

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

8. Study on meiotic division of F₁ hybrid of *O. sativa* (L.) × *O. brachyantha*, A Chev. et Roehr⁽¹⁾

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In the pursuit of obtaining method or methods whereby the good characters of species growing in the wildness can be transferred to the cultivated species, attempts were made to try to double the chromosomes of the F₁ of the interspecific hybrids, but failed with repeated trials in the past few years. In 1963, backcross method was tried and triploids were produced in back-crossing the recurrent parent *O. sativa* with the F₁ hybrids of *O. sativa* × *O. officinalis*. Detailed study was made to find out the mechanism involved in the formation of the unreduced gametes (Li *et al* in press). In this case, it was the result of non-synchronized division of mitosis and cytokinesis. As a result, triads instead of tetrads were formed.

O. brachyantha is found in W. Africa and was classified in the section Granulata by Ghose and others. After successful hybridization with *O. paraguayensis*, *O. sativa*, and *O. minuta* (Li *et al* 1961, Wu *et al* 1963), *O. brachyantha* was designated as genome F. Back crossing was attempted in 1963 involving many spikelets, but no seeds were obtained. It would be of interest therefore to study the meiotic division of F₁ of this intersectional hybrid in detail to find out whether or not unreduced gametes are produced. If so, what is the mechanism involved?

Material and Methods

The F₁ plants was kept alive by asexual reproduction. Propiano-carmin was used throughout this study. Trace of ferric chloride was used as a mordant in order to facilitate the staining of spindles.

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Results

Pollen fertility

In December 1963, one panicle of this hybrid was fixed and studied. Few well stained pollen grains were found among thousands of defective ones. After being stained with KI solution. Unfortunately, no detailed counts were made.

In May 1964, when the panicles were fixed in the greenhouse where the temperature was rather high, no good pollen was found among thousands of defective ones examined. However after the plants were removed from the greenhouse and were kept in a room only with diffused light where the temperature was not so high (below 30°C), the panicles were refixed and examined. It was found that there was 0.089% of well stained pollen grains found in a total of 4494 grains counted. It would be of interest to mention here that the diameter of the grains varied somewhat and the average diameter were smaller than that of the *O. sativa* parent used, ratio being 1:0.83.

In the case of *O. sativa* × *O. officinalis*, the average size of the unreduced pollen grains was the same as that of the recurrent parent, it would be correct to assume that these grains are diploid gametes also. Of course, the frequency was very much lower than that of *O. sativa* × *O. officinalis*.

Meiotic division of the hybrid

In general, the meiotic division of the hybrid can be divided by the following types:

a. Division being more or less regular

In our earlier studies, chromosomes of A and F genomes did not pair with one another (Wuu *et al* 1963), so there were practically no bivalents found (only 0.03 per cell). In the first division, the chromosome distributed randomly to the poles with more or less long spindles (fig. 1-2). However, cytokinesis might or might not have taken place at this stage and in most of the microsporocytes examined, cytokinesis was very much delayed. When first cytokinesis was delayed (fig. 5), the two second metaphase plates were not arranged side by side always (fig. 6), but at right angles to each other (fig. 5) or in other odd way. As a result, the tetrad was of various types (fig. 7-9). In most of microsporocytes observed, the first and second cytokinesis were completed almost simultaneously. The sporads thus produced would be sterile because they were unbalanced either in number or in constitution of chromosomes.

b. First division with split chromosomes

The chromosomes were found to be split at diakinesis, (fig. 10). These microsporocytes were found in a slide with microsporocytes more or less in first division. Again the nucleus seen was too big for any typical restitution

nucleus. Furthermore, the percentage of stainable pollen was so small. It could be assumed therefore that this kind of microsporocytes was still in diakinesis with chromosomes split already at this stage. Why this was so, no explanation can be offered. If it was the start of the second division, then, there would be more unreduced sporads formed because dyads would be formed (fig. 12). As it was, 24 chromosomes migrated to one pole at first anaphase. Since there were no second division figures with random distribution of chromosomes found, the fate of these dyads was unknown.

c. Longitudinally compressed spindle leading to the formation of restitution nucleus.

It seemed to be correct to identify that whenever the nucleus of the microsporocyte was very small and compact and with distinct chromosomes, invariably the spindles formed in subsequent stages would be longitudinally compacted ones (fig. 13). It was highly probable that subsequent divisions would lead to the formation of restitution nucleus (fig. 14, 15). The fate of the microsporocytes with restitution nucleus was unknown. It might be predicted that some of these microsporocytes would go into second division something like fig. 11 and 12, and dyads would be formed to yield unreduced sporads. Since good pollen was so few, this possibility did not appear to be highly probable.

d. Chromatic connection found at the end of first division

Sometimes, there was a big sized chromatic bridge formed at first telophase (fig. 16). When this microsporocyte went to second metaphase, the plates were formed very close to each other just across the wall (fig. 17), or two plates would merge into one, stimulating first division.

Explanation of plate figures

A. Division more or less regular

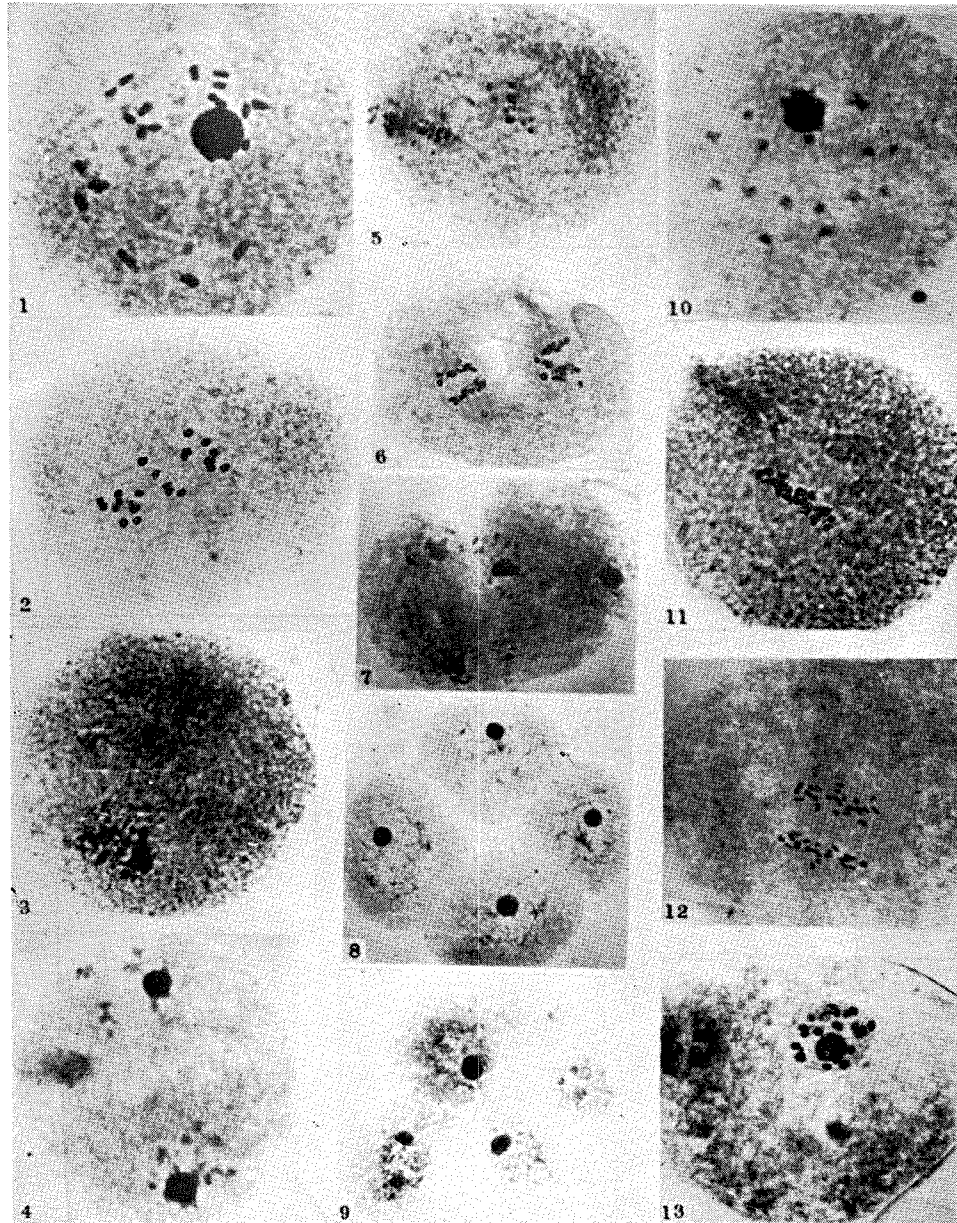
1. Diakinesis with 24 univalents.
2. MA-I with 24 univalents distributed randomly to the poles. Few lag on the equator.
3. TI with phragmoplast expanding to the periphery.
4. Interkinesis with delayed cytokinesis.
5. MII with metaphase plates oriented at right angles to each other. Cytokinesis was much delayed.
6. AII a cross wall was formed in the middle. The spindles were arranged at right angle to each other.
7. TII spindles were perpendicularly arranged.
8. Quartet with each cell of more or less equal size.
9. Quartet with unequal cells.

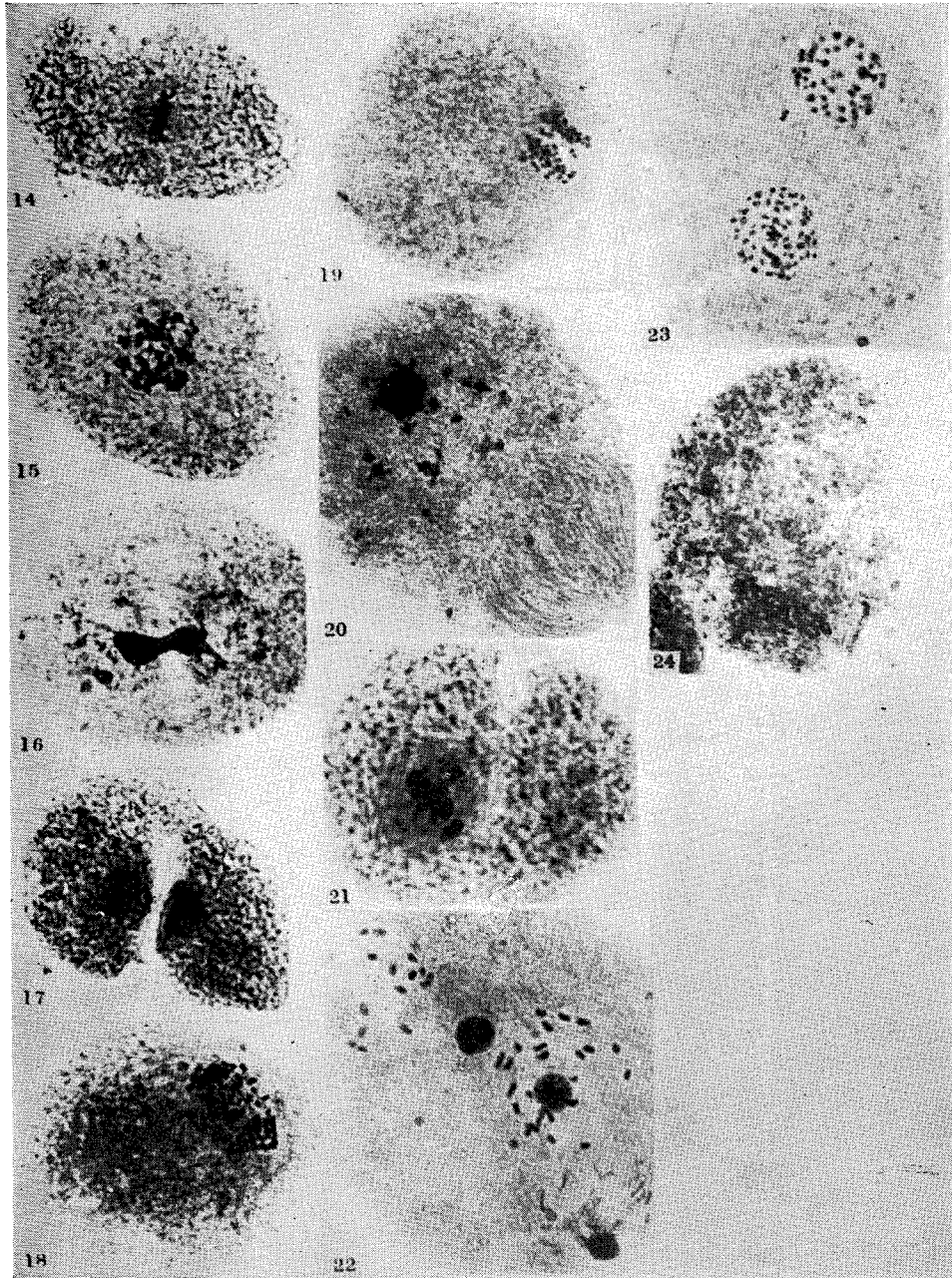
B. First division with split chromosomes

10. Diakinesis with split chromosomes.
11. MI split chromosomes were lined up on the equator.
12. AI with 24 chromatids in each pole.

C. Longitudinally compressed spindle leading to the formation of restitution nucleus

13. A small nucleus in diakinesis.





e. Microsporocytes with eccentrically placed nucleus

In this hybrid, when the nucleus was found to be eccentrically placed in a microsporocyte, the spindle was found to be formed with the chromosomes, but not away from them (fig. 18, 19). Invariably, the spindle was the longitudinally compressed type and the chromosomes were split at this division presumably to be the first division. Otherwise there would be plenty of unreduced pollen found. The fate of this sporads was unknown.

f. Microsporocytes with eccentrically placed nucleus and with its spindle formed at the other end.

Of close to hundred slides prepared for study, only in one slide, did we find quite a few microsporocytes with nuclei eccentrically placed and their spindles were formed at the other end. In other slides, very occasionally did we find such microsporocytes. Sometimes, the spindle was formed at the center of the microsporocyte. Others it might be formed off-center (fig. 20). Like the hybrid in *O. sativa* × *O. officinalis* (Li *et al* in press), triads would be formed (fig. 21) giving rise to two unreduced sporads and one anucleated one.

g. Syncytes

Syncytes (fig. 22, 23) were very frequently found in this hybrid. Some of them were binucleated, others multinucleated, and still others with fused nucleus (fig. 24). No division figure further than MI was ever been observed.

Conclusion and Summary

The meiotic division of *O. sativa* × *O. brachyantha* was characterized with some seemingly normal divisions which were characteristic of the hybrid with unpaired chromosomes leading to the formation of tetrads. In most of the microsporocytes, the first cytokinesis was delayed and was not completed till the end of second division.

14. MI with longitudinally compressed spindle.

15. Restitution nucleus.

D. Chromatic connection formed at the end of first division

16. TI with large chromatic bridge.

17. MII, the two metaphase plates were very close to each other.

E. Microsporocytes with eccentrically placed nucleus

18-19. AI with chromatids separating in an eccentrically placed nucleus.

F. Nucleus eccentrically placed. Spindle formed at the other end. Non-synchronized division both in space and time

20. Nucleus at interkinesis with split chromosomes, spindle was formed at the other end.

21. A dyad with a nucleated and an anucleated cell. The nucleated cell was at MII leading to the formation of a triad with diploid gametes.

G. Syncytes

22-23. Syncytes with two nuclei.

24. Syncyte with fused nucleus at MI.

With longitudinally compressed spindle formed around a small and compact nucleus, restitution nucleus would be formed. This might lead to the formation of unreduced sporads in a dyad.

In a few instances found, the phenomenon of non-synchronization of nuclear division and spindle formation in first division both in space and time was very evident. Of course, this would lead to the formation of unreduced sporads in a triad formed.

Pollen fertility was found to be less than 0.01%. It would be of interest to find out whether or not fertile ovules with unreduced number of chromosomes would be available. Having these it would make the back-cross method possible. Perhaps elaborate back-crossing program in addition to the employment of embryo-culture technique might give a ray of hope in the future in order to obtain any triploid plants. This is in progress.

Oryza sativa L. 及其近緣種之細胞遺傳學的研究

8. *O. sativa* L. × *O. brachyantha* A. Chev. et Roehr.

第一代雜種減數分裂之研究

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O. sativa × *O. brachyantha* 種間雜種的減數分裂和正常的減數分裂相似，雖然分屬於 A 和 F genome 的染色體不配對，結果仍形成四分子。大多數小孢子母細胞的第一次細胞質分裂遲延，一直到第二次核分裂終了時細胞質分裂才完成。

在小而緊聚的核四周有縱方向被壓縮的紡錘體，而致在第一次分裂後仍形成原來的核 (Restitution nucleus)。結果在小孢子母細胞 meiosis 後形成二分子 (dyad)，而有染色體數不減的 sporads 的形成。

在少數情形下，可以發現在不同時間和細胞內的不同位置進行核分裂和形成紡錘體。這樣在小孢子母細胞分裂完後形成三分子，其中二個為染色體數不減的 sporads，另一個則不含染色體。

花粉率為略少於 0.01%。假如含有未減數染色體的可孕胚珠 (Ovules) 也同樣存在時，則可以用來作回交。胚胎培養 (embryo culture) 或可有助於得到三倍體的植株。

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