

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

10. Study on Meiosis and Unreduced Gametes Formation of the hybrid *O. sativa* L. \times *O. australiensis* Domin.⁽¹⁾

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In the cytogenetics laboratory of the Institute of Botany, attempts have been made in trying to introduce the good characters of wild species of *Oryza* into the cultivated rice, *Oryza sativa*. Repeated attempts for the production of amphiploids of the interspecific hybrids have failed. Fortunately, in the interspecific hybrid of *Oryza sativa* \times *O. officinalis* some unreduced gametes were produced. By backcrossing, seeds were produced and seedling grown from them were proved to be triploids (Ho and Li in press). The mechanism involved in the production of unreduced gametes were reported in detail (Li *et al.* 1964). The same results were also observed in the meiosis of *Oryza sativa* \times *O. brachyantha* (Yang *et al.* in press).

With *O. australiensis*, the genome was designated as EE (Li *et al.* 1961, 1963a). In the hybrid of *O. sativa* and this species, more good pollen grains were encountered with than with the other two hybrids, *O. sativa* \times *O. officinalis* (AC) and *O. sativa* \times *O. brachyantha* (AF). Therefore a detailed study of this hybrid in regard to its meiosis as well as the mechanism involved in the production of unreduced gametes was made and will be reported in this paper.

Material and Methods

The microsporocytes were fixed in Farmer's fluid. Propianocarmine was used for staining. Regular smear method was used for preparation and sometimes a trace of ferric-hydroxide in 45% propionic acid was added for better spindle staining.

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Results

1. Pollen Fertility

Pollen grains were fixed in alcohol and observed by I₂-KI staining method. Out of 3,420 pollen grains counted 69 well-stained grains were observed. The pollen fertility was 2.02%. Some well-stained pollen grains were shown in figure 1.

2. Results of Back-crossing

According to the amount of good pollen that could be obtained from this hybrid, it was presumed that more seeds would be obtained from this hybrid when it was backcrossed to its recurrent parent, *O. sativa*. To our surprise, only two seeds were produced out of more than two-thousand spikelets used in effecting this backcrossing. Of this two seeds produced, one germinated. Presumably it would be a triploid. The reason for the production of so few seed in backcrossing is still unknown.

3. Meiosis

In the smear preparation of the microsporogenesis of this hybrid, the chromosomes of *Oryza australiensis* could be well-differentiated from those of *O. sativa* by being much larger in size and much dark in staining at diakinesis and metaphase. These were well studied by Shartry and Ranga Rao (1961) and (Li *et al.* 1963b). In our material, we agreed fully with their observations.

a. Pollen mother cells with a large nucleus and a normal spindle

It was amazing to find that in the majority of pollen mother cells, the first division was found to be rather normal (diag. 1-4, fig. 2-6). The univalent chromosome of *O. australiensis* would migrate randomly to the poles ahead of *O. sativa* which would lag at equatorial plate either split or unsplit at this stage, and then migrated to their respective poles while split or migrated to the poles at random while unsplit (diag. 2, fig. 4, 5).

The first division seemed to be rather normal up to the first telophase. However, at later stages very few tetrads were found in many slides examined (fig. 9). Presumably, these dyads would stop at this stage and would proceed no further. Of 558 microspores counted, only 8.6% of them were found to be tetrads; the remainder 91% to be dyads. Evidently these daughter cells of the dyads would soon degenerate because of the unbalanced condition in chromosome number (± 12) as well as chromosome constitution, namely, the chromosomes of *O. australiensis* being mixed with those of *O. sativa*.

It was observed in these pollen mother cells with nucleus of normal size, the formation of the spindle was rather normal in both divisions (diag. 2, 5, fig. 7) no matter whether there be only one or two division as far as the spindle mechanism was concerned, and would lead to the completion of well synchronized nuclear division and cytokinesis. The fate of the sporads of a

tetrad (diag. 6, fig. 8) would lead to the formation of sterile microspores because of the unbalanced chromosome condition both in number and in constitution.

b. Pollen mother cells with a minute nucleus and a compact spindle

When pollen mother cells were observed to have a minute nucleus (diag. 7, fig. 10) as compared with normal pollen mother cells, in majority of the cases this would lead to the formation of a compact spindle (diag. 8, 12, fig. 11, 12) giving rise to a restitution nucleus after the completion of the first division. The restitution nuclei were easily identified to have four nucleoli in the interphase (diag. 9). Post-restitution nucleus division was normal (diag. 10, fig. 14, 15) leading to the formation of unreduced sporads (diag. 11).

In some other instances, after the formation of the restitution nucleus, cytokinesis might have partly completed and the cross-wall was formed up to both sides of the restitution nucleus. In a normal second division as we could observe, the equatorial plate would be perpendicular to the cross-wall (diag. 5, fig. 7). In the post-restitution nucleus division, however, the orientation of the equatorial plate would be quite haphazard. When the equatorial plate was formed parallel with the cross-wall formed after the first division (diag. 14, fig. 16), then a dyad was formed (diag. 15). This mechanism of unreduced-gamete formation has not been reported before as the authors are aware of. Sometimes, the equatorial plate was formed perpendicular to the cross-wall as in the case of normal second division (diag. 15, fig. 17), then two kinds of division results could be obtained. A tetrad was formed with daughter nuclei placed very close together in the two poles (diag. 18) or a dyad was formed (diag. 17, fig. 19). However, in many instances, the equatorial plate was oriented in somewhere intermediate between these two extremes just described. In respect to the orientation of the equatorial plate the out come would be a dyad or a tetrad, equal or unequal in size of the sporads obtained.

c. Non-synchronization of nuclear division and spindle formation

In this hybrid sometimes we found nonsynchronization of nuclear division and spindle formation (diag. 19-23, fig. 20-24) to occur just as it was found in the hybrid of *O. sativa* × *O. officinalis* (Li *et al.* 1964). Since this was described in detail by Li *et al.* (1964), it would be superfluous for us to describe this further. Unfortunately, in figure 20 the orientation of the spindle was wrongly placed but this was the only one found when we took the photomicrographs.

Discussion

It is of paramount importance that diploid gametes must be produced in the hybrid of *O. sativa* with its related diploid wild species such as *O. officinalis*, *O. brachyantha* and *O. australiensis* so that back-cross method can be applied

for breeding work. In the case of hybrid of *O. sativa* × *O. officinalis*, the production of unreduced gametes is effected by the nonsynchronization of nuclear division and spindle formation. In the case of the hybrid of *O. sativa* × *O. brachyantha* restitution nuclei are formed leading to the formation of the dyads after two successive meiotic divisions as well as the nonsynchronization of nuclear division and spindle formation. In this hybrid, however, we found that in addition to these, at the post-restitution nuclear division the orientation of the equatorial plate would be parallel with the cross-wall which is already partly formed, thus leading to the formation of a dyad after the completion of the second division. This phenomenon is decidedly a new mechanism in the formation of diploid gametes encountered in interspecific hybrids of *Oryza*.

We pointed out already that about 91% of the division figures in the end of the supposedly second division remain to be dyads. Our assumption is that most of these dyads are the original dyads which never divide further and would constitute the sterile microspores formed later.

In this hybrid we find the existence of two kinds of pollen mother cells: one with normal sized nucleus leading to the formation of normal spindle, and the other with a minute nucleus leading to the formation of a compact spindle and the final formation of a restitution nucleus. This observation would be at variance from what was described by Snyder (working with *Paspalum* natural hybrids, 1961) and Walters (working with the *Bromus* hybrid, 1958). They found that the chromosomes (univalents) would return to the equator to form the restitution nucleus after they migrated first to their respective poles at the first division. After returning, the spindle was also shortened accordingly. Decidedly, in the case of our hybrid, the difference in the size of the nucleus and the length of the spindle later formed were distinctly divergent from one another. From our observation the restitution nucleus could only be formed in these pollen mother cells with minute nuclei and never from those pollen mother cells with normal sized nucleus.

Summary

The different stages of meiosis of this hybrid of *O. sativa* × *O. australiensis* were observed to be of the same nature as those being described by Shastry and Ranga Rao (1961) and Li *et al.* (1961, 1963a, b) except that most of the dyads would stop from further division. About 2.02% of good pollen were observed, presumably to be unreduced gametes. The mechanism involved in the production of these unreduced gametes were;

1. Pollen mother cells with a minute nucleus leading to the formation of a compact spindle, then the formation of restitution nucleus and finally the dyad with two unreduced gametes.

2. The orientation of the equatorial plate at post-restitution nucleus division was parallel to the cross-wall leading finally to the formation of a dyad with two unreduced gametes.

3. Nonsynchronization of the nuclear division and spindle formation leading to the formation of triad with two unreduced gametes.

Oryza sativa L. 及其近緣種之細胞遺傳學研究

10. *O. sativa* L. × *O. australiensis* Domin. 雜種減數分裂 及具未減數染色體的配偶子形成之研究

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O. sativa × *O. australiensis* 第一代雜種之減數分裂情形經詳細研究，結果和 Shastry 等氏 (1961) 及李先聞等 (1961, 1963a, b) 的報告大致相同，但在減數分裂之第一次分裂後形成的二分子大部分不再繼續分裂，所以在減數分裂終了時大部分為二分子，僅極少數為四分子。根據觀察結果約有 2.02% 好花粉產生，而這些花粉當為具未減數染色體的配偶子。這些具有染色體數不減的配偶子形成原因如下：

1. 凡具有一小核的花粉母細胞，到第一次減數分裂中期形成一個緊縮的紡錘體，以後染色體不分開遂形成 restitution nucleus，再經第二次減數分裂 (second meiotic division) 產生二分子，各為具未減數染色體的配偶子。

2. Post-restitution nucleus division 的中期，若產生的赤道板和第一次細胞質分裂的細胞壁平行或在相同位置，那麼經這次分裂後也會形成二分子，各為具未減數染色體的配偶子。

3. 核分裂和紡錘體形成的時間空間都不一致，經減數分裂的二次分裂，後一個為空細胞，另兩個則為具有未減數染色體數的配偶子。

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Explanation of Plates

- Fig. 1. Showing some well-stained pollen grains with unreduced number of chromosomes.
- Fig. 2. Diakinesis, showing chromosomes widely spread in the nucleus.
- Fig. 3. Metaphase I with 24 univalents-large chromosomes are of *O. australiensis*.
- Fig. 4. MI-AI, 24 univalents randomly migrated toward two poles with *australiensis* chromosomes ahead of the *sativa* chromosomes. Notice the length of spindle.
- Fig. 5. MI-AI, showing large *australiensis* chromosomes migrated to the poles first, with lagging *sativa* chromosomes split at the equatorial plate.
- Fig. 6. Dyad.
- Fig. 7. MII with incomplete cross-wall.
- Fig. 8. Tetrad.
- Fig. 9. Microspores showing mostly dyads and a few tetrads.
- Fig. 10. Diakinesis with a compact and small nucleus.
- Fig. 11. MI-AI, showing spindle compact and short.
- Fig. 12. Restitution nucleus formation with short and compact spindle.
- Fig. 13. Restitution nucleus at interkinesis with 4 nucleoli
- Fig. 14. Early anaphase of post-restitution nucleus division.
- Fig. 15. Anaphase of post-restitution nucleus division, separation of chromatids, some migrated to the two poles ahead of others.
- Fig. 16. Post-restitution nucleus division spindle formed at the same orientation it should be formed if there were a first division. First cytokinesis was incomplete with a cross-wall cut the cell only peripherally.
- Fig. 17. Post-restitution nucleus division with diffused spindle. Cytokinesis half completed with spindle parallel to the cross-wall half completed.
- Fig. 18. Post-restitution nucleus division, metaphase, cross-wall at one side of the dividing nucleus.
- Fig. 19. Post-restitution nucleus division, telophase, following the stage as depicted in fig. 17. Dyad is formed.
- Fig. 20. Spindle formed independently of the nucleus both in space and in time. Nucleus is in interkinesis after the formation of a restitution nucleus.
- Fig. 21. MII, chromosomes in one of the cells only, with its spindle formed perpendicular to the cross-wall.
- Fig. 22. MII, chromosomes in one of the cells only, with equatorial plate perpendicular to the cross-wall.
- Fig. 23. Early telophase II, the first cytokinesis is incomplete.
- Fig. 24. Triad, most of the chromosomes in 2 sporads. There are a few chromosomes in the third sporad.
- Fig. 25. Multipolar microsporocyte.



