sigma^2_e + k\sigma_g^2$
	Within line	$\sigma^2_e$
$X_2$	Among lines	$\sigma^2_e + k\sigma_g^2 + (k+2)\sigma^2_{x_2}$
	Within line	$\sigma^2_e + 2\sigma^2_{x_2}$
$X_3$	Among lines	$\sigma^2_e + k\sigma_g^2 + (k + \frac{1}{2})\sigma^2_{x_3}$
	Within line	$\sigma^2_e + \frac{1}{2}\sigma^2_{x_3}$

$\sigma_e^2$ ....Environmental variance

$\sigma_g^2$ ....Genetic variance due to impurity of the original seeds

$\sigma^2_{x_2, x_3}$ ....Induced genetic variance

$k$ .....Plant number per line ( $= \frac{\sum r_i - \sum r_i^2 / \sum r_i}{n-1}$ , where  $r$ =No. of plants in each line,

$n$ =No. of lines)

### Plate I.

Fig. 2. Representative seedlings of *L. luteus* germinated in darkness (right) and in light (left) after 12 days.

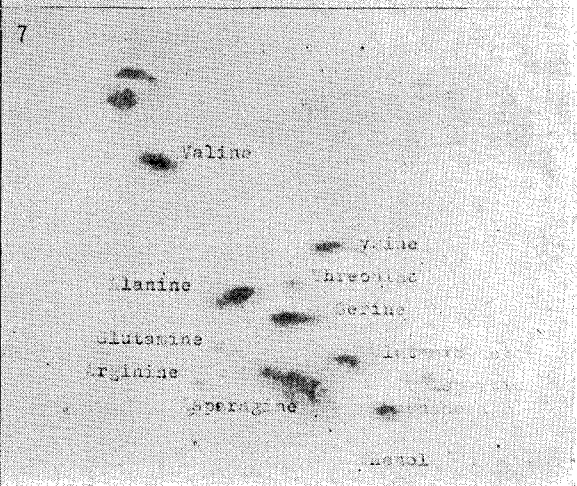
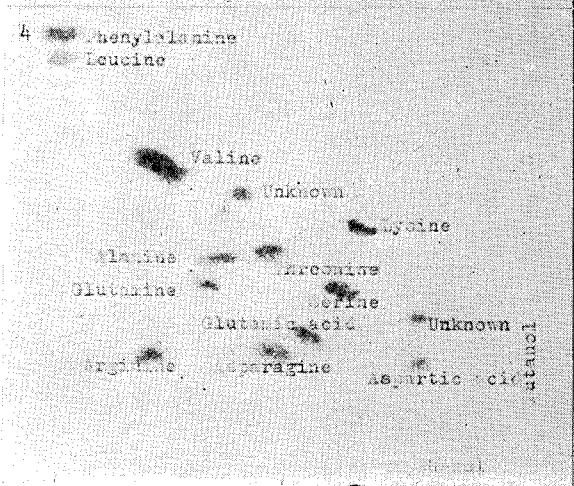
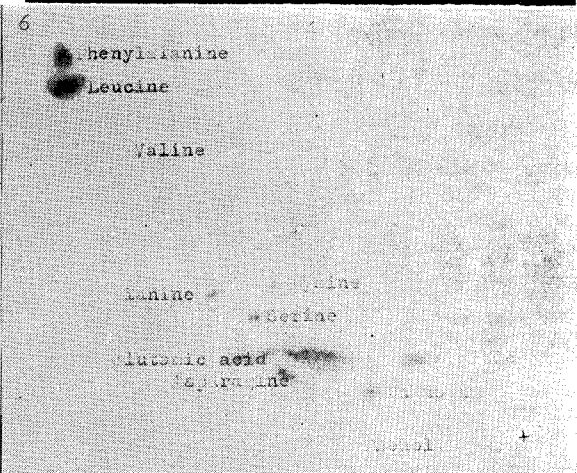
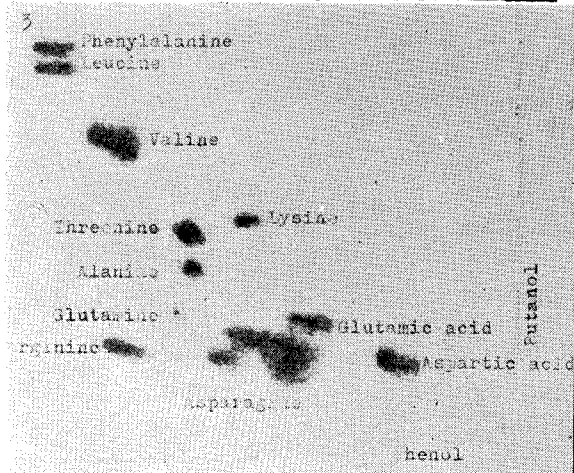
Fig. 3. Paper chromatogram showing asparagine and amino acids in the alcohol-soluble fraction of lupine seedlings germinated in darkness.

Fig. 4. Paper chromatogram showing asparagine amino acids in the alcohol-soluble fraction of lupine seedlings germinated in light.

Fig. 5. Representative seedlings of *L. luteus* germinated with glucose (left) and without glucose (right) after 12 days.

Fig. 6. Paper chromatogram showing asparagine and amino acids in the alcohol-soluble fraction of lupine seedlings germinated with glucose.

Fig. 7. Paper chromatogram showing asparagine and amino acids in the alcohol-soluble fraction of lupine seedlings germinated without glucose.



## Plate II.

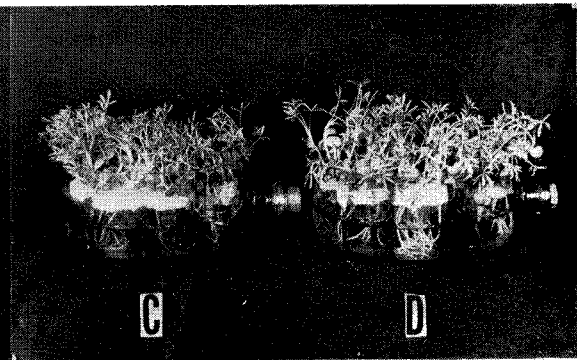
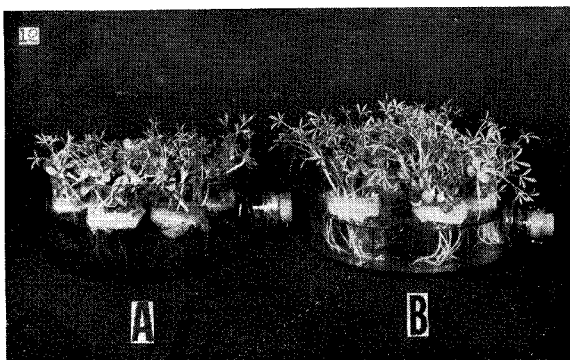
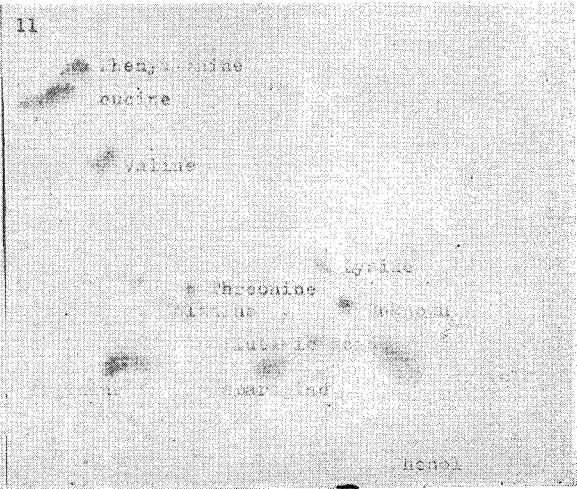
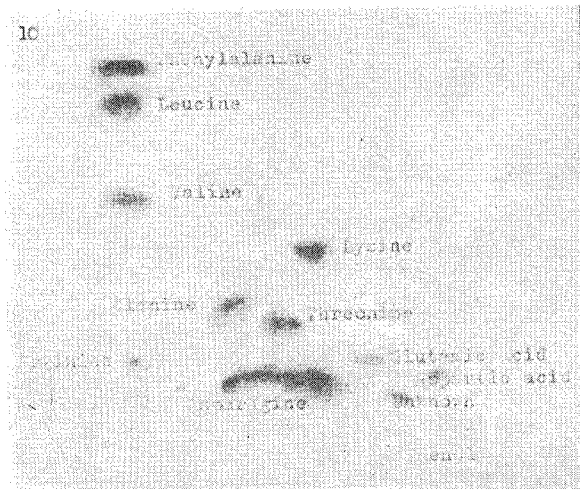
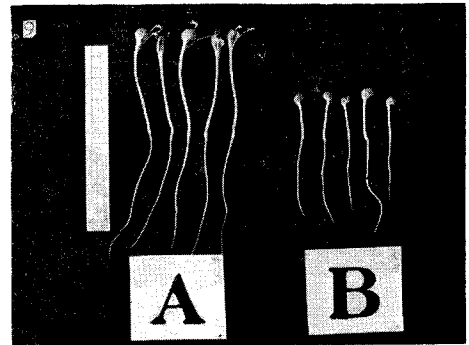
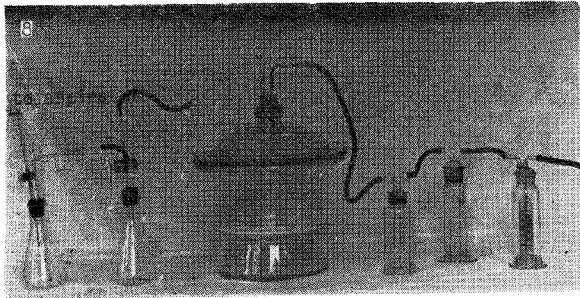
Fig. 8. Construction of apparatus for anaerobic culture in darkness. Description in the text.

Fig. 9. Representative seedlings of *L. luteus* germinated in aerobic condition (A) and anaerobic condition (B) after 10 days.

Fig. 10. Paper chromatogram showing asparagine and amino acids in the alcohol-soluble fraction of lupine seedlings germinated in aerobic condition.

Fig. 11. Paper chromatogram showing asparagine and amino acids in the alcohol-soluble fraction of lupine seedlings germinated in anaerobic condition.

Fig. 12. Lupine seedlings cultured on different culture solution. Distilled water (A). Urea (B). Sodium Nitrate (C). Ammonium Chloride (D).





Using the formulas given in Table 3, the variance components  $\sigma^2_x$ 's and others were estimated from the data for  $X_2$  and  $X_3$  separately. An example of computation (for heading date in  $X_3$ ) is given below.

				(Expected)	
Control:	{	Among lines	$\sigma^2_e + 4.99 \sigma^2_g$	= 5.959	5.990
		Within line	$\sigma^2_e$	= 2.431	2.587
16 Kr:	{	Among lines	$\sigma^2_e + 4.97 \sigma^2_g + 5.47 \sigma^2_{x_3:16Kr}$	= 9.270	9.313
		Within line	$\sigma^2_e + 0.5 \sigma^2_{x_3:16Kr}$	= 2.863	2.892
20 Kr:	{	Among lines	$\sigma^2_e + 4.76 \sigma^2_g + 5.26 \sigma^2_{x_3:20Kr}$	= 20.495	20.514
		Within line	$\sigma^2_e + 0.5 \sigma^2_{x_3:20Kr}$	= 3.727	3.982
25 Kr:	{	Among lines	$\sigma^2_e + 4.97 \sigma^2_g + 5.47 \sigma^2_{x_3:25Kr}$	= 11.341	11.381
		Within line	$\sigma^2_e + 0.5 \sigma^2_{x_3:25Kr}$	= 3.023	3.081
30 Kr:	{	Among lines	$\sigma^2_e + 4.97 \sigma^2_g + 5.47 \sigma^2_{x_3:30Kr}$	= 11.980	12.064
		Within line	$\sigma^2_e + 0.5 \sigma^2_{x_3:30Kr}$	= 3.589	3.144

The least squares solution of these ten equations gave the following estimations of variance components:

$$\sigma^2_e = 2.587 \pm 0.111$$

$$\sigma^2_g = 0.682 \pm 0.050$$

$$\sigma^2_{x_3: 16 Kr} = 0.610 \pm 0.063$$

$$\sigma^2_{x_3: 20 Kr} = 2.791 \pm 0.064$$

$$\sigma^2_{x_3: 25 Kr} = 0.988 \pm 0.063$$

$$\sigma^2_{x_3: 30 Kr} = 1.113 \pm 0.063$$

The expected value of variances thus obtained seem to be in fair agreement with observed ones.

Assuming that  $\sigma^2_{x_3}$  is  $\frac{9}{16} D$ , the values of  $D$  due to different doses of X-rays are estimated as follows:

$$D_{16Kr} = 1.08$$

$$D_{20Kr} = 4.96$$

$$D_{25Kr} = 1.76$$

$$D_{30Kr} = 1.98$$

The data for plant height and panicle length were treated in the same manner. The results of computations are shown in Table 4.

Table 4 shows that in  $X_2$ , the radiation-induced variance increased in accordance with the dosage of X-rays, but in  $X_3$ , it reached the maximum at 20 Kr and then decreased. The results of computation for heading date, given above as an example, also support this conclusion, though  $X_2$  data were not available for this character.

Table 4. Values of variance components estimated.

Item	X <sub>2</sub> Plant height	X <sub>2</sub> Panicle length	X <sub>3</sub> Plant height	X <sub>3</sub> Panicle length
$\sigma^2_e$	16.396±1.808	1.865±0.201	13.222±0.963	2.103±0.141
$\sigma^2_g$	4.235±0.631	0.214±0.070	8.012±0.436	0.376±0.064
$\sigma^2_{x_2:16 \text{ Kr}}$	0.610±0.690	-0.039±0.077	1.330±0.545	-0.113±0.080
$\sigma^2_{x_2:20 \text{ Kr}}$	2.681±0.704	0.093±0.078	6.159±0.555	0.289±0.081
$\sigma^2_{x_2:25 \text{ Kr}}$	3.218±0.699	0.146±0.078	2.156±0.545	0.116±0.080
$\sigma^2_{x_2:30 \text{ Kr}}$	6.296±0.700	0.450±0.078	0.479±0.545	0.057±0.080
D <sub>16Kr</sub> *	5.49	-0.31	2.36	-0.20
D <sub>20Kr</sub>	21.45	0.74	10.95	0.51
D <sub>25Kr</sub>	25.74	1.17	3.83	0.21
D <sub>30Kr</sub>	50.37	3.60	0.85	0.10

$$* \sigma^2_{x_2} (X_2) = \frac{1}{8} D, \quad \sigma^2_{x_3} (X_3) = \frac{9}{16} D.$$

Heritability values for line means were estimated from the data in Table 4 by the formula

$$h^2 = \frac{\sigma^2_x}{\frac{1}{k} \sigma^2_e + \sigma^2_x}$$

As an example, the results for plant height are shown in Fig. 1. The figure clearly shows that the heritability value became highest at 20 Kr in X<sub>3</sub>.

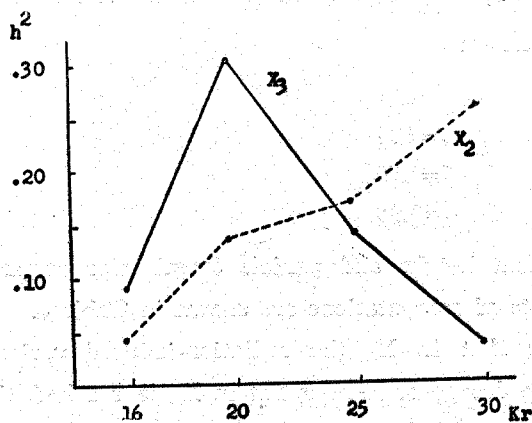


Fig. 1. Heritability values for plant height relative to the doses of X-rays. (Values expected when  $k=1$ )

### Discussion

It is a well known fact that in rice, yield, as well as characters correlated with it, generally show a low heritability value, so that line selection is more preferable than individual selection. The theoretical studies of radiation-induced variances by the writers have shown that when the  $X_2$  is grown in lines, the variance of lines contains only one-eighth of the fixable genetic variance induced by radiation, while in  $X_3$ , it is 9/16 among line means. This indicates that in so far as selection is aimed at characters with a low heritability, we had better grow the  $X_2$  in bulk and separate the  $X_3$  into lines for selection.

It then follows that the dosage of radiation should be determined so as to obtain the highest heritability value of  $X_3$  lines for the desired character. The results of the present study indicate that 20 Kr of X-rays for dormant rice seeds might be such a recommendable dosage. A question may arise, however, as to why the induced genetic variance in  $X_3$  has markedly decreased in plots irradiated at high doses.

In this experiment, when seeds were taken from  $X_2$  plants, sterile or extremely dwarf ones were not selected, while such plants were large in number in plots irradiated at high doses. This might be the reason for the reduction of genetic variance in those plots. However, it seems that even though no such artificial selection was made, natural selection would work in the same direction, because sterile or dwarf plants would produce less seeds than normal ones. We may assume further that inter-cell competition in  $X_1$  plants, and gametic selection or certation in  $X_1$  and  $X_2$  plants, may also eliminate unadaptable mutants, reducing the variability of the progeny of irradiated plants. It is possible that the rate of the reduction increases with the dosage of radiation.

For breeders, such a natural elimination of unadaptable mutants might be a favorable process. For promoting this process, it seems that at least one segregating generation should be grown in bulk under the pressure of natural selection. Further investigations of irradiated populations from the view-points mentioned above may furnish us with information on the basis of which a sound scheme of radiation-breeding may be established.

### Summary

Seeds of a representative "Japonica" rice variety of Taiwan, Taichung No. 65, were X-rayed at different doses, and the resultant variations in several agronomic characters were surveyed in  $X_2$  and  $X_3$ . For analysing the data, formulas were constructed to obtain the expected radiation-induced variances in those generations. The formulas proved that in  $X_2$ , the fixable part of the induced variance is distributed in the variances among and within lines

in a 1:3 ratio, but in a 3:1 ratio in  $X_3$ . Analysis of the data by those formulas showed that in  $X_2$ , the induced variance increased in accordance with the dosage, but in  $X_3$ , the largest value appeared at 20 Kr and decreased at higher doses. Heritability values also showed the same tendency. It was pointed out that selection of  $X_3$  lines might be preferable for breeders, and the reduction of induced variances in plots irradiated at high doses, which might be due to elimination of unadaptable mutants, should be further investigated.

## X光線不同照量對水稻遺傳變異之統計遺傳的研究

高國楠 胡兆華 張文財 岡彥一

本研究係以水稻臺中 65 號為供試材料，將種子以不同的線量處理之，以統計遺傳的方法，就其處理第二代及第三代數農藝性狀加以調查分析。就本研究使用公式證明，X光線所引起的固定的遺傳變異，系統間及系統內於  $X_2$  為 1:3，而於  $X_3$  則為 3:1。又放射線所引起的變異量於  $X_2$  係隨照射線量之增加而增大，於  $X_3$  則以 20 kr 為最大，遺傳力亦顯示出同樣之趨勢（見第 1 圖）。

本研究指出於育種行選拔以於  $X_3$  系統時較為恰當。至於高線量反使變異量減少的原因，可能係由於不適於生存的突變被淘汰所致。關於此點尚須進一步之研究。（摘要）

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