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Taxonomic status of *Oryza glumaepatula* Steud. II. Hybridization between New World diploids and AA genome species from Asia and Australia

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Abstract

Intraspecific and interspecific crosses were made using *O. glumaepatula* Steud., *O. rufipogon* Griff., *O. nivara* Sharma et Shastry, *O. meridionalis* Ng, and other diploid accessions from the New World to determine biosystematic relationships among the New World, Asian, and Australian AA genome species. All intraspecific and interspecific combinations produced seeds and hybrids, but at different levels of success. The *O. glumaepatula*, *O. rufipogon*, and *O. nivara* intraspecific hybrids were generally fertile with mean pollen stainability ranging from 75.2% to 80.1% and mean spikelet fertility varying from 32.1% to 87.7%. The *O. meridionalis* intraspecific crosses showed 3.8% pollen stainability and 0.1% spikelet fertility. Interspecific hybrids showed varying fertilities. Crosses of *O. glumaepatula* with the New World diploid accessions IRGC 103812 and 105561 produced highly fertile hybrids with 68.0% to 89.7% pollen stainability and 70.8% to 86.6% spikelet fertility. In crosses between *O. glumaepatula* and the Asian species and other diploid New World accessions IRGC 100961, 103810, and 104386, sterile hybrids were produced with pollen fertility ranging from 0 to 35.1% and spikelet fertility from 0 to 8.2%. IRGC 103810 and 104386 formed fertile hybrids when crossed to *O. nivara* and *O. rufipogon*, which were also interfertile. Interspecific crosses of *O. meridionalis* with the other species produced highly sterile hybrids.

Introduction

In the genus *Oryza* L., six wild species share a similar AA genome following the taxonomic treatment by Vaughan (1989). These are *Oryza rufipogon* Griff. and *O. nivara* Sharma et Shastry from Asia, *O. longistaminata* A. Chev. et Roehr. and *O. barthii* A. Chev. from sub–Saharan Africa, *O. meridionalis* Ng from Australia, and *O. glumaepatula* Steud. from South America. Juliano et al. (1998) showed by multivariate analysis of morphological characters that a group of samples that matched the original description of *O. glumaepatula* by Steudel in 1854 (Chevalier, 1952) collected from the lower Amazon River in Brazil, and a few from French Guiana and Surinam were morphologically distinct from *O. rufipogon* and *O. nivara* and thereby justified its status as a good taxonomic species. Some New World diploids, however, lacked the flat and narrow grain and long and wide sterile lemmas characteristic of the 'typical' *O. glumaepatula* and these either formed a distinct cluster or grouped with the Asian species indicating that the New World diploids are not a homogeneous group. The taxonomic status of these other accessions remains unclear.

Wild rice samples from South America, described as *O. glumaepatula* have recently been used as a source of cytoplasmic male sterility in rice (IRRI 1994) and a possible source of resistance to black-streaked dwarf fijivirus (Yang et al., 1991). The elucidation of the genetic relationship of *O. glumaepatula* with the other AA genome species should provide additional information on its possible performance in hybridization with the cultigen. The biosystematic relationship of *O. glumaepatula* with the other AA genome species has yet to be systematically studied. We conducted hybridization studies to evaluate the relationship between South American diploids confirmed as *O. glumaepatula* (Juliano et al., 1998) and the other AA genome species, *O. nivara*, *O. rufipogon*, *O. meridion-*

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Table 1. Spikelet and pollen fertilities of parental species used in the hybridization of AA genome *Oryza* species from the New World, Asia, and Australia

Species	IRGC Acc. No.	Origin	Mean fertility %	
			Pollen	Spikelet
O. glumaepatula[1]	100924	Brazil	88.4	–[2]
	100968	Surinam	85.9	50.2
	100970	Brazil	91.8	–[2]
	105465	French Guiana	48.7	47.8
	105687	Brazil	97.9	89.2
	105689	Brazil	75.6	66.6
New World diploids	100961	Cuba	11.0	0.8
	103810	Venezuela	93.8	64.6
	103811	Venezuela	95.6	–[2]
	103812	Venezuela	94.5	21.4
	104386	Brazil	67.1	64.3
	105561	Colombia	77.3	21.3
O. nivara	100593	Taiwan	79.1	44.9
	105391	Thailand	91.9	77.7
	106185	India	89.0	69.1
O. rufipogon	100588	Taiwan	95.2	59.6
	105567	Indonesia	93.9	51.7
	106135	India	89.0	69.1
O. meridionalis	101147	Australia	76.1	76.0
	105300	Australia	81.3	65.5

[1] IRGC accession 105672 from Brazil was included in hybridization but was not examined for pollen and spikelet fertilities due to insufficient number of panicles.
[2] Spikelet fertility was not obtained due to limited number of panicles.

alis, as well as the other New World diploid accessions which did not appear to be 'typical' *O. glumaepatula*.

Materials and methods

Establishment of parental materials

The parental species used in the hybridization program included seven accessions of *O. glumaepatula*, three accessions each of *O. nivara* and *O. rufipogon*, two accessions of *O. meridionalis*, and six other diploid accessions from the New World (Table 1). The seeds were obtained from the International Rice Genebank (IRG) at the International Rice Research Institute (IRRI), Philippines. Seed dormancy was broken by heat treatment at 50 °C for 7 days and hull removal. Seeds were germinated in March 1995 on MS medium (Murashige & Skoog, 1962) for two weeks. The seedlings were transferred to culture solution (pH5) (Yoshida et al., 1976) and allowed to grow in an indoor cabinet for two weeks under controlled conditions (21 °C/29 °C, 70% RH, 12H/12H 1000 μ ϵ $m^{-2}s^{-2}$ light intensity). The seedlings were transplanted singly to 30 cm pots and maintained under ambient conditions at about 30 °C in the IRG screenhouse.

Morphological and fertility characterization

Each accession was represented by 5 to 10 plants and scored for selected morphological characters that would assist in the identification of any hybrid progeny. To confirm the hybrid nature of progenies, their isozyme patterns based on 10 enzyme systems were compared to parental materials (data not presented). Pollen fertility was based on the frequency of stainable mature pollen grains from five individual spikelets in I_2-KI solution for 3 min. Pollen grains were considered fertile only when round, filled, and fully stained. For each plant, natural spikelet fertility was obtained by deriving the ratio of filled grains to total spikelets from five individually bagged panicles. For each accession, mean pollen and spikelet fertilities were obtained from all plants used in hybridization studies.

Hybridization and establishment of hybrids

Intraspecific and interspecific crosses were made in June to September 1995 according to the combinations shown in Tables 2 to 7. Since there were different morphological groups within the New World diploids, these were treated as interspecific crosses. Emasculation and pollination were carried out following the procedures described by Naredo et al. (1997). The seeds were harvested 10 days after pollination and immediately germinated on MS medium and maintained similarly as the parental materials. At most, five F_1 hybrids were maintained for each cross and scored for selected morphological characters and compared to their parents. Pollen and spikelet fertilities were obtained similarly as the parental materials.

Results

Crosses and development of hybrids

Thirteen intraspecific (Table 2) and 79 interspecific crosses (Tables 3–7) were made with a total of 21,255 spikelets pollinated. All intraspecific cross-

Table 2. Seed set from intraspecific crosses of *O. glumaepatula* and other AA genome *Oryza* species from Asia and Australia and fertilities of hybrids.

Hybrid combination	No. of pollinated spikelets	Seed set		F_1 hybrids		Fertility of F_1 hybrids (%)	
		No.	%[1]	No.	%[2]	Pollen	Spikelet
O. glumaepatula × *O. glumaepatula*							
100970 × 105687	70	34	48.6	8	11.4	78.4	88.7
105465 × 105687	149	18	12.1	1	0.7	67.1	96.8
105465 × 105689	146	50	34.2	25	17.1	87.8	75.0
105687 × 100970	94	38	40.4	17	18.1	68.8	92.6
105687 × 105465	111	16	14.4	8	7.2	81.2	91.8
105689 × 105465	28	11	39.3	6	21.4	75.2	81.2
		Mean	31.5		12.6	76.4	87.7
O. nivara × *O. nivara*							
100593 × 105391	471	99	21.0	11	2.3	85.9	23.1
105391 × 100593	116	47	40.5	29	25.0	78.5	2.2
106185 × 105391	44	4	9.1	2	4.5	61.1	71.1
		Mean	23.5		10.6	75.2	32.1
O. rufipogon × *O. rufipogon*							
100588 × 106135	323	72	22.3	43	13.3	78.0	61.7
106135 × 100588	109	5	4.6	2	1.8	82.1	75.8
		Mean	13.4		7.6	80.1	68.8
O. meridionalis × *O. meridionalis*							
101147 × 105300	158	20	12.6	12	7.5	4.1	0
105300 × 101147	104	10	9.6	10	9.6	3.5	0.2
		Mean	11.1		8.6	3.8	0.1

[1] % seed set = seed set/no. of spikelets pollinated
[2] % hybrid = no. of hybrid/no. of spikelets pollinated

es successfully produced seeds (Table 2). In the *O. glumaepatula* × *O. glumaepatula* crosses, seed set ranged from 12.1% (105465 × 105687) to 48.6% (100970 × 105687) with a mean of 31.5%. The *O. nivara* × *O. nivara* crosses produced seeds ranging from 9.1% to 40.5% with a mean of 23.5%. The *O. rufipogon* × *O. rufipogon* and *O. meridionalis* × *O. meridionalis* crosses had a mean seed set of only 13.4% and 11.1%, respectively.

As with the intraspecific crosses, most interspecific crosses also produced seeds except for one *O. glumaepatula* × *O. nivara* cross, 100970 × 106185 (Table 3), the cross between a New World diploid and *O. nivara*, 105561 × 100593 (Table 5), and the cross between *O. rufipogon* and one of the New World diploids, 106135 × 103810 (Table 6).

Crosses of *O. glumaepatula* as the female parent showed a wide variation in seed set ranging from 0 to 54.1% (Table 3). Seed set was generally high in crosses with IRGC accessions 100924, 100968, 105672, and 105687 and low in crosses with IRGC 100970 and 105465. A high seed set ranging from 45.4% to 54.1% with a mean of 48.4% was observed in crosses with three other diploids from the New World (IRGC 103810, 105561, and 103812). In crosses with *O. nivara* (IRGC 100593, 105391, and 106185), seed set was highly variable ranging from 0 to 51.8% with a mean of 25.9% and with *O. rufipogon* (IRGC 100588 and 106135) from 4.5 to 44.7% with a mean of 22.5%. The *O. glumaepatula* × *O. meridionalis* (IRGC 105300) crosses showed a low seed set ranging only from 6.8% to 15.4%.

Mean seed set in crosses with *O. glumaepatula* as the male parent was generally lower than in the reciprocal crosses (Table 4). In crosses with the other New World diploids, seed set varied from 2.2% to 39.8% with a mean of 15.2%. Seed set ranged from 10.2% to 53.1% with a mean of 32.7% in crosses with

Table 3. Seed set of interspecific crosses of *O. glumaepatula* as female with other New World diploids and AA genome *Oryza* species from Asia and Australia and fertilities of hybrids.

Hybrid combination	No. of spikelets pollinated	Seed set		F_1 hybrids		Mean fertility%	
		No.	%	No.	%	Pollen	Spikelet
O. glumaepatula × New World diploids							
100968 × 103810	733	335	45.7	58	–[1]	2.6	0.7
100968 × 105561	695	376	54.1	49	–[1]	89.7	79.6
105687 × 103812	44	20	45.4	2	4.5	72.1	78.8
		Mean	48.4			54.8	53.0
O. glumaepatula × *O. nivara*							
100924 × 106185	56	29	51.8	11	19.6	2.9	0.5
100968 × 100593	365	135	37.0	54	–[1]	2.9	4.6
100970 × 106185	30	0	0				
105465 × 105391	231	32	13.8	10	4.3	0.2	8.2
105687 × 106185	96	26	27.1	3	3.1	8.4	8.2
		Mean	25.9			3.6	5.4
O. glumaepatula × *O. rufipogon*							
100968 × 100588	157	31	19.7	9	5.7	0.2	0
100968 × 106135	193	53	27.5	4	2.1	9.4	0.9
100970 × 106135	106	20	18.9	5	4.7	21.3	2.9
105465 × 100588	168	11	6.5	5	3.0	0	0.8
105465 × 106135	358	16	4.5	12	3.4	21.0	3.6
105672 × 106135	53	19	35.8	16	30.2	19.2	6.9
105687 × 106135	161	72	44.7	15	9.3	8.0	3.9
		Mean	22.5			11.3	2.7
O. glumaepatula × *O. meridionalis*							
100970 × 105300	103	7	6.8	1	1.0	0	0.2
105465 × 105300	143	22	15.4	10	7.0	0	1.0
105687 × 105300	163	19	11.6	4	2.4	0	1.0
		Mean	11.3			0	0.7

[1] Percentage of hybrids was not estimated because not all seeds were germinated.

O. nivara. In crosses with *O. rufipogon,* this varied from 0.8% to 48.1% with a mean of 15.8%, and with *O. meridionalis* seed set ranged from 2.0% to 27.7% with a mean of 11.5%.

The interspecific crosses of the other New World diploids as female with the Asian or Australian species produced seeds ranging from 0 to 36.4% (Table 5). In crosses among the New World diploids, seed set ranged from 3.7% to 22.1%. Seed set in crosses with *O. nivara* varied greatly from 0 to 32.6% with a mean of 11.4% and in *O. rufipogon* from 2.0% to 36.4% with a mean of 14.4%. The cross with *O. meridionalis* showed only 4.4% seed set. In reciprocal crosses, the seeds produced ranged from 0 to 43.6%. When used as male parent (Table 6), crosses with the *O. nivara* accessions (IRGC 100593 and 105391) showed high seed set varying from 33.1% to 43.6% while with *O. rufipogon* as female, the highest seed set was only 15.7% (105567 × 103811).

Differences in seed set were observed in reciprocal crosses between the two Asian species (Table 7). Seed set was generally higher (mean = 34%) in *O. nivara* × *O. rufipogon* crosses than in *O. rufipogon* × *O. nivara* crosses (mean = 9.3%). Crosses of *O. nivara* with *O. meridionalis* resulted in less than 5% seed set.

There was a large discrepancy between the total seed set and the number of hybrids obtained in both intraspecific (Table 2) and interspecific crosses (Table 3–7). In the *O. glumaepatula* intraspecific crosses, the mean frequency of hybrids was only 12.6%. The

Table 4. Seed set of interspecific crosses of *O. glumaepatula* as male with other New World diploids and AA genome *Oryza* species from Asia and Australia and fertilities of hybrids.

Hybrid combination	No. of spikelets pollinated	Seed set		F_1 hybrids		Mean fertility %	
		No.	%	No.	%	Pollen	Spikelet
New World diploids × *O. glumaepatula*							
100961 × 100968	428	18	4.2	2	0.5	18.0	0.5
100961 × 105465	90	2	2.2	0			
103810 × 100968	1520	319	21.0	67	4.4	2.7	0.3
103812 × 105465	100	6	6.0	3	3.0	79.6	86.6
103812 × 105687	112	16	14.3	2	1.8	68.0	70.8
104386 × 105689	133	53	39.8	35	26.3	0.2	0.4
105561 × 100968	273	52	19.0	37	13.6	80.0	84.4
		Mean	15.2			41.4	40.5
O. nivara × *O. glumaepatula*							
100593 × 100968	168	43	25.6	29	17.3	0.8	0.1
105391 × 100968	39	4	10.2	1	2.6	1.3	0
105391 × 105465	96	51	53.1	17	17.7	2.4	3.9
106185 × 105465	211	69	32.7	49	23.2	0	0.6
106185 × 105687	281	118	42.0	57	20.3	3.3	1.5
		Mean	32.7			1.6	1.2
O. rufipogon × *O. glumaepatula*							
100588 × 100968	245	19	7.8	8	3.3	2.3	0.5
100588 × 105689	233	35	15.0	21	9.0	0.5	0
105567 × 105687	99	7	7.1	4	4.0	1.6	0
106135 × 100924	55	13	23.6	5	9.1	17.0	4.7
106135 × 100968	67	2	3.0	2	3.0	35.1	2.0
106135 × 100970	119	1	0.8	1	0.8	12.7	0.4
106135 × 105465	80	17	21.2	14	17.5	5.4	0.8
106135 × 105672	27	13	48.1	3	11.1	14.7	3.7
		Mean	15.8			11.2	1.5
O. meridionalis × *O. glumaepatula*							
105300 × 100970	123	6	4.9	2	1.6	0	0
105300 × 105465	198	4	2.0	2	1.0	0	1.2
105300 × 105687	130	36	27.7	30	23.1	0	0.5
		Mean	11.5			0	0.6

O. rufipogon intraspecific hybrids produced the lowest mean number of hybrids at 7.6%. In the interspecific crosses the highest frequency of hybrids (30.2%) was obtained from the *O. glumaepatula* × *O. rufipogon* cross (105672 × 106135) although the hybrid frequencies in some crosses were not estimated because not all seeds were germinated. The difference in seed set and actual number of hybrids was due to abortion of seedling growth upon germination in medium, complete failure to germinate, and in some cases, absence of an embryo.

Morphological characterization of parents and hybrids

Hybrids were obtained from all intraspecific and interspecific combinations. However, some crosses of New World diploids with *O. glumaepatula* (100961 × 105465), with *O. meridionalis* (103812 × 105300), and with *O. nivara* (103812 × 106185), and the *O. nivara* × *O. meridionalis* cross (106185 × 105300) failed to produce hybrids.

Table 5. Seed set of interspecific crosses between other New World diploids as female and AA genome *Oryza* species from Asia and Australia and fertilities of hybrids.

Hybrid combination	No. of spikelets pollinated	Seed set		F_1 hybrids		Mean fertility %	
		No.	%	No.	%	Pollen	Spikelet
New World diploids × New World diploids							
100961 × 103810	378	14	3.7	4	1.1	1.4	0.2
103810 × 105561	874	193	22.1	52	6.0	0.7	1.2
105561 × 103810	373	60	16.1	27	7.2	5.0	4.4
		Mean	14.0			2.4	1.9
New World diploids × *O. nivara*							
100961 × 100593	1091	96	8.8	33	3.0	5.3	12.8
100961 × 105391	372	12	3.2	5	1.3	22.1	5.4
103810 × 100593	542	177	32.6	44	8.1	80.6	69.4
103812 × 106185	122	14	11.5	0			
104386 × 105391	308	38	12.3	12	3.9	52.6	7.3
105561 × 100593	41	0	0				
		Mean	11.4			40.2	39.5
New World diploids × *O. rufipogon*							
100961 × 100588	559	42	7.5	23	4.1	7.8	2.1
100961 × 105567	95	2	2.1	1	1.1	0	0.6
100961 × 106135	651	13	2.0	3	0.5	29.6	13.8
103810 × 106135	143	52	36.4	31	21.7	87.2	62.3
103811 × 105567	107	12	11.2	3	2.8	4.7	0.5
103811 × 106135	163	15	9.2	4	2.4	7.2	0
103812 × 106135	113	13	11.5	4	3.5	0	4.2
104386 × 100588	226	79	35.0	41	18.1	65.9	70.5
		Mean	14.4			25.3	19.2
New World diploids × *O. meridionalis*							
103812 × 105300	160	7	4.4	0			

The hybrid nature of the progenies was confirmed using morphological and isozyme markers (data not presented). In some of the intraspecific crosses, determining the hybrid nature of the plant was difficult because the parents were morphologically very similar. In the cross 105687 × 105465, for example, the parents shared most characters, except for apiculus color, awn color, and panicle type. The hybrid showed the female trait for the last two traits but had a distinct red apiculus characteristic of the male parent. Determining the hybrid nature of plants derived from interspecific crosses was easier because the parents were morphologically dissimilar. The hybrids of the *O. nivara* × *O. glumaepatula* cross 100593 × 100968, for example, showed a character intermediate to both parents (ligule color), the female parent character for panicle exsertion, and most of the male traits.

Fertility of parents and intraspecific hybrids

Table 1 shows the fertility of the parental accessions. Spikelet fertility in most of the parents was lower than the frequency of stained pollen grains. Pollen stainability among *O. glumaepatula* accessions was generally high (>75%) except for IRGC 105465 which had only 48.7% pollen stainability and also showed low spikelet fertility of 47.8%. The New World diploid accession IRGC 100961 was highly sterile with 11.0% stainable pollen grains and 0.8% spikelet fertility. The other New World diploids showed high pollen stainability although spikelet fertility varied only from 21.3% to 64.6%. *O. nivara*, *O. rufipogon*, and *O. meridionalis* exhibited good fertility with more than 75% stainable pollen grains. Spikelet fertility in these species ranged from 44.9% (IRGC 100593) to 77.7% (IRGC 105391).

Table 6. Seed set of interspecific crosses between other New World diploids as male and AA *Oryza* genome species from Asia and Australia and fertilities of hybrids.

Hybrid combination	No. of spikelets pollinated	Seed set		F_1 hybrids		Mean fertility %	
		No.	%	No.	%	Pollen	Spikelet
O. nivara × New World diploids							
100593 × 103810	402	133	33.1	54	13.4	76.7	72.3
100593 × 105561	101	44	43.6	1	1.0	3.7	3.6
105391 × 104386	149	52	34.9	41	27.5	83.5	0.1
		Mean	37.2			54.6	25.3
O. rufipogon × New World diploids							
100588 × 104386	232	30	12.9	18	7.8	79.8	68.5
105567 × 103811	108	17	15.7	9	8.3	2.1	1.1
106135 × 103812	236	16	6.8	8	3.4	3.6	8.2
106135 × 103810	56	0	0				
		Mean	8.8			28.5	25.9
O. meridionalis × New World diploid							
105300 × 103812	439	69	15.7	43	9.8	0.2	0.1

Table 7. Seed set of interspecific crosses among Asian and Australian AA genome *Oryza* species and fertilities of hybrids.

Hybrid combination	No. of spikelets pollinated	Seed set		F_1 hybrid		Mean fertility %	
		No.	%	No.	%	Pollen	Spikelet
O. nivara × *O. rufipogon*							
100593 × 100588	240	134	55.8	32	13.3	80.3	68.5
100593 × 106135	363	157	43.2	40	–[1]	58.9	82.6
105391 × 100588	341	93	27.2	44	12.9	63.1	10.6
105391 × 106135	51	13	25.5	7	13.7	83.9	55.0
106185 × 106135	126	23	18.2	18	14.3	80.4	74.8
		Mean	34.0			73.3	58.3
O. rufipogon × *O. nivara*							
100588 × 100593	300	39	13.0	5	1.7	76.3	80.7
100588 × 105391	489	67	13.7	28	5.7	76.9	34.1
106135 × 100593	122	4	3.3	2	1.6	62.7	78.2
106135 × 105391	188	10	5.3	5	2.7	86.7	84.0
106135 × 106185	96	11	11.4	5	5.2	91.0	64.3
		Mean	9.3			78.7	68.3
O. meridionalis × *O. nivara*							
105300 × 106185	147	5	3.4	5	3.4	12.8	4.2
O. nivara × *O. meridionalis*							
106185 × 105300	146	1	0.7	0			

[1] Percentage of hybrids was not estimated because not all seeds were germinated.

The range of pollen and spikelet fertility in most intraspecific hybrids (Table 2) was comparable to the fertility of the parents, except for the *O. meridionalis* intraspecific hybrids which were highly sterile. Among the *O. glumaepatula* intraspecific hybrids, pollen stainability ranged from 67.1% to 87.8% and spikelet fertility from 75.0% to 96.8%. The *O. nivara* intraspecific hybrids had 75.2% mean pollen stainability. The spikelet fertility in these hybrids was lower than the frequency of stained pollen grains, especially in the hybrid 105391 × 100593 which was highly sterile with only 2.2% spikelet fertility. The *O. rufipogon* intraspecific hybrids showed a mean pollen stainability of 80.1% and mean spikelet fertility of 68.8%.

Fertility of interspecific hybrids

The F_1 interspecific hybrids showed more variable fertility than the hybrids from intraspecific crosses. Tables 3 and 4 show the fertility of hybrids obtained from reciprocal crosses of *O. glumapeatula* with other New World diploids and the Asian and Australian AA genome species. In reciprocal crosses involving *O. glumaepatula* and the New World diploid accessions IRGC 105561 and 103812, fertile hybrids were obtained with pollen stainability ranging from 68.0% (103812 × 105687) to 89.7% (100968 × 105561) and spikelet fertility from 70.8% (103812 × 105687) to 86.6% (103812 × 105465). Hybrids of *O. glumaepatula* with the other New World diploids, IRGC 100961, 103810, and 104386 were highly sterile with the highest pollen stainability at only 18% and spikelet fertility of 0.7%. The *O. glumaepatula* and *O. nivara* hybrids exhibited 0 to 8.4% pollen stainability and 0 to 8.2% spikelet fertility. The *O. glumaepatula* and *O. rufipogon* crosses produced hybrids with 0 to 35.1% pollen stainability and 0 to 6.9 spikelet fertility. The interspecific crosses of *O. glumaepatula* with *O. meridionalis* produced entirely pollen sterile hybrids although most crosses showed about 1% spikelet fertility.

The fertility of hybrids obtained from crosses of the other New World diploids with the Asian and Australian AA genome species are presented in Tables 5 and 6. Reciprocal crosses between the two New World diploid accessions IRGC 103810 and 105561 and the cross 100961 × 103810 produced highly sterile hybrids. IRGC 103811, 103812, and 105561 also produced highly sterile hybrids with less than 10% pollen and spikelet fertility when crossed with *O. rufipogon*, *O. nivara*, or *O. meridionalis*. IRGC 100961 produced highly sterile hybrids with the Asian and Australian

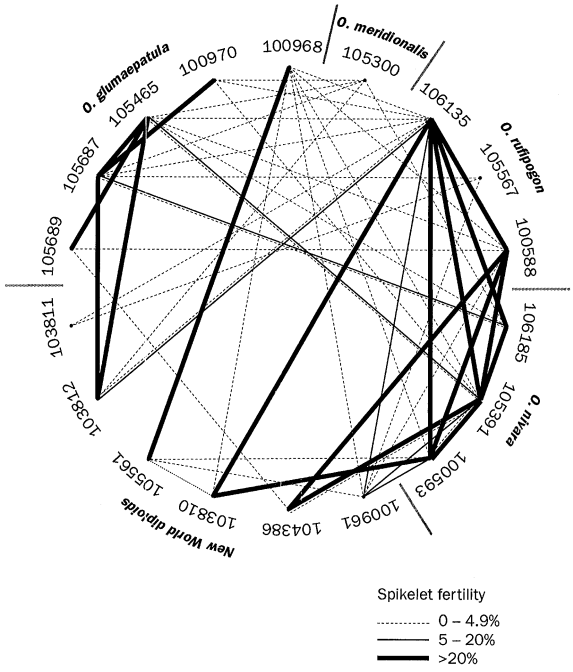

Fig 1. Crossing polygon of AA genome species from Asia, Australia, and the New World expressed in terms of spikelet fertility.

species, although a cross with *O. nivara*, 100961 × 105391 produced hybrids with 22.1% pollen fertility and hybrids obtained from the cross with *O. rufipogon*, 100961 × 106135 showed 29.6% pollen fertility and 13.8% spikelet fertility. When IRGC 103810 and 104386 were crossed to *O. rufipogon* and *O. nivara*, the hybrids formed were generally fertile, with pollen stainability ranging from 52.6% (104386 × 105391) to 87.2% (103810 × 106135) and spikelet from 0.1% (105391 x 104386) to 72.3% (100593 × 103810). These were comparable to the fertility of *O. nivara* and *O. rufipogon* hybrids which showed a pollen stainability range of 58.9% to 91.0%, and spikelet fertility range of 10.6% to 84.0% (Table 7). The hybrids produced from the *O. meridionalis* × *O. nivara* cross were highly sterile although there were some differences in fertilities between reciprocal crosses (Table 7).

Discussion

The results from this study clarify the relationship between *O. glumaepatula* with the other New World diploid wild rices, and the Asian and Australian AA genome species. Crossability of *O. glumaepatula* with these species was highly variable as indicated by dif-

ferences in seed set within combinations, although comparatively low seed set was observed in crosses with *O. meridionalis* than with the other New World diploids, *O. nivara,* and *O. rufipogon*. However, the relative ease by which seeds and hybrids were obtained from all combinations does indicate a relatively close genetic relationship among the species. This is confirmed by the normal pairing of chromosomes in their hybrids indicating close genomic relationships (Lu et al., 1998).

Figure 1 summarizes these relationships expressed in terms of spikelet fertility. The *O. glumaepatula* and *O. rufipogon* or *O. nivara* hybrids were highly sterile with only a few hybrids showing greater than 5% fertility. The reproductive isolation of *O. glumaepatula* from the Asian species confirms its status as a distinct species as was previously proposed on the basis of morphological studies (Juliano et al., 1998) and RAPD analysis (Martin et al., 1997). The figure also shows the clear isolation of *O. meridionalis* from both *O. glumaepatula* and the two Asian AA genome species. Crosses of the Australian taxon with *O. glumaepatula* produced almost sterile hybrids with less than 1% mean fertility and the hybrids produced with the cross from *O. nivara* showed only slightly higher fertility. This suggests a relatively distant relationship between *O. meridionalis* and the other species studied supporting the earlier report of the Australian taxon showing the maximum genetic distance from all AA genome species by isozyme analysis (Second, 1986). In general, AA genome rice species from different continents have developed strong isolation mechanisms.

The fertility of hybrids produced from crosses of the other New World diploid samples with *O. glumaepatula*, *O. nivara*, and *O. rufipogon*, however, were highly variable as shown in Figure 1. Although accessions IRGC 103812 and 105561 show *O. rufipogon*-like characters (Juliano et al., 1998), these produced fertile hybrids with *O. glumaepatula* but not with *O. rufipogon* or *O. nivara*. The strong reproductive isolation of these accessions from the Asian taxa precludes their classification as *O. rufipogon,* and perhaps are better considered as variants only of *O. glumaepatula*. Two other New World diploid accessions IRGC 103810 and 104386 formed highly sterile hybrids with *O. glumaepatula,* but were interfertile with the Asian taxa. These samples, with *O. nivara*-like characters (Juliano et al., 1998), are clearly not *O. glumaepatula* and might have been co-introduced into South America as a weedy type along with the cultigen. This idea is supported by the report that the large subunit of the Fraction 1 protein of IRGC 103810 is similar to that in *O. sativa* (Pental and Barnes 1985). The other New World diploid, IRGC 100961 formed sterile hybrids with *O. glumaepatula* and other South American diploids. However, hybrids formed with the Asian taxa were relatively more fertile, with some showing more than 5% spikelet fertility. The passport data for this accession revealed that it was collected from a muddy swamp adjacent to rice fields. The collection was identified as a natural hybrid between '*O perennis*' and *O. sativa*, which explains its affinity to the Asian species.

Our results have shown that interspecific hybridization is a good method to study species relationship among the AA genome *Oryza* species. We have confirmed the status of *O. glumaepatula* and other diploid accessions from the New World (IRGC 103812 and 105561) as an independent species. The study also showed the possible presence of natural hybrids or weedy types in the New World.

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