

KARYOLOGICAL STUDIES OF TRIPLOID RICE PLANTS

I. Chromosome pairing in autotriploid of *Oryza sativa* L.

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Triploid plants of rice are distinguished from diploid by their possession of broad leaves, large spikelets, and awns which appear even though the diploid progenitor is awnless. Their seed fertility is less than 1%, while empty seeds develop due to parthenocarpy in 30% to 40% of the spikelets. In rice, it is difficult to get triploids from hybridization between tetraploid and diploid (Okura, 1940; Morinaga and Kuriyama, 1959). Spontaneous triploids are however found in rice fields at a low frequency. Such a plant was found first by Nakamori (1932), and then by Ramiah *et al.*, (1933), Ichijima (1934), Shiga (1935), etc. Observations of meiotic chromosomes were reported by Morinaga and Fukushima (1935) and Ramanujam (1937).

Triploid plants of rice are of interest in relation to the following points: 1) How do the chromosomes pair in meiosis? 2) How is the secondary association? And, 3) how can we get heteroploid plants, especially trisomics, from them? The first and second problems are dealt with in this paper.

Materials and Methods

The triploid plants of different origins were used. One was found in a pure lines of Norin no. 8 (a Japanese variety), which had been obtained from a haploid plant by chromosome doubling. The other was found in an induced-mutant line (X_8 generation) of Taichung no. 65. They have been preserved by ratooning together with their diploid and haploid sibs. Both of them showed 36 chromosomes in root-tip cells.

For observing PMC's, young panicles taken at 10-11 A. M. were fixed with Farmer's fluid for 24 hours, and were preserved in 75% alcohol. Slides were prepared by the ordinary aceto-carmine smear method.

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Results of Observation

In diakinesis through first metaphase, the number of chromosome bodies varied from 12 to 20 among cells. Those chromosomes could be distinguished into trivalent, bivalent and univalent by their size and shape. Since the two triploid plants, obtained from Norin no. 8 and Taichung no. 65, showed no difference in the mode of pairings, the pooled data are presented in this paper.

At diakinesis, one to two trivalents were found to be attached to the nucleolus. Two cells at this stage had a H-shaped hexavalent, as showed in Fig. 1. From late diakinesis to first metaphase, the chromosomes showed secondary associations. An example for this is shown in Fig. 2, in which are seen 12 trivalents. Ten of them form two groups of three and two groups of two, while the remaining two are attached to the nucleolus. Considering the latter two as a group, we can enumerate five groups of trivalent chromosomes. This figure may then be regarded as representing the putative maximum secondary association in rice, $2(3)+3(2)$. Fig. 3 also shows a cell with similarly high secondary association. In Fig. 5, a metaphasic cell is shown having 10 trivalents 2 bivalents and 2 univalents, not only trivalents but also univalents come near other and appear as if in secondary association. The writer considers it to be secondary association of univalents as found in haploid plants. Secondary associations were also found in cells at second metaphase, though observations were not made in detail.

The frequencies of PMC's containing trivalents, bivalents and univalents in different combinations, observed in diakinesis through first metaphase, are given in Table 1. As shown in the table, the average number per cell of trivalents was about 10. The cells with 12 trivalents were 23.5% of the total observed number. When the number of trivalents is less than 11, the remaining chromosomes will either form bivalents or remain as univalents. In this case, it seemed that pairings could occur between non-homologous chromosomes. For instance, when 10 trivalents are formed, the remaining 6 chromosomes will consist of two groups of three homologous ones, as denoted by (a, a, a, b, b, b). They were found to form 3 bivalents resulting in a pairing type $10_{III}+3_{II}$. Then, one of the bivalents must be of the constitution a-b, or, the three bivalents may be a-a, b-b and c-c. Under this assumption, it must be that one of the trivalents is a-b-c, or two are a-d-d and b-c-e, or three are a-d-d, b-e-e and c-f-f. Thus, the pairing type $10_{III}+3_{II}$, $9_{III}+4_{II}+1_I$, $8_{III}+6_{II}$, etc., shown by an asterisk in Table 1, would involve pairings between non-homologous chromosomes. The table shows that such non-homologous pairings occurred in about 10% of PMC's.

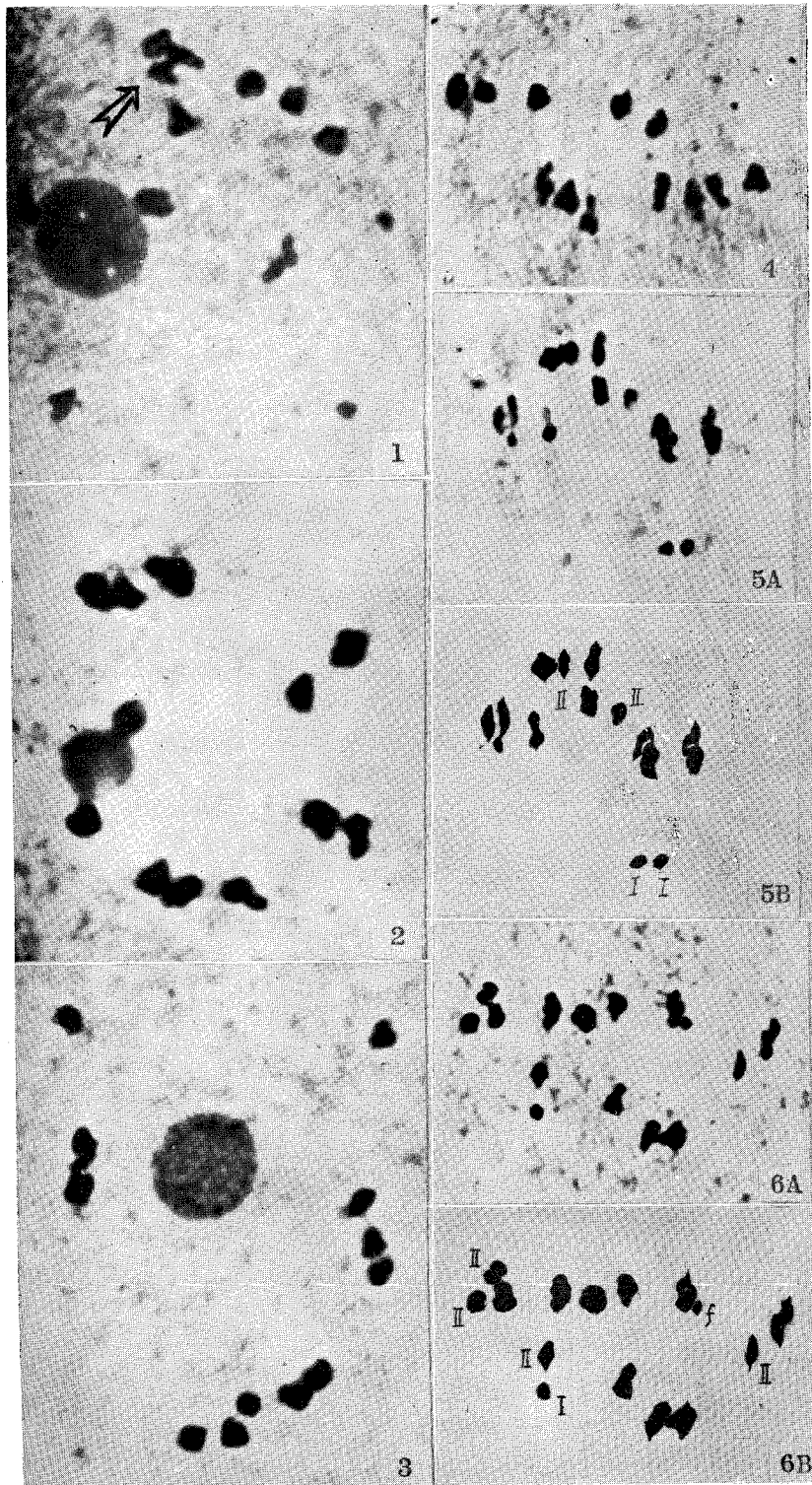


Table 1. Frequencies of PMC's with different pairing types, observed at diakinesis and meta-I in two triploid rice plants

| Chromosome configuration | | | Norin no. 8 | | Taichung no. 65 | | Total no. of cells observed | % |
|--|----|----|-------------|--------|-----------------|--------|-----------------------------|-------|
| | | | Dia | Meta-I | Dia | Meta-I | | |
| 12 | | | 19 | 34 | 25 | 26 | 104 | 23.5 |
| 11 | 1 | 1 | 14 | 32 | 23 | 35 | 104 | 24.4 |
| 10 | 0 | 3 | 1} 15 | 2} 34 | 1} 24 | | 4 | |
| 10 | 3* | | 4 | 6 | 3 | 2 | 15 | 21.3 |
| 10 | 2 | 2 | 6} 16 | 26} 37 | 15} 18 | 19} 23 | 66 | |
| 10 | 1 | 4 | 6 | 5 | | 2 | 13 | 15.4 |
| 9 | 4 | 1* | 6 | 4 | 4 | 2 | 16 | |
| 9 | 3 | 3 | 9} 16 | 28} 37 | 4} 8 | 5} 7 | 46 | 68 |
| 9 | 2 | 5 | 1 | 4 | | | 5 | |
| 9 | 1 | 7 | | 1 | | | 1 | 8.1 |
| 8 | 6* | | 1 | | | | 1 | |
| 8 | 5 | 2* | 5} 12 | 1 | 1} 4 | 5 | 7 | 36 |
| 8 | 4 | 4 | 4 | 12} 15 | 3} | | 24 | |
| 8 | 3 | 6 | 2} | 2} | | | 4 | 4.3 |
| 7 | 6 | 3* | 2} | 2} | | 1} 4 | 5 | |
| 7 | 5 | 5 | 3} 5 | 7} 10 | | 3} | 13 | 19 |
| 7 | 4 | 7 | | 1} | | | 1 | |
| 6 | 8 | 2* | 1} | | | | 1 | 1.8 |
| 6 | 6 | 6 | | 2} | | | 2 | |
| 6 | 5 | 8 | 1} 3 | 3} | | | 4 | 8 |
| 6 | 4 | 10 | 1} | | | | 1 | |
| 5 | 8 | 5* | | 1} | | | 1 | 5 |
| 5 | 7 | 7 | | 2} | | | 2 | |
| 5 | 6 | 9 | | 1} | 5 | | 1 | 5 |
| 5 | 5 | 11 | | 1} | | | 1 | |
| Total no. of cells | | | 86 | 177 | 79 | 100 | 442 | 100.0 |
| Average no. of III | | | 9.84 | 9.87 | 10.73 | 10.08 | 9.95 | |
| No. of cells with non-homologous pair. | | | 19 | 14 | 8 | 5 | 46 | 10.0 |
| % | | | 22.9 | 7.91 | 10.13 | 5.00 | 10.41 | |

Chromosome pairing in PMCs of autotriploid *Oryza sativa* L.

- Fig. 1. Diakinesis, showing a H-shape hexavalent (indicated by an arrow), eight trivalents, two bivalents and two univalents.
- Fig. 2. Diakinesis, showing two groups of three trivalents and three groups of two trivalents in secondary association.
- Fig. 3. Diakinesis, showing a 2(3)+1+1 configuration of trivalents.
- Fig. 4. Metaphase I, showing 12 trivalents.
- Fig. 5. Metaphase I, ten trivalents, two bivalents and two univalents in secondary association are found. (B. camera lucida drawing from A)
- Fig. 6. Metaphase I, nine trivalents, four bivalents, one univalent and a fragment are found.

Discussion

The mode of pairing of chromosomes in triploid rice has been observed by several workers. Morinaga and Fukushima (1935) reported that 12 trivalents were formed in PMC's. Ramanujam (1937) reported that cells with 10 trivalents were most frequent, while a cell had a hexavalent. A pairing type involving non-homologous pairings, as found by the present writers, was described by Ichijima (1934), that was $5_{III}+10_{II}+I_1$.

In the writers' materials, the average number of trivalents per cell was about 10, as found by Ramanujam. It is known that in autotetraploid rice the average number of quadrivalents is 7 to 8 (Cua, 1950; Oka *et al.*, 1955). Failure of chiasma formation may be responsible for the shortage of tri- or quadrivalents in these autopolyploids. When trivalents were not formed in full number, it was pointed out that pairings could occur between non-homologous chromosomes. This suggests that in the genome of rice, the constituent members have some partially homologous segments. The non-homologous pairing in triploid may then be compared with the intra-genome pairing found in haploid plants (Hu, 1957, 1960). Besides this, the occurrence of a hexavalent chromosome in a few cells, as well as pronounced secondary association between trivalents, were found. These also suggest the presence of partial homology between members of a genome.

The senior writer formerly pointed out that the pattern of intra-genome pairings found in haploid rice was consistent with that of secondary association in diploid, and the frequency distribution did not fit the Poisson distribution. It was concluded, accordingly, that these phenomena could not be due to random association of chromosomes (Hu, 1957, 1960). He found further that different species of *Oryza* had almost the same pattern of secondary association, the maximum type being $2(3)+3(2)$. He then considered, reviewing various previous papers on secondary association, that the genomes of those species might have partially homologous chromosomes, and as had been inferred by previous workers, the basic number of the genus *Oryza* might be five (Hu, 1962). It may be inferred that in the origin of the genus, the secondarily balanced consisting of 12 chromosomes might have been established by natural selection which favored readjustment of the chromosomes towards diploidization.

Summary

Using two autotriploid plants of rice, one obtained from Norin no. 8 and the other from Taichung no. 65, the pairing of chromosomes in PMC's was observed. The two plants showed no apparent difference. The average number

of trivalents per cell was about 10. It was pointed out that such pairing type as $10_{III}+3_{II}$, $8_{III}+6_{II}$, etc. were found in about 10% of cells, in which pairings between non-homologous chromosomes must be involved. Two cells with a hexavalent, as well as pronounced secondary association between trivalents, were also found. Referring to the senior writer's previous observations, these phenomena would be indicative of the presence of partial homology within the genome of this plant.

稻三倍體植物之核學的研究

第一報 栽培稻 *Oryza sativa* L. 同質三倍體 之染色體接合

胡兆華 何克明

觀察水稻農林八號及臺中六五號三倍體植物之花粉母細胞染色體之接合，結果兩品種間無差異，平均一細胞當三價染色體數為 10 個。約有 10% 之細胞其染色體接合型式為 10 個三價體與 3 個二價體，8 個三價體與 6 個二價體等，此種接合必包括非相同染色體接合在內。此外，發現有兩個細胞顯出 1 個六價體，又於三價體間見有明顯的次級接合，參照筆者（胡）前在種種水稻單倍體植物，二倍性及四倍性野生稻種之核學的觀察，此種接合現象可謂係稻染色體組其內具有部分相同性。（摘要）

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