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Hybridization of AA genome rice species from Asia and Australia I. Crosses and development of hybrids

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Abstract

Interspecific and intraspecific crosses of rice species were made involving the AA genome wild species *Oryza meridionalis* Ng from Australia, *O. nivara* Sharma et Shastry, *O. rufipogon* Griff, the weedy type *O. sativa* f. *spontanea* Rosch., and the cultigen *O. sativa* L. from Asia. Seed set and the number of hybrids obtained from both interspecific and intraspecific crosses were low and no significant differences were observed between the interspecific and intraspecific crosses. In the combination *O. meridionalis* × *O. nivara* and *O. meridionalis* × *O. rufipogon*, considerable differences in reciprocal crosses were observed, whereas higher seed set and more hybrids were obtained when *O. meridionalis* was used as the female parent. Pollen stainability and seed fertility of hybrids were very low, averaging between 1% and 9% in both interspecific and intraspecific combinations, except in the *O. nivara* intraspecific cross of 105386 × 106111, whose hybrid showed 83.0% pollen stainability and 66.5% seed fertility.

Introduction

The genus *Oryza* includes cultivated rice (*O. sativa* L.) which is the staple crop for more than half of the world's population. All species sharing the basic AA genome are closely related to this cultigen, among the most valuable germplasm resources for rice improvement. Transfer of agronomically useful traits by interspecific hybridization and other genetic approaches has been achieved in a number of *Oryza* species (Khush et al., 1977; Jena & Khush, 1990; Amante-Bordeos et al., 1992; Multani et al., 1994; Ishii et al., 1994). However, the biosystematic relationship of the AA genome species are not yet fully understood, and their taxonomic status within the genus *Oryza* is the subject of disagreement and confusion, reflected by the continual revision of the species in the genus by different authors (Morishima et al., 1992; Vaughan, 1994). In his taxonomic treatment of *Oryza*, Vaughan (1989) recognized six wild species in the genus having AA genome, namely *O. rufipogon* Griff and *O. nivara* Sharma et Shastry distributed in Asia, *O. barthii* A. Chev. and *O. longistaminata* Chev. et Roehr. in Africa, *O. glumaepatula* Steud. in South America, and *O. meridionalis* Ng in Australia. However, more recently, Vaughan (1994) has treated *O. glumaepatula* only as a form of *O. rufipogon* that became naturalized in South America.

The taxonomic status of the Asian AA genome wild species of rice has been a long-standing problem. Sharma & Shastra (1965) identified the Asian annual type as *O. nivara* which in their opinion was morphologically distinct from its perennial counterpart *O. rufipogon*. The two species also differ in their habitat preference with the annual type favoring temporary swamps which become dry during the dry season and the perennial type growing in deep swamps which remain moist throughout the year (Oka, 1988). The classification of *O. rufipogon* and *O. nivara* as separate species gained support from Chang (1976) and Ng et al. (1981a). However, Morishima & Barbier (1990) considered *O. rufipogon* and *O. nivara* only as ecotypes of *O. rufipogon* because of the continuous variation that apparently exists between them (Morishima et

al., 1992). So far, there is still no generally acceptable taxonomic treatment of the Asian AA genome species.

An annual Australian species which was referred to as *O. perennis* together with other AA genome species from Asia and Africa by Morishima (1969) formed a reproductively isolated group from the Asian forms. Unlike the Asian species in which continuous hybridization among wild, weedy and cultivated races occurs, this wild form from Australia remains geographically and genetically isolated from the cultivars and other Asian AA genome taxa (Chang, 1976). After futher study, Ng et al. (1981a, b) recognized this Australian form as an independent species *O. meridionalis*, on the basis of its unique morphological characteristics. These include the typical slender grains and distinctive longer, thicker, and rougher awns, in addition to its reproductive isolation from the Asian taxa. This taxonomic treatment has received general acceptance (Vaughan, 1989, 1994; Wang et al., 1992), but the biosystematic relationship of *O. meridionalis* with the Asian AA genome species is not clearly understood. Ng et al. (1981a), without presenting any experimental data, claimed that there were strong reproductive barriers between *O. meridionalis* and the other species. The objective of the present study was to determine the biosystematic relationship of the Asian AA genome species with the Australian *O. meridionalis* through evaluation of their crossability, and fertility of F1 hybrids.

Materials and methods

The materials used in the crossing program included five accessions of *O. rufipogon*, two accessions each of *O. meridionalis* and *O. nivara*, and one accession each of *O. sativa* L. and *O. sativa* f. *spontanea* Rosch (Table 1). These were conserved in the International Rice Genebank (IRG) at the International Rice Research Institute (IRRI), Philippines. Seed dormancy was broken by heat treatment at 40°C for 7 days followed by hull removal. Seeds were germinated in June 1993 on MS medium (Murashige and Skoog, 1962) or in petri dishes lined with moist filter paper. The plants were maintained at about 30°C in the IRG nursery screenhouse where hybridization was conducted in October 1993 at 11.7 h average daylength. Both intraspecific and interspecific crosses were made according to the combinations shown in Tables 2 and 3, generally using at least three plants per accession except in combinations in which only one or two plants were available for

Table 1. Accession numbers and origins of parental species

Species	Accession Number	Origin
O. meridionalis	105281	Western Australia
	105289	Queensland, Australia
O. nivara	106111	West Bengal, India
	105386	Tak, north Thailand
O. rufipogon	106080	West Bengal, India
	105953	West Java, Indonesia
	106288	Lake Murray, Western Province, Papua New Guinea
	106169	Lai Chau, Vietnam
	105303	Queensland, Australia
O. sativa f. *spontanea*[a]	105564	Indonesia
O. sativa (cv. Peta)	35	Indonesia

[a]Referred to in the later Tables as *O. spontanea*

pollination. The female parents were emasculated late in the afternoon using a vacuum emasculator running at three fourths horsepower giving a suction of about 500 mm Hg (Jennings et al. 1979) and panicles were immediately covered with glassine bags to avoid contamination with alien pollen. Pollinations were carried out the following morning. Pollinated panicles were again covered with glassine bags and mature hybrid seeds were collected after shattering of the panicles occurred.

Hybrid seeds were germinated on MS medium immediately after harvest. F1 plants were maintained alongside the parental lines in the IRG nursery screenhouse. Isozyme patterns based on five enzyme systems, namely, Phosphoglucose Isomerase (PGI, EC 5.3.1.9), Aminopeptidase (AMP, EC 3.4.11.1), Shikimate Dehydrogenase (SDH, EC 1.1.1.25), Endopeptidase (ENP, EC 3.4.21–24.–), and Malate Dehydrogenase (MDH, EC 1.1.1.37), were analyzed to confirm the hybrid status of the putative F1 plants, and their life cycle habits were also compared with the parents. Plants that died immediately after flowering were considered as annuals, those that had two or three flushes of flowering were treated as intermediates and plants that survived after two years were considered as perennials. Pollen fertility of parents and hybrids was determined by staining mature pollen grains from five spikelets with I-KI solution for 3 minutes. Only round, filled, and well-stained grains were considered viable. Seed set of parents and F1 plants was recorded under open

Table 2. Seed set and number of hybrids obtained from interspecific crosses among Asian and Australian AA genome rice species

Hybrid combination	No. of Spikelets pollinated		Seed set		Plants	
			No.	%[a]	No.	%[a]
O. meridionalis × *O. nivara*						
105281 × 106111	34		4	11.7	3	8.8
105281 × 105386	259		56	21.6	21	8.1
105289 × 106111	62		4	6.4	2	3.2
105289 × 105386	239		16	6.6	0	0
Total	594	Mean		11.6		5.0
O. nivara × *O. meridionalis*						
106111 × 105281	123		1	0.8	0	0
106111 × 105289	99		2	2.0	0	0
105386 × 105281	59		0	0		
105386 × 105289	41		0	0		
Total	322	Mean		0.7		0
O. meridionalis × *O. rufipogon*						
105281 × 106080	111		38	34.2	0	0
105281 × 106288	261		4	1.5	0	0
105281 × 105953	52		6	11.5	1	1.9
105281 × 106169	185		10	5.4	4	2.2
105289 × 106080	170		17	10.0	3	1.8
105289 × 105303	77		17	22.1	0	0
105289 × 106288	73		9	12.3	2	2.7
105289 × 106288	64		5	7.8	0	0
105289 × 106169	133		15	11.3	1	0.8
Total	1126	Mean		12.9		1.0
O. rufipogon × *O. meridionalis*						
105386 × 105381	59		0			
105386 × 105289	41		0			
106080 × 105281	33		0			
106080 × 105289	83		12	14.4	1	1.2
105303 × 105281	49		0			
105303 × 105289	126		2	1.6	1	0.8
105953 × 105281	136		1	0.7	0	0
105953 × 105289	78		5	6.4	1	1.3
106169 × 105281	223		1	0.4		
106169 × 105289	229		0	0		
Total	1057	Mean		2.4		0.3
O. spontanea × *O. meridionalis*						
105564 × 105281	172		31	18.0	6	3.5
105564 × 105289	141		57	40.4	7	4.5
Total	313	Mean		29.2		4.0
O. meridionalis × *O. sativa*						
105281 × 35 (Peta)	104		6	5.8	0	0
105289 × 35 (Peta)	158		9	5.7	0	0
Total	262	Mean		5.8		0
O. sativa × *O. meridionalis*						
35 (Peta) × 105281	442		4	0.9	0	0
35 (Peta) × 105289	619		24	3.9	0	0
Total	1061	Mean		2.4		0

[a] As a proportion of spikelets pollinated

Table 3. Seed set and frequency of hybrids obtained from intraspecific crosses between Australian and Asian AA genome rice species

Hybrid combination	No. of Spikelets pollinated	Seed set		Plants	
		No.	%[a]	No.	%[a]
O. meridionalis × *O. meridionalis*					
105281 × 105289	219	45	20.6	11	5.0
105289 × 105281	237	27	11.4	3	1.3
Total	456	Mean	16.0		3.2
O. nivara × *O. nivara*					
105386 × 106111	121	2	1.6	1	0.8
O. rufipogon × *O. rufipogon*					
106080 × 106169	215	13	6.1	1	0.5
106169 × 106080	120	0	0		
Total	335	Mean	3.1		0.2

[a] As percentage of pollinated spikelets

pollination conditions to evaluate their fertility. Fertilities of 50% and higher were considered as normal.

Results

Crosses and development of hybrids

A total of 4735 spikelets were pollinated in the interspecific crosses and more than 900 spikelets in the intraspecific crosses. The results of seed set and frequency of mature hybrids produced from the interspecific and intraspecific crosses are presented in Tables 2 and 3, respectively. Seed set was generally low in the interspecific hybrids and varied from 0 to 40% in different crosses. The frequency of actual hybrids, ranging from 0 to 8.8%, was even lower than the seed set. The *O. meridionalis* and *O. sativa* combination did not produce any hybrids (Table 2). Different interspecific combinations, especially the reciprocal crosses in *O. meridionalis* × *O. nivara* and *O. meridionalis* × *O. rufipogon*, showed considerable differences in both seed set and number of hybrids. When *O. meridionalis* was used as the female parent, higher seed set and number of hybrids was obtained than when it was used as the male parent. Of the five intraspecific crosses made, four produced seeds at low frequencies ranging from 1.6 to 20.6% with the highest values in the *O. meridionalis* crosses and the lowest in the *O. nivara* cross. In the different crosses, number of hybrids obtained was even lower with values ranging only from 0.5 to 5.0% (Table 3). Variations in seed set and frequency of hybrids were observed in different crosses of the same combinations, but these did not differ between interspecific and intraspecific crosses.

Hybrids from both interspecific and intraspecific crosses developed into mature and vigorous plants. Comparison of the isozyme banding patterns of parents and F1s revealed that parents that were polymorphic for an enzyme gave F1 hybrids exhibiting heterozygote banding patterns (Figure 1). For instance, the interspecific hybrid of *O. rufipogon* (Acc. 105303) and *O. meridionalis* (Acc. 105289) showed both the parental types for SDH and ENP. The intraspecific *O. rufipogon* hybrid from the cross 106080 × 106169 also showed heterozygote bands for PGI. The morphological characters of F1 hybrids were intermediate between their parents. The habit of the F1 hybrids generally followed that of their parents. A cross between annual parents resulted in annual hybrid formation, annual plants crossed to perennial plants produced hybrids with semi-perennial to perennial life cycles, whereas only perennial hybrids were obtained from crosses between two perennial parents. The F1 intraspecific *O. nivara* hybrid, 105386 × 106111 showed an intermediate life cycle (Table 4).

Fertility of hybrids

Very low pollen stainability and seed fertility were observed in all interspecific hybrids with large variation among crosses within the same hybrid combina-

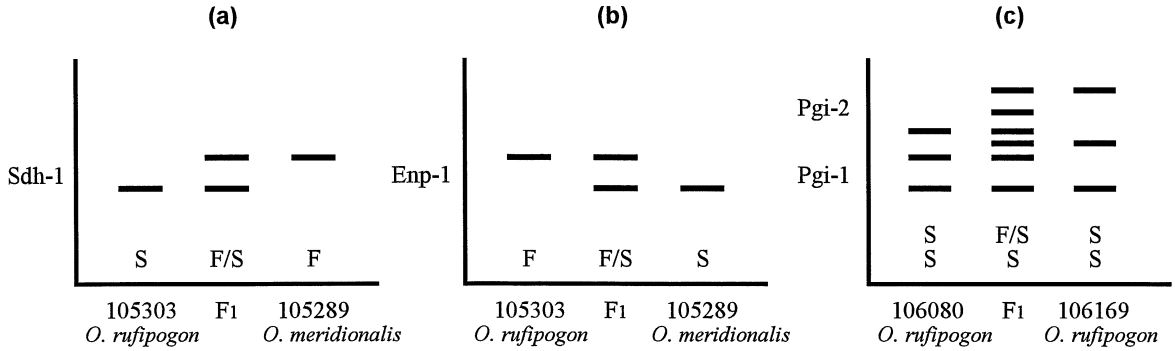

Figure 1. Zymogram of (a) Shikimate Dehydrogenase (Shd-1), (b) Endopeptidase (Enp-1) and (c) Phosphoglucose Isomerase (Pgi-1 and Pgi-2) isozyme patterns in the parents and F1 hybrids. S = Slow bands, F = Fast bands.

tion (Table 4). The average pollen stainability ranged from 1.8 to 15.1% while that of seed fertility varied from 1.0 to 8.7%. The *O. meridionalis* × *O. rufipogon* combination obtained the highest value and the lowest value was in the *O. meridionalis* × *O. nivara* combination. There were no differences in the average fertility between hybrid combinations obtained from reciprocal crosses. Intraspecific hybrids displayed unexpectedly low fertility with the exception of the *O. nivara* intraspecific hybrid, 105386 × 106111 that had 83.0% pollen stainability and 66.5% seed fertility. In contrast, parental accessions showed normal fertility with pollen stainability ranging from 50.4 to 85.6% and seed fertility from 54.0 to 94.5% except for the highly sterile *O. rufipogon* accession 105953, which showed only 10.4% stainable pollen and 1.27% seed fertility and the highly sterile *O. nivara* accession 105386, which had 78.8% pollen stainability but a low seed set of 16.0%.

Discussion

The evaluation of basic biological characteristics and biosystematic relationships of the wild species of rice is essential for their effective utilization in rice breeding. To meet this requirement, many studies including interspecific hybridization and genome analysis have been undertaken during recent decades (Nezu et al., 1960; Li et al., 1962; Second, 1985; Morishima, 1986; Oka, 1988; Morishima et al., 1992). Our results indicate that except for the hybrid combination of *O. meridionalis* × *O. sativa*, interspecific hybridization between *O. meridionalis* and the other Asian AA genome species can be achieved without any intervention such as embryo rescue, although production of hybrids is low, mainly because of the failure of some hybrid seeds to germinate. This partially suggests that species isolation where unviable or weak hybrid seeds were obtained from interspecific hybridization. The ease of hybridization demonstrates that introduction of useful genes from the Australian species to the Asian AA genome species through sexual hybridization is possible, and that a comparatively close relationship does exist between *O. meridionalis* and the Asian AA taxa. In our experiments, all interspecific hybrids were not completely sterile when they were selfed. This result is different from that reported by Ng et al. (1981a), in which the F1 hybrids between *O. meridionalis* and *O. rufipogon* or *O. nivara* were claimed to be completely sterile. However, the high sterility of the F1 hybrids with an average of less than 10% of seed set, compared with the high fertility of their parental species (Table 4), does indicate strong reproductive isolation between the species studied. This is one of the factors that Morishima (1969) considered to distinguish the geographical groups (*sensu* different species in current taxonomic classification) from one another. Except for the only *O. nivara* × *O. nivara* hybrid with 66.5% of seed set, all the intraspecific combinations produced few hybrid plants ($<$5% in average) that showed high sterility with less than 5% of seed set, indicating genetic differences between populations of the same species.

The complexity of the reproductive systems of these AA genome species hinders the use of hybridization data to predict the relationships of the species. In other words, it is not justifiable to use data from crossing and hybrid fertility alone to define the biosystematic relationship of the AA genome *Oryza* species.

Table 4. Pollen stainability and spikelet fertility of parents and hybrids from interspecific and intraspecific between Asian and Australian AA genome rice species

Parent and hybrid combinations			% Fertility		Life cycle[a]
			Pollen	Seed	
Parents					
O. meridionalis	105281		50.4	72.3	A
	105289		54.2	72.3	A
O. nivara	106111		85.6	87.1	A
	105386		78.8	16.0	I
O. rufipogon	106080		64.6	78.4	P
	105953		10.4	–[b]	P
	106288		–	54.0	P
	106169		82.2	79.1	P
	105303		85.0	–	P
O. spontanea	105564		74.3	70.7	I
O. sativa (cv. Peta)	35		65.7	–	A
Interspecific crosses					
O. meridionalis × *O. nivara*					
105281 × 106111			3.3	0.2	A
105281 × 105386			1.6	2.9	A
105289 × 106111			0.5	0	A
		Mean	1.8	1.0	
O. meridionalis × *O. rufipogon*					
105281 × 106169			2.0	2.0	I
105289 × 106080			10.1	0.4	I
105289 × 106169			3.9	3.7	I
105289 × 106288			43.6	28.7	I
		Mean	14.9	8.7	
O. rufipogon × *O. meridionalis*					
106080 × 105289			1.4	0	I
105303 × 105289			44.0	19.5	I
105953 × 105289			0	0.4	I
		Mean	15.1	6.6	
O. spontanea × *O. meridionalis*					
105564 × 105281			0.9	0.7	I
105564 × 105289			17.2	12.8	I
		Mean	9.1	6.8	
Intraspecific crosses					
O. meridionalis × *O. meridionalis*					
105281 × 105289			3.1	1.0	A
105289 × 105281			2.0	0.2	A
		Mean	2.6	0.6	
O. nivara × *O. nivara*					
105386 × 106111			83.0	66.5	I
O. rufipogon × *O. rufipogon*					
106080 × 106169			16.5	1.9	P

[a]A = Annual, I = Intermediate, P = Perennial, [b]Data not available

It is more appropriate to combine the results from different studies at different levels, such as evaluation of morphological variation, genome analysis, and molecular markers, to gain a more accurate picture of the evolutionary processes of this valuable but complex group of species.

The considerable differences in crossability especially within the same combinations indicate diversity in the genetic basis for crossability. The large variation in seed set and frequency of hybrids obtained within the *O. meridionalis* × *O. rufipogon* combination reflects the high interpopulational variation of the parents, particularly of the perennial species like *O. rufipogon*, which was also noted by Morishima & Barbier (1990). This has important implications in crossing programs between these species, since accessions with normal or high fertility are usually included in order to obtain sufficient hybrids. It will also be possible to select highly compatible accessions to facilitate genetic introgression between species in rice breeding by wide hybridization. Differences in reciprocal crosses must also be considered in crossing programs as demonstrated when *O. meridionalis* was used as a female parent, producing more hybrids. The genetic basis for differences in crossability and fertility of hybrids is complicated and needs further study.

References

Amante-Bordeos, A., L.A. Sitch, R. Nelson, R.D. Dalmacio & N.P. Oliva 1992. Transfer of bacterial blight resistance from the tetraploid wild rice *Oryza minuta* to cultivated rice, *Oryza sativa*. Theor Appl Genet 84:345–354.

Chang, T.T. 1976. The origin, evolution, cultivation, dissemination, and diversification of Asian and African rices. Euphytica 25:425–441.

Ishii, T., D.D. Brar, D.S. Multani & G.S. Khush. 1994. Molecular tagging of genes for brown planthopper resistance and earliness, introgressed from *Oryza australiensis* into cultivated rice, *O. sativa*. Genome 37:217–221.

Jena, K.K. & G.S. Khush, 1990. Introgression of genes from *Oryza officinalis* Well ex Watt to cultivated rice, *O. sativa* L. Theor Appl Genet 80: 737–745.

Jennings, P.R., W.R. Coffman & H.E. Kaufmann 1979. Rice Improvement. International Rice Research Institute, Los Baños, Philippines.

Khush, G.S., K.C. Ling, R.C. Aquino & V.M. Aguiero 1977. Breeding for resistance to grassy stunt in rice. In: Proc 3rd Int. Congr SABRAO, Canberra, Australia. Plant Breed Papers 1(4):3–9.

Li, H.W., T.S. Wang, C.C. Chen & W.H. Wang, 1962. Cytogenetic studies of *Oryza sativa* L. and its related species. 2. A preliminary note on the interspecific hybrids within the section *Sativa* Roschev. Bot. Bull Acad. Sin. 3:209–219.

Morishima, H. 1969. Phenetic similarity and phylogenetic relationship among strains of *Oryza perennis*, estimated by methods of numerical taxonomy. Evolution 23:429–443.

Morishima, H. 1986. Wild progenitors of cultivated rice and their population dynamics. In: Rice Genetics: Proceedings of the International Rice Genetics Symposium pp. 3–14.

Morishima, H. & P. Barbier, 1990. Mating system and genetic structure of natural populations in wild rice *Oryza rufipogon*. Plant Species Biol 5:31–39.

Morishima, H., Y. Sano & H. Oka, 1992. Evolutionary studies in cultivated rice and its wild relatives. Oxford Surveys in Evol Biol 8: 135–184.

Multani, D.S., K.K. Jena, D.S. Brar, B.G. delos Reyes, E.R. Angeles & G.S. Khush, 1994. Development of monosomic alien addition lines and introgression of genes from *Oryza australiensis* Domin to cultivated rice *O. sativa* L. Theor Appl Genet 88:102–109.

Murashige, T. & F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco cultures. Physiol Plant 15:473–497.

Nezu, M.T., T.C. Katayama & H. Kihara, 1960. Genetic study of the genus *Oryza*. I. Crossability and chromosomal affinity among 17 species. Seiken Zihô. 11:1–11.

Ng, N.Q., T.T. Chang, J.T. Williams & J.G. Hawkes, 1981a. Morphological studies of Asian rice and its related wild species and the recognition of a new Australian taxon. Bot J Linn Soc 16:303–313.

Ng, N.Q., J.G. Hawkes, J.T. Williams & T.T. Chang, 1981b. The recognition of a new species of rice (*Oryza*) from Australia. Biol J Linn Soc 82:327–330.

Oka, H.I., 1988. Origin of Cultivated Rice. Japan Scientific Societies Press, Tokyo.

Second, G., 1985. A new insight into the genome differentiation on *Oryza* L. through isozymic studies. In: A.K. Sharma & A. Sharma (eds.), Advances in Chromosome and Cell Genetics, pp. 45–78, Oxford and IBH Publ. Co., New Delhi.

Sharma, S.D. & S.V.S. Shastry, 1965. Taxonomic studies in genus *Oryza* L. III. *O. rufipogon* Griff. *sensu stricto* and *O. nivara* Sharma et Shastry *nom. nov*. Indian J Genet Plant Breed 25:157–167.

Vaughan, D.A., 1989. The genus *Oryza* L.: current state of taxonomy. IRRI Research Paper Series. 138.

Vaughan, D.A., 1994. The wild relatives of rice: A genetics resources handbook. IRRI, Manila, Philippines.

Wang, Z.Y., G. Second & S.D. Tanksley, 1992. Polymorphism and phylogenetic relationships among species in the genus *Oryza* as determined by analysis of nuclear RFLPs. Theor Appl Genet 83:565–581.