# ANALYSIS OF RECIPROCAL TRANSLOCATIONS IN RICE

## —MUTATIONS IN RICE INDUCED BY X-RAYS. V.—

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With the view to utilizing reciprocal translocation method for genic analysis proposed by Burnham (1934, 1946), Yamashita (1940) and Kramer (1954), the writer has made X-ray irradiation of rice seeds (Taichung No. 65, a representative of ponlai rice in Taiwan) since 1954. The semi-sterile plants produced by X-ray irradiations, when selfed, segregated into 1 fertile and 1 semi-sterile plants. In the subsequent generations, the semi-sterile plants continue to segregate in this manner, while the normal plants bred true. When the semi-sterile plants were crossed with original strain, the $F_1$ also showed 1 fertile and 1 semi-sterile segregation ratio. Furthermore, ring of four chromosomes were detected cytologically in the PMCs of these strains; therefore, the semi-sterility was found to be attributable to reciprocal translocations between two non-homologous chromosomes. When the fertile plants produced from a RT heterozygote were back crossed to the original strain, the progeny was either semi-sterile or fertile, and the segregation of fertile and sterile plants in the same cross was never observed. Since there were only semi-sterile individuals, it was then apparent that the male parent of the former type would be a translocation homozygote, while that of the latter would be of the same chromosomal structure as the original strain as reported in the previous paper (Hsieh *et al*. 1959).

The present report deals with results of analysis of interrelations of translocated chromosomes by cytogenetical observation from the $F_1$ plants of intercrosses of RT homozygotes. RT analysis was made regarding the following respects: In $F_1$s between two RT lines, 1) if all the chromosome paired normal, the parental lines would have translocations in the same chromosomes; 2) if a quardrivalent chromosome was formed, the chromosomes concerned in translocations in both lines would be the same though it is possible that their translocations are situated in different arms; 3) if a ring of six chromosomes was formed, the parental lines would have one chromosome in common in their translocations; 4) if two rings of four were found, the parental lines would have quite different translocations.

Cytological observations were made with pollen mother cells of $F_1$ plants

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of intercrosses. Spikelets were taken at 11-12 AM. Farmer's fluid was used as the fixative and the ordinary aceto-carmine smear methods was applied.

### Frequency of types of pairing in intercrosses of RT homozygotes

Intercrosses among fourteen RT homozygotes designated as BF lines were made in order to estimate their interrelations. The frequencies of $1(\cdot)4$, $2(\cdot)4$, and $1(\cdot)6$ varied according to crosses. Sometimes, a giant ring, which might be an octavalent, was found; such was counted as two rings of four. The frequency of occurrence of a ring of four ranged from 10 to 100%, and those of two rings of four and one ring of six ranged from 20 to 80%.

In order to test whether rings occur independently of each other in intercrosses, the theoretical frequencies of occurrence of ring chromosomes were calculated as follows:

Let $a$ be the frequency of one ring of four in the $F_1$ of $a$ RT line with the original strain, and $b$ be the frequency of the other RT line, the frequencies of cells with zero, one and two rings in the intercross of the two are shown by the following formula:

$$\{a+(1-a)\}\ \{b+(1-b)\} = ab \qquad\qquad 2(\cdot)4 \text{ or } (1(\cdot)6)$$
$$+(1-a)b+(1-b)a \qquad 1(\cdot)4$$
$$+(1-a)(1-b) \qquad\qquad 0\ (12_{II})$$

For instance, in the cross $a\ (20\%) \times b(13\%)$, the frequency of $2(\cdot)4$ is expeced to be approximately 3%. Therefore, for cytological analysis of interrelations of RT lines, it is desirable to use lines with a high frequency of rings. Table 1 shows the results of a comparison between calculated and observed numbers of ring chromosomes. The large chi-square values in some crosses indicated that significant differences occur between the expected and observed numbers.

Table 1. Frequency of rings of four and six in $F_1$s of intercrosses of RT homozygotes

| Crosses | percentage of ring of four in parents | | $(1-a)b+(1-b)a$ | $ab$ | | $\dfrac{(1-a)}{(1-b)}$ | Total | $X^2$ |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | $(a)$ | $(b)$ | $1(\cdot)4$ | $2(\cdot)4$ | $1(\cdot)6$ | $0$ | | |
| BF2-2 × BF11-5 | 40.5 | 89.3 | 5 (5.74) | 5 (3.6) | | 0 (0.64) | 10 | 1.37 |
| BF2-2 × BF12-8 | 40.5 | 91.0 | 27 (33.5) | 31 (21.4) | | 0 (3.1) | 58 | 9.09* |
| BF2-2 × BF14-1 | 40.5 | 82.5 | 36 (34.3) | 15 (20.4) | | 10 (6.3) | 61 | 3.81 |
| BF5-5 × BF 6-4 | 60.7 | 34.2 | 46 (45.8) | 20 (17.9) | | 20 (22.3) | 86 | 0.49 |
| BF5-5 × BF12-8 | 60.7 | 91.0 | 36 (21.5) | | 16 (28.7) | 0 (1.8) | 52 | 17.20* |
| BF6-4 × BF 7-7 | 34.2 | 96.0 | 67 (57.5) | 17 (29.2) | | 5 (2.3) | 89 | 9.84* |
| BF6-4 × BF11-5 | 34.2 | 89.3 | 10 (13.8) | 11 (6.7) | | 1 (1.5) | 22 | 3.92 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| BF6-4 × BF12-8 | 34.2 | 91.0 | 76 (68.0) | 27 (38.6) | | 5 (6.4) | 108 | 2.54 |
| BF6-4 × BF14-1 | 34.2 | 82.5 | 28 (32.0) | 25 (15.0) | | 0 (6.1) | 53 | 13.15* |
| BF11-5 × BF16-1 | 89.3 | 58.0 | 16 (20.5) | 28 (24.4) | | 3 (2.1) | 47 | 1.90 |
| BF11-5 × BF20-2 | 89.3 | 60.5 | 14 (17.14) | 25 (22.14) | | 2 (1.72) | 41 | 0.99 |
| BF14-1 × BF16-1 | 82.5 | 58.0 | 56 (50.2) | | 50 (53.5) | 6 (8.3) | 112 | 1.33 |

Note:1)　　　　Number in parenthesis shows expectation.

2)　　*　....The observed number did not fit to the expectation.

## Analysis of reciprocal translocations

From the result of observation of PMCs and Table 1, the interrelations of fourteen RT lines are summarized in Table 2. In the Table 2, a number in parenthesis shows the pairng types assumed on the basis of their pollen fertility. As the data in Table 2 show, some lines such as BF2-2, BF6-4, BF16-1 and BF20-2, showed the same pattern of pairings in crosses with other lines, and no ring was found in their intercrosses. Therefore, these lines may be considered to have the same translocation. This is true also for BF5-5 and BF7-7, and for BF11-5, BF12-8 and BF14-1. However, when the lines BF3-1, BF4-6, BF8-2, BF13-8 and BF17-2, were intercrossed, the $F_1$s showed 12II, and when they were crossed with other lines such as BF5-5, BF7-7, BF11-5, BF14-1 and BF12-8, the $F_1$s showed 1(·)4 while no 2(·)4 or 1(·)6 was found. Probably, such lines were not true RT lines.

Table 2.　Interrelations of translocation lines, estimated from the results of cytological observations of intercrosses.

| | BF 4-6 | BF 5-5 | BF 6-4 | BF 7-7 | BF 8-2 | BF 11-5 | BF 12-8 | BF 13-8 | BF 14-1 | BF 16-1 | BF 17-2 | BF 20-1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BF 2-2 | | 2(·)4 | $(12_{II})$ | 2(·)4 | (1(·)4) | 2(·)4 | 2(·)4 | (1(·)4) | 2(·)4 | $12_{II}$ | | $(12_{II})$ |
| BF 3-1 | $12_{II}$ | | 1(·)4 | 1(·)4 | $(12_{II})$ | 1(·)4 | 1(·)4 | $12_{II}$ | | 1(·)4 | $(12_{II})$ | |
| BF 4-6 | | 1(·)4 | 1(·)4 | 1(·)4 | $(12_{II})$ | | | | 1(·)4 | | $(12_{II})$ | |
| BF 5-5 | | | 2(·)4 | $(12_{II})$ | | 1(·)6 | 1(·)6 | 1(·)4 | 1(·)6 | 2(·)4 | 1(·)4 | 2(·)4 |
| BF 6-4 | | | | 2(·)4 | 1(·)4 | 2(·)4 | 2(·)4 | 1(·)4 | 2(·)4 | $12_{II}$ | 1(·)4 | $(12_{II})$ |
| BF 7-7 | | | | | 1(·)4 | 1(·)6 | 1(·)6 | 1(·)4 | 1(·)6 | 2(·)4 | 1(·)4 | (2(·)4) |
| BF 8-2 | | | | | | 1(·)4 | 1(·)4 | $(12_{II})$ | 1(·)4 | 1(·)4 | | |
| BF11-5 | | | | | | | $(12_{II})$ | | $12_{II}$ | 2(·)4 | | 2(·)4 |
| BF12-8 | | | | | | | | 1(·)4 | $(12_{II})$ | (2(·)4) | | 2(·)4 |
| BF13-8 | | | | | | | | | 1(·4) | 1(·)4 | $12_{II}$ | |
| BF14-1 | | | | | | | | | | 1(·)4 | 1(·)4 | (2(·)4) |
| BF16-1 | | | | | | | | | | | 1(·)4 | $12_{II}$ |

Table 3. Result of RT analysis

| | BF 2-2<br>BF 6-4<br>BF16-1<br>BF20-2 | BF 5-5<br>BF 7-7 | BF11-5<br>BF12-8<br>BF14-1 | |
|---|---|---|---|---|
| BF 2-2<br>BF 6-4<br>BF16-1<br>BF20-2 | | 2(·)4 | 2(·)4 | $RT_1$ |
| BF 5-5<br>BF 7-7 | | | 1(·)6 | $RT_2$ |
| BF11-5<br>BF12-8<br>BF14-1 | | | | $RT_3$ |
| | $RT_1$ | $RT_2$ | $RT_3$ | |

According to the above analysis, the RT lines were grouped on the basis of their behavior in respective crosses, as shown in Table 3, reciprocal translocations apparently different from one another are only three among 14 analyzed. They were further designated as $RT_1$, $RT_2$, and $RT_3$ respectively.

Assuming that from two certain chromosomes which had been in structure $1-\bar{1}$ and $2-\bar{2}$ originally, a reciprocal translocation, 1-2 and $\bar{1}-\bar{2}$ was induced by X-ray irradiation, and the semi-sterile plants were such a structutre as $\dfrac{1-\bar{1},\ 2-\bar{2}}{1-2,\ \bar{1}-\bar{2}}$, a ring of four could be formed. In the cross between $RT_1$ and $RT_2$ showed two rings of four, and the interchanged chromosomes were different from each other, then it may have such RT constitution as $\dfrac{1-\bar{1},\ 2-\bar{2}}{1-2,\ \bar{1}-\bar{2}} + \dfrac{3-\bar{3},\ 4-\bar{4}}{3-4,\ \bar{3}-\bar{4}}$. In the cross of $RT_2$ × $RT_3$, one ring of six was formed, then the RT constitution may be assumed to be $\dfrac{3-\bar{3},\ 4-\bar{4},\ 5-\bar{5}}{3-4,\ \bar{3}-5,\ \bar{4}-\bar{5}}$. In this case, the common chromosome is $4-\bar{4}$. In the cross of $RT_1$ × $RT_2$, the RT constitution may assumed to be 2(·) 4 = $\dfrac{1-\bar{1},\ 2-\bar{2}}{1-2,\ \bar{1}-\bar{2}} + \dfrac{4-\bar{4},\ 5-\bar{5}}{4-5,\ \bar{4}-\bar{5}}$. According to the above analysis, the interrelation of the 3 RT lines can be summarized as shown in Table 4.

Table 4. The chromosome interrelations of RT lines

| | Chromosome number | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| $RT_1$ | 0 | 0 | | | |
| $RT_2$ | | | 0 | 0 | |
| $RT_3$ | | | | 0 | 0 |

### Relation between the ocurrence of ring chromosomes and pollen fertility

At diakinesis and first metaphase of a translocation heterozygote, a quardrivalent in the form of "Zigzag" or "Open". In the former, alternate chromosomes proceed to the same pole; in the later, adjacent ones moved together. If these three types of disjunction were equally frequent and no cross over occurred, a 66.6% pollen sterility would be expected as follows:

$$
\begin{array}{lll}
\text{"Zigzag" ring (alternate)} & 1/3 = 33.3\% & \text{Fertile} \\
\text{"Open" ring} \left\{ \begin{array}{l} \text{adjacent—I} \\ \text{adjacent—II} \end{array} \right. & \left. \begin{array}{l} 1/3 = 33.3\% \\ 1/3 = 33.3\% \end{array} \right\} & 66.6\% \text{ Sterile}
\end{array}
$$

When the "Zigzag" and the "Open" types were equally frequent, a 50% pollen abortion will be expected. As shown in Table 5, the percentage of good pollen actually found ranged from 30% to 60%, the average being 50%. This suggests that the "Zigzag" and the "Open" ring might be equally frequent. This value approaches those reported by Oka (1953) and Hwang (1958). Based on this value, when two rings of four are formed, the pollen fertility is expected to be 25%. The observed value was 22.9%, a little lower than the expectation.

When a ring of six is formed, fertility (alternate-segregation) may be estimated as follows:

Let $R$ represent the number of chromosomes associated with the ring ($2R = 6$ for a ring of six), the number of different modes of disjunction may be $_{2R}C_R$. It may be assumed that alternate disjunction occurs with the frequency $\dfrac{2}{_{2R}C_R} = \dfrac{2}{_6C_3} = \dfrac{6 \times 2}{6 \times 5 \times 4} = \dfrac{1}{10}(\because R = 3)$. That of adjacent segregation is $1 - \dfrac{1}{10} = \dfrac{9}{10}$. From this, if we extend the principle of semi-sterility in the simplest case (i. e. ring of 4), then the frequency of alternate-disjunction in a ring of six is estimated to be $\dfrac{2}{2+9} \times 100 = 18.1\%$, and that of adjacent disjunction is 81.9%. This value approaches the observed fertility 17.1%. The theoretical value for 1(·)4 calculated by Hwang (1958), based on the frequency of bivalents and zigzag type quardrivalents, was 49.6%. He also used Yamashita's (1948) formula[2] and calculated the values for 2(·)4 and 1(·) 6 to be 22% and 17% respectively. They showed good fits to the values obtained in the present study.

Table 5.  Relation between pairing types and pollen
fertility in intercrosses

| | Good pollen in per cent | | | | | | | | | | | Total | Average |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | lines | (%) |
| 0 | | | | | | | | | 2 | 5 | 3 | 10 | 91 0 |
| 1(·)4 | | | | 1 | 4 | 16 | 6 | | | | | 27 | 50.0 |
| 2(·)4 | | 2 | 8 | 7 | | | | | | | | 17 | 22.9 |
| 1(·)6 | | 3 | 3 | 1 | | | | | | | | 7 | 17.1 |
| Total | | 5 | 11 | 9 | 4 | 16 | 6 | | 2 | 5 | 3 | 61 | |

(2)  Yamashita's (1958) formala:  $Y = Kdt$: where $Y$ is pollen fertility, $t$ is the number of translocation, $K$ is constant for chromosome separation (usually 1) and $d$ is frequency of zigzag type ring.

As mentioned above, the percentage of good pollen is lowered in proportion with the number of ring chromosomes. For instance, the fertility of a plant having a ring of eight is expected to be 5.6%. Therefore, it would be difficult to establish larger rings such as ring of ten or more.

It will be concluded, according to the above mentioned observations, that at least three different RT lines out of 14 were found. It was identified further that five chromosomes were involved in three reciprocal translocation lines. Artificially induced segmental interchanges may be useful for establishing linkage groups as pointed out by Anderson (1945), Kramer (1954) and Burnham (1956). It is also useful to locate given genes on a chromosome by using linkage relationships with a certain marker gene to the relevant chromosomes. The writer is now on the way to prepare more strains such as reported in the present work for linkage analysis and other genetical studies.

## Summary

Fourteen reciprocal translocation homozygotes obtained through test cross of RT heterozygotes which were induced by X-rays were intercrossed. The interrelation of interchanged chromosomes of these lines was studied cytologically by the aceto-carmine smear technic. RT analysis was made regarding the following respects: In $F_1$s between two RT lines; 1) if all the chromosome paired normally, the parental lines would have translocations in the same chromosomes, 2) if a quardrivalent chromosome was formed, the chromosomes concerned in translocations in both lines would be the same though it is possible that their translocations are situated in different arms, 3) if a ring of six chromosomes was formed, the parental lines would have one chromosome in common in their translocations and 4) if two rings of four were found, the parental lines would have quite different translocations.

The result of RT analysis made according to this rule showed that at least three different RT lines out of 14 were identified, and five chromosomes out of 12 were involved in different reciprocal translocations within the materials. The percentage of good pollen in plant having 1(·)4, 2(·)4 and 1(·)6 were 50%, 22.9% and 17.1% respectively and showed good fits to the expectation. The more RT strains are being isolated and the linkage analysis is now under way.

# 水　稻　染　色　體　相　互　轉　座　之　分　析

## 水稻經X線處理後所發生之突然變異之第五報

### 謝　順　景

　　水稻經X線處理後其後代中產生多種半不稔體 (Semi-sterile plant)。此種半不稔稻自交後分離出稔實個體及半不稔個體其比例爲 1 ： 1 。若將所分出之半不稔稻再行自交則其後代同樣分離爲，稔實及半不稔個體。稔實稻固定，不再發生分離現象，但半不稔稻則繼續以 1 ： 1 之比例分離。經檢查其花粉母細胞結果，發現有頻度甚高之環形四價染色體 (Ring of four chromosome)，故認爲半不稔性係由染色體之相互轉座所致。由相互轉座異型體所分出之稔實個體若回交於正常系統，則其後代非全部爲半不稔體，卽全部爲稔實個體，前者卽爲相互轉座同型體 (RT homozygote)。

　　爲明瞭轉座染色體間之相互關係乃將14個相互轉座同型體互交，並按如下標準分析染色體相互間之關係。(1) 若染色體之配對完全正常，則染色體之轉座發生在同一條染色體之相同位置上。(2) 若形成一個四價染色體，則表示兩個系統之轉座染色體相同，但轉座發生在不同的臂 (arm) 上。(3) 若形成一個六價染色體，則轉座染色中一對爲相同。(4) 若形成兩個四價染色體，則雙親之轉座染色體完全不同。根據此種原則進行染色體相互轉座之分析結果，發現14個相互轉座系統中，可歸納爲不同的三羣，此三羣之染色體轉座，發生於十二條染色體中之五條染色體上。

　　具有一個四價，二個四價及一個六價染色體植株之正常花粉率各爲50％，22.9％及17.1％，此值與期待值甚爲符合。上述材料正準備作連鎖分析之用。(摘要)

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