Molecular characterization of a Latin American *Pyricularia grisea* population

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Irrigated rice in the Southern Cone of Latin America is grown in southern Brazil, Argentina, and Uruguay. Although rice blast has been present in that region for a long time, it became a major constraint to rice production starting in 1996.

The MGR586 probe was used to generate DNA fingerprints (Eco*RI*-RFLPs, restricted fragment length polymorphism) from monoconidial isolates of *Pyricularia grisea* collected in southern Brazil, Argentina, and Uruguay from cultivars and breeding lines during the rice seasons of 1994, 1997, and 1998. A total of five different MGR-fingerprint lineages have been found in this region, but only one has been isolated on the tropical cultivars that are the most extensively grown. Even in breeding nurseries where other different isolates have been identified, the DNA fingerprints of isolates on tropical cultivars consistently belong to the same lineage. This suggests a specific susceptibility of those cultivars to that particular fungal lineage but, at the same time, also suggests resistance to the other lineages found in the region.

Comparisons made with other isolate fingerprints from Latin America showed a high similarity between the El Paso 144-associated lineage in the Southern Cone and a lineage from Colombia, for which a source of incompatibility (resistance gene) is known. It is possible that the addition of that gene to El Paso 144 would complete the spectrum of resistance (i.e., exclude all regional pathogen lineages) necessary to provide a more durable protection.

Irrigated rice in the Southern Cone of Latin America is grown in southern Brazil, Argentina, and Uruguay. Until approximately 1980, medium-grain types, with Italian (japonica) background, were cultivated in a large area. During the next decade, an indica long-grain cultivar (IRGA409) was released in southern Brazil, whereas a Texas variety, Bluebelle, was mostly grown in Uruguay and Argentina. Following the increasing demand from Brazil of indica-type rice, several cultivars closely related to the Brazilian cultivar IRGA409 were grown commercially in Uruguay and Argen-

tina. Although rice blast has been present in that region for a long time, it became a major constraint to rice production in 1996. The spread of this disease has followed the diffusion of closely related germplasm. Consequently, the national breeding programs have started a novel approach for durable resistance based on the hypothesis of lineage exclusion, in which resistances are combined based on preventing infection by families of the blast fungus that exist in the region.

The first step in breeding for blast resistance is to identify the genetic components and structure of the pathogen population. Inoculation of differential cultivars helps to describe pathotype variation, but differences in assay conditions, intermediate readings on the differentials, and different scoring procedures make this method hard to standardize. On the other hand, the molecular characterization of *Pyricularia oryzae* monoconidial isolates yields an objective description of the individuals and their relatedness.

A family of *Magnaporthe grisea* repeated sequences (MGR sequences) was found in a high copy number (approximately 40 to 50 copies per genome) in isolates that infect rice (Hamer et al 1989). One of these repeated sequences, MGR586, has been used for phylogenetic analyses, pathotype variation and stability, estimates of strain relatedness (Levy et al 1991b), and genetic mapping of important genes in this fungus (Hamer 1991). A representative set of blast fungus isolates on rice collected in the southeastern region of the United States over a 30-year period was used to study pathotype variation and evolution (Levy et al 1991b). Another study was conducted in Colombia, where a large collection of isolates from a "blast hot spot" was analyzed (Levy et al 1991a). These and other studies in Asia (Borromeo et al 1993, Chen et al 1995, Zeigler et al 1995) support the hypothesis that isolates from a defined region form several discrete lineages, each of which has a specific and limited virulence spectrum. Such a relationship between lineages and virulence coupled with the identification of the resistant hosts for each lineage should help design a strategy for durable resistance.

Materials and methods

Isolates were taken from lesions present on field-collected infected plants of different rice cultivars grown commercially and in experimental nurseries in Argentina, southern Brazil, and Uruguay. Samples were collected during the summers of 1994, 1997, and 1998 and most of the lesions collected were from neck-infected panicles after flowering. Plant tissue was thoroughly washed and placed in a high-moisture chamber to facilitate sporulation of *P. oryzae*. A monoconidial isolate was obtained using light microscopy and grown on rice polish agar plates. Samples of each isolate were stored on dried filter papers and a small piece was cultured in 250-mL flasks with 125 mL of complete liquid media to obtain enough fungus tissue for DNA isolation.

DNA was isolated following the CTAB protocol of Zeller and Levy (1994). Three micrograms of chromosomal DNA were digested with Eco*RI* and fractionated by electrophoresis on 0.8% agarose gels. A nonpathogenic strain of DNA digested with the same restriction enzyme was used as a reference standard that exhibited an

MGR-DNA profile of 30 RFLPs (restriction fragment length polymorphisms) of known length ranging from 0.9 to 38.2 kb (Levy et al 1993). The electrophoresed DNA was blotted to Hybond$^+$ membranes (Amersham Corp.). Electrophoresis and Southern blotting were carried out according to Sambrook et al (1989). A nonradioactive-labeled probe, MGR586, using the ECL (Amersham Corp.) direct nucleic acid labeling and detection system was hybridized to DNA blots to obtain the MGR-DNA fingerprints on blue-light-sensitive film.

The MGR-DNA RFLPs were visually scored as present or absent to build up the RFLP profile of each isolate. Groups on the basis of obvious similarities were made and variation within a profile group was evaluated from pairwise comparisons of the proportion of shared RFLPs between fingerprints. Nei and Li's index of genetic similarity (*Sxy*) for RFLP comparisons was calculated. The small range of RFLP variation within groups and the large distance between groups allowed the use of a consensus MGR-DNA profile fingerprint for each group in order to describe the structure of the population sampled.

Results

A total of five MGR fingerprint groups have been found on the luminographs from the rice-infecting isolates collected in southern Brazil, Argentina, and Uruguay during the 1994, 1997, and 1998 rice seasons (Table 1). Two groups occurred more frequently than the others did throughout years, localities, and cultivars. The isolates from tropical cultivars expressed a common profile of about 66 bands (Fig. 1) and an *Sxy* ranging from 0.992 to 0.86 (Table 2), indicating that they belonged to one lin-

Table 1. Cultivars and breeding lines collected by year and country.

Cultivar	Year	Country	Cultivar	Year	Country
Maybelle	1994	Argentina	El Paso144	1994	Argentina
Tacuarí	1997	Uruguay	El Paso144	1997	Uruguay
L1070	1997	Uruguay	Chui	1997	Brazil
Lacasine	1994	Argentina	Bluebelle	1996	Argentina
Tacuarí	1998	Argentina	El Paso144	1998	Argentina
Jackson	1997	Argentina	Yerbal	1997	Brazil
H121	1994	Argentina	Don Juan	1997	Argentina
Epagri107	1998	Argentina	El Paso144	1997	Argentina, Brazil
Chui	1996	Argentina	IRGA416	1997	Brazil
H205	1994	Argentina	Bluebelle	1998	Uruguay
IRGA409	1998	Argentina	El Paso144	1994	–
5CT	1996	Argentina	IRGA417	1997	Brazil
Tacuarí	1994	Argentina	Caraguatá	1998	Uruguay
RP2	1998	Argentina	L1119	1997	Uruguay
IRGA409	1997	Brazil	IRGA417	1996	Brazil
El Paso227	1994	Argentina	Tacuarí	1998	Uruguay
El Paso144	1998	Uruguay	Fany	1997	Uruguay
El Paso144	1997	Brazil			

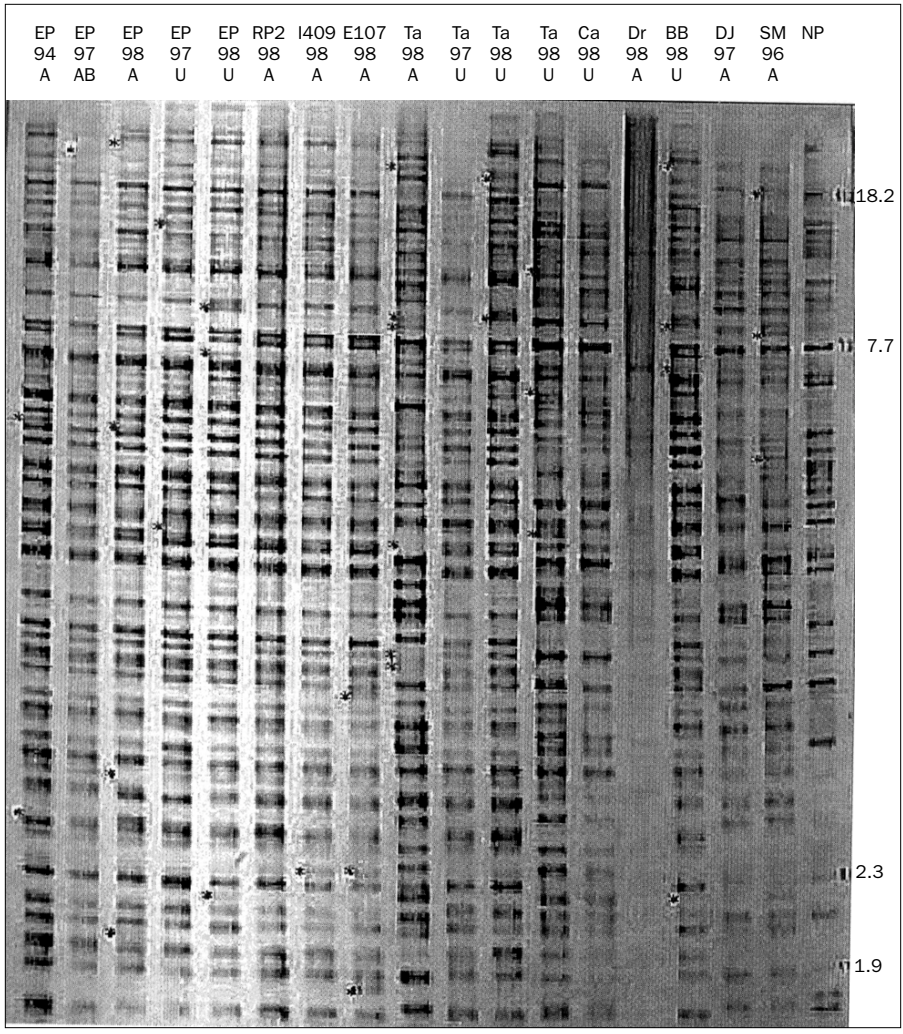

Fig. 1. MGR fingerprints of *Pyricularia grisea* isolates from Brazil, Argentina, and Uruguay.

eage. The second group was formed by three related lineages commonly found on American cultivars (Fig. 2), each 95% similar to each other but all >70% different from the lineage on tropical cultivars. The isolates from the Argentinean cultivar Don Juan showed only the profile belonging to a lineage common to all American varieties, whereas the isolates from the old Texas cultivar Bluebelle showed both profiles—but in different years.

The other three MGR fingerprints have been found on isolates from a Brazilian cultivar, Chui (not shown), and two Uruguayan cultivars, INIA Tacuarí and INIA

Table 2. Index of genetic similarity (*Sxy*) in % (Nei and Li) of representative isolates of *Pyricularia grisea* from southern Brazil, Argentina, and Uruguay.

Cultivar	Year	EP	EP	EP	EP	EP	RP2	I409	E107	Ta	Ta	BB	Ta	Ca	DJ	SM	Ta
EP144	1994	1															
EP144	1997	98	1														
EP144	1998	96	96	1													
EP144	1997	97	98	96	1												
EP144	1998	96	97	95	96	1											
RP2	1998	98	99	97	99	98	1										
IRGA409	1998	98	99	96	98	97	99	1									
Epagri107	1998	96	97	95	96	96	98	99	1								
Tacuarí	1997	98	99	97	99	98	99	99	98	1							
Tacuarí	1998	97	99	96	97	98	99	98	96	99	1						
Bluebelle	1998	96	96	97	96	99	97	96	95	97	97	1					
Tacuarí	1998	26	26	24	24	29	26	27	27	26	27	30	1				
Caraguatá	1998	26	26	25	25	29	27	28	28	28	28	31	98	1			
Don Juan	1997	12	10	10	10	13	10	12	12	10	12	13	70	71	1		
SM	1996	12	10	10	10	15	10	11	11	10	13	15	68	69	97	1	
Tacuarí	1998	25	23	23	23	26	24	23	23	24	23	26	74	75	75	74	1

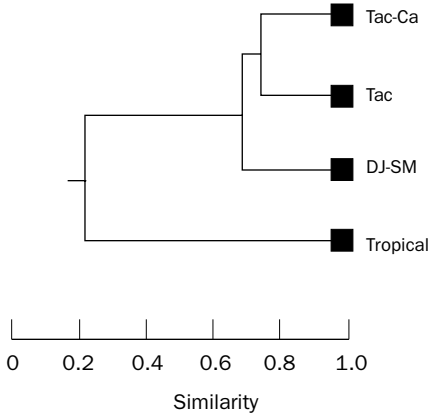

Fig. 2. Dendrogram of the rice blast isolates from the Southern Cone population (Tac = Tacuarí, Ca = Caraguatá, DJ = Don Juan, SM = San Miguel isolates).

Caraguatá. Only one MGR fingerprint was found on isolates from the most commercially grown cultivars, El Paso 144 and IRGA409. These cultivars have a common origin from a CIAT (Centro Internacional de Agricultura Tropical) population. Also, isolates from other tropical cultivars such as IRGA417, IRGA416, and Chui showed the same profile (not shown).

The five MGR fingerprint groups allowed the identification of discrete groups of *P. oryzae* isolates and defined lineages on the basis of a low index of genetic similarity (Table 2, Fig. 2) between members of different lineages and the high values of the members in each lineage.

Conclusions

MGR-RFLP has been used successfully in understanding the population structure of the rice blast fungus in different regions. In this study, five discrete lineages were detected in southern Brazil, Argentina, and Uruguay. However, only one was isolated from tropical cultivars, which are the most extensively grown. The presence of other isolates belonging to different lineages at the same sites and times but on other hosts suggests a specific susceptibility of those tropical cultivars to that particular fungal lineage. At the same time, this association also suggests resistance to the other lineages found in the region. Only one isolate from the tropical cultivar Chui (not shown) showed a profile completely different from that of the others.

Comparisons made with other *P. oryzae* isolate fingerprints from Latin America showed a high similarity between the El Paso 144 associated lineage in the Southern Cone and a lineage (SRL-2) from Colombia, for which a source of incompatibility (resistance gene *Pi-ta* from Yashiro-mochi) is known. We are testing the possibility that the addition of that gene to El Paso 144 will complete the spectrum of resistance

(i.e., exclude all regional pathogen lineages) necessary to provide a more durable protection.

Since El Paso 144 and IRGA409 are the cultivars that have the largest area planted in the region, a backcross program began in 1999 to introduce the *Pi-ta* gene from Yashiro-mochi into El Paso 144.

References

Borromeo ES, Nelson RJ, Bonman JM, Leung H. 1993. Genetic differentiation among isolates of *Pyricularia* infecting rice and weed hosts. Phytopathology 83:393-399.

Chen DH, Zeigler RS, Leung H, Nelson RJ. 1995. Population structure of *Pyricularia grisea* at two screening sites in the Philippines. Phytopathology 85:1011-1020.

Hamer JE, Farrall L, Orbach MJ, Valent B, Chumley FG. 1989. Host species-specific conservation of a family of repeated DNA sequences in the genome of a fungal plant pathogen. Proc. Natl. Acad. Sci. USA 86:9981-9985.

Hamer JE. 1991. Molecular probes for rice blast disease. Science 252:632-633.

Levy M, Correa-Victoria FJ, Zeigler RS, Xu S, Hamer JE. 1991a. Organization of genetic and pathotype variation in the rice blast fungus at a Colombian "hot spot." Phytopathology 81:1236-1237.

Levy M, Romao J, Marchetti MA, Hamer JE. 1991b. DNA fingerprinting with dispersed repeated sequence resolves pathotype diversity in the rice blast fungus. Plant Cell 3:95-102.

Levy M, Correa-Victoria FJ, Zeigler RS, Xu S, Hamer JE. 1993. Genetic diversity of the rice blast fungus in a disease nursery in Colombia. Phytopathology 83:1427-1433.

Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular cloning: a laboratory manual. 2nd edition. Cold Spring Harbor, N.Y. (USA): Cold Spring Harbor Laboratory Press.

Zeigler RS, Cuoc LX, Scott RP, Bernardo MA, Chen DH, Valent B, Nelson RJ. 1995. The relationship between lineage and virulence in *Pyricularia grisea* in the Philippines. Phytopathology 85:443-451.

Zeller K, Levy M. 1994. Protocols of the Workshop "Integración de fitopatología, mejoramiento y biología molecular para el desarrollo de una resistencia estable al añublo del arroz, *Pyricularia grisea*. CIAT, Cali, Colombia, 10-19 octubre 1994.

Notes

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