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

4. Interspecific Crosses Involving *O. australiensis* with *O. sativa* and *O. minuta*[1]

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O. australiensis Domin is the wild rice found in Australia, and was placed in the section *Sativa* by the classification of Roschevicz (1931). It can be crossed with many species in this section (Gopalakrishnan 1959, cited by Richharia, 1960; Nezu *et al.*, 1960; Morinaga and Kuriyama, 1960; Morinaga *et al.*, 1960, 1962; Li *et al.*, 1961; and Hu, unpubl.). However, its genomic designation has not been ascertained as yet. This paper deals with the presentation of the results obtained from additional crosses involving *O. australiensis* with two other species and the eventual evaluation of all the data obtained so far.

Materials and Methods

O. sativa Linn. (2n=24), *O. minuta* Presl (2n=48) and *O. australiensis* Domin (2n=24) were used as the parent species in this experiment. The last two species were kindly furnished by Dr. H. I. Oka of the Japanese National Institute of Genetics.

In our crossing work, hot water (43°C) emasculation for 7 minutes method was used. Embryo culture was also applied for the inmature embryos which were excised from the young seeds at the age of 7–9 days after pollination. The young seedlings were transplanted to pots and grown in the green house. In order to inhibit flower initiation of the plants in winter, light period was extended for about 3–4 hours until 9 o'clock every evening. Heating was provided throughout the winter of 1961. PMC's were fixed in 1:3 acetic-alcohol and stained with acetocarmine. Smear technique was applied exclusively in this study.

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(2) Director and Assistant Research Fellows respectively.

Results

1. *Results of crossing*

Crossing was attempted in large scale involving these two crosses. Most of the hybrid seeds of *O. minuta*×*O. australiensis* were characterized by their degenerated size, reaching about one third of the normal grain of *O. minuta*. By comparing the pollinated florets to the true hybrids obtained in these two crosses, we found that with *O. minuta*×*O. australiensis* it was more successful than with *O. sativa*×*O. australiensis*. The crossing results are listed in Table 1.

Table 1. *The results of interspecific crosses of* O. sativa×O. australiensis *and* O. minuta×O. australiensis

Crosses	No. of pollinated florets	No. of embryos cultured	No. of seedlings transplanted	No. of true hybrids obtained	No. of adult plants raised	Cross-ability*
O. sativa× *O. australiensis*	1,173	22	8	1	1**	0.058%
O. minuta× *O. australiensis*	1,263	331	many	29	27	2.137%

* Crossability = $\frac{\text{No. of true hybrids}}{\text{No. of pollinated florets}} \times 100$

** This hybrid was found to be triploid cytologically, see next paragraph.

2. *Cytological studies of the hybrids*

(1) *O. sativa*×*O. australiensis*

In *O. sativa*×*O. australiensis* hybrid, 80 cells in MI were observed. It was suprisingly found that this plant was a triploid resulting from the fusion of unreduced male gamete of *O. australiensis* with a normal egg of *O. sativa*. (see later discussion)

From Table 2, it can be seen that the chromosome association at MI in this triploid hybrid was extremely variable. Univalents, bivalents, and multivalents were all observed. Predominantly, however, were the bivalents. Most of these were large sized closed types. Occasionally, however, there were some bivalents made of much smaller chromosomes and were of the open type and furthermore they were apt to disjoin precociously at this stage. Since, outwardly, this triploid hybrid looks very much like the male parent, *O. australiensis*, the diploid gamete must have originated from the male parent.

Some outstanding morphological differences between the parents *O. sativa* and *O. australiensis* and their diploid and triploid hybrids are presented below:

O. australiensis: *O. australiensis* is a tall plant with lax inflorescences and long and narrow leaves; culm somewhat prostrate and spreading; ligule short; auricle absent; awn of the lemma about 4–5 cm long; the tip of the palea extended with an apiculus elongation.

Table 2. *Chromosome associations at MI in the hybrid of* O. sativa×O. australiensis* *(triploid)*

IV			III	II			I		Frequency
AAAA	AAAS	AASS	AAS	AA	SS	AS	A	S	
				12	1			10	15
				11	1		2	10	10
			1	11	1			9	9
				12	2			8	7
				12				12	7
			1	10	1		2	9	7
				11			2	12	4
				12	3			6	4
				11	2		2	8	2
			1	10	3		2	5	2
		1		10	1		2	8	1
1				10	1			10	1
	1		1	9			1	10	1
		1		10			2	10	1
		1	1	10	1			7	1
		1		11	1			8	1
			2	9	1		2	8	1
			1	11	2			7	1
			1	10			2	11	1
			1	10	2			9	1
				10	1		4	10	1
				11	3		2	6	1
				11		2		10	1
Total	1	1	4	25	893	90	2	65	746	80
Mean	0.01	0.01	0.05	0.31	11.16	1.13	0.03	0.81	9.36	
Range	0-1	0-1	0-1	0-2	9-12	0-3	0-2	0-4	5-12	

* A and S represent chromosomes of *O. australiensis* and *O. sativa* respectively.

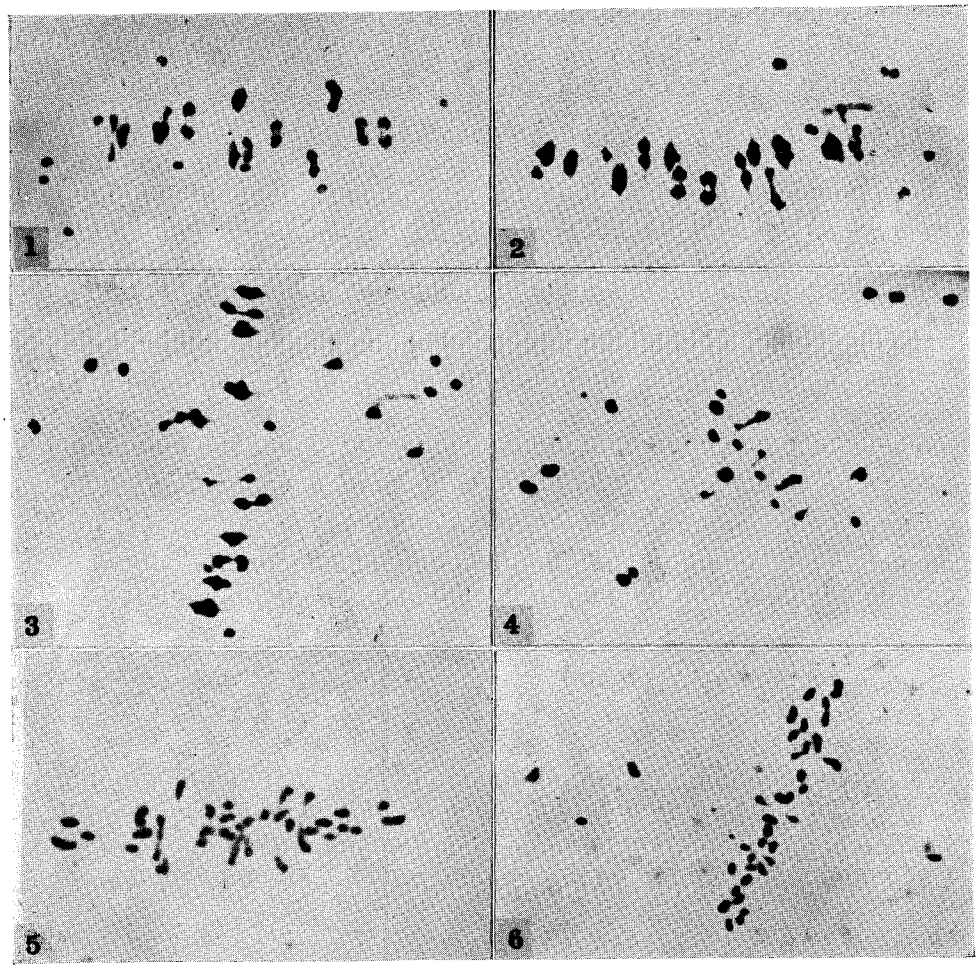
O. sativa: The plant height and leaf-blade are shorter than *O. australiensis;* culm erect; ligule long and acute; auricle small; panicle dense and dropping; spikelet awnless; the tip of the palea without an apiculus elongation.

O. sativa×*O. australiensis*

Diploid hybrid: Plant morphology is intermediate between its parents; culms erect; leaf-blade as long as *O. sativa;* ligules intermediate between its parents; auricle smaller than that of *O. sativa;* panicles erect; rachis much shorter than *O. australiensis.*

Triploid hybrid: Plant morphology is more similar to *O. australiensis* than to *O. sativa* as in plant hight, shape of panicle, length of leaf-blade, spreading culms; the awn and the apiculus elongation of the palea are longer than those of *O. australiensis;* ligules as long as those of *O. sativa* or even longer; auricle slightly larger than *O. sativa.*

If this argument could hold, then we could assume that the bivalents (about 12) were doubled chromosomes of *O. australiensis,* and the smaller ones those of *O. sativa.* And those precociously dividing smaller bivalents were the chromosomes of *O. sativa.* In Table 2, the chromosomes were thus divided by



Figs. 1-6. Chromosome associations at MI in the hybrids. ×1115. 1, *O.sativa*×*O. australiensis* (3n). 1_{III} (AAS)+12_{II} (11AA+1SS)+9_{I} (S). 2, *O. sativa*×*O. australiensis* (3n). 14_{II} (12AA+2SS)+8_{I} (S). 3, *O. sativa*×*O. australiensis* (3n). 12_{II} (11AA+1SS)+12_{I} (2A+10S). 4, *O. sativa*×*O. australiensis* (2n). 4_{II} (3AS+1SS)+16_{I} (9A+7S). 5, *O. minuta*×*O. australiensis*. 6_{II} (1AM+5MM)+24_{I} (11A+13M). 6, *O. minuta*×*O. australiensis*. 3_{II} (1AM+2MM)+30_{I} (11A+19M).

their size, and A and S were assigned to them to represent the chromosomes of *O. australiensis* and *O. sativa* respectively.

Of the bivalents found, the mean frequency of the doubled *O. australiensis* chromosomes was 11.16 per cell. Pairing of the non-homologous chromosomes of *O. sativa* was 1.13. Of the univalents found, naturally most of them would belong to *O. sativa,* the mean frequency being 9.36. However, there was a mean frequency of 0.81 univalents found presumably to be of *O. australiensis* origin. In many cells studied, one trivalent was found and the association was found to be two large sized chromosomes and one small one.

Dr. C. H. Hu of Chung Hsing University kindly furnished us with a hybrid plant *O. sativa*×*O. australiensis*, a diploid 2n=24. The results of the cytological study are shown in Table 3.

From Table 3, it can be seen that the bivalents found per cell were 2.4, and 19.2 for univalents and there were also found some trivalents but very rarely. From these results, it can be concluded that the genomes of *O. sativa* and *O. australiensis* would be different from one another. From triploid hybrid it was learnt that the larger chromosomes were those of *O. australiensis.* Accordingly, in the diploid hybrid of *O. sativa* and *O. australiensis* the 12 larger chromosomes were assumed to belong to *O. australiensis.* In general, the chromosomes of *O. australiensis* were 2-4 times as big as those of *O. sativa* as an average at MI. Thus between the chromosomes of *O. sativa* and *O. australiensis* the classification could be easily made. However, in some cells, the largest chromosome of *O. sativa* in comparison with the smallest of *O. australiensis* might give confusion to a clear cut classification. In some such cells, 13 instead of 12 larger chromosomes were counted. When cases like this were found, there were two chromosomes more or less of the same size and of intermediate size. In a few cells, however, 3 chromosomes were of such intermediate size. Altogether there were 45 chromosomes of intermediate size met. Since there were 150 cells studied, and since there were 24 chromosomes in each cell, the error was only 1.2% (45/24×150).

Since the identity of the chromosomes could be ascertained almost with certainty, the partners of the bivalents might be also identified. From 150 cells studied, there were altogether 366 bivalents found. Of these,

AA: 35/366= 9.6%
AS: 248/366=67.8%
SS : 83/366=22.7%

From these results, we can see that about two thirds of the bivalents were the result of allosyndesis, the other third of autosyndesis. It seems that there were more pairing between the chromosomes of different genomes rather than that done intragenomically.

Because of the appreciable difference in the size of the chromosomes making up AS bivalent, they were heteromorphic of the extreme type. These bivalents were found to be open types as a rule. On the contrary, the AA bivalents were also heteromorphic in most of the cases found. About 50% of them were found to be closed type. Similarly, the SS bivalents were also heteromorphic in most of the cases and they were all of the open type (Fig. 4).

Mention should be made here that in many cells studied, the bivalents would remain on the equator with the chromosomes of *O. sativa* at MI, whereas

Table 3. *Chromosome association at MI in the hybrid* O. sativa×O. australiensis* (*diploid*)

III			II			I	Frequency
AAS	ASS	SSS	AA	AS	SS	A+S	
						24	18
				1		22	14
			1			22	2
					1	22	11
1						21	1
				2		20	16
				1	1	20	12
					2	20	2
			1	1		20	2
				3		18	10
				2	1	18	10
			2		1	18	1
			2	1		18	1
			1	1	1	18	4
			1		2	18	1
				1	2	18	2
			1	2		18	2
		1		2		17	1
				2	2	16	1
				3	1	16	8
1	1			1		16	1
				4		16	5
			1	1	2	16	1
				2	2	16	1
			1	3		16	2
			1	2	1	16	4
			2	1	1	16	1
				5		14	2
			1	4		14	2
			1	3	1	14	2
				4	1	14	3
			1	2	2	14	1
	1			2	2	13	1
			1	4	1	12	2
			2	4	1	10	1
			2	2	3	10	1
				6	1	10	1
Sub-total	2	2	1	35	248	83	2,853	150
Total	5			366				
Sub-mean	0.01	0.01	0.007	0.23	1.66	0.55	19.02	
Mean	0.033			2.4				
Sub-range	0–1	0–1	0–1	0–2	0–6	0–3	10–24	
Range	0–2			0–7				

* A and S represent chromosomes of *O. australiensis* and *O. sativa* respectively.

the chromosomes of *O. australiensis* seemed to get to the poles ahead of the chromosomes of *O. sativa*. However, this was not a constant phenomenon.

(2) *O. minuta*×*O. australiensis*

In *O. minuta*×*O. australiensis* hybrid, there were 128 cells examined. The results are presented in Table 4.

It was assumed that there was little affinity between the chromosomes of

Table 4. *Chromosome association at MI in the hybrid* O. minuta×O. australiensis

	IV	III	II	I	Frequency
			0	36	1
			1	34	3
			2	32	12
			3	30	11
		1	2	29	1
			4	28	26
		1	3	27	3
			5	26	23
	1	1	4	25	2
			6	24	16
			4	24	1
		1	5	23	3
			7	22	16
		1	6	21	1
			8	20	6
			9	18	1
		1	8	17	1
			10	16	1
Total	1	11	601	3,359	128
Mean	0.007	0.085	4.70	26.24	
Range	0-1	0-1	0-10	16-36	

O. minuta and *O. australiensis,* though the number of bivalents ranged from 0–10, with the mean of 4.70 per cell. The chromosomes of *O. australiensis* were seemingly larger than those of *O. minuta,* but the difference was not so clear-cut as that between *O. sativa* and *O. australiensis.* These bivalents, were rather of mixed nature, i.e., they were the results of: (1) pairing between the nonhomologous chromosomes of *O. minuta.* (2) pairing between the nonhomologous chromosomes of *O. australiensis.* (3) pairing between the chromosomes of *O. minuta* and *O. australiensis.* Through detailed cytological observations we obtained roughly the frequencies of 72.21%, 6.32%, and 21.46% for the three kinds of pairing respectively as listed above. The majority of bivalents came from autosyndesis of the genomes BC of *O. minuta.* Just as in the case of *O. minuta*×*O. brachyantha* (Wuu *et al.*, 1963), the chromosomes of genomes B and C had some affinity among themselves. Multivalents were found, but they were of rare occurrence. Both pollen fertility and seed fertility were found to be nil in all these two hybrids.

Discussion

Most of the rice workers, such as Roschevicz (1931), Chevalier (1932), Ghose *et al.* (1956) and others, classified the species *O. australiensis* as a member of section *Sativa* of *Oryza.* Gopalakrishnan (1959, cited by Richharia, 1960) studied cytologically the hybrid *O. sativa*×*O. australiensis* and found that there was no homology between the chromosomes of these two species. For this reason, he

postulated that *O. australiensis* should be removed out of the *Sativa* section. Morishima and Oka (1960) adopted the techniques of factor analysis and matrix of correlation to study the degree of resemblance among 16 species in the genus *Oryza*. Based on the calculation of 42 characters, they constructed two tree-like diagrams (from correlation matrix I and II). According to them, *O. australiensis* was linked with *O. subulata* group from correlation matrix I diagram. But on the other hand, it also related to the *Officinalis* group from correlation matrix II diagram. They finally postulated that section *Sativa* Roschev. might be divided into three sections, viz., *Sativa, Officinalis* and *Australiensis* on condition that *O. australiensis* was ranked as a section.

Nezu *et al.* (1960) studied hybrids *O. sativa*×*O. australiensis* and *O. officinalis* ×*O. australiensis* cytologically, the metaphase figures of these two hybrids examined were 0.2 II+23.6 I and 0.5 II+23 I respectively. Since chromosome pairing rarely occurred in these hybrids, they concluded that *O. australiensis* has neither A nor C genome. Morinaga *et al.* (1960) crossed *O. minuta* with *O. australiensis,* they found 36 univalents in this hybrid. As the genomes of *O. minuta* are BBCC, so *O. australiensis* has neither genome B nor C.

Li *et al.* (1961) reported that in hybrids *O. paraguaiensis*×*O. australiensis* and *O. australiensis*×*O. alta,* more bivalents were found at MI. The means per cells were 7.81 (range 2–12) and 6.175 (range 2–11) respectively. From these results, Li *et al.* (1961) conjectured that *O. australiensis* is partially homologous to one of the genomes of *O. paraguaiensis* or *O. alta.* However, from our studies later (Li *et al.,* 1962), it was found that the mean bivalent number at MI per cell of *O. sativa*×*O. latifolia* were 6.26 (range 0–11) in one cross and 4.64 (range 1–8) in another. For *O. paraguaiensis*×*O. sativa* var. *spontanea* the mean was 3.55 (range 0–8). All these involved the crossing of genome A with CD. Nezu *et al.* (1960) also found similar results in the hybrids *O. sativa indica*×*O. minuta,* the mean bivalent number at MI was 4.5 (range 0–9) per cell and it was 4.7 (range 1–8) when *japonica* was used instead of *indica.* These crosses involved the genomes A and BC. It was reported earlier that when there was size difference in the chromosomes of the two parents concerned, more than 2/3 of the heteromorphic bivalents of *O. minuta*×*O. australiensis* were presumably from the pairing of B and C genomes. It would be highly possible therefore that pairing between chromosomes of C and D genome might be also frequent as the chromosomes of genomes B and C in a hybrid. If this would be true, then the majority of the numerous bivalents found in the hybrids of *O. paraguaiensis*×*O. australiensis* or *O. australiensis*×*O. alta* might be pairing between the chromosomes of genome C and D. Unfortunately, however, there seemed to have no appreciable size difference between the chromosomes of *O. australiensis* and those of *O. paraguaiensis* or *O. alta.* Therefore detailed analysis

of the pairing condition could not be ascertained therefore.

From this brief review, together with the evidences presented in this paper, we found that the genome of *O. australiensis* may be different from any other known genomes, i. e., A, C, BC, CD, with the exception of genome F of *O. brachyantha* which has not been crossed successfully with *O. australiensis* up to the present. But based on the difference of chromosome size, plant morphology as well as the failure of hybridization between them here, we make a tentative proposition that the genome of *O. australiensis* is also different from F. Consequently, *O. australiensis* may be assigned to a separate genome, and E is suggested.

Summary

1. The only hybrid plant obtained from *O. sativa*×*O. australiensis* was a triploid. In plant morphology, this hybrid resembled *O. australiensis* very closely. Cytologically, the paired chromosomes (approaching 12 bivalents) were the larger size ones. Doubled gamete was assumed to have come from the male parent. Some multivalents were also found.

2. A diploid hybrid of the same cross was also studied. The bivalents found were 2.40 per cell. With the help of the size difference between the chromosomes of *O. sativa* and *O. australiensis,* (the average size of the chromosomes of the latter was 2–4 times as big as that of the former) about 2/3 of the bivalents were pairing of chromosomes of *O. sativa* and *O. australiensis* (allosyndesis), and the other third autosyndesis. Trivalents were also found.

3. In the hybrid *O. minuta*×*O. australiensis,* the mean number of bivalents found was 4.70. With the help of the size difference again, more than two thirds were assumed to be pairing of the chromosomes of *O. minuta.*

4. Genome E was suggested for *O. australiensis.*

Oryza sativa L. 及其近緣種之細胞遺傳學研究

4. *O. australiensis* 與 *O. sativa, O. minuta* 的種間雜種

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1. 在 *O. sativa* 與 *O. australiensis* 的雜交試驗中，作者等得到一株三倍體的雜種。外部形態上，這雜種較近似於 *O. australiensis;* 細胞學上，此雜種有大約 12 對較大的二

價體和 12 個較小的單價體。有時亦可見到少數的多價體。

2. 二倍體的 *O. sativa*×*O. australiensis* 亦曾做細胞學的觀察與研究，以資與三倍體的雜種對照。此二倍體的雜種在第一中期平均每個細胞有 2.40 個二價體。由於 *O. sativa* 與 *O. australiensis* 的染色體在第一中期時大小的差異甚大，故可用以判斷此等二價體的配對情形。大約三分之二的二價體是 *O. sativa* 與 *O. australiensis* 染色體的異源配對 (*allosyndesis*)，另外三分之一是同源配對 (*autosyndesis*)。

3. *O. minuta*×*O. australiensis* 每個細胞的平均二價體數是 4.70。利用 *O. minuta* 與 *O. australiensis* 第一中期時染色體大小上的差異，知道三分之二的二價體是由來於 *O. minuta* 染色體的同源配對 (*autosyndesis*)。

4. 作者等建議 *O. australiensis* 的染色體組符號爲 EE。(摘要)

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