CYTOGENETICAL STUDIES ON THE GENUS *ORYZA*
3. CHROMOSOME PAIRING IN THE INTERSPECIFIC HYBRID WITH
THE ACD GENOMES¹⁾

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Since F₁ hybrid of the cross, *O. sativa* × *O. latifolia*, was cytologically investigated by Gotoh and Okura (1935), results of several investigations on chromosome pairing at diakinesis and metaphase I in the F₁ hybrids between the A and CD genomes have been reported (Table 1). Almost all of the *sativa* chromosomes failed to pair at meiosis with the chromosomes of *O. latifolia* (Hirayoshi 1937; Morinaga 1941, 1964; Morinaga *et al.* 1960, 1962; Nezu *et al.* 1960), with the exception of the data described by Li *et al.* (1962). It was generally recognized that there was no homology between the A and CD genomes.

Table 1. Range of chromosome pairings per cell in F₁ hybrids between A and CD genomes as reported in literature

Authors	Number of			Cross-combinations
	bivalents	trivalents	tetravalents	
Gotoh & Okura (1935)	Degenerated			Taichu-65 × <i>O. latifolia</i>
Hirayoshi (1937)	0- 3**			<i>japonica</i> × "
	0**			<i>indica</i> × "
Morinaga (1941)	Degenerated			<i>O. sativa</i> × "
Morinaga & Kuriyama (1960)	0- 2**			" × <i>O. paraguayensis</i>
	0			" × <i>O. latifolia</i>
Nezu <i>et al.</i> (1960)	0			<i>O. latifolia</i> × <i>O. sativa</i>
Li <i>et al.</i> (1962)	0- 4(0.96)*	0	0	<i>O. sativa</i> × <i>O. latifolia</i>
	0-11(6.26)*	0-1(0.18)*	0-1(0.06)*	" × <i>O. paraguayensis</i>
	0- 8(4.64)*	0-2(0.11)*	0-1(0.03)*	" × <i>O. latifolia</i>
	0- 5(0.56)*		0	" × "
				<i>O. sativa</i> f. <i>spontanea</i> × <i>O. paraguayensis</i>
	0- 8(3.55)*	0-1(0.03)*	0-1(0.01)*	<i>O. paraguayensis</i> × <i>sativa</i> f. <i>spontanea</i>
	0- 7(0.96)*	0	0	<i>O. sativa</i> f. <i>spontanea</i> × <i>O. latifolia</i>
	—	—	—	" × <i>O. alta</i>
Morinaga <i>et al.</i> (1962)	0			<i>O. sativa</i> × <i>O. grandiglumis</i>
	0			<i>O. glaberrima</i> × "
Morinaga (1964)	0- 3			<i>O. sativa</i> × <i>O. latifolia</i>
	0- 5			" × " (Guiana)

() * shows mean number of chromosome pairings per cell.

** the case which bivalents were scarcely observed.

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On the other hand, from the pachytene analysis in the F_1 hybrids of *O. sativa* $\times$ *O. officinalis* (Shastri *et al.* 1960, 1961; Katayama 1964, 1965a), *O. sativa* $\times$ *O. minuta*, and *O. sativa* $\times$ *O. punctata* (Katayama 1965b, unpubl.), it was suggested that the A genome of *O. sativa* was homologous or partially homologous to the C genome of *O. officinalis*, *O. minuta*, and *O. punctata*.

It is important to determine the relationship between the A and CD genomes on the basis of the chromosome pairing at pachytene stage and number of bivalent chromosomes at diakinesis and metaphase I in the F_1 hybrids.

In this paper, the results of cytological observations in meiosis of the F_1 hybrids between the A and CD genomes are described, and the relationships among the A, C, BC and CD genomes are proposed.

MATERIALS AND METHODS

Twenty-three F_1 hybrids between taxa possessing the A genome (*O. sativa*, *O. sativa* f. *spontanea*, *O. sativa* v. *fatua*, *O. breviligulata*, *O. perennis*, and *O. glaberrima*) and those with the CD genomes (*O. alta*, *O. latifolia* and *O. grandiglumis*), and their cross-combinations which were employed as materials in the present experiment were tabulated in Table 2. Fourteen species and strains and 2 F_1 hybrids used in this experiment were furnished by the National Institute of Genetics at Mishima and the National Institute of Agricultural Sciences at Hiratsuka.

Table 2. List of cross-combinations between the A and CD genomes used in this experiment

F ₁ plant no.	Cross-combinations		
	(AA)		(CCDD)
1**	<i>O. sativa</i> (647)	$\times$	<i>O. alta</i> (WOO17)
8**	" (563)	$\times$	<i>O. latifolia</i> (WOO19)
35	" (P. T. B. 10)*	$\times$	" (WOO19)**
36	"	$\times$	<i>O. grandiglumis</i> (WO613)**
43	" (Gendjah Ratji)*	$\times$	"
32, 47	<i>O. sativa</i> f. <i>spontanea</i> *	$\times$	"
33, 34	"	$\times$	<i>O. latifolia</i> (WOO19)**
93	" (Y 15)*	$\times$	" (Y19)*
94	"	$\times$	" (WOO19)**
31	<i>O. sativa</i> v. <i>fatua</i> (Y 4)*	$\times$	" (Y19)*
37	"	$\times$	<i>O. grandiglumis</i> (WO613)**
29, 39	<i>O. breviligulata</i> (Y 8)*	$\times$	<i>O. latifolia</i> (WOO19)**
30	"	$\times$	" (Y19)*
40	"	$\times$	<i>O. grandiglumis</i> (WO613)**
38	" (Af 104)**	$\times$	"
42	" (Af 99)**	$\times$	"
41	<i>O. perennis</i> (W 107)**	$\times$	"
45	<i>O. glaberrima</i> (Y 9)*	$\times$	"
89	" (W 416)**	$\times$	"
91	"	$\times$	<i>O. latifolia</i> (WOO19)**

* Material obtained from the National Institute of Agricultural Sciences.

** Material obtained from the National Institute of Genetics.

Crosses were made using the method described by Katayama (1965a). The F_1 plants were kept from November to the end of March in a phytotron, and then grown in a glasshouse in April. From the beginning of May, they were moved to the outside and kept under a controlled day-length of 9 hours.

After fixation of the PMCs with acetic alcohol (3 : 1), the acetocarmine squash technique was used for preparation of slides for cytological observations.

RESULTS

In 1963, 1191 flowers were pollinated from which 4 perfect and 617 imperfect grains were obtained. A total of 184 grains were chosen at random from 20 cross-combinations, and sown on a culture medium in test tube. Of 109 plants which survived, 106 were ascertained as true F_1 hybrids. The remaining 3 plants were not cytologically observed. In some hybrids, $F_1\#30$, $F_1\#33$, $F_1\#38$, $F_1\#41$ and $F_1\#48$ ($F_1\#30$ refers to abbreviation for F_1 plant no. 30), the meiotic figures could not be observed because of degeneration of pollen mother-cells. Of F_1 hybrids of 20 cross combinations between the A and CD genomes, those of 16 were cytologically examined in the present experiment. The results are shown in Table 3. Size of PMCs in the F_1 hybrids of *O. grandiglumis* was remarkably larger than that in other hybrids. The hybrids were completely sterile.

1. Pachytene and Diplotene stages

The number of pachytene cells analyzed in the ACD hybrids was small, because the stage was not easy to analyze as compared with the F_1 hybrids between the A and C or A and BC genomes. In the PMCs analyzed, however, about 12 bivalents showed almost complete pairing throughout the length (Fig. 1) except some chromosome threads occasionally showed asynaptic regions.

Table 3. Frequency of chromosome pairings per PMC in F_1 hybrids between A and CD genomes

F ₁ plant no.*	Pachytene $12_I + 12_{II}$	Bivalents						Trivalents			
		Diakinesis			MI			Diakinesis		MI	
		Range	Mean	Total	Range	Mean	Total	Range	Total	Range	Total
1		0- 4	1.00	36	0- 2		6				
8		0-12	2.69	75	0- 4	1.21	14				
35					2-11	4.63	61				
36		0-10	4.67	53	0- 8	2.69	64	0- 1	1		
43					0- 7	3.39	61			0- 1	1
32					0- 6	2.52	50			0- 1	1
47					0- 6	1.70	76			0- 1	1
33		0- 6		11	0- 7	2.91	24				
34	5				2-10	5.81	48				
29		2- 9		8	0- 6	3.04	23				
39		1- 8	5.38	17	3-11	6.11	38			0- 1	2
30		0- 6		5	0- 6	2.81	16				
40					0- 6	2.90	99			0- 1	3
38		2- 4		6	0- 3		8				
41		0- 5	1.67	21	0- 5	1.85	50			0- 1	2
45		0-10	4.78	57	0- 7	2.90	62	0- 1	2		

* refer to Table 2 for cross-combinations.

Figs. 1-4. Various stages of chromosome pairings in ACD hybrids.
 1. Pachytene stage, showing complete pairing (F₁#46-1).
 2. Late diplotene stage, showing bivalents with chiasmata (F₁#36-5). 3. MI, showing 3_I + 30_I (F₁#47-2). 4. MI, showing 10_I + 16_I (F₁#34-4).

The bivalents having chiasmata (Fig. 2) were clearly observed at a late diplotene stage in one F₁ hybrid (F₁#36). On the basis of this observation, it is assumed that almost all of the bivalents observed at diakinesis and metaphase I are allosyndetic being formed between chromosomes of the A genome and one of the two genomes, namely C.

2. Diakinesis and Metaphase I

From Table 2, the number of bivalents per cell in 20 or more PMCs ranged from 0-4 (mean=1.00) in F₁#1 to 0-12 (mean=2.69) in F₁#8 at diakinesis and from 0-5 (mean=1.86) in F₁#41 to 3-11 (mean=6.11) in F₁#39 at metaphase I, respectively. Even in the F₁ hybrids which gave less analyzable PMCs than 20, the number of bivalents per cell varied widely. The number of chiasmata per bivalent ranged from 1 to 3 at early diakinesis. Moreover, in some F₁ hybrids, F₁#36, F₁#43, F₁#32, F₁#47, F₁#39, F₁#40, F₁#41 and F₁#45, one V-shaped trivalent was observed in a few cells (Fig. 4).

Univalents were either concentrated on the equatorial plate (Fig. 3), or randomly distributed about it (Fig. 4), or were distributed along the spindle. There is no recognizable difference in size among univalents. The bivalents generally have a tendency to be oriented on the equatorial plate at metaphase I (Figs. 3 and 4). Most of the bivalents observed at metaphase I were loose-dumbbell-shaped with a single chiasma.

Fig. 5. A diagrammatic representation of species relationships in the *Sativa* section. — shows complete homology — shows partial homology shows complete homology (Morinaga 1937; Morinaga & Kuriyama 1960; Morinaga *et al.* 1962; Li *et al.* 1962)

DISCUSSION

From the morphological and cytological viewpoints, Roschevicz (1931) grouped *O. sativa*, *O. latifolia*, and *O. grandiglumis* into the same section, Section *Sativa* Roschev. Ghose *et al.* (1956), Sampath and Rao (1951), and Morishima and Oka (1960) divided these species into two groups, *Sativa* and *Officinalis*.

In the F₁ hybrid, *O. sativa* (Taichu No. 65) × *O. latifolia*, Gotoh and Okura (1935) were not able to observe meiotic divisions, because of the degeneration of the PMCs. On the basis of the cytological observations at metaphase I in the F₁ hybrid, *O. sativa* (*indica*) × *O. latifolia*, Hirayoshi (1937) suggested that the genome of *O. sativa* was not homologous to either one of the two genomes of *O. latifolia*. In the first meiotic

division of the F_1 hybrid, $O. sativa \times O. latifolia$, no regular bivalent formation was noticed by Morinaga (1941). A few bivalents occasionally found may be reasonably accounted as the result of autosynopsis. From these facts, Morinaga concluded that the A genome of $O. sativa$ was not homologous to either one of the two genomes of $O. latifolia$. Nezu *et al.* (1960) failed to find any bivalent at metaphase I in the F_1 hybrid, $O. sativa \times O. latifolia$, indicating that two genomes of $O. latifolia$ were different from the A genome of $O. sativa$.

In the F_1 hybrid, $O. sativa \times O. latifolia$, however, Li *et al.* (1962) found as many as eleven bivalents at meiosis. They proposed that the A genome of $O. sativa$ was partially homologous to one of the two genomes of $O. latifolia$.

In the present experiment, about 12 bivalents per cell at pachytene, 0 to 12 at diakinesis, and 3 to 11 at metaphase I were observed. These findings are in a close agreement with those obtained by Li *et al.* (1962).

From the chiasma formation at diplotene stage and early diakinesis in the F_1 hybrid between the A and CD genomes, the author assumes that almost all of the chromosome pairings at pachytene and of the bivalent chromosomes at later stage, diakinesis and metaphase I, were not non-homologous pairing but homologous pairing.

On the basis of the pachytene analysis in the F_1 hybrids of $O. sativa \times O. officinalis$ (Shastry *et al.* 1960, 1961; Katayama 1964, 1965a), $O. sativa \times O. minuta$ or $O. sativa \times O. punctata$ (Katayama 1965b, unpubl.), it was reported that the A genome of $O. sativa$ was homologous or partially homologous to the C genome of $O. officinalis$, $O. minuta$ and $O. punctata$. As it was mentioned before, the author observed that the pairing between the A genome of $O. sativa$ and one of two genomes of $O. latifolia$ was complete at pachytene stage, and that the number of bivalents ranged from 0 to 12 at diakinesis and from 3 to 11 at metaphase I, indicating that the A genome of $O. sativa$ was partially homologous to the C genome of $O. latifolia$ or $O. grandiglumis$.

The trivalents which were seldom formed in the F_1 hybrids, $O. sativa \times O. grandiglumis$, $O. sativa$ f. *spontanea* $\times O. grandiglumis$, $O. breviligulata \times O. latifolia$ and $O. breviligulata \times O. grandiglumis$, may arise from the pairing of the chromosomes among the A, C and D genomes.

On the basis of the results obtained from the F_1 hybrids between the A and C genomes (Shastry *et al.* 1960, 1961; Katayama 1964, 1965a), A and BC (Katayama unpubl.), and A and CD, the author proposes that the bivalents found in these cross-combinations have arisen from partial homology between the A and C genomes. A diagrammatic representation of species relationships among the A, C, BC and CD genomes is given in Figure 5.

It would be one of the most important and interesting problems to discover rice taxa with the BB and DD genomes.

SUMMARY

In order to elucidate the relationship between the A and C genomes, 16 F_1 hybrids between the A (*O. sativa*, *O. sativa* f. *spontanea*, *O. sativa* v. *fatua*, *O. perennis*, *O. breviligulata* and *O. glaberrima*) and CD (*O. alta*, *O. latifolia* and *O. grandiglumis*) genomes were studied cytologically. About 12 completely paired chromosomes were observed at pachytene stage, 0-12 bivalents at diakinesis, and 3-11 at metaphase I. These findings suggest that one of the CD genomes in *O. latifolia* is partially homologous to the A genome in *O. sativa*.

On the basis of the pachytene analysis in the F_1 hybrid, *O. sativa* × *O. officinalis* (Shastry *et al.* 1960, 1961; Katayama 1964, 1965a) and the results obtained from the F_1 hybrids, *O. sativa* × *O. minuta* and *O. sativa* × *O. punctata* (Katayama 1965b, unpubl.), the author postulates that bivalents observed in the F_1 hybrids, *O. sativa* × *O. latifolia* and *O. sativa* × *O. grandiglumis*, were derived from partial homology between the A and C genomes.

Judging from these results so far obtained cytologically in the meiosis of the F_1 hybrids between the genomes AA and CC, AA and BBCC and AA and CCDD, the author offers the species relationship in the section *Sativa* as shown diagrammatically in Figure 5.

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