Genetic Resources and Crop Evolution **44:** 25–31, 1997.

Hybridization of AA genome rice species from Asia and Australia II. Meiotic analysis of *Oryza meridionalis* and its hybrids

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Received 18 December 1995; accepted 12 March 1996

Key words: chromosome, hybrid, meiosis, *Oryza*, wild rice

Abstract

To determine the genomic constitution of *Oryza meridionalis* Ng (2n=2x=24) and to estimate genomic affinity between Asian and Australian wild species of rice containing the AA genomes, chromosome pairing was analyzed at metaphase I in *O. meridionalis*, *O. rufipogon* Griff. (2n=2x=24), *O. nivara* Sharma et Shastry (2n=2x=24), *O. sativa* f. *spontanea* Rosch, and their artifical hybrids. The *Oryza* parental species and their F1 hybrids showed normal meiosis, but slightly reduced chiasma fequency was observed in the hybrids. It is concluded from the cytological analysis that (i) the Australian *O. meridionalis* contains the AA genome which has very high affinity to that in the Asian AA genome wild species of rice; (ii) chromosome structual changes, such as inversions and reciprocal translocations, have occurred in the genomes of the different species studied.

Introduction

Hybridization between different species and detailed analysis of chromosome pairing in meiosis of the parental species and their F1 hybrids have played a remarkably important role in the understanding of biosystematic relationships of the rice genus (*Oryza* L.) during the last few decades (Morinaga, 1941, 1943; Nezu et al., 1960; Li et al., 1962; Ogawa & Kayayama, 1971; Nowick, 1986). The genomic constitution of species in the genus *Oryza* has been proposed through the intensive cytogenetic studies, and it is now accepted that five basic genomes, i.e., AA, BB, CC, EE, and FF, comprise the diploid *Oryza* species which are the major components of the genus. The genomic make-up of two diploid species, *O. granulata* Nees et Arn. ex Watt. and *O. meyeriana* (Zoll. et Mor. ex Steud). Baill, is still not determined, but judging from their distinct morphology and some preliminary cytological studies (Katayama & Onizuka, 1979; Ni et al., 1988), the two species most likely to contain another basic genome rather different from any of the known genomes in the genus. The tetraploid species are known to be composed of two genomic combinations, i.e. BBCC and CCDD, where the origin of the DD genome is still unknown (Morinaga, 1941, 1943; Katayama, 1967). Three tetraploid species, *O. ridleyi* Hook f., *O. longiglumis* Jansen, and *O. schlechteri* Pilger, have not sufficiently been studied cytologically (Katayama & Onizuku, 1979; Katayama, 1992; Naredo & Vaughan, 1992), but they probably do not fall into any of these genomic groups since they are morphologically quite distinct.

To date, eight *Oryza* species have been assigned the AA genome; these are the two cultigens, *O. sativa* L. and *O. glaberrima* Steud., and their six diploid wild relatives, *O. rufipogon* Griff., *O. nivara* Sharma et Shastry, *O. barthii* A. Chev., *O. longistaminata* A. Chev. et Roehr., *O. glumaepatula* Steud., and *O. meridionalis* Ng. The taxonomy of these species may need to be revised when more data from various studies have been accumulated. The AA genome wild species in *Oryza* have the closest genetic relationship with rice cultigens and in this sense represent the most accessible germplasm resources for the improvement of rice cultivars through transfer of agronomically favorable traits from wild to cultivated rice species. However, the genomic relationship between the different AA

Table 1. IRGC accession numbers and origins of parental species and the hybrids

Species and hybrids	IRGC accession number	Origin
O. meridionalis	105281	Western Australia
	105289	Queensland, Australia
O. rufipogon	106080	West Bengal, India
	106169	Lai Chau, Vietnam
O. nivara	106111	West Bengal, India
	105386	Tak, North Thailand
O. spontanea	105564	Indonesia
O. meridionalis × *O. nivara*	105281 × 106111	*
	105281 × 105386	*
	105289 × 106111	*
O. meridionalis × *O. rufipogon*	105281 × 106169	*
	105289 × 106080	*
	105289 × 106169	*
O. rufipogon × *O. meridionalis*	106080 × 106289	*
*O. spontanea*** × *O. meridionalis*	105546 × 105281	*
O. nivara × *O. nivara*	105386 × 106111	*
O. meridionalis × *O. meridionalis*	105289 × 105281	*

*All hybrids were made in the screenhouse of Genetic Resources Center, IRRI, Los Baños, Philippines (Naredo et al. 1996).
**Referred in the text as *O. sativa* f. *spontanea*

genome species has not been adequately and systematically assessed. In order to utilize the germplasm resources in the AA genome wild species more effectively, a better understanding of the biosystematic relationship of these species is needed.

Oryza meridionalis is a diploid annual species from the northern part of Australia (Ng et al. 1981b). This species is geographically isolated from the Asian AA genome *Oryza* species, except for *O. rufipogon* and *O. sativa* which occur in the same region as *O. meridionalis* in Australia (G. Second, 1987, unpublished trip report; Vaughan, 1994). Morphologically, *O. meridionalis* is distinct from all other AA gemone rice species (Ng et al., 1981a). However, the biosystematic relationship of *O. meridionalis* with the Asian *Oryza* species are poorly understood particularly through assessment of meiotic chromosome pairing in their hybrids. Although some crosses were made among *O. sativa*, Africa AA genome wild rice species and *O. perennis* from different continents, the knowledge of *O. meridionalis* as an independent species was lacking and the taxonomic delimitation of *O. perennis* was ambiguous (Chu et al., 1969). This could have affected the appropriate interpretation of species relationship in the AA genome group. Even in the absence of cytological evidence, *O. meridionalis* was assigned to the AA genome by some authors (Ng et al., 1981b; Vaughan, 1989; Naredo et al., 1996). In an earlier study Naredo et al. (1996) presented results of crosses, development of hybrids, and the complexity of reproductive isolation between *O. meridionalis* and the Asian genome rice species. This study further assessed the biosystematic relationship of the Australian *O. meridionalis* and the Asian AA genome rice species through analysis of the chromosome pairing in meiosis of the parental species and their F1 hybrids.

Materials and methods

The hybrids between the AA genome *Oryza* species used in this study were those reported in an earlier hybridization experiment (Naredo et al., 1996), and the hybridization included two accessions each of *O. meridionalis*, *O. rufipogon*, and *O. nivara* and one accession of *O. sativa* f. *spontanea* (Table 1).

Meiotic chromosomes configurations of the parents and hybrids were analyzed on pollen mother cells (PMCs). For the meiotic preparation, immature spikelets were collected and placed in Farmer's fixa-

Table 2. Meiotic configuration at metaphase I in the Asian and Australian AA genome *Oryza* species

Species and accession No.	2n=	No. of cells observed	Chromosome configurations*				Chiasmata /cell
			I	II			
				Total	Rings	Rods	
O. meridionalis							
105281	24	50	–	12.00 (12)	11.50 (10–12)	0.50 (0–2)	23.50 22–24)
105289	24	23	–	12.00 (12)	11.61 (9–12)	0.39 (0–3)	23.60 (21–24)
O. rufipogon							
106080	24	50	–	12.00 (12)	11.70 (10–12)	0.30 (0–2)	23.70 (22–24)
106288	24	50	–	12.00 (12)	11.72 (10–12)	0.28 (0–2)	23.72 (22–24)
O. nivara 106111	24	29	–	12.00 (12)	11.27 (7–12)	0.72 (0–5)	23.26 (19–24)

*Meiotic configurations are expressed as mean and range (enclosed in parentheses) values.

tive (absolute ethanol : acetic acid = 3 : 1) for 24 hours at 4°C and then transferred to 70% ethanol and stored at 4°C until used. The spikelets were refixed for 4 hours in Carnoy's II solution (absolute ethanol : chloroform : acetic acid = 6 : 3 : 1) with a few crystals of ferrous chloride ($FeCl_2 \cdot 4H_2O$) added to increase the staining intensity, and then transferred to 70% ethanol for 24 hours. The spikelets were stained in hydrochloric acid-carmine (Snow 1963) at 50°C for 24 hours and at room temperature for at least 3 days. The stained anthers were squashed in 45% acetic acid and sealed with modified Hoyer's medium as described by Lu & Bothmer (1990). Meiotic pairing was analyzed at metaphase I (MI) only in PMCs with complete chromosomes.

Results

All the AA genome *Oryza* parental species exhibited a consistent chromosome number of 2n=2x=24 in PMCs with normal meiosis (Table 2). Meiotic pairing of the representative accessions was very high with predominantly ring bivalent formation at MI. The two *O. meridionalis* accessions showed an average of 23.50 and 23.60 chiasmata per PMC. The two *O. rufipogon* accessions had an average of 23.70 and 23.72 per PMC, whereas the only *O. nivara* accession presented a slightly lower average chiasma frequency at 23.26 per PMC. No univalent or multivalent formations were detected at MI in any of the parental species. Chromosomes were equally segregated to the two poles at anaphase I and no micronuclei were found in the tetrads.

The two intraspecific hybrids showed perfect bivalent formation, and always 2n=2x=24 in the PMCs (Table 3). Meitoic configuration of the two hybrids were comparable to those of parental species, although with a slight reduction in the chiasma frequency in the *O. meridionalis* × *O. meridionalis* combination. The *O. nivara* × *O. nivara* hybrid showed an average of 11.54 ring bivalents and 23.54 chiasmata per PMC. Ring bivalents in the *O. meridionalis* × *O. meridionalis* hybrid occurred at an average of 9.22 per PMC and chiasma number was recorded at 21.22 per PMC. No univalents and multivalents were scored in either of these intraspecific hybrids. Chromosomes were equally segregated to the two poles at anaphase I and one to two chromatid bridges in company with fragments were observed in both hybrids. No micronucleus was observed in the tetrads.

The interspecific hybrids consistently showed 24 chromosomes. High bivalent formation was observed at the diakinesis and metaphase I (Figure 1, A and B), although with a slight reduction in chiasma frequency due to the presence of univalents and/or increase in rod bivalents, compared to their parental species, with the exception in the cross *O. sativa* f. *spontanea* × *O. meridionalis* (Table 3). Few lagging univalents were

Table 3. Meiotic configuration at Metaphase I in the inter- and intraspecific hybrids

Hybrid combination and accession No. (♀ × ♂)	2n=	No. of cells observed	Chromosome configurations*						Chiasmata /cell
			I	II			III	IV	
				Total	Rings	Rods			
O. nivara × *O. nivara*									
105386 × 106111	24**	33	–	12.00 (12)	11.54 (10–12)	0.45 (0–2)	–	–	23.54 (22–24)
O. meridionalis × *O. meridionalis*									
105289 × 105281	24**	50	–	12.00 (12)	9.22 (6–12)	2.78 (0–6)	–	–	21.22 (18–24)
O. meridionalis × *O. nivara*									
105281 × 106111	24	50	0.06 (0–2)	11.90 (10–12)	7.08 (2–11)	4.82 (1–10)	0.02 (0–1)	0.02 (0–1)	19.12 (14–23)
105281 × 105386	24	50	0.04 (0–2)	11.50 (4–12)	7.30 (1–12)	4.24 (0–8)	–	0.24 (0–4)	19.68 (16.24)
105289 × 106111	24**	39	–	11.85 (8–12)	9.23 (3–12)	2.61 (0–5)	–	0.08 (0–2)	21.43 (19–24)
O. meridionalis × *O. rufipogon*									
105281 × 106169	24**	50	0.12 (0–2)	11.82 (9–12)	10.34 (7–12)	1.48 (0–5)	–	0.06 (0–1)	22.42 (19–24)
105289 × 106080	24	50	0.06 (0–2)	11.78 (10–12)	7.26 (3–11)	4.52 (1–9)	0.02 (0–1)	0.08 (0–1)	19.42 (15–23)
105289 × 106169	24	50	–	11.80 (10–12)	8.20 (5–11)	3.60 (1–7)		0.10 (0–1)	20.38 (17–23)
O. rufipogon × *O. meridionalis*									
106080 × 105289	24	50	–	12.00 (12)	8.92 (6–12)	3.08 (0–6)	–	–	20.92 (18–24)
O. spontanea × *O. meridonalis*									
105564 × 105281	24**	50	–	11.96 (12)	11.58 (10–12)	0.38 (0–2)	–	0.02	23.62 (22–24)

*Meiotic configurations are expressed as mean and range (enclosed in parentheses) values.
**One to two chromatid bridges were observed at anaphase I.

observed at anaphase I and II. Micronuclei were found in a few tetrads of some hybrids.

The hybrid *O. meridionalis* × *O. nivara* crosses dislayed an average of 0–0.06 univalents, 11.50–11.90 total bivalents, 0–0.02 trivalents, and 0.02–0.24 quadrivalents per PMC. An average of 19.12–21.43 per PMC was recorded in this combination. One to two chromatid bridges in addition to fragments were observed at anaphase I in the IRGC 105289 × IRGC 106111 hybrid (Table 3).

The reciprocal combinations between *O. meridionalis* and *O. rufipogon* showed a similar degree of chromosome pairing, although only one hybrid was analyzed from the *O. rufipogon* × *O. meridionalis* cross. The *O. meridionalis* × *O. rufipogon* cross had an average of 0–0.12 univalent, 11.78–11.82 bivalents, 0–0.02 trivalents, and 0.06–0.10 quadrivalents per PMC. Chiasma frequency varied from 19.42–22.42 per PMC. The *O. rufipogon* × *O. meridionalis* cross showed an average of 12.00 bivalents and no univalents or multivalents were scored. One to two chromatid

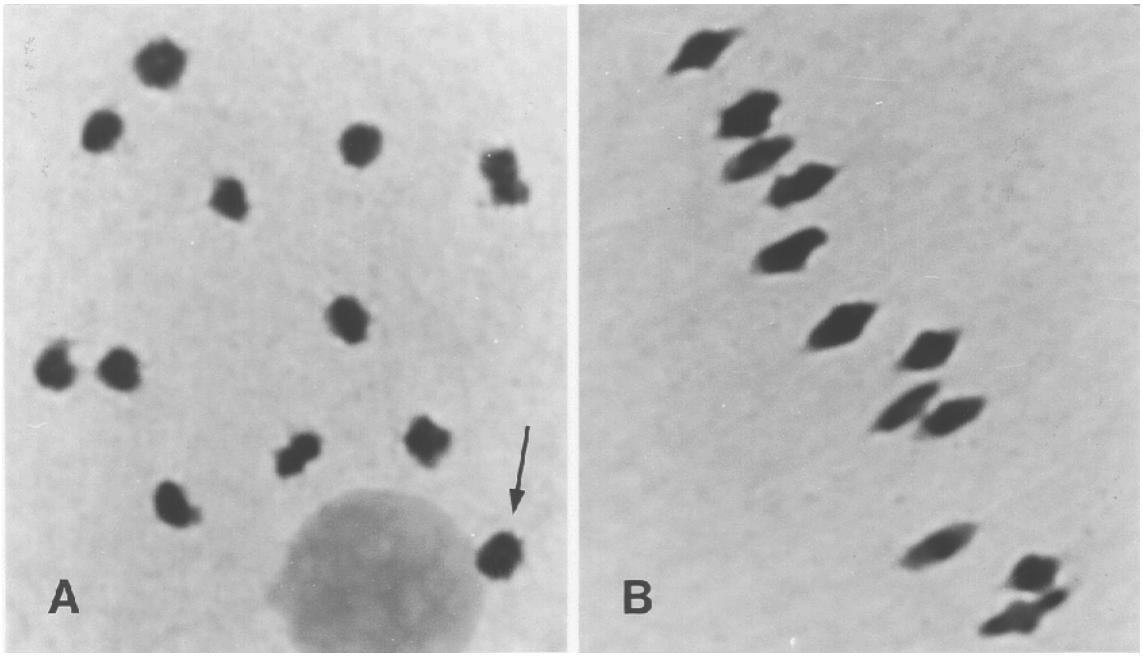

Figure 1. A and B. Meiotic chromosome pairing in the *O. meridionalis* × *O. nivara* hybrid. A. Diakinesis showing 12 ring bivalents, one bivalent is attached to the nucleolus indicating the satellite chromosomes (arrow). B. Metaphase I showing 12 ring bivalents.

bridges accompanied with fragments were observed at anaphase I in the IRGC 105281 × IRGC 106169 hybrid.

The *O. sativa* f. *spontanea* × *O. meridionalis* hybrid showed predominant ring bivalent formation, occurring at an averge of 11.58 per PMC. No univalents were formed but one quadrivalent was noted in one out of fifty cells observed. Chiasma frequency occurred at an average of 23.62 per PMC. One to two chromatid bridges in company with fragments were observed in PMCs at anaphase I.

Discussion

Oryza meridionalis is a diploid annual species endemic to northern Australia. This species is geographically isolated from all other closely related AA genome species or rice occurring in Asia, Africa, and South America. Comparative morphological studies of the AA genome species from Asia and Australia demonstrated considerable differences of *O. meridionalis* from *O. nivara* and *O. rufipogon*, particularly with regards to the typically slender spikelets, distinctly longer, thicker, and rougher awns, and considerably smaller anthers of *O. meridionalis* (Morishima, 1969; Ng et al., 1981a). It is also believed that *O. meridionalis* was genetically isolated from the Asian AA genome *Oryza* species. The crossability between *O. meridionalis* and *O. nivara*, *O. rufipogon* and fertility of their F1 hybrids are generally low (Morishima, 1969; Chu et al., 1969; Naredo et al., 1996), indicating reproductive isolation between the Australian and Asian AA genome *Oryza* species. The results given by isozyme analysis also showed a distinct grouping of the Australian *O. meridionalis* from all the other AA genome *Oryza* species (Second, 1985). The assessment of the meiotic pairing of the hybrids of *O. meridionalis* with *O. nivara*, and *O. rufipogon*, therefore, will provide more information for the understanding of genomic affinity between the Australian and Asian AA genome species of rice.

Although the genomic symbol of *O. meridionalis* has been cited or suggested in many publications as AA (Ng et al., 1981b; Vaughan, 1989), and even our research group followed this convention (Naredo et al., 1996), none of the papers has provided supportive experimental evidence for this genomic designation to *O. meridionalis*. In a short note, the meiotic chromosome behaviour of a hybrid between *O. meridionalis* and *O. nivara* was reported by a Chinese scientist (Pan, 1983). However, *O. meridionalis* was identified as a

strain of Australian *O. nivara*, so no significant conclusion was made, even though normal meiosis and high metaphase I chromosome pairing were observed in the hybrid. The designation of the AA genome to *O. meridionalis* must be based on chromosome pairing data.

Meiosis of *O. meridionalis* was normal with nearly full chromosome pairing, but the frequency of meiotic pairing in the hybrid between different populations of *O. meridionalis* was somewhat reduced with a significantly higher number of rod bivalents. This indicates a slight genetic modification of the genomes in the different *O. meridionalis* populations, which was also demonstrated by the low fertility (2.6% of stainable pollen and 0.6% of seed set) in the same hybrid (Naredo et al., 1996). In comparison with its parents, the intraspecific hybrid between *O. nivara* populations showed nearly no reduction of meiotic pairing and almost normal fertility with 83.0% stainable pollen and 66.5% of seed set (Naredo et al., 1996). The results suggest that the extent of genetic modification differs from population to population and may be reflected by the meiotic pairing and fertility data of the intraspecific hybrids.

Compared with the respective parental species, the frequency of chromosome pairing was slightly reduced with the presence of the univalents and higher number of rod bivalents in the hybrids, suggesting a very minor modification of the genomes in the respective *Oryza* species. In addition, the differentiation of the AA genomes in the *Oryza* species studied is probably caused by chromosome structural changes, such as inversions and reciprocal translocations, reflected by the presence of multivalents at metaphase I and chromatid bridges at anaphase I of the interspecific hybrids in this study.

The meiotic pairing pattern of *O. meridionalis* × *O. nivara*, *O. sativa* f. *spontanea* × *O. meridionalis*, and reciprocal *O. meridionalis* × *O. rufipogon* hybrids were similar, although with some variations. The high frequency of chromosome pairing in the hybrids between *O. meridionalis* and other AA genome *Oryza* species strongly supports the suggestion that *O. meridionalis* does carry the AA genome. *Oryza meridionalis*, *O. nivara*, *O. sativa* f. *spontanea*, and *O. rufipogon* which are very closely related share nearly identical AA genomes. On the other hand, morphological and isozyme studies have indicated the unique status of *O. meridionalis*, and results from hybridization studies also highlight strong reproductive isolation between the Australian *O. meridionalis* and the Asian AA genome species. Since the AA genome in *O. meridionalis* has a high genomic affinity to that in the Asian AA genome *Oryza* species, we suggest that it is not necessary to differentiate this genome as A^mA^m as published by Vaughan (1989).

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