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

3. Two Intersectional Hybrids, *O. sativa* Linn. × *O. brachyantha* A. Chev. et Roehr. and *O. minuta* Presl × *O brachyantha* A. Chev. et Roehr.[1]

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Dividing the *Oryza* genus into 4 sections by the Russian botanist R. J. Roschevicz (1931) was a great contribution to the knowledge of rice research. Since then, most of the rice workers adopted this universal classification in their investigations. On the other hand, however, taxonomy has been improved through the use of both cytological and statistical knowledge during the past thirty years. Henceforth Roschevicz's classification of rice was inevitably being modified or partly corrected by many workers, such as Chevalier (1932, reviewed by Yeh, unpubl.), Ghose *et al.* (1956), Richharia (1960) and Morishima and Oka (1960). Most of them came to the same conclusion that the genus *Oryza* should better be divided into three groups, viz., *Sativa, Officinalis,* and *Granulata* (or miscellaneous group) instead of four sections of Roschevicz. But for the sake of convenience, the present authors still adopted Roschevicz's taxonomical system in this paper.

Since the success of getting a hybrid *O. paraguaiensis* × *O. brachyantha* by Li *et al.* (1961), the authors were thus inspired and attempted to carry out a more intensive crossing program involving the species *O. brachyantha.* By using embryo culture method as being used before (Li *et al.,* 1961), two more intersectional hybrids, *O. sativa* × *O. brachyantha* and *O. minuta* × *O. brachyantha,* were obtained. Some brief cytological and morphological evidences about these two hybrids are presented in this paper.

Materials and Methods

Three parent species, *O. sativa* Linn. (2n = 24), *O. minuta* Presl (2n = 48) and *O. brachyantha* A. Chev. et Roehr. (2n = 24) were used in this hybridization experiment.

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The former two belonged to the section *Sativa* Roschev., the latter, section *Coarctata* Roschev., respectively. Part of these species were furnished by Dr. H. I. Oka of the Japanese National Institute of Genetics, to whom the authors are indebted. Hot water emasculation method, 43°C. for 7 min., was applied throughout the whole crossing experiment. For about 7–9 days after hybridization, the embryos were excised from the seeds and cultured on White's medium in test tube under room temperature throughout the young seedling stage. When seedlings reached the height of about 10–12 cm., they were then transplanted to pots in the green house. Artificial light and heating system were provided in order to regulate flowering date and keep the favourable growth temperature throughout the winter of 1961.

The 3:1 alcohol-glacial acetic fixative and acetocarmine smear method were used exclusively in the studies of PMC's.

Results

1. *Crossability of the parent species.*

Since the three parent species belonged to two different sections according to Roschevicz's classification, so the chance of making a successful hybridization was very rare. The crossability of these two crosses can be seen in Table 1.

Table 1. *Results of interspecific crossing of rice by using artificial medium culture of the immature embryos.*

Cross combinations	No. of pollinated florets	No. of embryos cultured	No. of seedlings transplanted	No. of seedlings died after transplanting	No. of true hybrids obtained	Crossability*
O. sativa × *O. brachyantha*	3,201	57	48	5	1	0.03%
O. minuta × *O. brachyantha*	713	33	10	—	5	0.70%

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

From these data, two facts are clearly manifested. Firstly, to obtain an intersectional hybrid was much more difficult than an intrasectional one (this comparison being made with our former results). Secondly, using tetraploid *O. minuta* as female parent, by comparison, might be more successful in respect to hybridization than using diploid *O. sativa*. Of 3,201 florets pollinated in the combination of *O. sativa* × *O. brachyantha*, only one true hybrid was fortunately obtained. On the other hand, of 713 pollinated florets of *O. minuta* × *O. brachyantha*, five hybrids were secured. The ratio of success of hybridization of the former to the latter was only 0.043.

2. *Cytological observations of the hybrids.*

Of the three species used in this experiment, two are diploid species with 2n=24 chromosomes, namely, *O. sativa* (Kuwada, 1909) and *O. brachyantha* (Morinaga and Kuriyama, 1954; Krishnaswamy *et al.*, 1954). The other species, *O. minuta*, is a tetraploid with 2n=48 chromosomes (Morinaga, 1934). Since 1934, Morinaga has carried out an extensive program on the studies of interspecific crosses and genomic analyses in the genus *Oryza*. Through hybridization of *O. sativa* with seven other diploid forms, namely, *O. glaberrima, O. breviligulata, O. perennis, O. cubensis, O. sativa* var. *fatua, O. sativa* var. *spontanea* and *O. formosana*, and based on the normal chromosome behaviour at first metaphase of these hybrids, he reached the conclusion that all these forms have the same genomic constitution and designated it as genome A.

The cross *O. sativa*×*O. minuta* was made by Okura in 1937. Since then, several other workers such as Morinaga (1940), Nandi (1938), Capinpin and Magnaye (1951) and others used the same cross or reciprocal one as material for their cytogenetic studies. In spite of much confusion in their respective reports, BBCC genomic symbol however was finally designated by Morinaga (1956) for *O. minuta*.

According to Morinaga and Kuriyama (1960), *O. paraguaiensis* has genomes CCDD. Li *et al.* (1961), through meiotic analysis of *O. paraguaiensis*×*O. brachyantha*, found that 36 univalents were most frequently met at MI. Based on the failure of crossing *O. brachyantha* with *O. sativa, O. officinalis, O. minuta*, etc., these authors concluded that *O. brachyantha* has a genome differed from any previously known genomes, namely, A, C, BC and CD, so they designated F for the genome of *O. brachyantha*.

From this brief review, we know that the three species used in our experiment were different in genomic constitution from one another.

In *O. sativa*×*O. brachyantha*, 350 microsporocytes were observed at first metaphase. Nearly all of the cells had 24 univalents (Fig. 8), the average bivalent number was only 0.03 (Table 2). There were nine cells with one bivalent and 22 univalents (Fig. 7). The highest bivalent numbers found in one and only one cell were two. Since the chromosomes of the two parents (at MI in particular) have no appreciable difference in size, whether the paired chromosomes are the result of autosyndesis or allosyndesis, no definite conclusion can be drawn from them.

In addition to the data presented in Table 2, there were four cells in which chromosome numbers were 48 instead of 24 (Fig. 9).

In *O. minuta*×*O. brachyantha*, much more complicated chromosome associations were met with at MI. Of 130 cells observed, 47 cells had 36 univalents, 82 cells had variable numbers of bivalents (Fig. 6), ranging from one to seven.

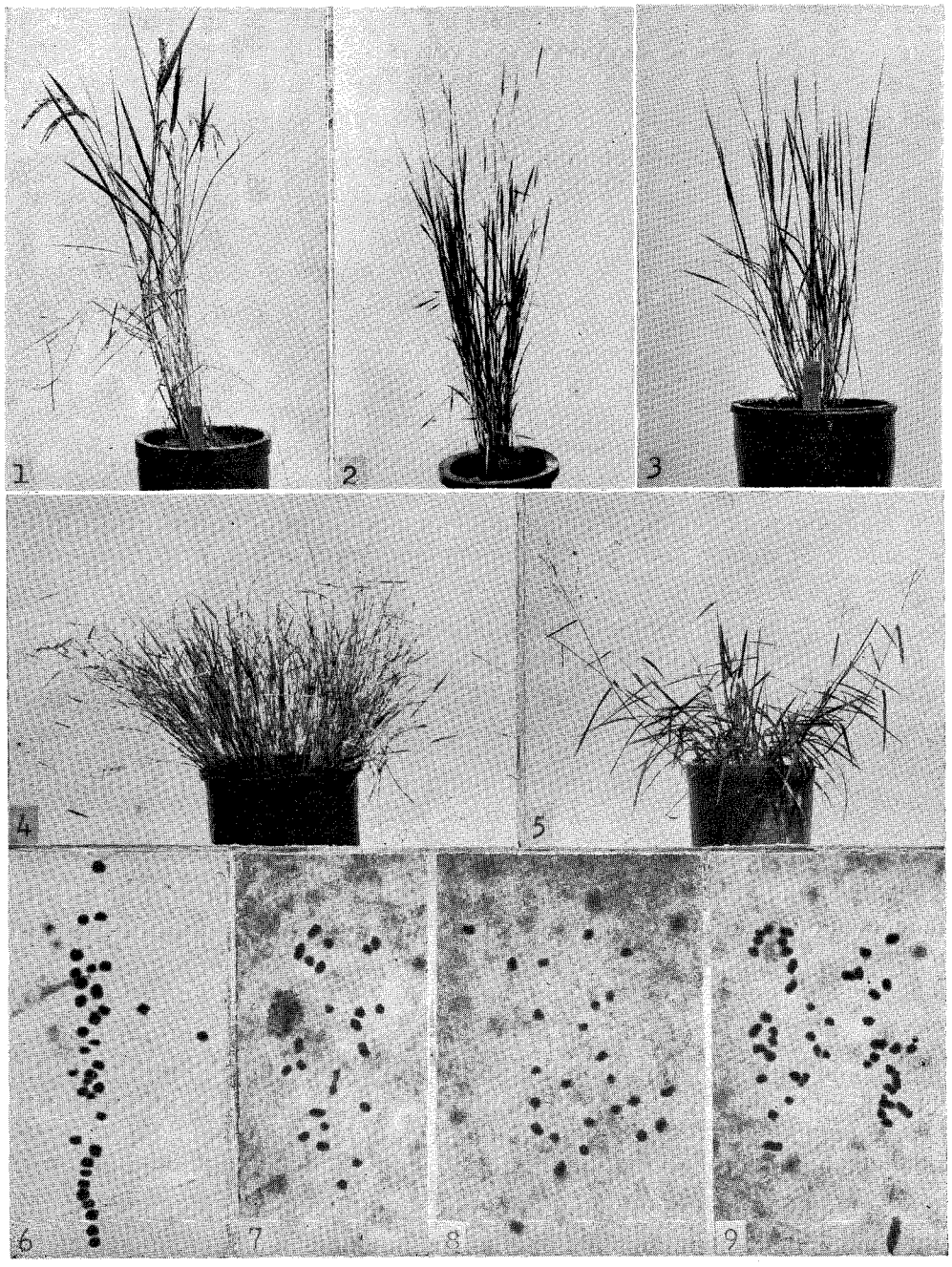

Fig. 1–5. Plants of parents and their F_1 hybrids. 1. *O. sativa*. 2. *O. sativa* × *O. brachyantha*. 3. *O. brachyantha*. 4. *O. minuta* × *O. brachyantha*. 5. *O. minuta*.

Fig. 6–9. Chromosome pairing at different stages of first meiosis in the hybrids. 6. *O. minuta* × *O. brachyantha*. MI, 1II+34I. The bivalent supposed to be come from autosyndesis of the chromosomes of *O. minuta*. 7. *O. sativa* × *O. brachyantha*. MI, 1II+22I. 8. *O. sativa* × *O. brachyantha*. MI, 24I. 9. *O. sativa* × *O. brachyantha*. AI, showing 48 chromosomes.

Multiple chromosome associations, such as pentavalents, tetravalents as well as trivalents were also found in different cells.

Table 2. *Meiotic observations of chromosome pairing in two interspecific hybrids of* Oryza.

Cross combinations	No. of cells observed	Chromosome configuration				
		V Range (Mean)	IV Range (Mean)	III Range (Mean)	II Range (Mean)	I Range (Mean)
O. sativa×*O. brachyantha*	350				0-2 (0.03)	20-24 (23.94)
O. minuta×*O. brachyantha*	130	0-1 (0.008)	0-1 (0.008)	0-1 (0.015)	0-7 (1.439)	23-26 (33.007)

As pointed out previously by Hu (unpubl.) that *O. brachyantha* had smaller chromosomes than those of other species of *Oryza*. The present writers also observed that there were about 12 small and 24 large chromosomes in the microsporocytes of the hybrids. Accordingly, bivalents were roughly divided into three groups, viz., (1) pairing of the chromosomes of *O. minuta*. (2) pairing of the chromosomes of *O. brachyantha*. (3) pairing of the chromosemes between *O. minuta* and *O. brachyantha*. The frequency of these three groups were 155 (82.89%), 22 (11.77%) and 10 (5.34%) respectively in a total of 130 cells observed. It seemed that pairing between chromosomes of *O. brachyantha* and *O. minuta* might not be a constant phenomenon. The mean number was only about 0.08 per cell. This indicated that there was little homology between the chromosomes of *O. minuta* and *O. brachyantha*. Most of the bivalents originated, it seemed, from autosyndesis of the chromosomes of *O. minuta*.

Pollens of these hybrids were stained by iodine-potassium iodide solution and examined microscopically. No normal pollens were found. All of them were wrinkled in shape and light-stained in color. Seed set was also found to be nil.

3. *Morphological descriptions of the hybrids.*

O. sativa×*O. brachyantha:* The female parent *O. sativa* was variety Taichung No. 65, a *Japonica* type characterized by its intermediate height; broad and awnless spikelets; oblong and intermediate sized empty glumes; long, acute, split ligule and colorless stigmas. *O. brachyantha* was characterized by its short, slender culms; long and narrow spikelets with straight, stout and lengthy awns; linear lanceolate empty glumes; minute ligule and purple stigmas. The vegetative parts of the F_1 hybrid were apparently more like *O. sativa* than *O. brachyantha,* such as plant height, length and width of leaf blade, shape of ligule, etc. Of the characters in the panicle, they resembled *O. brachyantha*

more closely. It had long and narrow spikelets with the same size of awns as that of the male parent. Empty glumes were lanceolate but slightly longer and wider than those of *O. brachyantha*. Stigma's color was dark purple. Some morphological measurements of the pertinent parents and their hybrids were listed in Table 4.

O. minuta×*O. brachyantha*: *O. minuta* was characterized by its procumbent growth habit; slantly round ligules; small spikelets with short, soft awns; semi-lax panicle; black, minute, triangular empty glumes. The F_1 hybrid strongly resembled *O. minuta* morphologically except with compact panicles and narrower and longer spikelets. The length of awn was intermediate between that of parents.

Table 3. *A list of measurements of some quantitative characters of* O. sativa, O. minuta, O. brachyantha *and their hybrids.**

	Height of plant (cm)	Length of culm (cm)	Length of panicle (cm)	Thickness of culm (mm)	Length of ligule (mm)	Length of awn (mm)	Length of spikelet (mm)	Width of spikelet (mm)	Terminal leaf blade	
									Length (cm)	Width (cm)
O. sativa	92.0	66.9	22.8	4.37	13.8	—	7.54	3.38	33.7	1.15
O. sativa×*O. brachyantha*	112.0	73.7	22.5	4.26	5.5	11.6	9.62	2.24	21.4	1.10
O. brachyantha	74.3	64.5	13.4	2.12	1.0	12.1	8.29	1.53	15.6	0.75
O. minuta×*O. brachyantha*	60.0	46.3	12.0	2.31	1.0	4.6	6.01	1.31	9.57	0.70
O. minuta	99.0	85.6	13.4	2.08	1.9	2.4	5.01	1.97	15.0	1.16

* Plants were grown in pots in the greenhouse.

Discussion

Up to the present, the species *O. brachyantha* has been successfully hybridized with three other species, namely, *O. sativa*. *O. minuta* and *O. paraguaiensis* by Li and his co-workers in Taiwan through 1960–1962. The designation of F for the genome of *O. brachyantha* is supported further by the present study. According to Chevalier's opinion (1932, reviewed by Yeh, unpubl.), *O. brachyantha* should be taken out of section *Coarctata* Roschev. and placed under section *Sativa* Roschev. He also divided *Oryza* into four sections, i.e., *Euoryza, Padia, Sclerophyllum* and *Rhynchoryza,* corresponding to *Sativa, Granulata, Coarctata* and *Rhynchoryza* of Roschevicz's system except for species *O. brachyantha*. Ghose *et al* (1956) divided the genus *Oryza* into *Sativa, Officinalis* and *Granulata* sections. It is of their opinion that *O. brachyantha* could be listed in section *Granulata* together with *O. granulata, O. ridleyi* and *O. coarctata*. The success of crossing *O. brachyantha* with three other species belonging to section *Sativa* Roschev. perhaps gives a further support to the postulate of Chevalier. However, more

considerations from different angles pertaining to this problem should be undertaken before any definite conclusion can be drawn.

An extensive study of inter-specific cross in *Oryza* was carried out by Nezu *et al.* (1960). They indicated that no hybrids were obtained in those crosses involving *O. brachyantha* as parent, such as *O. granulata*×*O. brachyantha, O. coarctata*×*O. brachyantha, O. ridleyi*×*O. brachyantha* and *O. subulata*×*O. brachyantha.* The number of pollinated florets of these crosses made, however, was rather small to draw any definite conclusion.

Summary

1. A large amount of interspecific crosses in the genus *Oryza* were carried out in the fall of 1961, from which several intersectional hybrid plants of *O. sativa*×*O. brachyantha* and *O. minuta*×*O. brachyantha* were obtained.

2. The crossability of these two crosses were 0.03% and 0.70% respectively. This indicated that obtaining an intersectional hybrid was much more difficult than an intrasectional one.

3. The majority of chromosomes in the microsporocytes of both hybrids observed were univalents. Most of the bivalents found in *O. minuta*×*O. brachyantha* were assumed to be pairing between the chromosomes of the genomes BC of *O. minuta.*

4. Both of these two hybrids strongly resembled their female parents morphologically, but their panicles were more like *O. brachyantha,* the male parent.

5. The designation of F for the genome of *O. brachyantha* by Li *et al.* (1961) was further supported by the present study.

O. sativa L. 及其近緣種之細胞遺傳學研究

3. 節間雜種 *O. sativa* L.×*O. brachyantha* A. Chev. et Roehr. 及 *O. minuta* Presl×*O. brachyantha* A. Chev. et Roehr.

武光東 尚 榮 呂嘉琳 周 涓 李先聞

1. 1961 年秋季，著者等從事于 *Oryza* 屬的種間雜交工作，得到兩個節間雜種： *O. sativa*×*O. brachyantha* 及 *O. minuta*×*O. brachyantha.*

2. 此二雜種的雜交成功率分別爲 0.03% 及 0.70%，與過去的結果相比較，知節間雜種之獲得，遠較種間雜種爲難。

3. 二雜種的染色體在花粉母細胞減數分裂第一中期，多數均爲不配對的單價體。少數的二價體，係由 *O. minuta* BC 組的染色體互相配對而來。

4. 二雜種的外部形態極似其母本 *O. sativa* 或 *O. minuta*, 但花序則較像父本 *O. brachyantha*。

5. 李先聞等過去定 *O. brachyantha* 之染色體組爲 F，本研究之結果，支持彼等之見解。

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