CYTOGENETICAL STUDIES OF ORYZA SATIVA L. AND ITS RELATED SPECIES

12. An Alien Additional Line Second Backcross Generation of *O. sativa* × *O. australiensis*[1]

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Introduction

In the past few years, we have worked on the isolation of alien additional lines involving the utilization of wild species such as *O. officinalis* (CC), *O. australiensis* (EE), *O. brachyantha* (FF), *O. breviligulata* (A^gA^g), and another cultivated species *O. glaberrima* (A^gA^g). The initial cross was made between *O. sativa* (either *japonica* or *indica* or both) and and any one of the wild species concerned. Then the hybrid was backcrossed to the recurrent parent *O. sativa*. In so doing spontaneous doubling took place in the hybrid (Li *et al.* 1965, 1966), thus triploids were obtained (AAX) with the exception that when *O. glaberrima* was used. Further repeated backcrossing of these triploids with the recurrent parent would aim in getting a complete set of alien additional lines with a particular wild species. However, repeated backcrossing of the triploid by the recurrent parent made only with partial success. Embryo culture was used exclusively for all these young embryos obtained but only a a few young mature plants were obtained. So far, there were five mature plants obtained from BC_2 of AAE (*O. sativa* × *O. australiensis*); one from AAF (*O. sativa* × *O. brachyantha*); and none from BC_2 of AAC (*O. sativa* × *O. officinalis*). Of the five plants involving AAE, one had 24 chromosomes, one had 25 chromosomes, two had 26 chromosomes and one had 27 chromosomes. Of the only one plant obtained from BC_2 of AAF, the chromosome number was 28. Since the plant with 25 chromosomes was the first alien additional line obtained, a detailed cytological study was made. This is reported hereby in this paper.

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Material and Method

The microsporocytes were fixed in Farmer's fluid. Propiano-carmine was used for staining. Regular smear method was used for preparation and sometimes a trace of ferric-chloride in propiano-carmine was added for better spindle staining.

Results

From Fig. 4 it can be seen, besides the twelve bivalents there was an extra chromosone. From Fig. 1 and Fig. 1a there were two nucleoli attached to each other, one large and one small in this late pachytene stage. There were two satellite chromosomes attached to these nucleoli. From further observation of the early diakinesis stage as shown in Fig. 2, there were two satellite chromosomes attached to the two different sized nucleoli. One of these chromosome was definitely double. The other one was single. A diagrammatical drawing is shown in Fig. 3a, a fused nucleolus with two satellite chromosomes attached. One is double, the other single. From these various observations, it can be concluded that the extra chromosome involved is the satellite chromosome of *O. australiensis.* Since the extra chromosome was single, it could only organize a smaller nucleolus whereas the doubled satellite chromosomes of *O. sativa* could organize a larger nucleolus. In anaphase, this extra chromosome might migrate to only one pole and was included in this very daughter nucleus was shown in Fig. 5. Or it might lag on the equatorial plate and would not be included in neither daughter nuclei as shown in Fig. 7. If the extra chromosome was included in the daughter nucleus the two nucleoli would be formed in the dyad stage as shown in Fig. 8. If the second division would follow the same pattern, the two sporads of the quartet stage would have two nucleoli as shown in Fig. 10. In case the extra chromosome was eliminated in the first division then microspores would have only one nucleolus in each. This is shown in Fig. 9. Sometimes only one sporad would have two nucleoli as shown in Fig. 11. indicating that some abnormal division took place in the second division. In Table 1, there were pollen and seed fertility of the recurrent parent and the trisomic plant. In regard to the pollen fertility, it can be seen that it was only 55.5% in trisomic plant as against 96.5% of the recurrent parent. With seed fertility, the difference was much qreater in *O. sativa* (98%) as against 20% of the trisomic plant. From the study of the quartet, the number of nucleoli formed in sporads was recorded in Table 11. There were 16.8% with all the sporads with one nucleolus, 20.4% with one sporad with two nucleoli, and the majority of the case was 62.8% with two sporads with two nucleoli, Assuming that all the pollen grains with two nucleoli were almost nonfunctional in competition with normal pollen grains, then from the frequecy found, of the sporads with different number of nuclecli, estimation could be made of the pollen fertility. This was recorded in the column of estimated percentage of pollen fertility. The total percentage would be 62.3% as against 55.5% of the actual observation made in the trisomic pollen. This would constitute a relative good fit. In Fig. 14, it shows the morphological difference of a branch of a panicle of the recurrent parent and that of the trisomic plant. The morphological difference of the spikelets of the two respective plants can be observed in Fig. 15. It can be seen that with the trisomic plant the nonflowering glume was larger and longer so that it overlapped the smaller and shorter one.

Table 1. *Pollen and seed fertility of O. sativa and its trisomic plant.*

plant	pollen fertility	seed fertility
O. sativa	96.5%	98%
trisomic plant	55.5%	20%

Table 2. *The frequency of quartets with different numbers of nucleoli in each sporad.*

quartet	frequency	estimate % of pollen fertility
4 with one nucleolus	16.8%	16.8%
3 with one nucleolus	20.4%	15.3%
2 with one nucleolus	62.8%	31.4%

Conclusion

An alien additional line was obtained from BC_2 generation of the triploid AAE of the original cross of *O. sativa* (Taichung 65) × *O. australiensis*. The extra chromosome was the satellite chromosome of *O. australiensis*. Morphologically, the spikelet of this trisomic plant differed greatly from that of the recurrent parent *O. sativa* (AA).

Oryza sativa L. 及其近緣種細胞遺傳學之研究

12. 對 *O. sativa* × *O. australiensis* 外來添加染色體系統 (alien additional line) 之研究

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自雜種 O. sativa (Taichung 65) × O. australiensis 經第一次回交所得的三倍體 (triploid) AAE. 再回交，於 BC_2 一代中獲得第一株添加一單價外來染色體之植株，其植株及穗的形態與親本相差很大，做細胞學的研究，在粗絲期 (Pachytene) 可看出添加了一核仁染色體 (Eig. 1.) 對花粉母細胞減數分裂過程之研究，發現四分子中，在一細胞裏有二個分立的核仁及一個核仁二種不同的現象，測其含一個核仁細胞之頻度，結果與花粉成熟 (Pollen feertility) 頻度非常吻合 (table. 1.)。

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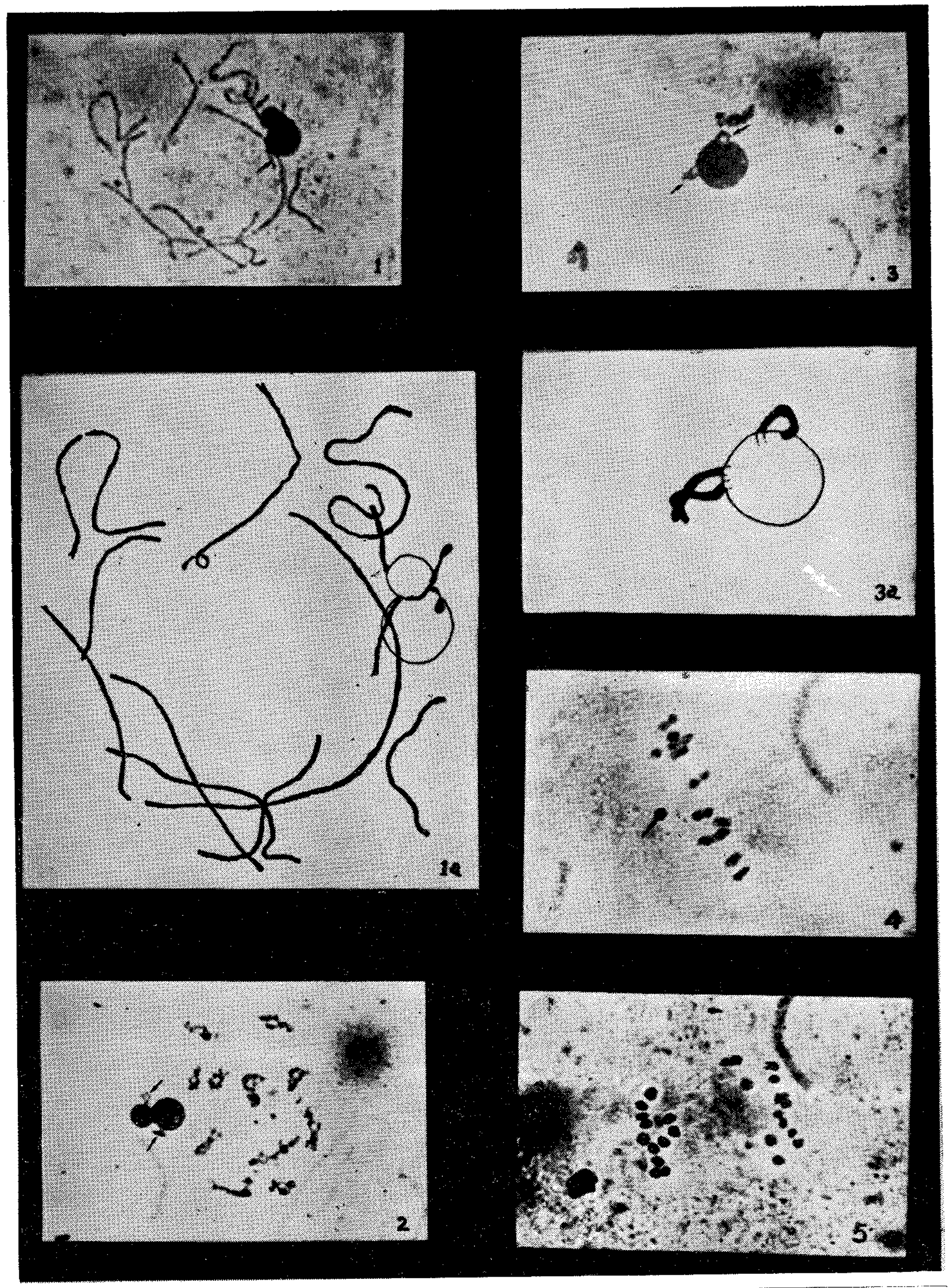

Explanation of the Plate Figures

Fig. 1. Pachytene. An extra satellite chromosome attached to the smaller nucleolus.
Fig. 1a. Camera lucida drawing of the above.
Fig. 2. Early diakinesis showing the 12 bivalents and a univalent with two nucleoli, one big and one single (lower).
Fig. 3. A single nucleclus with two satellite chromosomes, one single (upper) and one double (lower).
Fig. 3a. Camera lucida drawing of the same.
Fig. 4. An extra chromosome of the *O. australiensis* one the left side of equatorialplate in metapiase 1.
Fig. 5. An extra chromosome goes to the right pole in anaphase 1.

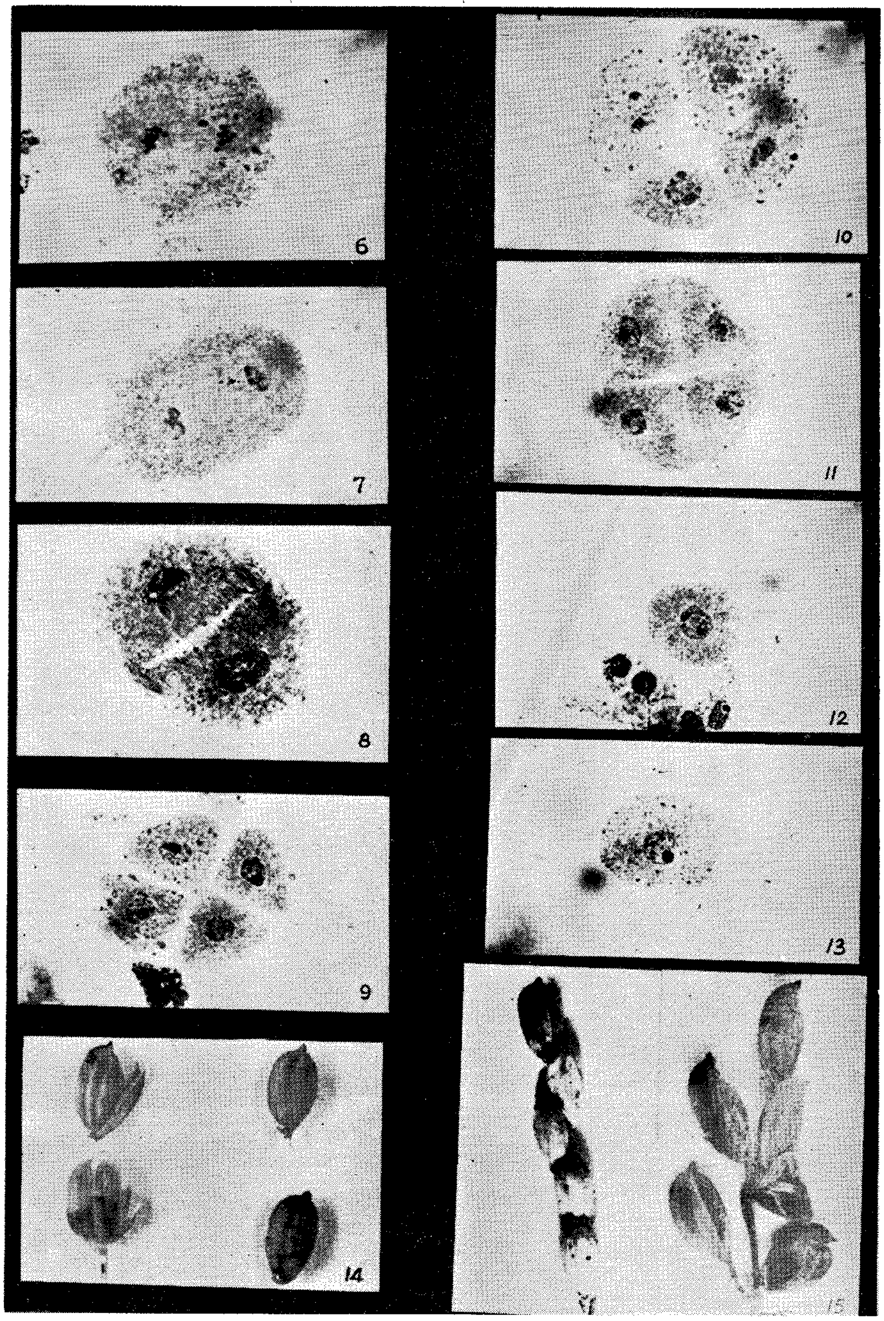

Fig. 6. An extra chromosome lags behind in late anaphase.

Fig. 7. The extra chromosome is not included in the daughter nuclei.

Fig. 8. There are two nucleoli organized by having two nucleolus organizer chromosomes in the right daughter nucleus.

Fig. 9. A normal quartet is formed after the loss of the extra chromosome.

Fig. 10. Quartet nuclei each with two nucleoli of equal size (left two).

Fig. 11. Only one nucleus with two nucleoli (lower left).

Fig. 12. A microspore with two nucleoli of equal size.

Fig. 13. A normal microapore with only one nucleolus.

Fig. 14. The morphlolgical difference of the single spikelits of the recurrent parent and the trisomic plant. One the trisomic spikiltes, the nonflowering glume was larger and longer so that it overlapped the smaller and shorter one.

Fig. 15. The morphological difference of a branch of a panicle of the recurrent parent and that of the trisomic plant.