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

7. Non-synchronization of mitosis and cytokinesis in relation to the formation of diploid gametes in the hybrids of *Oryza sativa* L. and *O. officinalis* Wall.[1]

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(Received Mar. 3, 1964)

In the past few years, attempts were being made to induce doubling of chromosomes by colchicine treatment in the the F_1 hybrids between species especially of different genomic constitution so that amphiploids could be produced, but failed in repeated trials. More trials were being made with new technique and the results obtained so far were encouraging. It was reasoned at the same time that in case diploid gametes could be produced spontaneously in the F_1 hybrids, and if back-crossing could be resorted to, then triploid progenies could be obtained. Thus in the spring of 1963 F_1 hybrid of *Oryza sativa*×*O. officinalis*, acession No. W002, (genomic constitution AC, 2n=24) was back-crossed with the recurrent parent *O. sativa*. To our surprise more than 50 seeds were obtained. Some of these were grown to maturity and they were found to be triploid with 36 chromosomes with the genomic constitution AAC. Since then, attempts were also being made in using *O. sativa* to back cross the F_1 hybrids such as *O. sativa*×*O. australiensis*, and *O. sativa*×*O. brachyantha*. Only one seed was obtained with the former but this sole seed failed to germinate even with good precaution.

A series of detailed studies was made to find out the nature of this spontaneous formation of diploid gametes in this hybrid and other hybrids and the results obtained with the hybrid *O. sativa*×*O. officinalis* are reported in this paper. Propiano-carmine was used exclusively in this study. To facilitate

(1) Paper No. 26 of the Scientific Journal Series, Institute of Botany, Academia Sinica. This study was supported by U. S. D. A. Agricultural Research Service Contract FC-TA-102 (A6-CR-4) and also supported partly by the National Council on Science Development.

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staining of the spindles, a trace of ferric hydroxide solution was added with good effect.

Results

1. *Pollen grains*

Pollen grains were fixed and examined by iodine method. It was found that with a total of 2591 grains counted only 12 were well stained as shown in Fig. 26, and the pollen fertility was found to be 0.46%. By comparison the average size of these grains was about the same as those of indica and japonica varieties which had haploid number of chromosomes.

2. *Meiosis in F_1 hybrid*

From our study, the meiosis in F_1 hybrid could be classified into 3 *types, i. e.*

a. Microsporocytes with centrically placed nucleus and with normal spindles.

b. Microsporocytes with centrically placed nucleus and with longitudinally compressed spindles.

c. The formation of diploid gametes as a result of eccentrically placed nucleus in the microsporocytes leading to non-synchronization of mitosis and cytokinesis.

a. Those with normal spindles

In this hybrid, the microsporocytes were either of the normal spindle type or of the other two abnormal types in a single spikelet. In other words, hardly these types were found to be mixed in the same slide.

Early prophase was studied in detail, but the results were not very satisfactory so they were not included in this report. At diakinesis, in most of the microsporocytes, the chromosomes were single and were well condensed. But in a few of them, the chromosomes were non-contracted and they looked very much like chromosomes in somatic cells such as in root tips.

At first metaphase, there was regular congression of the chromosomes at the equatorial plate (Fig. 1). Some univalents migrated poleward ahead of others. These along with the bivalents which seemed to be less easy to separate especially the ones with double chiasmata (Fig. 2) lagged behind on the plate. Later, after most of the univalents reached the poles, the laggards were migrating poleward. Presumably the chromosomal fiber, connecting with the kinetochore of each chromosome, was leading the way and dragging and pulling one of the arms along. Consequently this arm would assume the attenuated form as a result of this imposition upon it (Fig. 3). In other microsporocytes, the chromosomes were split and the chromatids were held together only by their kinetochores. Randomly they moved to the poles and were not separated (Fig. 4). In others, the two chromatids separated in the first division (Fig. 5).

In some microsporocytes, the two daughter nuclei were still linked by chromatic material, presumably by the laggards, forming seemingly a restitution nucleus (Fig. 6). Phragmoplast was formed regularly leading to the formation of the cell plate. It seemed that the second division was rather normal (Fig. 8, 9, and 10), leading to the formation of tetrad with four cells mostly of unequal sizes. No multinuclei were ever found. Nor micronuclei and polyads were seen. Those microspores thus produced were of approximately 12 chromosomes and were apt to degenerate because they were unbalanced either in number or in constitution.

b. Microsporocytes with centric nucleus and longitudinally compressed spindle

Since many preparations had this type of meiosis, naturally, this type would be the more predominant type of division.

At MI, the chromosomes congressed at the equator (Fig. 12). And the chromatids if each chromosome was split into two at this time, migrated to opposite poles even though these pole centers were very close to each other and the spindle formed was very short (Fig. 14). The phragmoplasts developed as usual leading to the formation of the cell plate which would bisect the microsporocyte into two halves. Each of these was nucleated (Fig. 15, 16, 17). In others, the expansion of the phragmoplast would not reach the periphery of the cell on the same side. Thus, an incomplete cross wall was formed.

Formation of restitution nucleus.

In-so-far as the pole centers were so close to each other, naturally the univalents or chromatides, even after random distribution or separation, would not be able to migrate far from the equator and away from each group and the two daughter groups were more or less connected. Ultimately, restitution nucleus was formed. Cell plate was consequently formed cutting through the center, midway between the pole centers (Fig. 15). Sometimes this bisection would split the restitution nucleus into two halves. In others, the restitution nucleus held its own intact with cross walls formed at both sides (Fig. 17).

Sometimes, the restitution nucleus was formed without showing apparent metakinesis after going through the motion of the disappearance of nuclear membrane and nucleoli, then the reoccurrence of nuclear membrane and the reinstatement of nucleoli (Fig. 16). Concurrently, the phragmoplast was expanded leading finally to the formation of the cross wall (Fig. 17).

Sometimes, when there was some delay in the formation of the spindle as shown in Fig. 18, the chromosomes seemed to be able to move poleward pre-

sumably led by their own kinetochore. Again, this was destined to form restitution nucleus. Quite frequently, one or more chromosomes would be left outside of this restitution nucleus.

In the second division, if the daughter nuclei were not distantly separated, MII in the two halves of the dyad could be seen in most of the cases to start simultaneously with the two metaphase plates more or less connected. There might be two separate spindles formed. However, in some cases, one end of the spindle might be fused if the daughter nuclei were very close to each other.

In the case when restitution nucleus was formed, a cell plate was formed which bisected this nucleus into two halves. The second metaphase looked very much like the first one. The metaphase plate was more or less connected and there might be two separate spindles. The chromosomes would split (Fig. 19, 20). Consequently, each daughter nucleus in the quartet would contain more or less the haploid number of chromosomes which again were unbalanced either in number or the constitution and they were also sterile.

In Fig. 20, most of the chromosomes was in one half of the dyad leaving only two chromosomes in the other half, and the cell plate was only half completed. The unequal distribution of the chromosomes in the two halves of the dyad was due to the off-center location of the dividing nucleus to start with.

c. Microsporocyte with eccentrically placed nucleus.

Occasionally, there were few microsporocytes with nuclei placed eccentrically and also away from the equator (Fig. 21, 22). Since the incidence was rare, any detailed study of their meiotic division was naturally excluded. As being shown in Fig. 18, when the formation of the spindle was delayed, these nuclei still would divide when the signal was being given. Since there was no spindle formed at the time, metakinesis would have to be omitted. None-the-less, the chromosomes might have migrated poleward somewhat. This was followed by chromosomal split, and reappearance of nuclear membrane and nucleoli and finally the whole nucleus would go into interkinesis. Concurrently, or soon afterwards, spindle was formed at the regular position irrespective of the absence of a nucleus, (Fig. 21). Then phragmoplast formation which was followed by cell plate formation (Fig. 23) was initiated. The ultimate result of this non-synchronized division of mitosis and cytokinesis both in space and in time would be the formation of a dyad with one nucleated half possessing all the chromosomes and the other anucleated.

MII was quite normal (Fig. 24), except for the fact that the spindle was rather out of place. It was perpendicular to the cross wall rather than parallel as it should be. AII was again normal with the chromatids migrating to their

respective poles. Spindle formation was not found in the anucleated half. Hence, when meiosis was completed a triad would be formed. Each of the nucleated cells (two) would have 24 chromosomes, but possess only roughly the quarter of the cytoplasm of the PMC, the same as each of the four cells would receive in a normal meiotic division. This probably would explain the fact that these diploid gametes thus formed were approximately of the same size as those of either indica or japonica varieties which were haploids.

Discussion

The results of a regular failure of the first or second division is the formation of unreduced or approximately unreduced gametes which function and yield polyploid progeny in the case of diploids, diploid progeny in the case of haploid (Darlington 1937, P. 417). In the past, many cytogeneticists worked on this problem (Darlington 1937, P. 417). In discussing the possible courses of meiosis in the total absence of pairing, Darlington (1937) made a diagram (Fig. 128A). One of the possible courses is when the chromosomes do not pair, they come back to the equator to form the restitution nucleus after migrated poleward initially and only dyads are obtained. Each would be diploid gametes. In the second row of the same diagram, a triad was formed given rise to two diploid gametes after the abnormal first cytokinesis. More recently, Walters (1958), working with *Bromus* hybrids, found that during metaphase, the numerous univalents moved to the poles and then returned to the equator, and the spindle increased and decreased in length. Anaphase might begin at anytime during the return of the univalents to the equator. When anaphase was too long delayed, the chromosome tended to remain at the equator and formed a restitution nucleus at telophase. Supernumerary cell division occurred during prophas II, primarily in those microsporocytes with restitution muclei. Snyder (1961), working with *Paspalum secans* found that in most microsporocytes the movement toward the poles was followed by a movement toward the equator. Consequently a restitution nucleus was formed. The post restitution division was usually regular and resulted in the formation of dyads of unreduced microspores. Unfortunately, no such dyads were depicted in any of the photomicrographs illustrated in the paper. Furthermore, if it were possible, a proposal like the omission of the first cytokinesis would have to be realized. Then the second cytokinesis would have to take place exactly at the place where the first cell plate would have formed originally. Or an assumption would have to be made that the formation of the first cell plate is delated till then.

In the F_1 hybrid of *O. sativa*×*O. officinalis*, the diploid gametes are produced by non-synchronized division of mitosis and cytokinesis both in space and in time. This will be described step by step as follows:

a. Nucleus eccentrically placed, away from the equatorial plate or at one end of the cell.

Van Wisselling (1909) found in *Spirogyra* that where the nucleus is originally suspended in the middle of the cell but is sometimes formed in a displaced position. When it is naturally displaced the division is correspondingly unequal (*vide* Mazia 1961, P. 314).

Presumably, in the pre-microsporocyte division of this hybrid the nuclei are displaced in some cells to start with, naturally giving rise to subsequent unequal division. Thus nucleus is displaced in some of the microsporocytes. Not only the nucleus is displaced, the cell size is distorted as well (Fig. 21).

b. Chromosome autonomy.

With this displacement of the nucleus away from the equatorial plane, the spindle will be formed at the regular site between the pole centers albeit much delated in time.

In discussing the autonomy of the chromosomes in mitosis, (Shrader 1953, pp. 311–312) maintained that the chromosomes themselves play an active part in the mitotic mechanism. Any movement beyond the initial separation is however associated with the presence of some part of the mitotic apparatus, even though the relationship is not always obvious. The forces that are involved in such an active participation of the chromosomes are still obscure (Shrader, 1953, pp. 311–312). In the F_1 hybrid of *sativa* and *offiicinalis,* mitosis take place in the absence of the spindle. Perhaps, it can take place as follows:

After the signal for the mitosis to initiate, this displaced nucleus will divide by itself precociously as compared with cytokinesis. A series of events would take place, such as the disappearance of nucleoli and nuclear membrane. Whether or not metakinesis would take place is rather problematic. Most probably, it is curtailed in the absence of the spindle. The poleward movement of the chromosomes which is led by the chromosome fiber of each univalent like-wise would be very much limited in extent (Fig. 18). Finally the chromosomes are split only to be held together by their unsplit kinetochores. With the reappearance of nuclear membrane and nucleoli, the stage is set for this nucleus to go into interkinesis (Fig. 23). Since there are only a few such displaced nuclei to work with, more work should be done to verify the validity of the description just being given.

c. Cytokinesis independent of chromosomes.

In a normal division, cytokinesis is governed by mitotic apparatus both in space and in time and the place of cytokinesis is normally perpendicular to the

midpoint of the spindle. Van Wisseling (1909 *vide* Mazia 1961, pp. 322–323) observed in *spirogyra* that when the spindle was displaced to one end of the dividing cell by strong centrifugation, mitosis took place at that end, but the new and normal cell wall appeared at the normal position midway between the ends of the cell. The spindle completed its division producing a binucleate cell besides its anucleate sister. It seems to the writers that after centrifuging, it is the nucleus which is much heavier than the spindle if it is formed then that goes to the end in Van Wisseling's experiment with *Spirogyra*. Indeed, Beams and King (1938, *vide* Mazia 1961, p. 425), found that if the plant cells are centrifuged at telophase, the heavier nuclei move toward the centrifugal pole while the phragmoplast is displaced peripherally. The results obtained by Van Wisseling would bear out this point strongly that mitosis and cytokinesis can proceed and lead to their completion autonomously. After a lengthen review of the experiments carried out by different investigators, mostly with animal material in artificial dissociation of the mitotic apparatus in relation to cytokinesis, Mazia (1961, pp. 328–329) concluded that the determination of cytokinesis seems to take place previous to the visible beginning of the division of the cell, and after the signal has been given, cytokinesis may proceed with some degree of autonomy. The geometric requirements would be satisfied that the centers

Explanation of plate figures

Figures 1-11 inclusive with normal spindles

Fig. 1. MI Showing one bivalent and 22 univalents with one univalent divides precociously.

Fig. 2. Early AI, showing two bivalents, one with two chiasmata. Several univalents have migrated poleward ahead of others. Most of the univalents as well as the bivalents lag behind.

Fig. 3. Late AI, many univalents have reached the poles but few still lag behind on the equator along with the bivalent. The chromosome fibers connecting with their respective kinetochores of each univalent in coordination with the continuous of the spindle, seem to lead the way in poleward movement, as x. The attenuated arms on the opposite side of the kinetochore of each univalents seem to be pulled along or dragged by the pulling force of chromosome fibers of each chromosome.

Fig. 4. AI, chromosomes being split are migrating to the poles after congression.

Fig. 5. AI (late), chromatids migrated to their respective poles.

Fig. 6. TI, some laggards linking the daughter nuclei together.

Fig. 7. AI, tripolar spindle.

Fig. 8. Interkinesis, showing chromosomes being randomly distributed at first division. The split chromosomes are held together by their unsplit kinetochores. One half of the dyad has many more chromosomes than the other.

Fig. 9. MII

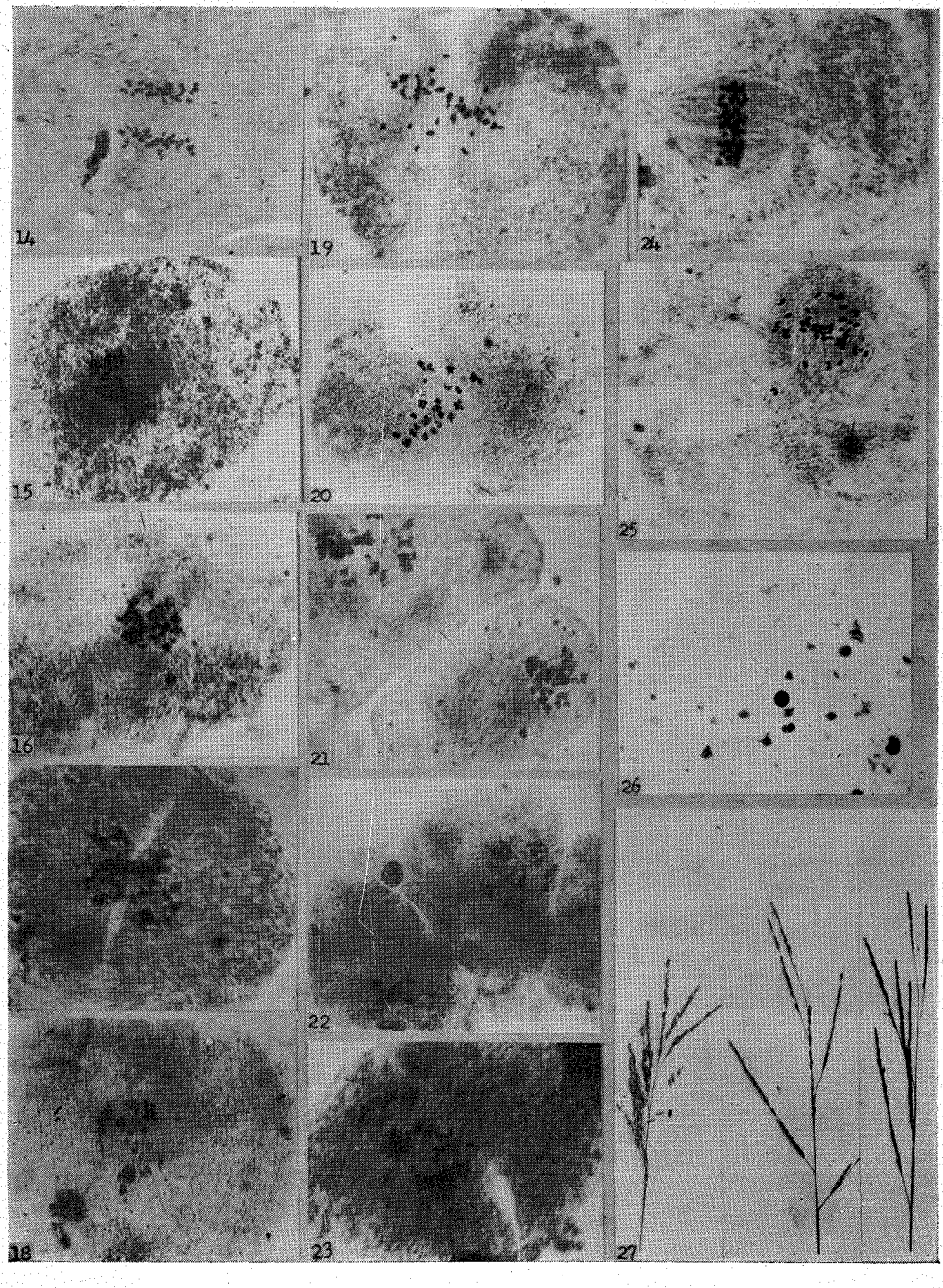
Fig. 10. Early TII

Fig. 11. Tetrad with unequal sizes of cells, two much larger than others.

Figures 12-20 Longitudinally compressed spindles with centrically placedsnucleus.

Fig. 12. MI showing 24 univalents with very compact spindle.

Fig. 13. Early AI, nucleus at one side but still on the equatorial plane.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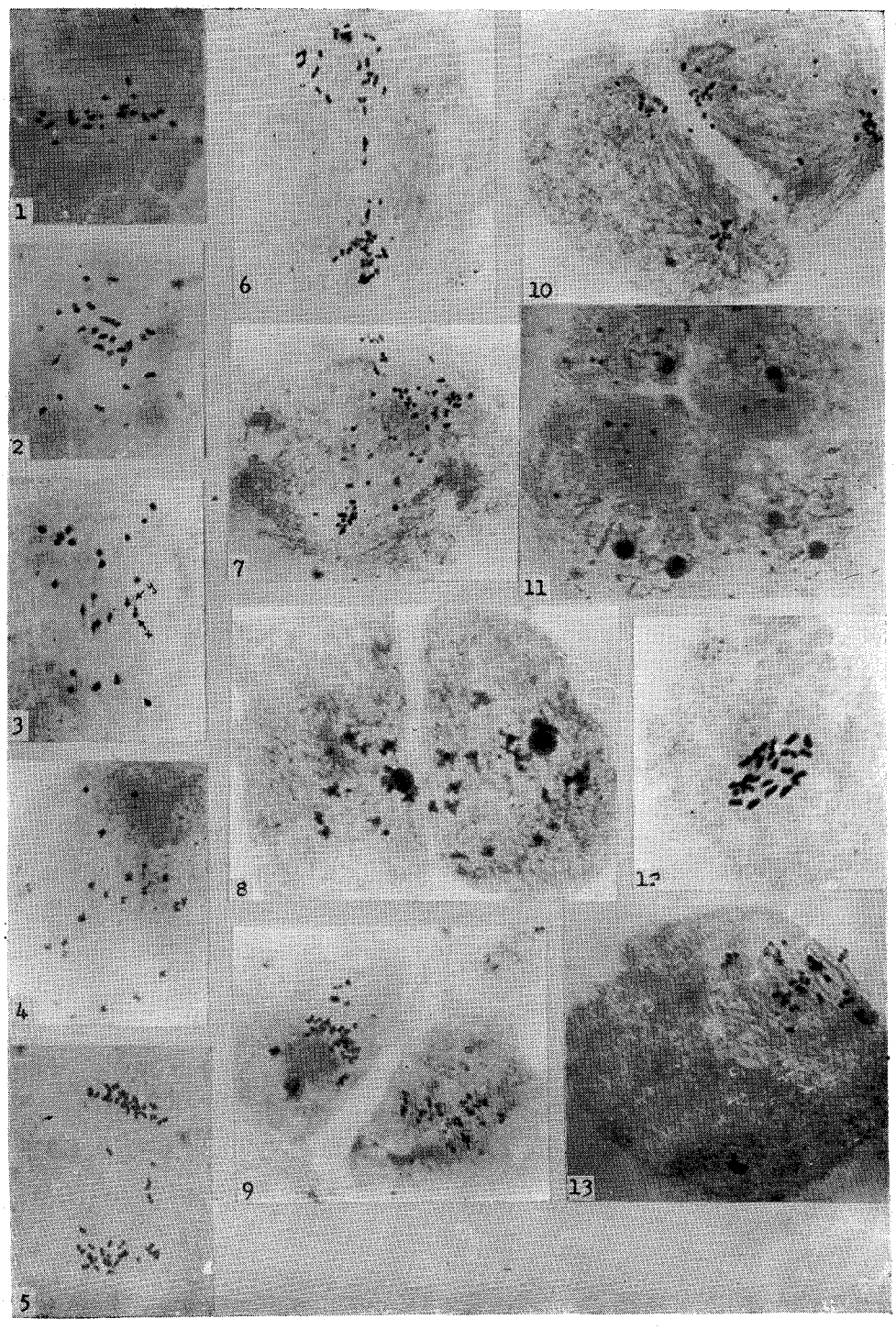
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determine the plane of division. The chromosomes can be excluded as essential for cytokinesis.

In the F_1 hybrid of *sativa*×*officinalis,* with the displaced nucleus, cytokinesis even though delayed would proceed normally in the absence of chromosomes at approximately the same site. This would signify that the spindle would be formed between the two centers despite the absence of the nucleus. It should be pointed out here in passing that the spindle formed in this nucleus displaced cells is of the longitudinally compressed type rather than the normal type. It seems therefore that our finding is at variance with the kind of division as being diagramed by Darlington (1937, Fig. 128A, p. 415) in which the cell plate formed is displaced since the nucleus is centrically placed. Consequently, the anucleated half is very much smaller than the nucleated half in the dyad formed. In the *Oryza* hybrids, however, the two halves of the dyad are more or less of the same size. This would signify that the nucleus is displaced and the cell plate is not.

Fig. 14. Late AI, chromosomes already split.
Fig. 15. Formation of restitution nucleus. Phragmoplast is expanding.
Fig. 16. TI, restitution nucleus with reocurrence of nucleolus and the phragmoplast expansion to the periphery. Chromosomes are split and are held together by the kinetochores.
Fig. 17. Restitution nucleus at interkinesis. Phragmoplast has reached the periphery, and the cell plate is formed, dividing this nucleus into two at the center.
Fig. 18. Restition nucleus without spindle. (delayed spindle formation in this case). Seemingly the chromosomes are migrating poleward autonomously, initiated by their own kinetochores. However the nucleus is centrically placed.
Fig. 19. MII, the split chromosomes are going to four poles. Somehow the restitution nucleus is still connected despite cytokinesis.
Fig. 20. Same as Fig. 19. Practically all the chromosomes are in one half of the dyad leaving the other with only 3 chromosomes.
Figures 21-25 Eccentrically placed nucleus (at one end of the cell) with spindle formed at the regular site.
Fig. 21. Microsporocyte at the bottom with eccentrically placed restitution nucleus in interkinesis and the spindle is formed at the regular site only very much delayed.
Fig. 22. Both microsporocytes showing restitution nucleus in interkinesis with spindle and phragmoplast formed at the regular site.
Fig. 23. Dyad with an anucleate half, and the other half with a restitution nucleus, in which the chromosomes are split. Cytokinesis seems to be incomplete.
Fig. 24. The restitution uncleus in nucleated half of the dyad is dividing. However, the cell plate is perpendicular to the axis of the spindle and is located about the middle of the photomicrograph. The right half of the dyad is only partly seen.
Fig. 25. Restitution nucleus in the nucleated half of the dyad at early AII. Again the spindle is not parallel with the cross wall as it should be located. The anucleated half is at the bottom and is only partly shown.
Fig. 26. Pollen grains mostly sterile, Fhere is only one good pollen.
Fig. 27. Panicles of *O. sativa,* var. Nanteh (left), *O. officinalis* (right) and the F_1 hybrid (middle).

d. Second division is normal.

This is essential for the formation of diploid gametes. As shown in the figures 24 and 25, the equatorial plate and spindle are wrongly oriented. The plate should be perpendicular to the cell plate rather than parallel. In the study of the F_1 hybrid of *O. sativa*×*O. brachyantha,* however, it is found that the orientation of the equatorial plate and the spindle in many of such cells are more or less regular (unpublished). It may be concluded therefore, that these cells are just the exceptions found.

From the results of back-crossing, diploid gametes are found in the ovules. Since megasporogenesis is not studied, it is surmised that the same abnormal division is also taken place in the ovules, so that diploid gametes are formed.

e. Existence of both normal and longitudinally compressed spindle.

The existence of these two distinct types of spindle is clear cut. In only a few preparations did we find the normal type. In most of the preparations, however compressed type is found. Attempts were not made however to find out which spikelets on the panicle would have the normal spindle and which have the other type.

f. Size of the pollen grains.

It was found that the size of these diploid pollen grains is more or less the same as haploid pollen grains of either japonica variety or indica varieties. This is explanable that these game es do receive only a quarter of the cytoplasm of the original microsporocyte despite the fact that they receive diploid number of chromesomes.

Summary

In back-crossing the F_1 hybrid of *Oryza sativa*×*O. officinalis* with the recurrent parent *O. sativa,* many seeds were obtained. On germination, these back-cross progenies were found to be triploid with 36 chromosomes.

Meiosis in microsporocytes of the F_1 hybrid was studied in detail. It was found that there were three different major types of meiotic division with practically non-pairing of the chromosomes, belong to A and C genomes at late prophase and metaphase:

1. The normal type with centric nucleus and normal spindle.
2. Centrically placed nucleus but with longitudinally compressed spindle.
3. Eccentrically placed nucleus with delayed formation at the regular site.

In the first two types of division tetrads varying in size and chromosome number might be obtained but were all sterile, in the third type triad would be

formed and two fertile diploid gametes were formed from one microsporocyte.

When the nucleus was displaced to one end, mitosis and cytokinesis would have taken place independently from one another both in space and in time.

Oryza sativa L. 及其近緣種之細胞遺傳學研究

7. *O. sativa* L. 和 *O. officinalis* 第一代雜種之核分裂及細胞質分裂的不同時間，不同空間和二元體配偶子形成的關係

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以 *Oryza sativa*×*O. offcinalis* 的第一代雜種回交輪廻親本 *O. sativa* 得到了很多種子。種植後發現其外形比 F_1 更類似 *O. sativa*。這些回交後代是三倍體，染色體數爲 36。

詳細研究第一代雜種的小孢子母細胞減數分裂，發現有三種主要的不同分裂法。屬於A和C genome 的染色體大致上在前期的晚期和中期都是不配對的。三種不同的分裂型爲：

1. 正常分裂型，細胞核在細胞的中央，有正常的紡綞體。
2. 細胞核在細胞的中央，但是紡綞體由縱的方向被壓縮。
3. 核不在細胞中央，紡綞體却在正常的位置遲延形成。

前二種分裂型四分子的大小和染色體數各異，但爲不孕的。在第三種情形形成三分子，其中二個爲得自同一小孢子母細胞可孕的二元體配偶子，另一個則不含染色體。

當細胞核在細胞的一端時核分裂（mitosis）和細胞質分裂（cytokinesis）無論就時間或空間來說都是各自獨立發生的。

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