STUDIES ON VARIETAL DIFFERENCES IN RADIOSENSITIVITY IN RICE VI. DIALLEL ANALYSIS OF RADIOSENSITIVITY WITH RESPECT TO REDUCTION IN ROOT LENGTH¹⁾

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The aim of the present experiment is to estimate the genetical control of radiosensitivity in rice varieties and to observe the influence of temperatures after flowering on the radiosensitivity of the seeds produced.

It is well known that the radiosensitivity of plants is controlled not only by nuclear and physiological factors but also by the genotype. In *Atriplex hortensis*, Johnson (1933) observed a greater radiosensitivity in red-colored and white varieties than in the green ones. Since then, many workers have demonstrated variations in radiosensitivity of plant species using various responses to radiation. Few attempts, however, have been directed to the genetic control of radiosensitivity. Genetically conditioned differences in the response to radiation in plants were reported first by Smith (1942), who, in *Triticum monococcum*, found a strain with about two times as high radiosensitivity as the others. Major genic (Lamprecht 1956; Takaki and Yamashita 1967) and polygenic (Gregory 1956; Davies 1962a, b; Lawrence 1963) control of radiosensitivity were recently reported in some plant species.

In the first paper of this series (Ukai 1967), the author reported that rice varieties significantly differed in radiosensitivity as measured by reduction in root length. In this study the genetic control of radiosensitivity was investigated by analyzing the data for root length of the control and irradiated F₂ plants in a diallel experiment of six rice varieties.

MATERIAL AND METHODS

In 1963, crosses between six varieties of *Oryza sativa* L. in all possible combinations were made according to a half diallel design. The varieties used are listed in Table 1. They were chosen on the basis of their differential radiosensitivity. In 1964, the parental and F₁ seeds were sown in a nursery bed and the seedlings were planted in pots. A set of 15 F₁ and 6 parental lines, each consisting of 4 plants, were grown. Within two days after the first flowering, the plants were divided into two groups, one being placed

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Table 1. Rice varieties used for experiments

Symbol	Variety	Type
P 1	Senshō	upland
P 2	Hamayū	lowland
P 3	Rikutō Nōrin 12	upland
P 4	Koshijiwase	lowland
P 5	Shirogane	lowland
P 6	Shimotsuki	lowland

in a 20°C and the other in a 30°C room of a "biotron". The flowering of parental varieties P2, P6 and their F₁ was promoted by a short-day treatment. At maturity all panicles were harvested from each plant.

From the 20°C and 30°C treatments, 30 seeds per line were sampled at random. The seeds were divided into two groups. One group of 15 seeds was used as the control, and the other was exposed to 26 kR of Cs-137 gamma-rays, dose-rate being 3 kR per hour. Prior to irradiation the seeds were stored in a desiccator containing saturated solution of NaClO₃, for more than two weeks until they showed a constant weight. The moisture content of the seeds was then about 14 per cent. Then, they were sealed in plastic bags and irradiated. Immediately after exposure the seeds were soaked in a solution of Usuplun for five hours for sterilization. After being washed with distilled water, they were germinated on filter paper in petri dishes at 30°C. Each petri dish was supplied with 10 ml of distilled water for the first 43 hours and 5 ml for the next 48 hours in addition. Ninety-six hours after irradiation, the length of seminal roots was measured. Two and three replications were made for the 20°C and 30°C treatments, respectively.

As shown in the first paper of this series (Ukai 1967), in rice, the sensitivity as measured by reduction in root length can be expressed by a quantity which is independent of the dosage applied. Namely, if the mean root length of the irradiated plot is expressed as a ratio to the control value, the dose response curves are of sigmoid type for all varieties tested within the dose range from 5 to 150 kR. As the growth of root is not completely inhibited even at the highest dose, we use as a parameter of reduction in root length the ratio of the mean difference in root length between the plot in question and the highest dose plot to the difference between the control and highest dose plots. (E_r). When E_r was plotted against dose D divided by D_{50r} , the dose where E_r is 0.5, no difference was observed between the dose response curves of the varieties tested. To investigate whether or not the following function fits to the observed values with an appropriate value of n , integers from 1 to 12 were substituted for n .

$$E_r = 1 - (1 - e^{-x})^n,$$

where x is a variable proportional to D . The function showed the best fit when n is 4. Dose response curve of a variety, therefore, can be approximated by the following function,

$$E_r = 1 - (1 - e^{-KD})^4 \quad (1)$$

where K is a quantity characteristic of a variety, called "coefficient of sensitivity". From the equation (1) we can estimate K by,

$$K = -D^{-1} \cdot \log_e [1 - (1 - E_r)]^{1/4}$$

In the present experiment K was estimated for each line from the E_r value obtained, and the sensitivity was expressed by $\log_{10} 100 K$. K was multiplied by 100 for avoiding negative logarithms. As shown in the previous paper (Ukai 1969), modifying factors such as water content increased K values of the varieties tested by a constant factor, irrespective of the initial radiosensitivity. Hence, a logarithmic transformation was used to introduce additivity.

The methods used for analysis of a diallel experiment were mainly the ones presented by Hayman (1954, 1958). The statistic presented here follow Hayman's notations.

RESULTS

The mean $\log 100 K$ values for parents and F_2 progenies from the 20°C treatment are given in Table 2, and those from the 30°C treatment are in Table 3.

In the 30°C treatment, the F_1 between P1 and P4 did not survive and an estimated value by the method presented by Taguchi (1960) was interpolated into the P1×P4 cell

Table 2. Diallel table for radiosensitivity after 20°C treatment.
Parental values being underlined.

female \ male						
	P 1	P 2	P 3	P 4	P 5	P 6
P 1	<u>.667</u>					
P 2	<u>.839</u>	<u>.972</u>				
P 3	<u>.719</u>	<u>.881</u>	<u>.761</u>			
P 4	<u>.819</u>	<u>.929</u>	<u>.826</u>	<u>.939</u>		
P 5	<u>.836</u>	<u>.887</u>	<u>.810</u>	<u>.886</u>	<u>.885</u>	
P 6	<u>.834</u>	<u>.970</u>	<u>.819</u>	<u>.899</u>	<u>.908</u>	<u>.933</u>

Table 3. Diallel table for radiosensitivity after 30°C treatment.
Parental values being underlined.

female \ male						
	P 1	P 2	P 3	P 4	P 5	P 6
P 1	<u>.707</u>					
P 2	<u>.760</u>	<u>.778</u>				
P 3	<u>.695</u>	<u>.700</u>	<u>.621</u>			
P 4	(.795)	<u>.760</u>	<u>.802</u>	<u>.849</u>		
P 5	<u>.763</u>	<u>.801</u>	<u>.739</u>	<u>.916</u>	<u>.750</u>	
P 6	<u>.738</u>	<u>.907</u>	<u>.615</u>	<u>.812</u>	<u>.797</u>	<u>.819</u>

of the diallel table. In the left diagram of Fig. 1, the points (V_r , W_r), the limiting parabola $W_r^2 = V_{0L0} \cdot V_r$, and the regression line of unit slope are shown; in the right diagram is shown the correlation between W_r and $W'r$. V_r , W_r and $W'r$ represents the variance of the r th array, the covariance between the parents and their offsprings in the r th array, and the covariance between the non-recurrent array means and the r th array, respectively. V_{0L0} is the variance of the parents.

Fig. 1. (V_r , W_r) and (W_r , $W'r$) graphs for radiosensitivity from 20°C treatment. Numbers indicate parental genotypes.
Left: (V_r , W_r) graph. The straight line represents unit slope.
Right: (W_r , $W'r$) graph. The line shows the best fitting regression.

From 20°C data, the regression of W_r on V_r was significant ($P < 0.01$) and had a slope not significantly differing from unit slope ($P = 0.2$ to 0.1). The significant regression line of unit slope suggested that the gene effect on radiosensitivity after the 20°C treatment fitted the Hayman's genetic model based on additive and dominance effects. There was no evidence for overdominance and non-allelic interaction. In the (W_r , $W'r$) graph, points representing the parents lie on the line of slope one half if gene distribution in the parents is random. The regression of $W'r$ on W_r was significant ($P < 0.01$) and its slope ($b = 0.41$) did not differ significantly from 0.5. Parents with rare genotypes are expected to have their points further below the regression line. The graph did not show such a parent.

The case with 30°C treatment was, however, quite different from the above. The slope of the regression of W_r on V_r was much lower than one ($b = 0.56$), though the deviation of the slope from one was insignificant (Fig. 2.).

In an attempt to identify a parent or parents causing the scattering of points in the (V_r , W_r) graph for the 30°C treatment, each parental array was eliminated in turn and new graphs were calculated from the remaining arrays. The greatest improvement in the regression line was caused by removal of parental arrays 4 and 6, but not by removal of any single parent (Fig. 3). A consistency to expectation of the (V_r , W_r) graph was observed. This indicated that the genetic differences among the four parents were almost entirely ascribable to additive and dominance effects, and that interaction

Fig. 2. (Vr , Wr) and (Wr , $W'r$) graphs for radiosensitivity from 30°C treatment. Numbers indicate parental genotypes.
Left: (Vr , Wr) graph. Right: (Wr , $W'r$) graph.

Fig. 3. (Vr , Wr) and (Wr , $W'r$) graphs from diallel analysis eliminating parental arrays 4 and 6.
Top: (Vr , Wr) graph. Bottom: (Wr , $W'r$) graph.

is mainly between P4 and P6 on one hand and the four parents on the other. Disturbances of the regression shown in the original (V_r , W_r) graph may be ascribed to non-allelic interaction and not to a variation due to error.

Further analysis was made for the 6×6 diallel table from 20°C treatment and the sub-diallel table from 30°C.

Genetic parameters Values for genetic parameters estimated from genetic expectations of variances and covariances are given in Table 4. Error variance was estimated from the variation between replications. The standard errors were calculated from the

Table 4. Estimates of genetic parameters and their standard errors for radio-sensitivity from the 20°C original and 30°C sub-diallel tables. Genetic expectations of variances and covariances, and mean of parents and F_2 table (m_{L0} , m_{L2}) are also shown.

Treatment	$\hat{D}$ $\times 10^{-4}$	$\hat{H}_1$ $\times 10^{-4}$	$\hat{H}_2$ $\times 10^{-4}$	$\hat{F}$ $\times 10^{-4}$	$\hat{h}^2$ $\times 10^{-4}$	$\hat{E}$ $\times 10^{-4}$	$\bar{V}_r$ $\times 10^{-4}$	$\bar{W}_r$ $\times 10^{-4}$	$\bar{W}'_r$ $\times 10^{-4}$	V_{0L0} $\times 10^{-4}$	m_{L0}	m_{L2}
20°C	114.49** ± 1.89	-6.68 ± 9.60	-7.68 ± 8.58	60.20** ± 6.53	-11.16 ± 5.77	5.16 ± 0.71	25.84	50.58	22.02	119.65	.860	.858
30°C sub-diallel	31.70** $\pm .99$	-4.56 ± 6.14	3.36 ± 10.73	-4.72 ± 3.84	124.00** ± 7.28	3.52 $\pm .47$	11.75	17.32	8.91	35.22	.714	.736

** significant at 1% level.

variance of ($V_r - W_r$). Significance by a t test was obtained for additive gene-effect (D) and distribution of genes (F) but not for dominance effects (H_1 and H_2) and sum of dominance deviation (h^2) in the 20°C treatment, while D and h^2 were significant but H_1 , H_2 and F were not, in the 30°C.

Degree of Dominance Dominance can be estimated from the (V_r , W_r) graph. The average dominance deviation in F_2 should be half that in F_1 , and the following relation holds,

$$W_r - V_r = \frac{1}{4} \left(D - \frac{1}{4} H_1 \right)$$

Letting the regression line of unit slope intersect the W_r axis at A and the parallel tangent to the limiting parabola intersect the same axis at B, we can obtain in a diallel table of F_2 progeny that $AB/OB = \frac{1}{4} H_1/D$, where O is origin. Estimates of V_r and W_r as plotted in Figs. 1, 2 and 3 have not been corrected for experimental error (E). These corrections do not alter interpretation of the graphs except that for the level of dominance. The corrections (E and E/n for V_r and W_r , respectively) shift all points in Figs. 1 and 3, 5.16×10^{-4} and 3.52×10^{-4} to the left and 0.86×10^{-4} and 0.88×10^{-4} downward so that lines of unit slope through the adjusted mean points ($\bar{V}_r$, $\bar{W}_r$) will intersect the abscissa at 20.4×10^{-4} and 2.93×10^{-4} for Figs. 1 and 3, respectively. The significance of regression of W_r on V_r and the intersection of line of unit slope with the W_r axis which was above $\frac{3}{4} AB$, suggested that incomplete dominance was

significant in both 20°C and 30°C plots. The insignificance of H_1 and H_2 from both treatments in Table 4 may be due to an overestimation of error E.

The proportion of dominant alleles to all parental alleles is given by $(V_R - \bar{V}_r)/(V_R - V_D)$ where V_R and V_D represent the variance of arrays estimated for completely recessive and dominant parents, which can be obtained from the value of V_r at intersection of the regression line of unit slope with the limiting parabola. A value of 0.686 was obtained for the 20°C treatment. This is an indication that about two thirds of alleles are dominant in the parents used. From the 30°C subdiallel table, the proportion of dominant alleles was calculated to be 0.513, which showed that the number of dominant and recessive alleles were almost equal in the parents.

Subtracting the overall parental mean (m_{L0}) from the overall F_2 mean (m_{L2}), the remainder gives an indication of the mean deviation of dominance as

$$4(m_{L2} - m_{L0}) = h.$$

A small value of h thus obtained (-0.008) and the insignificance of h^2 from the 20°C treatment showed that dominant alleles with positive and negative effects were equal in number, while from the 30°C treatment a positive value of h (0.87) was obtained and h^2 was significant at one per cent level, indicating that dominant alleles with positive effects were predominant in all of the alleles.

The parameter F provides an indication of the relative frequencies of dominant and recessive alleles, being positive when there are more dominant than recessive alleles. The value of F from the 20°C treatment was positive and significantly differed from zero, indicating an excess of dominant alleles, while F from the 30°C treatment was

Fig. 4. Relationship of the mean values from the 20°C treatment (X) to those from the 30°C treatment (Y). The lines are for $Y=X$, and the regression line of unit slope. ○ parent; ● F_1

insignificant, showing that the numbers of dominant and recessive alleles were equal in the parents. These results agreed with those above obtained.

Sensitivity of the parents and F_2 progenies were compared between the 20°C and 30°C treatments (Fig. 4). It can be seen from the graph that the mean radiosensitivity in the 30°C treatment is generally lower than that in the 20°C treatment. The regression line of unit slope intersected the Y axis at -0.094 . Therefore, the ratio of the radiosensitivity expressed by K for the 20°C treatment to that for the 30°C treatment was $10^{0.094}=1.24$ on the average. There was no difference between the parents and F_2 progenies with regard to the change in sensitivity with temperatures.

Analysis of control root growth was made from the data of the 20°C and 30°C treatments. Neither correlation between V_r and W_r or between W_r and W_r' was significant, indicating complete absence of genetic variation in the root growth of controls. Correlation between control growth and radiosensitivity shown by $\log 100 K$ was not significant, either.

DISCUSSION

The present investigation showed that, in rice, a larger part of radiosensitivity as determined by root growth depression is controlled by genetic factors. Moreover, it is interesting to note that epistasis was observed after a 30°C treatment but not after a 20°C treatment. In addition, direction of dominance differed between the low and high temperatures. These facts show that the temperature after flowering much influences not only on mean radiosensitivity but on the genetic behavior of radiosensitivity.

As previously reported (Ukai 1968a, b), the sensitivities as measured by plant height and by the number of dividing cells in root tips gave a close correlation with that in terms of root growth depression. The genetical result observed in this study, therefore, may possibly be applicable to the former sensitivities. Radiation induced pollen- and seed-sterilities and the frequency of chromosome bridges at root tips, however, may not be controlled by the genetic system presented here since these radiation effects showed little or no varietal differences.

So far, few attempts have been made to investigate the influence of plant environment on the radiosensitivity of seeds of the subsequent generation. Yamaguchi (1965) indicated that rice seeds harvested at a high altitudinal location with cool climate showed higher resistance to X-rays than those from lower altitude. A similar result was obtained by Ohba and Simak (1961) using pine seeds collected from different provinces in Sweden. These authors attributed variation in radiosensitivity to differences in catalase content associated with the length of the growing season. In contrast, the present study showed that the seeds harvested at a high temperature exhibited higher radio-resistance than those harvested at lower temperature. The reason for differential radiosensitivity of rice seeds from different altitudes may be found in other environmental factors than temperature.

From the experiments with sensitive varieties which showed a decrease in sensitivity with the increase in soaking time from 1 to 6 hours and with drying back of the seeds after 12 hours of soaking, Ukai (1968c) suggested the presence of a sensitizing factor in

the seeds. As an explanation for the difference in radiosensitivity between the 20°C and 30°C treatments, it may be conceivable that the sensitizing factor suggested is produced after flowering with a lower amount at a high than at a low temperature. More detailed experiments are required for a decisive conclusion.

SUMMARY

The results of diallel analysis of radiosensitivity in six rice varieties were presented. The criterion of radiosensitivity used was reduction in root length following an exposure of the seeds to gamma-rays. It was found that: (1) The temperature under which the seeds ripened had a strong influence not only on mean radiosensitivity but also on the genetical behavior of radiosensitivity in the subsequent generation. (2) After a 20°C treatment, radiosensitivity was determined by a genetic system of additive effect and incomplete dominance. No evidence for nonallelic interaction was obtained. (3) After a 30°C treatment, however, a non-allelic interaction was observed, which was due to two particular parental lines; their removal recovered additivity between loci of the remaining parents. (4) After the 20°C treatment, dominance was in the direction of both increasing and decreasing sensitivity, while dominance after 30°C treatment was in the direction of increasing. (5) The proportion of dominant alleles to all parental alleles also differed according to the temperature treatment. (6) No correlation was found between the radiosensitivity shown by $\log 100 K$ and the root length of the control.

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