

INTERSPECIFIC RELATIONSHIPS IN GENUS *ORYZA* OF THE SOUTHEAST ASIA-PACIFIC REGION

Joventino D. Soriano
Institute of Biology
University of the Philippines
Diliman, Quezon City, Philippines

ABSTRACT

The chromosomal structure, crossability, hybrid seed viability and morphological features of four species of genus *Oryza* believed to be indigenous to the region were studied. Except for tetraploidy in *O. minuta*, no other marked karyological difference was found among the various species. Interspecific crosses and hybrid seed viability were good only in certain combinations while the four species were more or less distinct morphologically. Based on the foregoing criteria, the *Oryza* species were classified into two regional groups.

Introduction

The genus *Oryza* is presently known to consist of some 21 or more species distributed in different parts of the tropical and sub-tropical regions. Two economically important crop plants belonging to this group are the so-called Asian rice (*O. sativa* L.) and African rice (*O. glaberrima* Steud.) The rest of the *Oryza* species are grass-like wild rices. The cytogenetic relationships among the cultivated and wild rices have been the subject of great interest among many plant investigators during the last 80 or so years in relation more or less to the origin of the staple crop species and understanding of a number of problems related to their improvement such as occurrence of intra- and interspecific sterility and the frantic search for new and useful germplasm needed for rice breeding work.

While previous studies on species interrelationships in the *Oryza* group have mostly dealt with plants occurring in distant geographical habitats, a regional approach might provide a more meaningful and undoubtedly less speculative analysis of the problem. The ancestors of present taxa then growing sympatrically at some given time invariably had a better opportunity for gene exchange than between those found in very distant lands. Many recent workers have recognized the important influence of environmental conditions in the success of interspecific crosses and their hybrids.

Materials and Methods

The four species of genus *Oryza* used in this study were the Australian wild rice (*O. australiensis* Domin.), *O. officinalis* Wall., *O. sativa* Linn., and the Philippine wild rice (*O. minuta* Presl.) Seeds of these species were originally obtained over a period of years from the International Rice Research Institute in Los Baños, Laguna through the kindness of Dr. Te-Tzu Chang and National Institute of Genetics in Misima, Japan. For *O. sativa* L., variety Peta, an *indica* rice was used. Seeds of a fifth species, *O. schlechterii* Pilger, reportedly an inhabitant of New Guinea, were not available for the study. Seeds of the four species were sprouted on moist tissue paper in petri dish and transplanted in pots at the Botany Experimental Garden, U.P. Diliman, Quezon City. Urea fertilizer was applied one week after transplanting. Reciprocal crosses were made following the hot water emasculation method. The florets were bagged after artificial pollination. Data on crossability and hybrid seed viability were obtained. Karyological analysis was done on selected metaphases in root-tips of germinating seeds of the four species employing a modified squash technique using aceto-carmin stain. Microscopic measurements were done with a micrometer and photomicrographs were magnified about 20,000 times.

Results and Discussion

Karyological features. Data on chromosome number, total chromosome length, chromosome morphology and number of satellites are shown in Table 1. Three diploid species such as *O. australiensis*, *O. officinalis* and *O. sativa* were found to have the same chromosome numbers of $2N=24$, while *O. minuta*, a tetraploid, has $4N=48$. The results of the present chromosome counts confirm those of earlier reports (Rau, 1929; Sampath and Ramanathan, 1949; Hu, 1964) based on root-tip examinations. Although chromosome counts have also been made on meiotic cells, particularly the pachytene stage where chromosomes are reportedly much longer and bigger than the mitotic figures (Morinaga, 1964; Shastri, 1964; Shastri and Rao, 1961; Li *et al.*, 1963) meiotic chromosomes do not stain very well and their centrometric attachments cannot be clearly located (Hu, 1964). A previous attempt by Yao, *et al.*, (1958) to analyze cryptic structural hybridity in intervarietal crosses using pachytene analysis did not succeed reportedly because of the difficulty of locating the centromeres. The mitotic genome, on the other hand, reflects the true somatic chromosome number directly, especially with the availability of suitable techniques some of which nowadays require very minimal pre-staining treatments (Marks, 1973).

Total chromosome lengths in the three diploid species ranged from approximately 36.2-41.3 micra. The differences in total chromosome lengths did not vary much from species to species although the chromosomes of *O. sativa* appeared to be

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Table 1. Some karyological features of four *Oryza* species

Species	Somatic chromo- some No.	Total chromosome length (micra)	No. of chromosomes			No. of satellites
			Median	Sub- median	Telo- centric	
<i>O. australiensis</i> Domin.	24	41.8 ± 0.56	10	10	4	2
<i>O. officinalis</i> Wall.	24	39.5 ± 0.72	10	10	4	2
<i>O. sativa</i> Linn.	24	36.2 ± 0.83	10	10	4	2
<i>O. minuta</i> Presl.	48	—	—	—	—	3-4

slightly smaller than those of *O. australiensis* and *O. officinalis*. This observation appears to support the view that *O. sativa* is probably a more recent derivative than *O. australiensis* and *O. officinalis* since long chromosomes indicate a more ancient origin (Soriano, 1985). The domestication of *O. sativa* had probably to wait for the occurrence of some gene mutations in these or other wild rices for such characters as large grains, non-shattering panicles, uniform maturity and other features of value to man.

Measuring, much less identifying, the individual chromosomes of the four *Oryza* species was not presently possible inspite of the recent encouragements from Khan (1975) as well as Kurata and Omura (1978), due mainly to the very small chromatic figures and absence of suitable chromosome landmarks or markers. Exceptions were the longest chromosomes (Chromosome 1), shortest chromosomes (Chromosome 12), and a pair of satellited chromosomes (Chromosome 10) in the three diploid species which with unending patience could be handily identified from the many mitotic figures. Li *et al.*, (1963) reports having encountered a similar predicament with mitotic chromosomes of *Oryza* species. A similar difficulty of identifying individual chromosomes of *O. sativa* due to absence of suitable markers was previously encountered some 27 years ago (Soriano, 1959) in connection with the identification of chromosomal segments involved in interchanges in X-irradiated material. On the other hand, Shastry *et al.*, (1960) encouraged the adoption of pachytene analysis on the *Oryza* species.

It was not likewise possible to determine the total chromosome length of *O. minuta* which has twice the number of chromatic figures even in the slightly enlarged mitotic cells of the tetraploid, both in materials that were pre-treated and not pre-treated with colchicine.

Regarding chromosome morphology, no apparent difference was found in the form or shape of the chromosomes in the three diploid species. In well-spread out and stained metaphase cells, there were 10 V-shaped or metacentric chromosomes with median centromere insertions, 10 J-shaped or acrocentric chromosomes with sub-median centromeric positions, and four rod-shaped chromosomes with more or less terminally attached centromeres. The number and attachment of the

two satellites appeared somewhat similar in the three diploid species, each satellite being attached to or near the tip of a short chromosome (Chromosome 10) by a relatively long and distinct stalk. In a previous paper, Kurata and Omura (1982) reported similar chromosome lengths and centromere locations in *O. officinalis* and *O. sativa*. In the tetraploid species, *O. minuta*, from three to four satellites were found in root-tip cells. As a good majority of the metaphases had four satellites each, it was assumed that one of the satellites in the other cells may have been accidentally deleted.

Crossability between species

Table 2 shows the degree of crossability of the four species as percentage of successful crosses. Six reciprocal crosses were made, which in most cases produced grains bearing features of one parent or a combination of both parents, except when *O. sativa* was the ovule parent when the hybrid seeds exhibited mainly the characteristics of cultivated rice. Deformed or empty caryopses were not considered good grains. Where the pistillate parent had the shattering character, as is the case with all the wild rices, care was observed to avoid any loss of grains by bagging the panicle.

Based on the data, the cross *O. officinalis* x *O. minuta* was the most compatible or crossable combination while *O. australiensis* x *O. officinalis* was the least successful, with only about 2.59% of the crosses producing good grains. Li *et al.*, (1963) successfully crossed *O. australiensis* with *O. officinalis* and other wild rice species but seed viability was similarly very low. In the cross between *O. sativa*

Table 2. Crossability and hybrid seed viability in four *Oryza* species

<i>Reciprocal crosses</i>	<i>Total crosses</i>	<i>Total good grains</i>	<i>% crossability</i>	<i>No. of viable seeds</i>	<i>% germination</i>
<i>O. australiensis</i> x <i>O. officinalis</i>	116	3	2.59	0	—
<i>O. sativa</i> x <i>O. australiensis</i>	145	16	11.03	3	18.75
<i>O. australiensis</i> x <i>O. minuta</i>	122	4	3.24	0	—
<i>O. sativa</i> x <i>O. officinalis</i>	133	2	1.50	0	—
<i>O. officinalis</i> x <i>O. minuta</i>	104	11	10.57	4	36.36
<i>O. sativa</i> x <i>O. minuta</i>	147	8	5.44	0	—

and *O. minuta*, grains were formed only when cultivated rice was the pistillate parent. This is believed due to differences in floral structure where *O. minuta* has very small spikelet parts.

The capacity of two species to cross and produce hybrid seeds is invariably an indication of close genetic affinity between them. The integrity of species is a fundamental and universal concept and even among very closely related species, only a low degree of crossability is expected. It is probable that crossability is dependent on a number of morphological, physiological and genetic barriers between the two parental species. Closely related species in general would have fewer of these differences than distantly or completely unrelated forms.

In the four *Oryza* species, the percentage of successful crosses after artificial pollination ranged from approximately 2.59-11.03%. Natural hybridization would probably be radically less successful considering possible differences in pollen dehiscence and various barriers to fertilization and embryo formation. A crossability value of less than 10% would probably be considered low under artificial hybridization as most of the unfavorable influence of environmental conditions have been minimized or entirely avoided.

Hybrid seed viability

The seed viability data (Table 2) may be used to indicate further the genetic affinity between two parental species. The capacity of a hybrid seed to germinate is undoubtedly related to the degree of cross compatibility between the two parents. None of the F_1 hybrid seeds resulting from the cross *O. australiensis* x *O. officinalis* was viable. Shastry *et al.*, (1961) found the F_1 hybrid plants from such cross to be completely sterile.

If an average of 14.6% germination of hybrid seeds is considered low (Morinaga, 1943), hybrid seed viability of about 18.75% from crosses of *O. australiensis* x *O. sativa* and 36.76% from *O. officinalis* x *O. minuta* are indeed good indications of genetic affinity between the parental species. With very low percentages of germinable hybrid seeds in most of the interspecific crosses, it is likely that much of the variability existing among these rices are probably due more to mutation than gene exchange. Mutations occur continuously in somatic and reproductive cells and may have accumulated throughout the long period of time the species has existed. Their perpetuation and expression of their phenotypic effects are undoubtedly subject to the various forces of natural selection.

Morphological features

The main vegetative features of the four *Oryza* species are shown in Table 3. The four species are phenotypically distinguishable by their growth habits. *O. australiensis* is a medium-sized plant with spreading purplish culms and long-peduncled panicle; *O. officinalis* is a relatively low, sparse plant with a few spreading light-green culms bearing slender panicles with brittle peduncles; *O. sativa* is a

tall green plant with many erect culms and leaves, compact panicles and large grains, and *O. minuta* is a very low-growing plant with radiating, almost procumbent, culms bearing dark-green leaves, fine panicles and very small spikelets. In a study of species interrelationships in Genus *Oryza*, Oka (1964) suggested that species resembling each other morphologically are highly correlated genetically.

Only *O. sativa* has compact and non-shattering panicles while all the wild rices have heavily shattering panicles. No sooner has a grain of wild rice attains a grayish or blackish color upon maturity than it falls to the ground with the slightest movement of the panicle. Thus, a wild rice panicle eventually becomes bereft of its grains as the plant approaches maturity. Indeed, the non-shattering character of cultivated rice is probably one of the most important mutations that occurred in the origin of the staple crop. Among the three wild rice species, *O. australiensis* produced the biggest grains while *O. officinalis* and *O. minuta* gave the smallest grains.

In a paper on the origin of cultivated rice, Richharia (1960) points out that some primitive features in genus *Oryza* are a diploid genome, long stamen, large ligules, tall plant growth, perennial habit, large leaves, long spikelets, pigmented stigma, marked trichomes and awns and wide geographical distribution. Based on these criteria, *O. minuta* would come out as a relatively recent form. Morinaga (1943) classified *O. minuta* and other species as members of Section *Sativa* Roschev. of genus *Oryza* based mainly on morphological similarities such as presence of intersecting rows of small tubercles in their flowering glumes and the linear-lanceolate shape of their empty glumes. However, many other species groupings in genus *Oryza* were based solely on genome analyses determined from chromosome associations in the F_1 hybrids (Katayama, 1966; Oka, 1964; Shastry, 1964;

Table 3. Some vegetative features of four *Oryza* species

Plant features	<i>O. australiensis</i>	<i>O. officinalis</i>	<i>O. sativa</i>	<i>O. minuta</i>
Growth habit	Medium plant, many spreading culms	Low plant, sparse growth, spreading culms	Tall plant, erect culms and leaves	Very low plant with radiating culms
Culms				
Length range (cm.)	35-50	50-30	55-75	10-25
Diam. range (cm)	6-10	3-5	8-15	3-5
Angle	Open	Open	Erect	Spreading
Color	Purplish	Light-green	Green	Dark-green
Leaf blade				
Length range (cm.)	36-48	10-20	35-62	10-24
Width range (cm.)	10-18	4-8	14-20	5-8
Color	Purplish tips	Light-green	Green	Dark-green

Sampath and Rao, 1951). This aspect of the interspecific relationships of the four *Oryza* species is under study and will be presented in future reports.

Regional species groups

Although the genus *Oryza* is world-wide in distribution, much of the uncertainties in species interrelationships are probably due to features which have undoubtedly been brought about by selection factors typical of the geographic region of their origin. The data on somatic chromosomes, crossability, hybrid seed viability and morphological features presented above indicate that although species differences occur and are profound enough for them to retain their individuality and integrity as species, they possess certain vital similarities and affinities that will allow their being classified into regional groups. Their sympatric existence for long periods of time has no doubt played a major role in preserving common morphological features as they have been more or less subjected to similar forces of natural selection. They possess a certain degree of crossability and germinability of their hybrid seeds that have more or less allowed a certain amount of gene exchanges between the different species.

The four *Oryza* species are thus classified into two groups based on the foregoing criteria, as follows:

- Regional Group I: *O. australiensis*
O. sativa
- Regional Group II: *O. officinalis*
O. minuta

Table 4. Reproductive structures of four *Oryza* species

Reproductive structure	<i>O. australiensis</i>	<i>O. officinalis</i>	<i>O. sativa</i>	<i>O. minuta</i>
Panicles				
Type	Open	Open	Compact	Intermediate
Size	Long	Short	Medium	Short
Shattering	Shattering	Shattering	Non-shattering	Shattering
Spikelets				
Length range (mm.)	4-5	3-4	10-12	2-3
Width range (mm.)	3-6	2-3	4-5	2-3
Grains				
Color	Black	Black	Brown	Black
Awn length	Long	Medium	Awnless	Long
100-grain wt. (gm.)	4-6	2-3	6-8	2-3

As no major karyological difference was found among the diploid species, the main criteria for the above relationships are crossability, germinability of hybrid seeds and similar morphological features. Thus, *O. australiensis* and *O. officinalis* belong to different groups due to their low percentage of crossability, inviability of their hybrid seeds and distinctly different morphological features. Using the same measures, *O. officinalis* and *O. minuta* gave a relatively high percentage of crossability of 10.57% and high viability of their hybrid seeds. Moreover, they are morphologically alike in panicle size and grain size.

The two regional groups given above were found to follow those proposed by previous workers (Morinaga, 1964; Sampath and Rao, 1951; Richharia, 1960; Katayama, 1966) based mainly on genomic analysis where *O. australiensis* and *O. sativa* were placed under the *Sativa* group of genus *Oryza* while *O. officinalis* and *O. minuta*, the *Officinalis* group.

Summary and Conclusions

1. The number and morphology of chromosomes of three diploid species, *O. australiensis*, *O. officinalis* and *O. sativa* were found to be essentially alike while *O. minuta* is a tetraploid with $4N=48$.

2. A relatively high percentage of the crosses, *O. australiensis* x *O. sativa* and *O. officinalis* x *O. minuta*, produced good seeds while other cross combinations gave a low seedset.

3. Hybrid seed viability from the cross *O. australiensis* x *O. sativa* and *O. minuta* x *O. officinalis* was relatively high while seeds from other cross combinations were not viable.

4. In vegetative and reproductive features, plants of *O. australiensis* and *O. sativa* resembled each other more than those of *O. officinalis* and *O. minuta* which likewise appeared similar.

5. The use of chromosomal structure, crossability, seed viability and morphological features in determining possible affinity among the four species is briefly discussed.

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