

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

1. Hybrids *O. paraguayensis* Wedd. × *O. brachyantha* Chev. et Roehr., *O. paraguayensis* Wedd. × *O. australiensis* Domin. and *O. australiensis* Domin. × *O. alta* Swallen

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Based on the surface structures of the lemma and palea, and the shape of spikelet, Roschevitz (1931) divided the genus *Oryza* into 4 sections, namely, *Sativa*, *Granulata*, *Coarctata* and *Rhynchoryza*. This system is adopted by most cytogenetists and taxonomists of rice.

Since 1935, the year when Gotoh and Okura successfully obtained the F₁ plants of *O. sativa* × *latifolia* and *O. sativa* × *cubensis*, a large amount of interspecific crosses have been made and the genome constitutions of some species have been identified by, among others, Morinaga (1943), Kihara (1959), Richharia (1960), Morinaga and Kuriyama (1960) and Yeh and Henderson (1960). Their results can be seen in Table 1. So far as the present authors are aware, all the studies carried out by those workers were confined to the section *Sativa* Roschev. No hybrid involving species belonging to the other 3 sections was ever obtained.

Failure in getting interspecific hybrids can be attributed to many causes. One of these is the inability of the hybrid zygote to grow into a normal embryo under the conditions of seed development after the zygote is obtained at the very initial stage. It was shown by other investigators that this kind of physiological barrier could be overcome. With this in mind, the authors tried embryo culture on a large scale. This was found to be very successful. Many crosses which could not be obtained otherwise were successfully cultured. Among these crosses, three triploid hybrids, *O. paraguayensis* × *brachyantha*, *O. paraguayensis* × *australiensis* and *O. australiensis* × *alta* which have not been reported by other investigators, were obtained. The hybrid *O. paraguayensis* × *brachyantha* is considered to be an intersectional hybrid, since *O. paraguayensis* belongs

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Table 1. Genomic constitution of the genus *Oryza*.

Species	Morinaga <i>et al.</i>	Richharia	Yeh & Henderson
<i>O. sativa</i>	AA	P ² P ²	A ₁ A ₁
<i>O. sativa</i> var. <i>fatua</i>			A ₁ A ₁
<i>O. sativa</i> var. <i>formosana</i>			A ₁ A ₁
<i>O. sativa</i> var. <i>spontanea</i>	AA		
<i>O. balunga</i>			A ₂ A ₂
<i>O. perennis</i>	AA	P ¹ P ¹	
<i>O. perennis</i> var. <i>cubensis</i>	AA		A ₂ A ₂
<i>O. perennis</i> var. <i>barthii</i>			A ₂ A ₂
<i>O. glaberrima</i>	AA	P ³ P ³	EE
<i>O. breviligulata</i>	AA	P ¹ P ¹	EE
<i>O. stapfii</i>		P ³ P ³	EE
<i>O. australiensis</i>	Not AA		
<i>O. officinalis</i>	CC		
<i>O. latifolia</i>	CCDD	O ¹ O ¹ O ² O ²	
<i>O. paraguayensis</i>	CCDD		
<i>O. alta</i>		O ¹ O ¹ O ² O ²	
<i>O. minuta</i>	BBCC	O ¹ O ¹ M ¹ M ¹	
<i>O. eichingeri</i>	BBCC		
<i>O. malamuzhiensis</i>		O ¹ O ¹ O ³ O ³	

to the section *Sativa* Roschev. and *O. brachyantha* to the section *Coarctata* Roschev. respectively. This paper is a brief report of the morphology and cytology of these hybrids.

Material and Methods

Parents used in the hybridization consisted of four species. These species were all sent by Dr. H. I. Oka of the Japanese National Institute of Genetics, who has been collecting wild species in the genus *Oryza* all over the world since 1957. Their names, accession numbers, etc. are shown in Table 2.

Table 2. Parents used in the hybridization.

Acc. No.	Oka's No.	Species name	2n
7	W023	<i>O. brachyantha</i> CHEV. <i>et</i> ROEHR.	24
15	W008	<i>O. australiensis</i> DOMIN.	24
20	W017	<i>O. alta</i> SWALLEN	48
25	W054	<i>O. paraguayensis</i> WEDD.	48

Hybridization was carried out by using hot-water method of emasculation. The hybrid seedlings were obtained through artificial culture of immature em-

bryos, ranging between 7-14 days of age after hybridization. White's medium was used. In addition, coconut milk was added. The young embryos were kept in a dark incubator at 30 (± 1)° C. The results of embryo culture are given in Table 3. Hybrid seedlings thus obtained were grown in an artificially heated green house from November 1960 through May 1961. Artificial lighting was also provided to regulate flowering of the hybrids.

Acetocarmine smears were used exclusively throughout this study.

Table 3. Results of artificial culture of interspecific-hybrid embryos of rice

Hybridization Combination	No. of embryos cultured	No. of embryos germinated	No. of embryos died after germination	No. of embryos not germinated	No. of embryos died after transplanting	No. of adult plants raised	No. of plants found to be selfed	No. of hybrid plants obtained
<i>paraguaiensis</i> × <i>brachyantha</i>	7	5	1	2	1	4	3	1
<i>paraguaiensis</i> × <i>australiensis</i>	2	2	1			1		1
<i>australiensis</i> × <i>alta</i>	4	4		1		3		3

Description of the Hybrids

O. paraguaiensis × *brachyantha*: *O. paraguaiensis* was characterized by its tall culms, and large and purple auricles. *O. brachyantha* was characterized by its short, slender culms; minute and colorless auricles and ligules; long and narrow spikelets with straight, stout and lengthy awns. The F₁ hybrid appeared to be more like *O. brachyantha* than *O. paraguaiensis*. It had short and slender culms; minute and colorless auricles and ligules; small leaf-blade and profused tillerings. However, the size and shape of its spikelets were intermediate between those of the two parents (Fig. 4: A, B and C). The glumes were subulate as those of *O. brachyantha*, but longer than those of both parents.

O. paraguaiensis × *australiensis*: *O. australiensis* was characterized by its apiculus elongation on the tip of the palea; narrow and blue-green leaves; bold spikelets; glabrous ligules and absence of auricles. The shape and color of the leaves of the hybrid were intermediate between those of the two parents. The auricles were light purple and slightly smaller than those of *O. paraguaiensis*. The ligules were fringed with hairs like those of *O. paraguaiensis*. The tip of the palea was found to be lacking in apiculus elongation.

O. australiensis × *alta*: As *O. paraguaiensis* × *australiensis*, the shape and color of the leaves of the hybrid were intermediate between those of the two parents. The auricles and ligules of *O. alta* were colorless and ciliated. The hybrid had colorless auricles and ciliated ligules similar to the male parent.

Cytology of the Hybrids

As previously reported by Sampath and Ramanathan (1949), Morinaga and

Kuriyama (1954, 1960), Nezu (1959) and Hu (unpubl.), *O. australiensis* and *O. brachyantha* had 24 chromosomes, and *O. paraguayensis* and *O. alta* had 48 chromosomes. In a comparative study of the karyotypes of some *Oryza* species, Hu (unpubl.) pointed out that *O. australiensis* had larger chromosomes and *O. brachyantha* smaller as compared with other species in the same genus which he studied. That *O. australiensis* having relatively larger chromosomes was confirmed by the present study of hybrids *O. paraguayensis* × *australiensis* and *O. australiensis* × *alta*. In sharp contrast to hybrids involving species other than *O. australiensis*, most of the bivalents found in the first metaphase plates of these hybrids were markedly heteromorphic (Figs. 7 and 8). Variation in size of chromosomes between species might be caused by some external factors as suggested by Navashin (1934) and Pierce (1937). In the present case, however, such variation was assumed to be due to genotypically controlled difference between different species.

Table 4. Chromosome associations at MI in the interspecific hybrids of *Oryza*

Hybrids	Plant number	No. of cells observed	III Range (Mean)	II Range (Mean)	I Range (Mean)
<i>O. paraguayensis</i> × <i>brachyantha</i>	55-1	200		0-3 (0.56)	30-36(34.89)
<i>O. paraguayensis</i> × <i>australiensis</i>	53-1	130	0-1(0.05)	2-12(7.81)	12-32(20.22)
<i>O. australiensis</i> × <i>alta</i>	71-1	103		2-11(6.75)	14-32(22.50)

In *O. paraguayensis* × *brachyantha*, 200 microsporocytes were observed at MI. It was found that most of the cells had 36 univalents, though one to three loosely paired bivalents were occasionally present. The mean numbers of bivalent and univalent per cell were 0.56 and 34.89 respectively (Table 4). It was apparent that there could be little homology between the chromosomes of *O. paraguayensis* and those of *O. brachyantha*. The occasional formation of bivalents might be accounted for either by assuming intragenomic or intergenomic association of chromosomes in the hybrid (see discussion). Intragenomic pairing was found to exist in the haploids of *O. glaberrima* and *O. sativa* (Hu, 1957 and 1960). Similarly, autosyndesis was the explanation offered by Morinaga (1943) for occasional pairing in the hybrid of *O. sativa* and *O. latifolia* and by Shama Rao and Seetharaman (1957) in *O. glaberrima* and *O. eichingeri* hybrid.

Chromosome pairing in *O. paraguayensis* × *australiensis* and *O. australiensis* × *alta* was very similar. The mean numbers of bivalents per cell at MI were 7.81 in *O. paraguayensis* × *australiensis* and 6.75 in *O. australiensis* × *alta* respectively (Table 4). It was found that most of the bivalents were heteromorphic. As shown in Figs. 7 and 8, 9 out of 10 bivalents were different in size and/or in shape. It was believed that the larger half of each unequal bivalent was derived from *O. australiensis*, while the smaller half from *O. paraguayensis* or *O. alta*. According to Hu (unpubl.), the lengths of the 12 chromosomes in

the root tip of *O. australiensis* (being pretreated with colchicine) ranged between 4.0-1.9 μ with a mean of 2.68 μ ; and those of *O. brachyantha* were 2.5-1.0 μ and 1.53 μ correspondingly. However, those of *O. paraguayensis* was not studied for direct comparison. Whether or not chromosomes of *O. australiensis* were actually larger than those of *O. paraguayensis*, no definite conclusion could be drawn at present. Nevertheless, this indicated that allosyndesis was probably the predominant type of chromosome pairing in these hybrids. In the hybrid *O. paraguayensis* \times *australiensis*, however, autosyndesis of both inter- and intragenomic pairings might also occur, but the frequency would be very low. It was evidenced further that association of three chromosomes was found with a mean of 0.05 per cell (Table 4).

In these hybrids, pollens were found to be completely sterile and no seeds were set. The sterility was assumed to be predominantly chromosomal in origin.

Discussion

Though crosses between *O. paraguayensis* and *O. alta* have not been made as yet, it may be assumed that these two species have the similar genomes CCDD. Both can be crossed with *O. latifolia* and the hybrid has normal chromosome pairing during meiosis (Morinaga and Kuriyama, 1960; Richharia, 1960). This conclusion is further supported by the present study of *O. paraguayensis* \times *australiensis* and *O. australiensis* \times *alta*.

The lack of homology between chromosomes of *O. australiensis* and those of genome A is clearly demonstrated by Kihara and Nezu (1960), Gopalakrishnan (1959, cited by Richharia, 1960) and Hu (unpubl.). In the present study, it was found that the genome of *O. australiensis* is partially homologous to perhaps one of the genomes of *O. paraguayensis* and *O. alta*. Evidence is yet to be obtained to show whether this homology is related to genome C or D. Naturally the cross of this species with *O. officinalis* is needed for a crucial test.

Based on statistic-taxonomical methods, Morishima and Oka (1960) subdivided the section *Sativa* of Roschevicz's classification into 2 groups, *Sativa* and *Officinalis*. The former is represented by those species with genome A. The latter consisted of *O. officinalis* and its related tetraploid species. This grouping agrees with that of Richharia (1960), whose classification is based on the morphology, geographical distribution and cytological behavior of rice species. These authors seemed to agree that it is difficult to decide whether *O. australiensis* should be included in *Sativa* or *Officinalis* since it is linked with both groups. In the present study, we found that *O. australiensis* is more related cytologically to *Officinalis* than to *Sativa*.

Hybridizations between *O. brachyantha* and *O. sativa*, *O. sativa* var. *spontanea*, *O. breviligulata*, *O. officinalis*, *O. minuta*, *O. latifolia* were tried, but no seedlings were obtained even embryo culture was resorted to. These

failures, together with the non-pairing condition in the hybrid in the present study involving genomes CD, may indicate a considerable degree of distinction between the genetic constitution of *O. brachyantha* and those of genomes A, C, BC and CD. For this reason, we suggest F for the genome of *O. brachyantha* (E was used by Yeh *et al.*, 1960).

According to Stebbins (1950, p 322), "Autosyndesis refers to the pairing of chromosomes derived from the same parental gametes of a particular plant, regardless of the similarity or difference from each other, while allosyndesis refers similarly to pairing between chromosomes derived from different parental gametes, such as normally occurs both in diploids and in polyploids." Taking, for example, the case of pairing in the hybrid *O. sativa* × *paraguaiensis*, we have:

	<i>O. sativa</i>	<i>O. paraguaiensis</i>
Genome:	A	C D
Chromosome:	A ₁	C ₁ D ₁
	⋮	⋮
	A ₁₂	C ₁₂ D ₁₂

In the case of autosyndesis, we can have intra-association, C₁C₂ or D₁D₂, or else inter-association C₁D₂. In practice, however, it would be very hard to differentiate them from one another.

Summary

1. In 1960, three interspecific hybrids of *Oryza* were obtained, *viz.*, *O. paraguaiensis* × *brachyantha*, *O. paraguaiensis* × *australiensis* and *O. australiensis* × *alta*. The use of embryo-culture technique might be the probable explanation offered for the success of these crosses.

2. The hybrids of *O. paraguaiensis* × *australiensis* and *O. australiensis* × *alta* were intermediate in external morphology between their parents, whereas the hybrid *O. paraguaiensis* × *brachyantha* was more like the male parent.

3. The genome of *O. australiensis* was found to be partially homologous to genome C or D of *O. paraguaiensis* and *O. alta*. It was evident that *O. australiensis* was more related to the group *Officinalis* than to the group *Sativa* of the section *Sativa* according to the classification of Roschevicz.

4. F was suggested for the designation of the genome of *O. brachyantha* based on the results of cytological study of the hybrid *O. paraguaiensis* × *brachyantha* and on the degree of crossibility in conducting interspecific hybridization.

5. The formation of heteromorphic or unequal bivalents in *O. paraguaiensis* × *australiensis* and *O. australiensis* × *alta* indicated that allosyndesis seemed to be the predominant type of chromosome pairing in these two hybrids.

Oryza sativa L. 及其近緣種之細胞遺傳學的研究

1. 種間雜種 *O. paraguayensis* Wedd. × *brachyantha* Chev. et Roehr.,
O. paraguayensis Wedd. × *australiensis* Domin. 及
O. australiensis Domin. × *alta* Swallen

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爲了瞭解稻屬 (*Oryza*) 各種間的親緣關係及其染色體組的構成，作者等自1960年開始從事於稻屬的種間雜交工作。雜交時使用溫湯去雄法，幼胚培養於 White 培養基中。經獲得的雜種甚多，其中：*O. paraguayensis* × *brachyantha*，*O. paraguayensis* × *australiensis* 及 *O. australiensis* × *alta* 等三種尙未有人發表過。本篇係就該等雜種之形態及細胞學做一簡略之介紹。

O. paraguayensis × *brachyantha* 在形態上趨近於父本，而 *O. paraguayensis* × *australiensis* 及 *O. australiensis* × *alta* 則介於兩親本之間。在減數分裂第一中期，*O. paraguayensis* × *brachyantha* 之染色體幾無配對現象。*O. paraguayensis* 之染色體組爲 CD。同時 *O. brachyantha* 與其他具有 A, C, BC 染色體組之種間雜交均未成功，此顯示 *O. brachyantha* 具有另一不同之染色體組 F (E 曾被 Yeh and Henderson 使用過，見表一)。*O. paraguayensis* × *australiensis* 及 *O. australiensis* × *alta* 在第一中期時有大約七、八個二價體，構成此等二價體之染色體在形狀及大小上均有差異 (heteromorphic)，大者似乎來自 *O. australiensis*，小者則來自 *O. paraguayensis* 及 *O. alta*，顯示在此等雜種中，染色體之配對爲 allosyndesis。亦可證明 *O. australiensis* 與 *O. paraguayensis*，*O. alta* 之親緣較近，而與 *O. sativa* 則較遠，因爲 *O. australiensis* 與 *O. sativa* 雜種之染色體幾無配對發生。(摘要)

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