

CYTOGENETICAL STUDIES OF *ORYZA SATIVA* L. AND ITS RELATED SPECIES

II. Study on Meiosis in Haploid *O. sativa*⁽¹⁾

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The meiotic behavior of haploid rice were described in the past by Morinaga and Fukushima (1934) and Hu (1957, 1960). It was stated that haploids were completely sterile, unless pollinated by normal pollen from diploid plants (Chang 1964). In our laboratory, one plant was obtained this way. It was cytologically observed and proved to be a diploid plant. In our former studies of the interspecific hybrid of rice, the mechanisms for unreduced gametes formation were studied in detail (Li *et al* 1964, Yang *et al* 1965, Ho and Li 1965). Since unreduced gametes were produced in the haploid plant of rice (var. Taichung, a *japonica* type), it would be of interest to find out the mechanisms involved in the unreduced gametes formation. This is reported here in this paper.

The microsporocytes were fixed in Farmer's fluid with 0.5% ferric chloride as a mordant. Propiano-carmin was used for staining. Regular smear method was used for preparations for cytological study.

Results and Discussion

1. Pollen Fertility

Of 7,533 pollen grains counted, 262 were well-stained with I₂-KI solution (fig. 1). The percentage of good pollen was found to be 3.48%. Perhaps identical situation would be found in macrosporogenesis. Accordingly, this value observed was quite close to the seed fertility of haploid plant after being pollinated with normal pollen as found by Morinaga (1964)

2. Frequency of bivalents in haploid microsporocytes

The chromosome pairing in microsporocytes of the haploid plant at diakinesis and metaphase-I was described by Hu (1957). The cells with

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chromosomes in juxtaposition to each other in pairs which were indicated as secondary association by Hu (1960) were also observed in our materials (fig. 3). In our study, only true bivalents either of close or of open shape (fig. 4, fig. 5) at metaphase-I were counted as true paired chromosomes. The results were listed in table 1 and were found to be quite similar to those observed by Hu (1960) in *O. Glaberrima* haploids. Out of 143 cells counted, the average frequency of bivalents per cell was 0.497.

Table 1. *Frequency of cells with true bivalents in the microsporocytes in haploid at metaphase-I*

Configuration	Type of bivalents	Frequency	Percentage
12 ^I		80	55.94
10 ^I + 1 ^{II}	open	49	34.27
10 ^I + 1 ^{II}	close	6	4.19
8 ^I + 2 ^{II}	open	4	2.8
8 ^I + 2 ^{II}	close	2	1.4
8 ^I + 2 ^{II}	open and close	2	1.4
Total		143	100.00

3. Meiosis in the haploid sativa

There were three major types of meiotic division in haploid microsporocytes.

a. Division being more or less regular

Most of microsporocytes were found to be with a large nucleus at diakinesis (fig. 2), which would then undergo normal meiotic division. Usually, metaphase-I plates could be observed either with only 12 univalents, or with univalents and one or two bivalents (fig. 3, 4, 5, 6). The bivalents usually congress at the equator. Whereas the univalents, it seemed, did not come at the equator at all but were rounded up randomly at the poles (fig. 4, 5). At the end of the first meiotic division, a dyad would be formed with each having $6 \pm$ chromosomes (fig. 7). At the second meiotic division, the chromosomes lined up more or less regularly at the equatorial plate (fig. 8) leading to a regular anaphase-II division (fig. 9). Subsequently tetrads with sporads of similar or of unequal size were formed (fig. 10, 11). These sporads with about 6 chromosomes would degenerate.

Occasionally, a PMC was found with the nucleus located at one side of the cell instead of at the center. In this case, the spindle was formed with displaced nucleus. At the end of the first meiotic division, an unequal dyad would be formed. After second metaphase (fig. 13) a tetrad with two large

sporads and two small ones was the end product (fig. 14). Again, these sporads with chromosomes fewer than 12 would be degenerated.

b. Unreduced gametes production due to the formation of restitution nucleus

In some of the microsporocytes, small nuclei with sticky chromosomes were observed (fig. 15). In this kind of cells, a small and compact spindle was formed at metaphase-I (fig. 16). This would lead to the formation of restitution nucleus (fig. 17, 18). Post-restitution nucleus division was normal. A metaphase figure with long spindle then followed. At anaphase, split chromatids moved toward their respective poles (fig. 19, 20). A dyad instead of the tetrad was formed. Each sporad with 12 chromosomes was the end product (fig. 21).

c. Unreduced gametes formation due to the non-synchronization of nuclear division and cytokinesis

Occasionally, microsporocytes were found to have eccentrically placed nucleus but a normally placed spindle (fig. 22, 23). Nuclear division seemed to precede cytokinesis. After the completion of cytokinesis, the cell was divided into two daughter cells. One was with an intact nucleus; whereas the other was devoid of any (fig. 24). At the second division, all the 12 chromosomes in one of the two daughter cells split and moved toward their respective poles (fig. 25). Then, a triad was found with two sporads containing 12 chromosomes in each and one sporad containing no nucleus (fig. 26). Or sometimes an unequal tetrad was formed, with two sporads containing 12 chromosomes and the other two empty ones having no chromosome at all (fig. 27).

These mechanisms of unreduced gametes formation were also found in the interspecific hybrids of *O. sativa* × *O. officinalis* (Li *et al* 194), *O. sativa* × *O. brachyantha* (Yang *et al* 1965) and *O. sativa* × *O. australiensis* (Ho and Li 1965).

Conclusion and Summary

The meiotic division of the haploid *O. sativa japonica* was studied in detail. The average true bivalent per cell was observed to be 0.497. Normal meiotic division took place in most of the microsporocytes of the haploid rice plant. At metaphase-I, the bivalents congressed at the equator regularly whereas the univalents seemed to be rounded up at the nearest pole without any congression. The microspores so produced having chromosomes fewer than 12 were all sterile. Occasionally, unreduced gametes were formed. Thus the percentage of well-stained pollen grains was found to be 3.43%. The mechanisms for unreduced gametes formation were similar to that observed in the inter-

specific hybrids of *O. sativa* × *O. officinalis*, *O. sativa* × *O. brachyantha* and *O. sativa* × *O. australiensis*. They were:

a. Unreduced gametes production due to the formation of restitution nucleus. Consequently dyads were obtained after two successive divisions. Each with an unreduced number of chromosomes (12).

b. Unreduced gametes formation due to the nonsynchronization of nuclear and cytokinesis. As a result, triads were obtained. Two of the sporads would have the unreduced number of chromosomes (12) but the other was devoid of a nucleus.

Oryza sativa L. 及其近緣種之細胞遺傳學的研究

II. 單元體 *O. sativa* 之減數分裂

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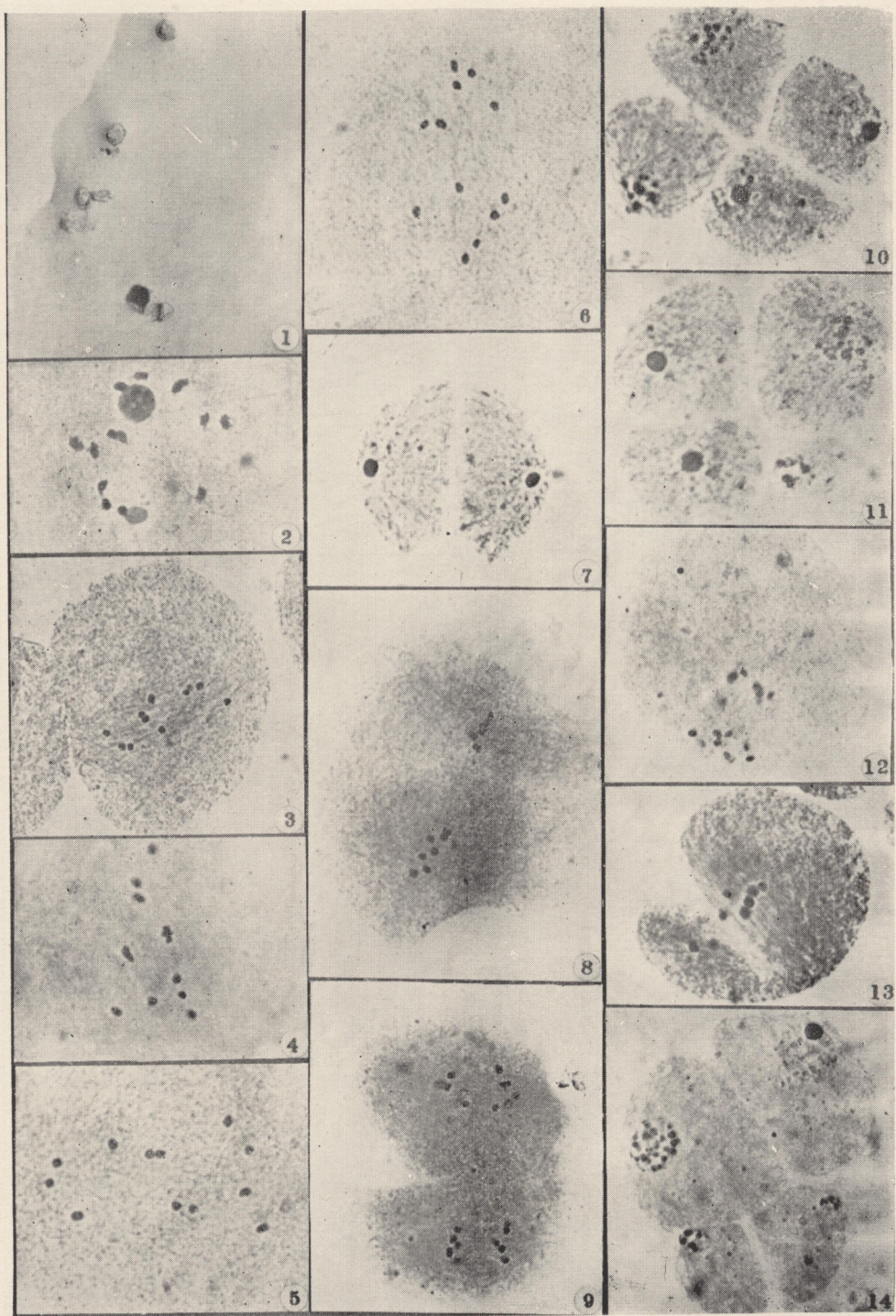
O. sativa (臺中65號) 單元體植株之小孢子母細胞減數分裂經詳盡的研究，其絕大多數是正常的。在第一次分裂中期每細胞含真正二價體數平均為 0.497 個。在後期時二價體在赤道板上，而單價體分散於二極。經正常減數分裂產生之小孢子含染色體數少於十二，皆為不孕者。有時會有染色體數不減的配偶子形成。花粉率為 3.48%。其形成染色體數不減的配偶子之原因與 *O. sativa* × *O. officinalis*, *O. sativa* × *O. brachyantha* 和 *O. sativa* × *O. australiensis* 等種間雜種相應，簡述如下：

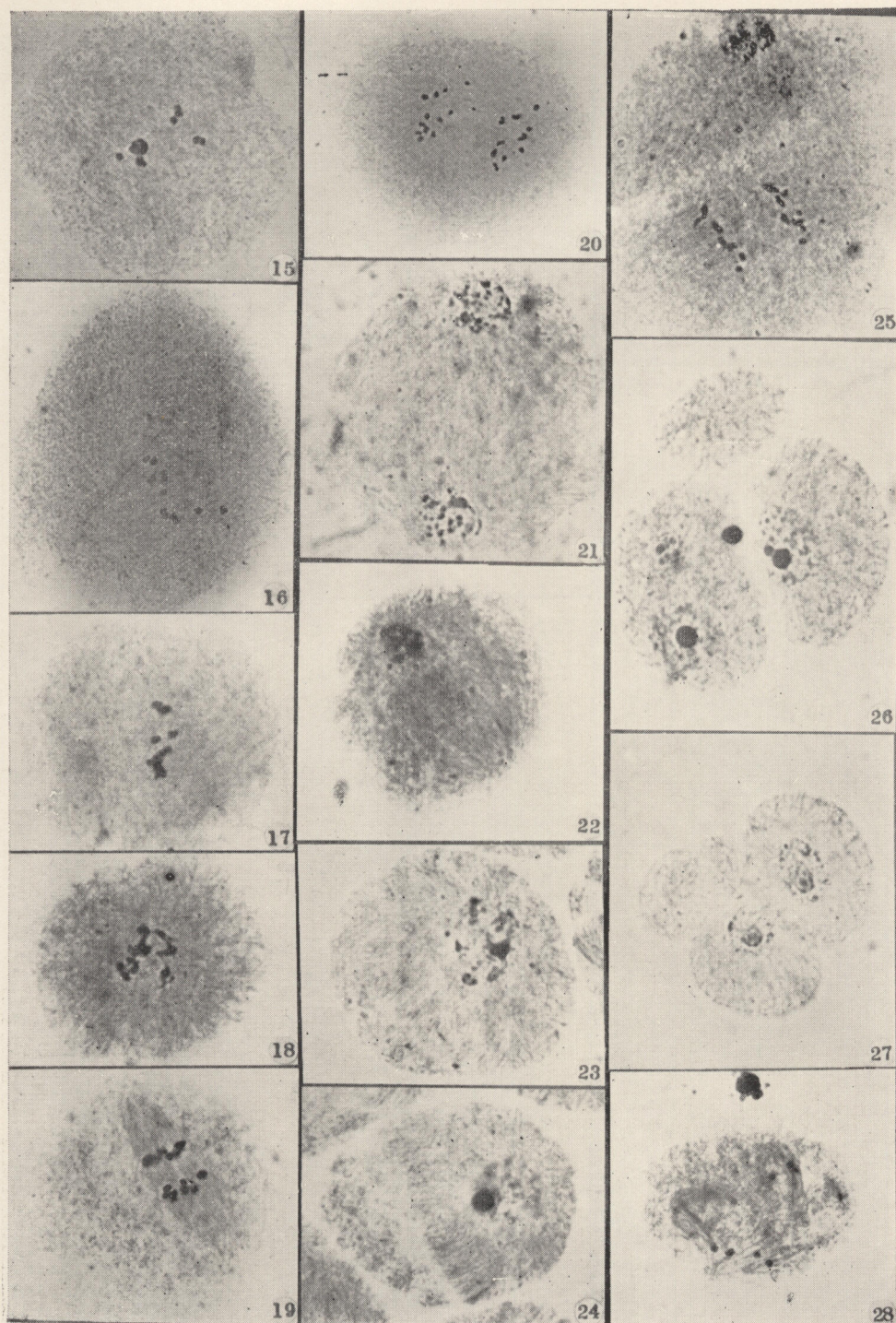
a. 由於 restitution nucleus 之形成而有染色體數不減之配偶子產生。經減數分裂之二次分裂後形成二分子。各含12個染色體。

b. 由於核分裂和細胞質分裂不一致而產生染色體數未減之配偶子。結果得三分子或四分子，其中二個小孢子各含12個染色體，其他則無染色體。

Explanation of the Plate Figures

- Fig. 1. Pollen grains mostly sterile, showing one well-stained pollen
 Fig. 2. Diakinesis with a large nucleus
 Fig. 3. MA-I, showing 12 univalents, eight of these seemed to have paired together showing secondary association
 Fig. 4. MA-I, a cell with two true bivalents and eight univalents
 Fig. 5. MA-I, a cell with 10 univalents and one bivalents of end to end pairing
 Fig. 6. MA-I, showing a cell with 12 univalents in 6-6 distribution
 Fig. 7. Interkinesis
 Fig. 8. M-II, a cell with seven split chromosomes in one half of the dyad and five in the other
 Fig. 9. A-II, dividing chromatids moving toward two opposite poles normally
 Fig. 10. Tetrad with sporads of similar size
 Fig. 11. Tetrad with sporads of unequal size
 Fig. 12. Nucleus in A-I, with chromosomes and spindle together at one side of the cell
 Fig. 13. M-II, Unequal sized halves of a dyad due to eccentrically placed nucleus at first meiotic division as shown in fig. 12
 Fig. 14. Tetrad with two large sporads and two small ones, the end product of fig. 13





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Explanation of the Plate Figures

- Fig. 15. A small nucleus at diakinesis, with more or less sticky chromosomes
- Fig. 16. M-I with compact short spindle and chromosomes split but they were held together by their respective centromeres
- Fig. 17. Formation of restitution nucleus
- Fig. 18. Restitution nucleus
- Fig. 19. Post-restitution nucleus division, anaphase, split chromatids moving toward two poles
- Fig. 20. Post-restitution nucleus division, anaphase, 12 chromosomes in one pole
- Fig. 21. Dyad with unreduced gametes
- Fig. 22. Interkinesis. Nucleus located at one end of the cell and the spindle formed at the regular position
- Fig. 23. Early prophase-II, the cell was divided into two halves
- Fig. 24. Same as fig. 23
- Fig. 25. A-II, with all the chromosomes in one of the sporads
- Fig. 26. One sporad with a nucleus of unreduced number of chromosomes presumably. The other sporad with two nuclei while in the third sporad, there was no nucleus.
- Fig. 27. Unequal tetrad having two sporads with nucleus of unreduced number of chromosomes and the other two with no nucleus whatsoever
- Fig. 28. Showing a microsporocyte with irregular multiple spindles