CSIRO PUBLISHING
www.publish.csiro.au/journals/app *Australasian Plant Pathology*, 2008, **37**, 357–364

DNA fingerprint and pathotype diversity of *Pyricularia oryzae* populations from Argentina

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Abstract. The genetic diversity of the rice blast pathogen, *Pyricularia oryzae*, was analysed in rice-growing provinces of Argentina. A total of 161 isolates of the fungus was collected from 15 rice cultivars at nine locations during 2000–05 and characterised using *Pot2*-DNA fingerprinting. Based on DNA analysis (isolates with ≥70% band similarity), five lineages were identified and designated A, B, C, D and E, with 11, 22, 4, 1 and 4 haplotypes identified, respectively. The predominant lineage, B, representing 38% of the collected isolates, was recovered from four cultivars in five locations. In contrast to lineages A and B, which did not contain a dominant haplotype, a single haplotype predominated in lineages C and E. Isolates representing all haplotypes were examined for virulence on a set of differential rice cultivars, near-isogenic lines and commercial cultivars commonly grown in Argentina, revealing 41 pathotypes and 24 international races. There was no significant association between DNA fingerprint similarities and pathotypes. Overall, these data indicated that populations of *P. oryzae* in Argentina are genetically simple and predominantly clonal yet have a high pathotype diversity.

Additional keywords: *Magnaporthe oryzae*, population structure, rice breeding.

Introduction

Rice blast caused by the fungus *Pyricularia oryzae* Cavarra, the anamorph of *Magnaporthe oryzae* B.C. Couch (Couch and Kohn 2002), is the most destructive disease of rice worldwide (Ou 1985). The pathogen produces necrotic lesions on leaves, nodes, necks and panicles of plants. Rice blast epidemics are often severe especially in temperate and subtropical ecosystems. Incorporation of resistance into commercial cultivars has been the preferred means of managing the disease. However, cultivars released as resistant often show high levels of susceptibility within a few years (Correa-Victoria and Zeigler 1995). Resistance breakdown has been attributed to extreme variability of virulence in the pathogen population, inappropriate use of rice resistance genes, or a combination of both (Zeigler *et al*. 1994). Successful breeding of cultivars with durable resistance to blast disease requires information on global and regional population diversity of *P. oryzae*, on genetic composition and on virulence spectra. In this way, molecular markers have greatly increased the knowledge about the fungal population diversity. Dispersed, repetitive DNA sequences such as *Magnaporthe grisea* repeat (MGR) have been used for characterising populations of the blast pathogen (Hamer *et al*. 1989). MGR 586 restriction fragment length polymorphism (RFLP) fingerprinting showed that many populations contain a limited number of lineages (Levy *et al*. 1991, 1993; Xia *et al*. 1993; Chen *et al*. 1995; Roumen *et al*. 1997; Xia *et al*. 2000). Because of difficulties involved in RFLP analysis of large numbers of samples, another simple method based on PCR was developed by George *et al*. (1998). This technique enables the amplification of *P. oryzae* genome sequences that lie between inverted-repeat transposable elements, named *Pot2*, distributed in ~100 copies throughout the fungal genome (Kachroo *et al*. 1994). A close correspondence between lineages based on *Pot2*-DNA fingerprinting and those obtained by RFLP was demonstrated (George *et al*. 1998).

P. oryzae is noted for expressing a large number of pathotypes, especially in rainfed-growing areas where the environment is most favourable for epidemic development (Ou 1980). The examination of virulence diversity of *P. oryzae* continues to be an active subject of research and different results have been reported on the degree of race variability. Although it was shown that some *P. oryzae* populations have a simple relationship between lineage and pathotype (Levy *et al*. 1991), other studies indicate that a complex relationship exists and a great diversity of pathotypes may occur within lineages for many rice cultivars (Correa-Victoria *et al*. 1994; Zeigler *et al*. 1995). Breeding for resistance may be successful if it is based on knowledge of *P. oryzae* population diversity. DNA fingerprinting, race composition, and near-isogenic lines (NILs) with major blast resistance genes for accurately assessing pathotypes, could contribute to this goal.

In Argentina, irrigated rice is mainly cultivated in three provinces, Corrientes, Entre Ríos and Santa Fe, covering an area of ~200 000 ha. Nearly all production is exported, making Argentina the second-ranked exporter of rice in Latin America (Fernández 2005). Blast is the most important rice disease in Argentina. Although rice blast was first reported in this country ~70 years ago (Fernandez Valliela 1978), information about the

 10.1071/AP08010 0815-3191/08/040357

pathogen in Argentina is limited to the determination of pathotype diversity of 12 isolates collected in different rice-growing regions, representing 10 races of international rice blast differentials (IRBDs) (Cordo *et al*. 1980).

The aims of the present study were to evaluate the genetic diversity of *P. oryzae* populations sampled on Argentinean rice cultivars between 2000 and 2005 using the *Pot2*-DNA fingerprinting approach, and to identify the pathotypes using IRBDs, local cultivars and NILs.

Methods

Collection of samples

Between 2000 and 2005, rice samples showing typical blast symptoms on leaves and panicles were collected from nine locations distributed in the Corrientes, Entre Ríos and Santa Fe provinces. Other samples included in this study were collected from nurseries of the Rice Experimental Station Julio Hirschhorn at Los Hornos, in Buenos Aires province (Fig. 1). At these sites, rice is cultivated as a single crop per year in an irrigated system. Climatic conditions, cultural practices and use of blast-resistant cultivars in some areas restrict blast epidemics, causing the epidemics to become sporadic. Samples were collected from cvv. Brillante, Carnaroli, Colonia Mascias 5 SCA, Diamante, El Paso 144, Fortuna, La Plata Mochi, Itapé and Yeruá, and from the experimental lines G 4001, G 4005, H 289 13–2-2–1, H 316, H 397 1–2-2–1 and H 397–6-1–2. In each year, leaf and neck panicle samples were collected during December and between February and May. Ten to 15 leaves and panicles were collected randomly in each field and at least five fields were sampled in each region. A total of 161 isolates of the fungus were collected from 15 rice cultivars and six lines at nine locations.

Isolation and maintenance of P. oryzae *cultures*

Infected samples were washed in running water for ~30 min and surface sterilised in 0.01% mercuric chloride solution. Sporulation was induced on leaves and necks by 24-h incubation in Petri dishes at $26 \pm 2°C$ and 100% relative humidity. Monoconidial isolates were obtained by transferring a germinated conidium to a Petri dish containing fresh, rice polish agar (Tuite 1969). Each culture was overlaid with several sterilised filter paper sections and incubated at $26 \pm 2°C$. After 10–14 days of incubation, the colonised filter paper sections were lifted from the agar surface, placed in small coin envelopes, allowed to dry for 3 days at room temperature and stored at $-20°C$ as described by Valent *et al*. (1986).

DNA preparation and PCR

Pyricularia grisea isolates were grown for 5–8 days at room temperature ($21 \pm 2°C$) in 50 mL of YEG medium (Valent *et al*. 1986) on a rotary shaker. Mycelia were harvested under vacuum, stored at $-20°C$ and then lyophilised. About 100 mg of powered mycelia were used for DNA isolation following a modified version of the cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson 1980) as follows. In total, 750 µL of extraction buffer (100 mM Tris-HCl, pH 8, 100 mM EDTA, 250 mM NaCl, 2% CTAB) and

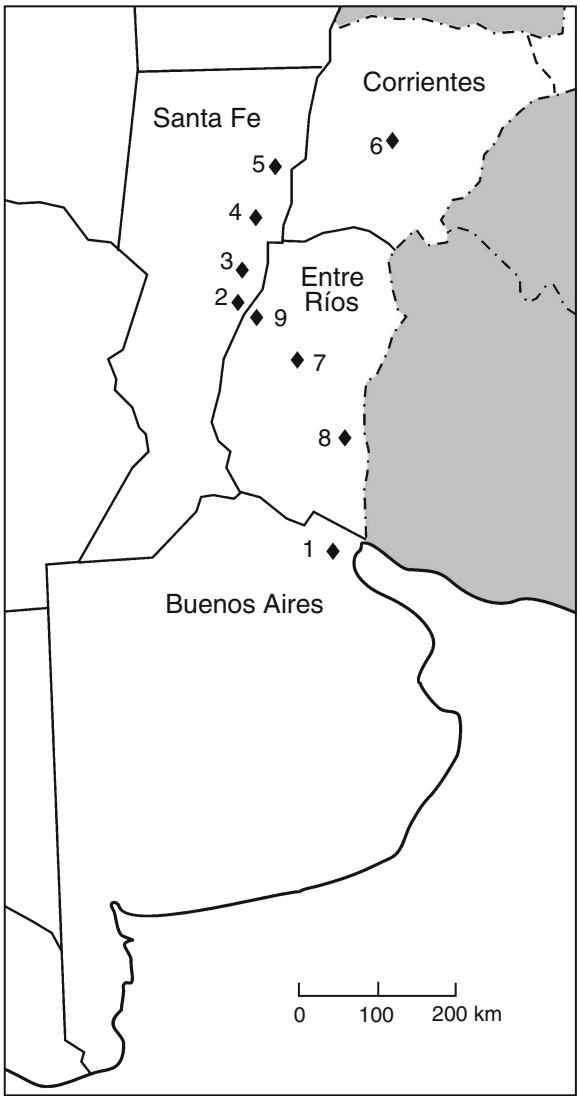

Fig. 1. Location of *Pyricularia oryzae* collection sites in the Argentinean provinces. (1) The Rice Experimental Station Julio Hisrchorn, Los Hornos, (2) Yataí, (3) San Jacinto, (4) San José, (5) San Joaquín, (6) Mercedes, (7) Villaguay, (8) Urdinarrain and (9) La Paz.

15 µL of 2-mercaptoethanol were added to the lyophilised mycelia and incubated at 65°C for 30 min. Cellular proteins were precipitated with 300 µL of 3 M potassium acetate (pH 4.8). After centrifugation at maximum speed in a microcentrifuge (Hermle Labortechnik GmbH, Wehingen, Germany), the supernatant was transferred to a new tube, and extracted with 500 µL of phenol : chloroform : isoamyl alcohol (25 : 24 : 1) and chloroform : isoamyl alcohol (24 : 1) until the interface became transparent. Nucleic acids were precipitated by adding 750 µL of cold isopropanol followed by incubation at 4°C for 30 min. After centrifugation at 17 500*g* for 5 min, the pellet was rinsed twice with 500 µL of 70% ethanol, air-dried and dissolved in 100 µL of Tris-EDTA buffer [10 mM Tris-HCl (pH 8) and 1 mM EDTA]. DNA quality and concentration were determined by electrophoresis on 0.9%

agarose gels. PCR amplifications were performed using the oligonucleotides *Pot2–1* and *Pot2–2* as primers reported by George *et al*. (1998). PCR reactions were conducted in a PTC-100 thermal cycler (MJ Research, Waltham, MA) in a final volume of 25 µL containing 100 ng of template DNA, 10 mM TRIS-HCl (pH 9.2), 25 mM KCl, 1.5 mM $MgCl_2$, 0.2 µM each of four dNTPs, 0.5 µM of each primer and 1 unit of Taq polymerase (Invitrogen, Carlsbad, CA). Thermal cycling conditions involved an initial denaturation step at 95°C for 2.5 min, four cycles of 94°C for 1 min, 62°C for 1 min and 65°C for 10 min, followed by 26 cycles of 94°C for 30 s, 62°C for 1 min and 65°C for 10 min and a final extension at 65°C for 15 min. Fifteen µL of PCR products were separated by electrophoresis in a gel containing 0.5% agarose and 0.75% Synergel (Diversified Biotech, Newton, MA) in 0.5 ×TBE buffer (90 mM TRIS-HCl, pH 8, 90 mM boric acid and 2 mM EDTA). Gels were run for 6 h at 120 V, stained with ethidium bromide and photographed with a FOTODYNE system (Hartland, WI).

Resolved bands ranging in size from 400 bp to ~5 kb were scored regardless of intensity. Only bands that amplified consistently in at least two independent experiments were scored and used for the analysis.

Data analysis

Pot2-DNA fingerprints were first scored visually and sorted into groups based on obvious similarities between amplified DNA profiles. These preliminary groups of isolates were then analysed together on the same gel to confirm fingerprint identity or similarity and to quantify the number of shared fragments among isolates. Lineages were assigned according to the similarity of *Pot2* amplification profiles. Forty-two isolates with different amplification patterns (haplotypes) were identified among five fingerprint lineages. DNA from isolates representing a particular lineage was electrophoresed together with representatives of other putative lineages to allow scoring of individual fragment positions. Isolates sharing more than 95% of their bands were considered a single haplotype (Roumen *et al*. 1997). To determine the genetic relationship among isolates, the presence or absence of fragments was scored manually for each isolate and was converted into binary data. A similarity matrix was calculated using the index of Nei and Li (1979) as $Sxy = 2n_{xy}/(n_x + n_y)$ in which n_{xy} is the number of fragments shared by a given pair of isolates and n_x and n_y are the number of fragments in isolates x and y. Cluster analysis based on the index and the unweighted pair group method of averages (UPGMA) method (Sneath and Sokal 1973) was performed using NTSYS version 2 software (Rohlf 1998) and a dendrogram was constructed. The robustness of clusters in the dendrogram was assessed by bootstrap analysis using PAUP version 4 software (Swofford 2000) and 1000 repeated samplings with replacement were conducted. Isolates that shared ≥70% of *Pot2*-DNA fingerprint bands were considered to be in the same lineage.

Virulence test and disease scoring

Forty-two isolates representing haplotypes from each of the five DNA fingerprint lineages were used in inoculation tests. Pathotypes were determined by a virulence assay including the eight cultivars of the IRBD (Ling and Ou 1969), seven NILs with known resistance genes, plus 12 selected commercial cultivars commonly grown in Argentina (Giarrocco *et al*. 2007). Cultivars Fanny and Oryzica Llanos 5 were included in each assay as a check for susceptibility and resistance efficiency, respectively. Ten seedlings of each rice cultivar were grown to the three to four leaf stage in the greenhouse at 26 ± 2°C and 12-h photoperiod in 10-cm plastic pots. Plants were fertilised with ammonium sulfate in three equal fractions (time of planting, 7 days later, and 1 day before inoculation) at an equivalent of 180 kg of nitrogen per hectare. Plants were spray-inoculated with aqueous conidial suspensions in 0.5% gelatin containing $1–5 \times 10^5$ conidia/mL prepared by harvesting conidia from the surface of 7–10-day-old cultures (Xia *et al*. 1993). Inoculated plants were maintained in a 100% humidity chamber placed in a greenhouse with a 12-h photoperiod and 24 ± 2°C. Disease reactions of the inoculated plants were scored visually 14 days after inoculation using a 0–5 scale system (International Rice Research Institute 1980). Host response was considered as compatible if the majority of seedlings exhibited fully sporulating lesions (lesion type 4 or greater). All other responses were judged as incompatible. The experiment was conducted three times with two replications repeated at the same time on each occasion. The virulence pattern was analysed based on the similarity of reaction type among isolates. A binary matrix indicating compatible and incompatible reactions of each isolate was utilised for constructing a matrix of similarity between all pairs of isolates according to the coefficient of Jaccard (Sneath and Sokal 1973). Based on these data, cluster analysis was conducted and a dendrogram was constructed using the UPGMA method of the NTSYS software.

The combined set of *Pot2* fingerprints and virulence data matrices were used to analyse the relationship between lineages and pathotypes. A matrix comparison with 1000 permutations was conducted using the Mantel test (Mantel 1967).

Results

Genetic structure of Argentinean P. oryzae populations

Pot2-DNA fingerprints revealed five lineages (named A–E) among the 161 field isolates tested, and each lineage contained 1–22 haplotypes (Fig. 2). The lineages were identified by scoring reproducible bands (4–17 in each sample) ranging in size from 400 bp to 5 kb. A total of 42 *Pot2*-DNA fingerprints identified among the five lineages were used to perform a cluster analysis (Fig. 2). The frequencies of the A, B, C, D and E lineages were 0.22, 0.39, 0.19, 0.05, and 0.14, respectively. The majority of isolates belonged to lineage B and they were found in five out of nine locations (Table 1). These isolates were sampled from four cultivars but with high frequency from Fortuna and Diamante (Table 2). Lineage A was the second largest fingerprint group, and isolates belonging to this lineage were found in five sampled sites (Table 1) and on seven cultivars (Table 2). Isolates of lineage C were present mainly on cv. Diamante (Table 2) and those of lineage E were recovered in three locations on five cultivars (Tables 1 and 2). Isolates of lineage D were found on an unknown cultivar and exclusively

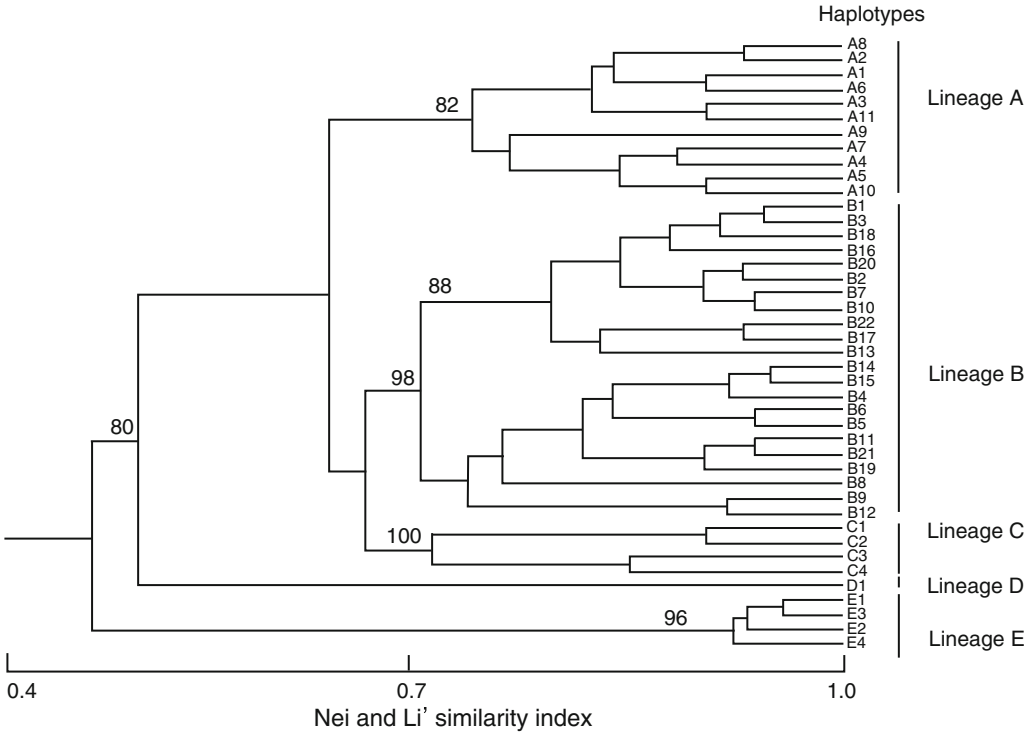

Fig. 2. Dendrogram constructed using the unweighted pair group method with arithmetic mean analysis and the index of Nei and Li (1979) of the binary matrix obtained for *Pot2*-PCR band comparison of 42 isolates of *Pyricularia oryzae*, representing all haplotypes included in the present study. The numbers of nodes represent the bootstrap value (%) based on 1000 iterations. Each group formed at ≥70% DNA profile similarity was designated as a lineage.

Table 1. Distribution of *Pot2*-DNA fingerprint lineages of *Pyricularia oryzae* in the sampled regions of Argentina

Province and location	No. of cultivars	No. of isolates	No. of haplotypes	Isolates in each clonal lineage				
				A	B	C	D	E
Buenos Aires, La Plata	4	29	11	17	0	4	8	0
Santa Fe, Yataí	4	9	2	1	0	0	0	8
Santa Fe, San Jacinto	2	17	4	0	9	8	0	0
Santa Fe, San José	3	67	13	9	25	19	0	14
Santa Fe, San Joaquín	1	16	5	0	16	0	0	0
Corrientes, Mercedes	1	5	2	0	5	0	0	0
Entre Ríos, Villaguay	3	15	2	8	7	0	0	0
Entre Ríos, Urdinarrain	1	1	1	0	0	0	0	1
Entre Ríos, La Paz	1	2	2	2	0	0	0	0

in the Buenos Aires province (Tables 1 and 2). In contrast, while different lineages were found on cvv. Fortuna, Diamante, La Plata Mochi, Brillante, and on an unknown cultivar, only one lineage was recovered from the remaining eleven cultivars (Table 2).

Haplotype diversity within DNA fingerprint lineages

Based upon *Pot2*-DNA fingerprint differences of 1–30%, 11, 22, 4, 1 and 4 haplotypes were found within lineages A, B, C, D and E, respectively (Fig. 2). While several non-predominant haplotypes within lineages A and B were represented, one haplotype was predominant within or unique to each of the fingerprint groups C, D and E (Table 3).

Pathotype diversity

A total of 41 distinct pathotypes was detected among the 42 isolates from the 29 tested cultivars. Twenty-four races representing eight international race groups (IA, IB, IC, ID, IE, IF, IG, and II-1) were detected among the isolates selected for this study (Table 4). The most frequently found races (each one representing 24% of the sampling), were II-1 (incompatible on all international tester genotypes) and IA (compatible with Raminad), which were recovered from six or seven cultivars, respectively. In decreasing order of frequency of occurrence, race groups IB (19%), IF and IG (9%), IC (7%), IE (5%) and ID (2%) were determined.

Table 2. Distribution of *Pot2*-DNA fingerprint lineages of *Pyricularia oryzae* on different rice cultivars and experimental lines grown in Argentina

Cultivar	No. of isolates	No. of haplotypes	Isolates in each lineage				
			A	B	C	D	E
Brillante	6	2	0	0	3	0	3
Carnaroli	7	1	0	7	0	0	0
Colonia Mascías 5 SCA	4	1	4	0	0	0	0
Diamante	43	9	9	18	16	0	0
El Paso 144	5	3	0	5	0	0	0
Fortuna	48	16	0	32	5	0	11
G 4001	7	1	0	0	0	0	7
G 4005	1	1	0	0	0	0	1
H 289 13–2-2–1	1	1	0	0	1	0	0
H 316	1	1	0	0	0	0	1
H 397–1-2–2-1	3	1	0	0	3	0	0
H 397–6-1–2	4	1	4	0	0	0	0
Itapé	4	2	4	0	0	0	0
La Plata Mochi	12	7	9	0	3	0	0
Unknown	14	2	6	0	0	8	0
Yeruá	1	1	1	0	0	0	0
Total	161						

Table 3. Haplotypes of *Pyricularia oryzae* found within each *Pot2*-DNA fingerprint lineage

Haplotype	No. of isolates (%)[A,B]				
	A	B	C	D	E
1	7 (18.9)	6 (9.7)	1 (3.2)	8 (100.0)	2 (8.7)
2	2 (5.4)	2 (3.2)	22 (71)		7 (30.4)
3	1 (2.7)	1 (1.6)	5 (16.1)		2 (8.7)
4	7 (18.9)	3 (4.8)	3 (9.6)		12 (52.1)
5	5 (13.5)	1 (1.6)			
6	1 (2.7)	2 (3.2)			
7	1 (2.7)	5 (8.0)			
8	1 (2.7)	8 (13.0)			
9	1 (2.7)	1 (1.6)			
10	6 (16.2)	2 (3.2)			
11	5 (13.5)	2 (3.2)			
12		1 (1.6)			
13		3 (4.8)			
14		1 (1.6)			
15		7 (11.4)			
16		1 (1.6)			
17		2 (3.2)			
18		4 (6.4)			
19		1 (1.6)			
20		7 (11.4)			
21		1 (1.6)			
22		1 (1.6)			

[A]Letters indicate *Pot2*-DNA fingerprint lineage.
[B]Number and percentage of isolates in each haplotype and lineage, based on *Pot2*-DNA fingerprinting.

No significant correlation was found between pathotype and *Pot2*-DNA fingerprint similarity ($r = -0.093$). Virulence patterns were distributed randomly with respect to genetic lineages. Typically, multiple races occurred in each of the five lineages analysed (Table 4). Higher race numbers were identified in lineages B, A and C.

Alternatively, both virulent and avirulent isolates to the known genes in the NILs were detected in the populations of *P. oryzae* (Table 5). In all the variable NIL-lineage combinations, compatible isolates comprised 14% of the isolates, and the number of incompatible isolates often exceeded half of those tested. Thirty-nine percent of the isolates were virulent for gene *Pi-4a* and the virulence frequencies were relatively low and in descending order were *Pi-2* (20%), *Pi-1* (16%), *Pi-3* (14%), IR 1529 (8%), *Pi-4b* (6%) and *Pi-1* + *Pi-33* (2%) (Table 5). Compatibility of all isolates from a lineage with a given NIL was not detected, although at least one isolate of each lineage was compatible with the *Pi-1*, *Pi-2*, or *Pi-4a* genes (Table 5).

Most commercial cultivars were highly resistant to the isolates assayed. Five of them were completely resistant to all isolates of each lineage. Fortuna, Diamante, Itapé and La Plata Mochi were the most susceptible cultivars, compatible with at least one isolate of each lineage (Table 6). Fortuna was the most susceptible cultivar and it was compatible with 60% of all assayed isolates. Cultivar Fanny was susceptible to all isolates and cv. Oryzica Llanos 5 was resistant to all isolates. Both cultivars were included as check comparisons.

Discussion

The knowledge of *P. oryzae* populations present in rice-growing regions in the world is relevant from an ecological, epidemiological and breeding perspective. Our results have shown that the *P. oryzae* population structure in Argentina is simple, composed of only five clonal lineages and has a low genetic diversity comparable to other studies conducted in the Americas, Europe and Asia that revealed population structures of *P. oryzae* (Levy *et al*. 1991; Han *et al*. 1993; Xia *et al*. 1993; Roumen *et al*. 1997; Don *et al*. 1999; Javan-Nikkhah *et al*. 2004; Piotti *et al*. 2005). Two lineages (A and B) were widely distributed across the rice-growing areas of Argentina, suggesting that they

Table 4. Race groups distribution of *Pyricularia oryzae* collected in Argentina during 2000–05

Lineage	No. of isolates	No. of rice cultivars[A]	International race groups
A	10	7	IA-52, IA-112, IA-128, IB-61, IB-64, IF-2, IG-2, II-1
B	16	5	IA-124, IA-127, IA-128, IB-14, IB-63, IB-64, IC-15, ID-16, IF-2, IF-4, IG-2, II-1
C	6	6	IA-88, IB-15, IC-16, IE-6, IE-8, IF-2, IG-2, II-1
D	4	1	IA-59, IF-4
E	6	4	IA-114, IA-126, IB-63, IB-64, IC-25, IE-8, II-1

[A]Number of cultivars from which the isolates assayed were collected.

Table 5. Frequency of compatible isolates from the five lineages of *Pyricularia oryzae* from Argentina on seven near-isogenic lines of rice

Cultivar	Resistance genes	Frequency of compatible isolates[A]					Mean %
		A[B]	B	C	D	E	
C104 LAC	*Pi*-1	0.10	0.12	0.16	0.25	0.16	16
C101 A51	*Pi*-2	0.10	0.19	0.16	0.25	0.33	20
C104 PKT	*Pi*-3	0.10	0.12	0.33	0.25	0.00	14
C101 PKT	*Pi*-4a	0.10	0.19	0.50	0.50	0.66	39
C105 TTP	*Pi*-4b	0.00	0.00	0.16	0.00	0.16	6
C 101 LAC	*Pi*-1 + *Pi*-33	0.00	0.12	0.00	0.00	0.00	2
IR1529	*Pi*-33 + unknown gene/s	0.30	0.12	0.00	0.00	0.00	8

[A]Calculated as the proportion of isolates tested inducing a susceptible reaction on a rice cultivar.
[B]*Pot2*-DNA fingerprint lineage.

may be the most broadly adapted lineages in the country. The low frequencies at which lineages C, D and E occurred may indicate that either they have persisted in the population only at a low frequency or that they have been recently introduced into the region. Although only one lineage was recovered from nine rice cultivars, the limited number of isolates assessed in our study precludes establishing a clear association between cultivar and lineage.

The low genotype diversity assessed by the *Pot2*-DNA fingerprint and the geographical distribution of the lineages may be ascribed to several factors. In Argentina, rice cultivation is relatively recent, being introduced in the late 1800s. Also, it could be considered that a relatively short growing season, the growth of relatively few cultivars in each province, and environmental conditions that favour only sporadic blast epidemics, together exert a strong selection pressure within *P. oryzae* populations. The highest blast diversity in Argentina was found in Santa Fe province. In total, 109 isolates from Fortuna and Diamante (56% belonging to lineage B), were collected at four locations, which cover 80% of the cultivable area. Every year a high level of disease is observed on these highly susceptible cultivars, probably favoured by excessive amounts of nitrogen fertiliser, conducive environmental conditions and some cultural practices. A different scenario is found in the Entre Ríos and Corrientes provinces, where more resistant cultivars are grown with lower levels of fertiliser (M. A. Marassi, pers. comm.), which

Table 6. Frequency of compatible isolates of five lineages of *Pyricularia oryzae* inoculated on rice cultivars commonly grown in Argentina

Cultivar	Frequency of compatible isolates[A]					Mean %
	A[B]	B	C	D	E	
Taim	0.10	0.06	0.00	0.00	0.00	3
CT 6919	0.00	0.00	0.00	0.00	0.00	0
Don Juan	0.10	0.06	0.00	0.25	0.00	8
San Miguel	0.00	0.00	0.00	0.00	0.00	0
RP2	0.00	0.00	0.00	0.00	0.00	0
El Paso 144	0.00	0.06	0.16	0.00	0.00	4
Yeruá	0.00	0.00	0.00	0.00	0.00	0
Itapé	0.40	0.37	0.66	0.25	0.33	40
Down	0.00	0.00	0.00	0.00	0.00	0
Fortuna	0.50	0.62	0.66	0.25	1.00	60
Diamante	0.30	0.5	0.50	0.25	1.00	51
La Plata Mochi	0.20	0.12	0.16	0.25	0.33	21
Fanny	1.00	1.00	1.00	1.00	1.00	100
Oryzica Llanos 5	0.00	0.00	0.00	0.00	0.00	0

[A]Calculated as the proportion of isolates tested inducing a susceptible reaction on a rice cultivar.
[B]*Pot2*-DNA fingerprint lineage.

could explain the smaller number of isolates and haplotypes obtained from those areas.

Within each lineage it is difficult to determine what processes have been involved in generating the different *Pot2*-DNA fingerprints. A sexual cycle does not seem to be a source of variation for rice blast disease in Argentina (Consolo *et al*. 2005), as reported in other regions of the world (Zeigler 1998). Other mechanisms for generating variation, such as mutations, parasexual recombination or transposition events could possibly be associated with the haplotype diversity we found. Different transposons, including *Pot2* inverted repeats, have been identified in the *M. oryzae* genome (Hamer *et al*. 1989; Valent and Chumley 1991; Dobinson *et al*. 1993; Sone *et al*. 1993; Kachroo *et al*. 1994, 1995; Farman *et al*. 1996) and some of these were demonstrated to be responsible of genetic and virulence variation. Thus, it could be speculated that different transposons may also contribute towards generating genome variability in Argentinean *P. oryzae* populations.

Although the International Race System is not very useful in describing a complete virulence spectrum of the pathogen, it is necessary to establish a reference pattern of races in the world (Xia *et al*. 1993). Our results on pathotype diversity examined across 42 isolates, selected to represent the different haplotypes among the five *Pot2* lineages, identified 24 races (up to 12 races within a lineage). Races recovered varied with cultivar, and several races could be recovered from one cultivar. These findings are in agreement with other studies, where virulence diversity within a lineage can be rather high (Levy *et al*. 1993; Xia *et al*. 1993, 2000). Since a low number of isolates representing each genetic group exhibited high pathotype diversity, a higher number of pathotypes could have been detected if all the isolates within a lineage had been tested. We found the lack of association between a DNA fingerprint and virulence is consistent with other findings, suggesting that isolates of the same pathotype are not closely related (Zeigler *et al*. 1995; Javan-Nikkhah *et al*. 2003; Park *et al*. 2003; Ninh Thuan *et al*. 2006). As reported in these previous studies, our results also suggest that lineage information is not a useful tool for predicting virulence phenotypes of *P. oryzae* isolates in Argentina.

Resistance to entire lineages is likely to be conditioned by individual resistance genes (Zeigler *et al*. 1995). Based on the analysis of resistance spectra of the NILs included in the present study, compatibility was variable among lineages evaluated. Combinations of at least two resistance genes occur in IR 1529 (*Pi-33* + unknown gene/s), which has conditioned resistance to three lineages and C101A51 (*Pi-1* + *Pi-33*), which has conditioned resistance to four lineages. According to these results, lines carrying more than one resistance gene may increase the resistance to all lineages. In this way, it is reported, and widely practised, that strategies for developing durable blast-resistant cultivars should include resistance gene combinations (Zeigler *et al*. 1994; Babujee and Gnanamanickam 2000; Gibbons *et al*. 2000; Hittalmani *et al*. 2000).

We found that compatibility of different lineages with commercial cultivars commonly grown in Argentina was low. Although there is no information about resistance genes present in those cultivars, it is clear that they carry either individual or combinations of resistance genes. This also seemed to be the case for the most susceptible cultivars (Fortuna, Diamante Itapé and La Plata Mochi), which were not compatible in relation to all isolates assayed.

The results generated in this study, based on a first survey of genetic and virulence diversity of *P. oryzae* populations in Argentina, have shown that although they have a simple genetic structure, they are highly diverse in pathogenicity. For this reason, breeding programs should be cautious in the development and deployment of *P. oryzae*-resistant cultivars in Argentina.

Acknowledgements

We thank F. Correa, F. Escobar, M. Duque and G. Prado (CIAT, Cali, Colombia), M. Reynoso and S. Chulze (UNRC) and R. Favot and M. Peart for help in the collection of samples. We also thank A. Vidal and M. Pinciroli for providing seeds. Authors G. L. Salerno and C. A. Cordo are career investigators of CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina) and CIC (Prov. Buenos Aires), respectively. Author V. F. Consolo is a fellow of CONICET. This research was supported by the Centro Argentino-Brasileño de Biotecnología (CABBIO-No. 64), Universidad Nacional de Mar del Plata (15E/142), CONICET and FIBA.

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Manuscript received 10 September 2007, accepted 5 January 2008