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

9. Study on Meiosis of the First Backcross Generation of (*Oryza sativa* L.×*O. officinalis* Wall.)×*O. sativa* L.[1]

KWEI-CHI HO and H. W. LI[2]

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In order to construct artificially *Oryza sativa* with the addition of all the possible 12 individual *O. officinalis* chromosome, many attempts have been made for years in our laboratory. One of these was to backcross the F_1 hybrid of *O. sativa indica*×*O. officinalis* (accession No. W 002), with genomic constitution AC ($2n=24$) to the recurrent parent *O. sativa* (AA). More than 50 seeds were obtained. Ten plants were grown to maturity. Outwardly, the panicles appeared to be between *O. sativa* and F_1 (fig. 25, 26). From the preliminary study of the microsporogenesis of this backcross generation, all were proved to be triploids. All had 36 chromosomes with the genomic constitution AAC. Hence the meiosis of the F_1 hybrid of *O. sativa*×*O. officinalis* was studied in detail and the mechanism for unreduced gametes formation was examined and was reported recently (Li *et al.* 1964).

Later a detailed study of the meiotic division of these triploids, the first backcross generation, was made and the observations were reported in this paper.

The microsporocytes were fixed in Farmer's fluid. Propiano-carmine was used for staining. Regular smear method was used for preparations except sometimes a trace of ferric hydroxide dissolved in 45% propianic acid was added for better spindle staining.

Results

1. *Chromosome pairing*

The genomic constitution of the first backcross generation of the (*O. sativa*×*O. officinalis*)×*O. sativa* was AAC as mentioned earlier. The frequency of the

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[2] Assistant Research Fellow and Research Fellow respectively.

chromosome pairing was counted in 81 metaphase I cells (table 1). The number of bivalents per cell varied from zero to 12. Each class was found to be more or less equally frequent. The two extremes with 12 bivalents and 12 univalents at one end and with 36 univalents at the other were shown in figures 2 and 9. The factor or factors controlling the pairing of homologous chromosomes to form bivalents was not known. The average number of bivalents per cell was 6.86.

Table 1. *The frequency of the microsporocytes of the first backcross generation of (O. sativa×O. officinalis)×O. sativa*

Bivalents/cell	0	1	2	3	4	5	6	7	8	9	10	11	12	total
Frequency	5	5	4	3	5	4	10	4	8	11	12	4	7	81
Total bivalents	0	5	8	12	20	20	60	28	56	99	120	44	84	556

2. *Meiosis*

Microsporogenesis in this first backcross generation was quite similar to that of the F_1 of *O. sativa*×*O. officinalis* (Li *et al.* 1964) except that the extremely long spindle was rarely found in this material. From our study, the major types of the microsporogenesis could be shown in diagram I, which was a modified version of those diagrams shown by Darlington (1937, p. 412, 415).

At diakinesis, in most of the microsporocytes some bivalents, although varied in number, were observed (fig. 1). At Metaphase I, the number of bivalents might be 12 (fig. 2, diag. 1) or zero (diag. 2, fig. 9) or somewhere in between (diag. 3, fig. 11).

When most of the homologous chromosomes of AA genome in this triploid (AAC) formed bivalents at metaphase I, the bivalents would migrate to the poles ahead of the univalents which lagged on the equatorial plate (diag. 4, fig. 3) at anaphase I. The univalents split at this stage but were held together by their respective centromeres. Later, the intact univalents might move to the two opposite poles. In either case, a dyad would be formed at the end of the first division (diag. 6). Each cell would have 12 chromosomes of A genome and one to 12 extra ones of C genome. At second meiotic division, the chromosomes lined up at the equatorial plate of each daughter cell and the chromatids separated from each other migrated toward the opposite poles (diag. 7, fig. 4). At the end of microsporogenesis, tetrads were formed, and each sporad would have 12 A chromosomes with a few C chromosomes (diag. 8, fig. 5). This was the type of meiosis that we would expect in the megasporogenesis because they might form the functional gametes.

With the microsporocytes having mostly univalents, the univalents might

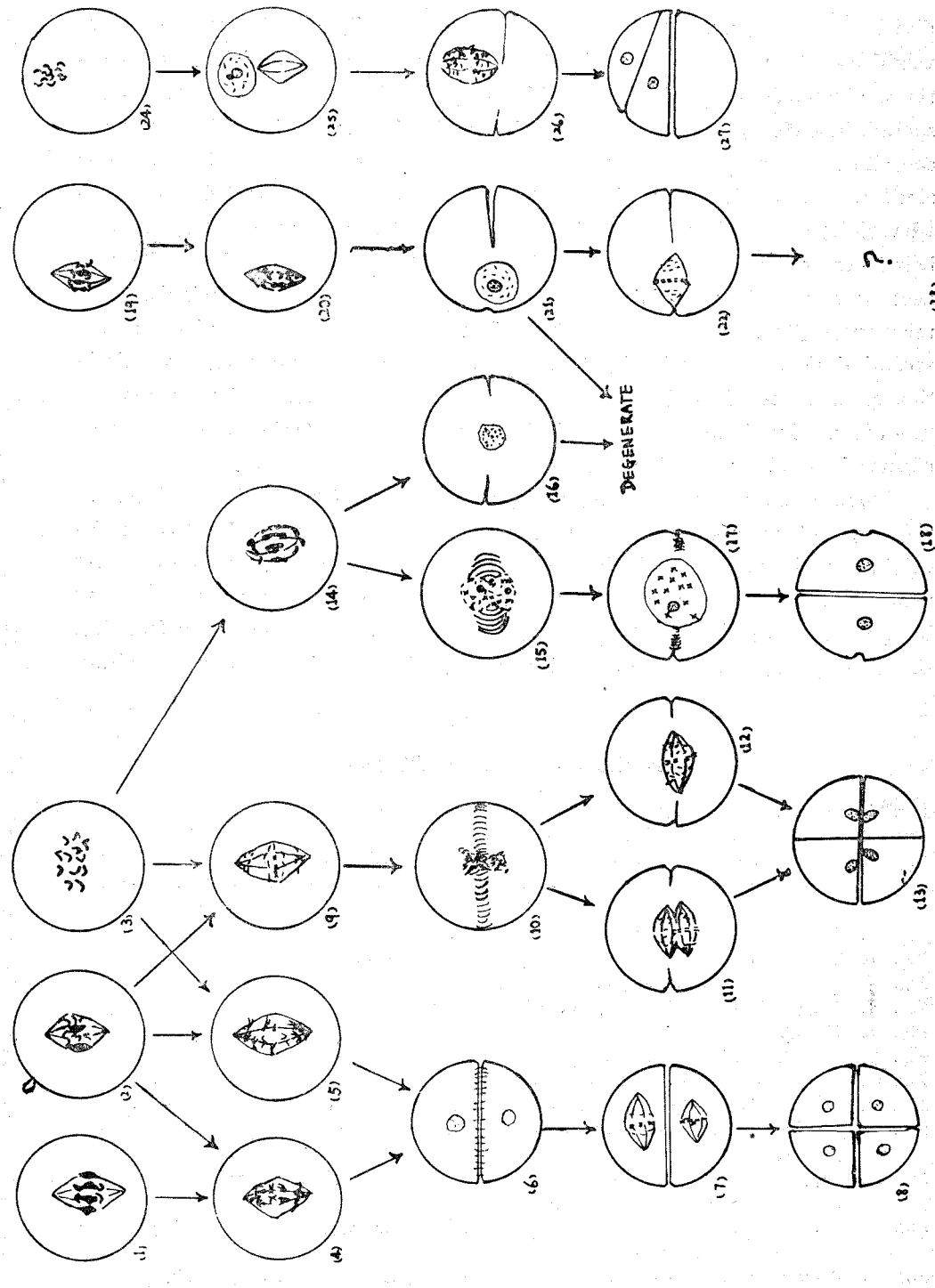


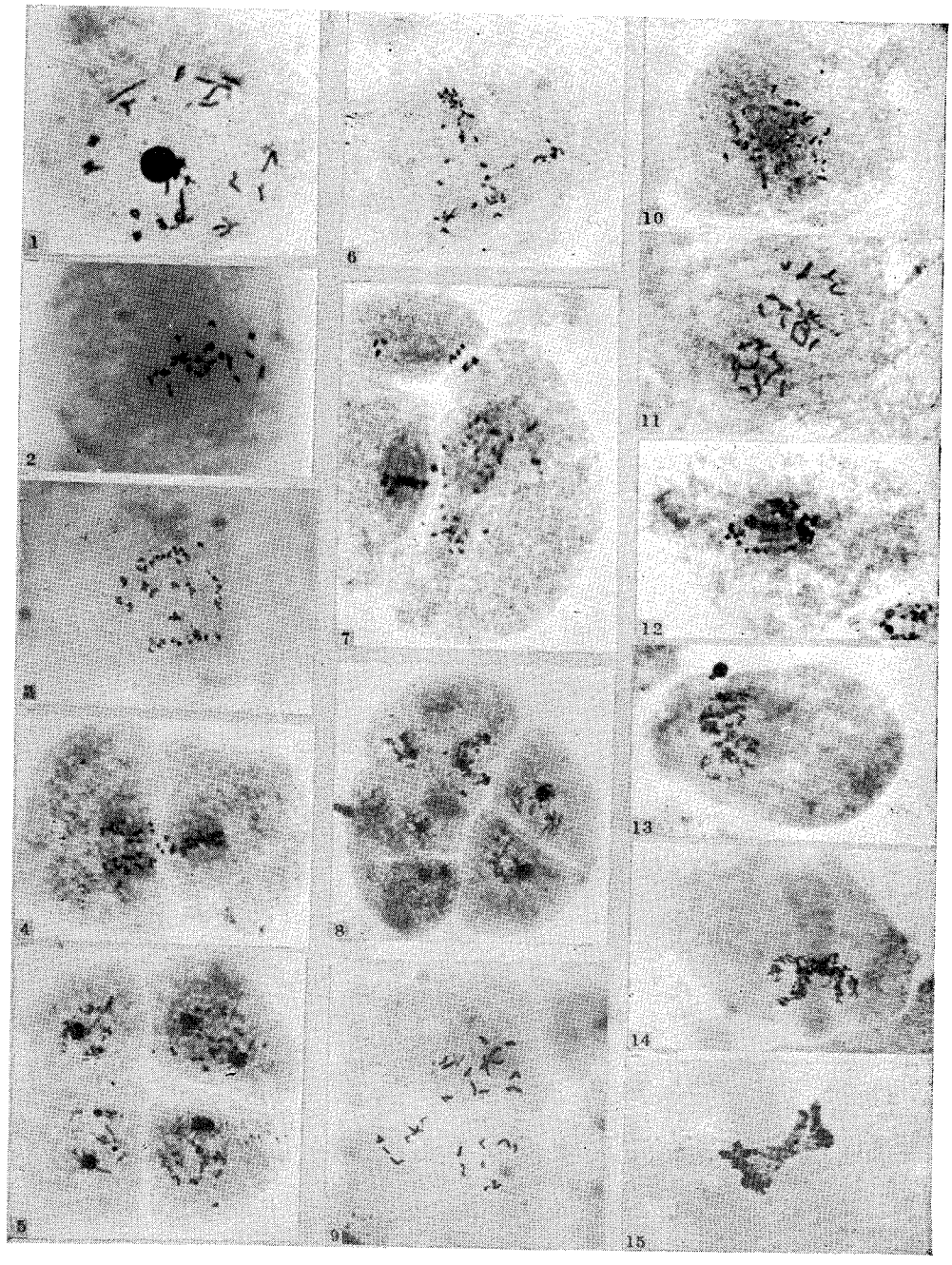
Diagram I. Possible major types of Meiosis of the Back-cross

either segretate at random (diag. 5) or they would split at the first meiotic division (diag. 9, fig. 10). In the former, theoretically, about 18 chromosomes would pass to each cell of the dyad at the end of the first division. An equational second division followed would lead to the formation of a tetrad. Each sporad, however would be either unbalanced in chromosomal number or in the constitution of chromosomes and were all non-functional. In the latter, a cell similar to a restitution nucleus would be formed at telophase I due to somewhat delayed cytokinesis (diag. 10, fig. 14, 15). At metaphase II, two spindles being very close to each other would be formed (diag. 11, 12). Since the connecting chromatic material was thin, the cross wall formed would bisect the microsporocyte into two with the cell plate passing through the spindle of the second division longitudinally (fig. 17). At the end of microsporogenesis in this case, a tetrad as shown in diag. 13 would be formed. In both cases, the sporads produced were sterile. A large proportion of microsporocytes was observed to belong to this type.

Some of the microsporocytes were found to form a restitution nucleus. In this type of microsporocytes, the nucleus and spindle were located at the original position. The spindle was somewhat longitudinally compressed. At anaphase I, the chromosomes did not separate clearly. As a consequence, many bridges were found (diag. 14, fig. 12). Possibly this was due to the fact that the chromosomes were more or less sticky. This kind of cells might either

Explanation of Plate Figures

Fig. 1. Diakinesis, showing 10 bivalents and 16 univalents.
Fig. 2. MI, showing 12 bivalents and 12 univalents.
Fig. 3. AI, chromatids split at this stage, 12 bivalents being separated and the chromosomes separated migrated to their respective poles while univalents still lagged on the equator.
Fig. 4. MII and AII, showing equational division.
Fig. 5. Tetrad with sporads more or less of the same size.
Fig. 6. A tripolar microsporocyte.
Fig. 7. Polyad formation in a tripolar microsporocyte.
Fig. 8. Hexad.
Fig. 9. Early MI, with 36 univalents.
Fig. 10. AI, univalents split and the chromatids migrated to their respective poles.
Fig. 11. MI, showing 9 bivalents and 18 univalents, chromosome are less condensed.
Fig. 12. Late AI, showing sticky chromosomes and bridges which would form restitution nucleus.
Fig. 13. TI, showing very short spindle in a somewhat eccentrically located nucleus, tending to the formation of a restitution nucleus.
Fig. 14. TI, restitution nucleus with slender connecting chromatic material showing distinct expanded phragmoplast.
Fig. 15. Restitution nucleus formation with slender connecting chromatic material between two groups.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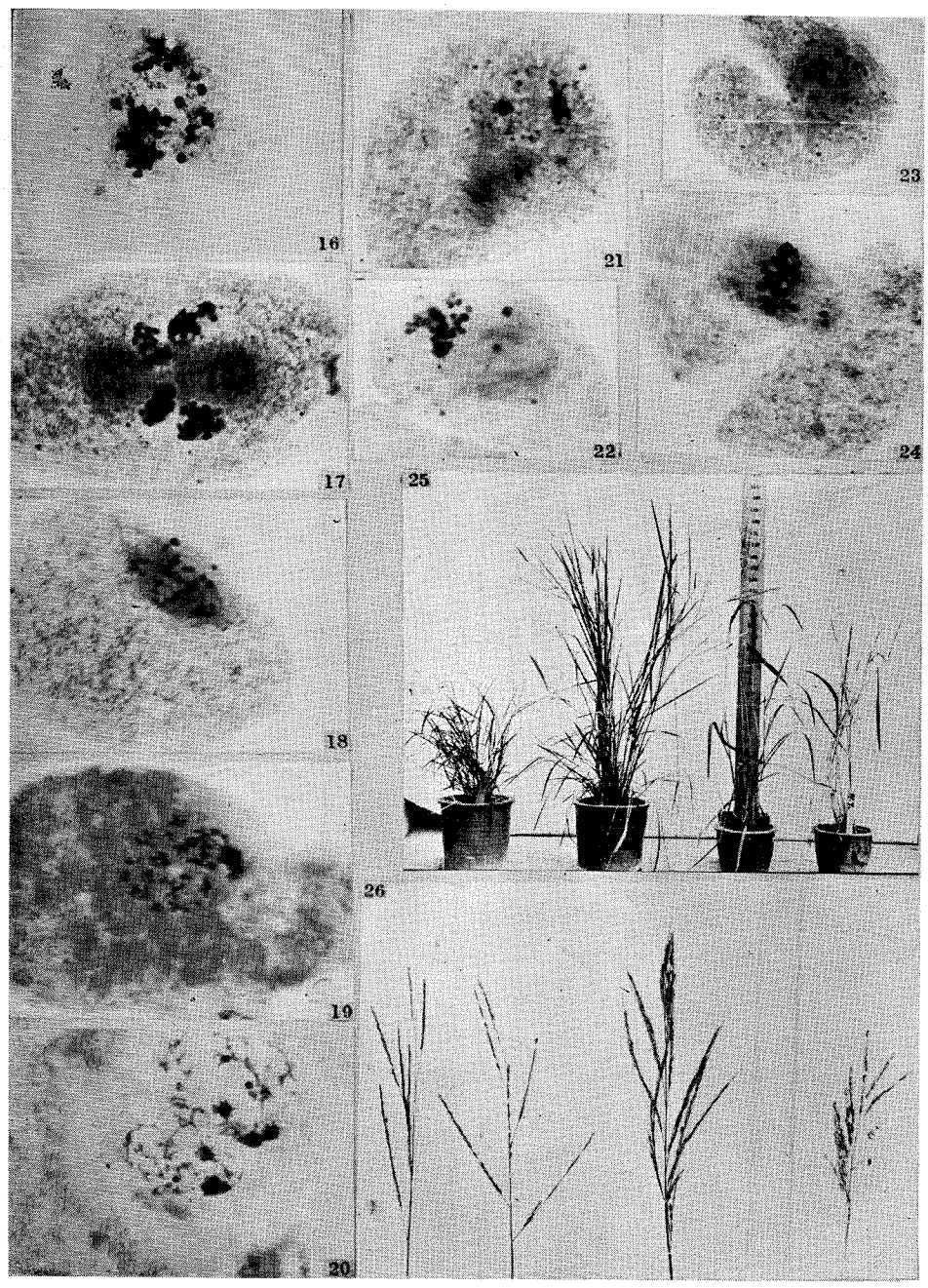
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degenerate (diag. 16) or form a restitution nucleus (fig. 13, 19, diag. 15). In the latter case the chromosomes would migrate to the two poles, which were very close to each other, to form two groups but were linked by connecting chromatic material (diag. 15, fig. 13, 20). Because of the fact that there was no interzonal space, after the first division the nucleus remained intact. At the second prophase, a cell with 36 cross-shaped univalents could be observed sometimes (diag. 17). Cytokinesis was found to be incomplete quite frequently (fig. 16). This kind of cells would sometimes undergo second meiotic division leading to the formation of a dyad (diag. 18). Consequently these sporads would have the unreduced number of chromosomes.

Another series of microsporogenesis as found in which the microsporocytes with both nuleus and spindle were eccentrically placed (diag. 19. 20, fig. 18). At the end of the first meiotic division, a restitution nucleus was formed (fig. 19) with incomplete cytokinesis (diag. 21). These cells might degenerate. Sometimes, a metaphase II spindle would form (diag. 22), and the cell would be bisected longitudinally by the delayed first cytokinesis.

One type of microsporogenesis which was very important for the formation of unreduced gametes in the F_1 megasporogenesis was also found in this material. This is the kind of microsporocytes with eccentrically placed nucleus and normal spindle (diag. 24 to 27). At metaphase I, the chromosomes were eccentrically placed and the spindle was formed at the original centric position (diag. 24, 25, fig. 21, 22, 23). In this case, chromosomes without spindle migrated autonomously but never moved far apart. Autonomous division of the chromosomes when there was no spindle involved was described to some extent by Shrader (1953) and Mazia (1961). Therefore a restitution nucleus was formed

Explanation of Plate Figures

Fig. 16. Prophase II, post-restitution nucleus prophase, showing 36 cross-shaped univalents with chromatids split but held together by their respective centromeres.
Fig. 17. TII, telophase of a restitution nucleus as shown in fig. 14, phragmoplasts expanded yet to reach the periphery however.
Fig. 18. AI, showing eccentrically placed nucleus and spindle, but a single chromosome was not involved in the division.
Fig. 19. Interkinesis of a restitution nucleus, somewhat eccentrically placed.
Fig. 20. Interkinesis of a restitution nucleus with periphery Phragmoplast.
Fig. 21, 22. Microsporocyte with eccentrically placed nucleus but centrically placed spindle.
Fig. 23. AII, dyad, anucleated in one cell, and the nucleated cell containing 36 split univalents moving apart from each other to the opposite poles.
Fig. 24. MII, dyad with an anucleated half and nucleated half at metaphase, two chrmosomes in the middle were not on the plate.
Fig. 25. Plants (left to right) of *O. officinalis*, F_1 of *O. sativa* × *O. officinalis*, the first backcross generation and *O. sativa* (Nanteh) respectively.
Fig. 26. Panicles (left to right) of *O. officinalis*, F_1, BC_1 and *O. sativa* respectively.

besides the regular spindle which was formed later or concurrently. Cytokinesis was delayed and the cell plate formed cut the microsporocyte into two daughter cells. In the nucleated cell, the 36 chromosomes split and the sister chromatids migrated toward the two opposite poles (diag. 26, fig. 23). A triad would be the end-product (diag. 27). One or two univalents was sometimes left out of the plate at the first division (fig. 24).

Occassionally, microsporocytes were found with three poles. Spindles were formed between any two of these poles. At anaphase I, chromosomes moved to the three poles at random (fig. 6). As a result, a triad with different number of chromosomes were formed. At metaphase II, spindles were formed in each cell of the triad and chromatids moved to the opposite poles as in regular division (fig. 7). Hexad was then formed at the end of microsporogenesis and they were all sterile.

Conclusion and Summary

The microsporogenesis of the first backcross generation of (*O. sativa*×*O. officinalis*)×*O. sativa* was more or less similar to that of the F_1 of *O. sativa*× *O. officinalis* except that rarely there were extremely long spindle found in the backcross generation.

The pairing of the twenty four homologous *sativa* chromosomes in this backcrossed generation with 36 chromosomes was not constant. An average, there were only 6.86 bivalents per cell instead of having 12 pairs.

The major types of microsporogenesis were studied (diag. I). The first column of the diagram showed the expected meiosis to take place in megasporocytes so that backcrossing to recurrent parent might construct artificially *Oryza sativa* with the possible 12 individual *O. officinalis* chromosomes of some of the ovules produced would have 12 A chromosomes plus one or more C chromosomes.

There were few other types of meiosis observed and they were described in detail. Restitution nucleus was formed. These might lead to the formation of unreduced gametes. Again as in the F_1 with non-synchronization of mitosis and cytokinesis triads instead of tetrads were formed leading to the formation of the unreduced gametes also.

Oryza sativa L. 及其近緣種之細胞遺傳學研究

9. (*O. sativa* L.×*O. officinalis* Wall.)×*O. sativa* L.

第一回交代的減數分裂之研究

何閏綺 李先聞

(*O. sativa*×*O. officinalis*)×*O. sativa* 第一回交代的 microsporogenesis 和 *O. sativa*×*O. officinalis* F_1 大致相同。但在第一回交代中很少有很長的紡綞體。

含有36條染色體的第一回交代，其中24個 *sativa* 同寶染色體 (homologous chromosome) 間的配對情形並不確定。平均每一細胞僅有 6.86個二價體而不是十二個。

經研究 microsporogenesis 主要分爲三型。圖解上的第一行乃是我們期望發生於 microsporocyte 者。因爲子房中有12個A染色體加一個或一個以上C染色體，故繼續回交可得含單個 *officinalis* 染色體的 *Oryza sativa*。

另有一些其他的減數分裂情形，亦經詳細描述。有 restitution nucleus 之形成，因而導致不減數的配偶子形成。跟 F_1 一樣由於核分裂和細胞質分裂不一致，結果產生三分子替代了四分子，亦可導致不減數配偶子之形成。

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