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Mating-type distribution and fertility status in *Magnaporthe grisea* populations from Argentina

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Abstract

Isolates of *Magnaporthe grisea* causing gray leaf spot on rice were collected in Argentina and analyzed for mating distribution and fertility. One hundred and twenty-five isolates of *M. grisea* were collected from rice plants between 2000 and 2003. Each isolate was tested for mating type through a polymerase chain reaction based assay. All *M. grisea* isolates from Argentina belonged to a single mating type, *MAT*1.1. The fertility status of isolates was determined using controlled crosses *in vitro*, pairing each isolate with GUY11 and KA9 (MAT1.2 standard hermaphroditic testers). Production of perithecia was scarce among isolates of the blast pathogen since a low percentage of them (7.2%) developed perithecia with only one of the fertile tester (KA9); all crosses failed with the other tester strain. Asci and ascospores were not observed. The presence of only one mating type and the absence of female fertile isolates indicate that sexual reproduction is rare or absent in *M. grisea* populations associated with rice in Argentina.

Key words: *Magnaporthe grisea*, mating type, perithecia, rice

Introduction

Magnaporthe grisea (Hebert) Yaegashi & Udagawa is a hermaphroditic, heterothallic ascomycete (anamorph: *Pyricularia grisea*) pathogenic to a large number of gramineae species [1]. Its greatest economic impact is as the casual agent of blast disease of rice, wheat and millets worldwide. The rice blast pathogen, is known for its high capacity for asexual reproduction [2]. This characteristic, combined with relatively simple population structures that were determined as DNA fingerprinting using the repetitive element MGR 586 [3, 4], suggested that *P. grisea* populations are generally composed of clonal lineages. Despite apparent clonality, *M. grisea* displays a high level of genetic variability. Mutation and migration generally were considered to be sources of such variation [5]. More recently, evidence has been presented showing that parasexual recombination may contribute to genetic variation, even in putatively clonal populations [6].

Sexual reproduction is known to be a significant source of genetic variation in many fungi [7]. So that, it is of importance to know the frequency of sexual reproduction in a population, since the genotypes that could arise due to recombination should be considered as targets when devising effective disease control.

Like other Ascomycetes, sexual compatibility in *M. grisea* is determined by the presence of two alleles (idiomorphs) at a single mating-type locus designated *MAT*1.1 and *MAT*1.2 and isolates of both mating types are required for the completion

of sexual reproduction [8]. *MAT*1.1 and *MAT*1.2 of *M. grisea* have been cloned and sequenced using a genomic subtraction strategy [9]. The perfect stage of *P. grisea* was first described by Hebert [10] in crosses between isolates from cereals and wild grasses. Since then, efforts have been made to produce perithecia successfully on artificial media under controlled conditions using hermaphroditic tester isolates from finger millet and rice [11, 12]. Although both mating types have been found in the same field at the same time it has not yet been possible to observe the perfect state in nature. Both mating types coexist in the majority of *M. grisea* populations from rice as reported in studies from different countries. Nevertheless, in some populations only one mating type is present [2, 13, 14]. The capacity of *M. grisea* isolates to produce perithecia is apparently controlled by genes at several loci and these segregate independently of mating type and of pathogenicity on different hosts [15].

High fertility is common among *M. grisea* strains from non-rice hosts [16]. Fertility in *M. grisea* field isolates was shown to range from total sterility (inability to mate with any other strain) to female sterility (ability to mate only as a male parent), to full fertility (ability to mate either as male or female parents) [17].

Mating-type distribution in a population has been used as criterion for examining the relationship among different isolates of the fungus and sexual recombination *in vitro* serves as a valuable tool for analyses of various genetic traits [18]. The analysis of mating-type distribution in a population, along with phylogenetic analysis, also provides insights into evolutionary history.

The present report is the first study of mating-type distribution and fertility status of the rice blast fungus *M. grisea* in Argentina.

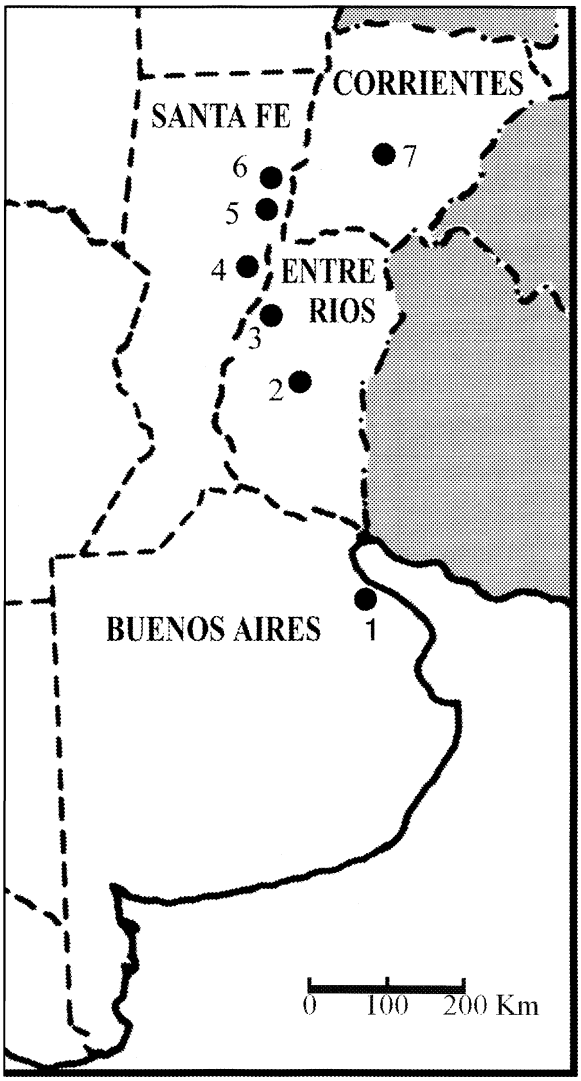

Figure 1. Map showing the sampling locations of *Magnaporthe grisea* isolates from rice in different provinces of Argentina. 1, The Rice Experimental Station Julio Hirschhorn, Los Hornos; 2, Villaguay; 3, La Paz; 4, San Joaquín; 5, Colonia San José; 6, San Jacinto; 7, Mercedes. Scale: 1 : 13,245,000.

Materials and methods

Collection of samples

Plants showing typical blast symptoms on leaf and panicles were collected during 2000–2003 from rice fields in Corrientes, Santa Fe, Entre Ríos Provinces which represent the major rice-growing areas of Argentina and from a nursery of The Rice Experimental Station Julio Hirschhorn, Los Hornos, Buenos Aires Province (Figure 1). Samples were assembled from Itapé, Fortuna, Diamante, La Plata Mochi, Carnaroli, El Paso 144 and Colonia Mascias 5 SCA cultivars.

Isolation and maintenance of M. grisea *isolates*

Infected samples were washed in running water for about 30 min, surface sterilized in 0.01% mercuric

chloride solution. Sporulation was induced on leaves and necks by 24 h incubation in Petri dishes at 26 ± 2 °C and 100% relative humidity. Monoconidial isolates were obtained by transferring a germinating conidium to a Petri dish containing fresh rice polish agar (rice polish 20 g/l, sucrose 5 g/l and agar 20 g/l). Each culture was overlaid with several sterilized filter paper sections and incubated at 26 ± 2 °C. After 10–14 days of incubation, the infested 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.

Mating-type assay

Magnaporthe grisea isolates of known and unknown mating type were grown for 5 days at room temperature (21 ± 2 °C) in 50 ml of complete medium broth [19]. Mycelia were harvested under vacuum, stored at −20 °C and then lyophilized. Genomic DNA was extracted following the CTAB (hexadecyltrimethylammonium bromide) extraction method [20]. PCR amplifications were performed running two different reactions using as primers the oligonucleotides reported by Xue et al. [21]: A1 (5′-AGCCTCATCAACGGCAA-3′) and A5 (5′-GGCACGAACATGCGCGATG-3′) for *MAT*1.1 and B15 (5′-CTCAATCTCCG TAGTAG-3′), B16 (5′-ACAGCAGTATAGCCTAC-3′) for *MAT*1.2. PCRs were done in a final volume of 20 μl containing 20 ng of template DNA, 10 mM Tris–HCl (pH 9.0 at 25 °C) 50 mM KCl, 2.5 mM of $MgCl_2$, 0.2 mM of each dNTP, 1 mM of each primer and 1 unit of *Taq* polymerase. Thermal cycling conditions involved an initial denaturation step at 95 °C for 5 min, 30 cycles of 95 °C for 1 min, 52 °C for 2 min and 72 °C for 2 min, followed by 72 °C for 5 min. PCR products were separated by electrophoresis in a 1% agarose gel at 90 V for 45 min, stained with ethidium bromide and photographed.

Cross compatibility and determination of fertility status

The compatibility between *M. grisea* isolates was determined by pairing standard hermaphroditic testers GUY11 and KA9 (kindly supplied by D. Tharreau, CIRAD, France) with all *M. grisea* isolates of unknown mating type. GUY11 and KA9 were MAT1.2. The tester KA9 (non-pathogenic to rice) was originally isolated from finger millet and produced numerous perithecia with isolates of the opposite mating type [13]. Crosses were made by pairing agar blocks of the field isolates and fertile tester strains about 2 cm apart on rice polish agar. The inoculated plates were sealed and incubated at 26 ± 2 °C for a week until the joining of mycelium of two paired individuals. At that point, they were placed under continuous white fluorescent light at 22 °C. After 20 days of incubation, the junctions between the mated individuals were examined under stereoscope for perithecial formation.

The relative degree of fertility was assessed by determining the number of perithecia produced on 1 cm^2 agar. Fertility levels of isolates were classified as high (> 20 perithecia), intermediate (10–19 perithecia), low (< 10 perithecia) or infertile (no perithecia). All crosses were conducted twice. Perithecia from each cross were examined under a microscope for the presence or absence of asci and ascospores by mounting three or four of them on a clean slide stained with cotton blue and gently squashed under a coverslip.

Results

Mating-type distribution of M. grisea *isolates in Argentina rice populations*

A total of 125 monoconidial isolates from rice were isolated from the blast samples and their mating type was identified using a PCR based assay (Table 1). DNA amplification products with expected sizes were produced from all isolates of known mating type (372 bp for MAT1.1 and 376 bp for MAT1.2). Isolates were entirely of one mating type (*MAT*1.1) in each population of *M. grisea* field isolates analyzed in this study (Figure 2).

Fertilility status of M. grisea *isolates*

A total of 250 crosses were made with the MAT1.2 tester strains GUY11 and KA9. Isolates that formed perithecia were designated as male, female, or hermaphroditic according to the nomenclature of Itoi et al. [22]. Male fertile isolates formed perithecia only on the side of the tester, while

Table 1. Mating-type distribution of *Magnaporthe grisea* populations from rice sampled between 2000 and 2003 in Argentina

Provinces	Location	Year of collection	Varieties sampled	Number of isolates	Mating type
Corrientes	Mercedes	2000/2001	El Paso 144	1	*MAT*1.1
	Mercedes	2002/2003	El Paso 144	1	*MAT*1.1
Santa Fe	Colonia San José, San Joaquín	2000/2001	Fortuna, Diamante	42	*MAT*1.1
	Colonia San José, San Jacinto	2002/2003	Fortuna, Diamante	46	*MAT*1.1
Buenos Aires	Los Hornos	2000/2001	La Plata Mochi	7	*MAT*1.1
	Los Hornos	2002/2003	La Plata Mochi, El Paso 144	10	*MAT*1.1
Entre Ríos	Villaguay	2000/2001	Carnaroli, Itapé, Colonia Mascías 5 SCA	15	*MAT*1.1
	La Paz	2002/2003	La Plata Mochi	3	*MAT*1.1

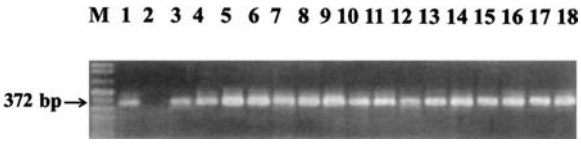

Figure 2. Polymerase chain reaction based mating-type assay of *Magnaporthe grisea* isolates of known and unknown mating type. The group of isolates tested represented all the regions when they were collected. PCR products separated after electrophoresis in a 1% agarose gel and stained with ethidium bromide. M = molecular size standard VIII-ladder (Boheringher); Lane 1 = positive control of amplification containing a MAT1.1 tester isolate (KA7) using A1 and A5 oligonucleotides; Lane 2 = amplification of a MAT1.2 tester isolate (GUY11) using A1 and A5 oligonucleotides; Lanes 3–18 = DNA fragments amplified from a representative set of field isolates from different regions.

female fertile isolates formed perithecia only on the side of the field isolate. Isolates that produced perithecia on both sides of the tester and the field isolate were considered hermaphrodites.

In positive control crosses between the standard hermaphroditic rice isolates fully developed perithecia containing asci and ascospores were observed approximately 20 days after inoculation. All field isolates failed to form perithecia in crosses with GUY11; however, some fertility was observed with isolate KA9 in 7.2% of isolates. Low fertility was observed (< 10 perithecia per cm^2) among the nine isolates that formed perithecia and all of them were female sterile. The perithecia ocurred singly or in groups, with the base partially or wholly embedded in the culture media. However, even when perithecia formed they were all found to be barren with no evidence of development of asci and ascospores.

Discussion

This is the first report describing the mating-type distribution and fertility status among populations of *M. grisea* isolates from rice in Argentina.

The majority of rice isolates analyzed for fertility status in this study failed to produce perithecia and, consequently, they are presumed to be sterile. Only a low percentage of the *M. grisea* isolates were sexually fertile, forming perithecia in laboratory mating experiments. The 125 isolates collected over a 4 years period belonged to a single mating type, *MAT*1.1, indicating a predominant asexual reproduction. The predominance of a single mating type as observed in this study has been previously reported in different works, analyzing worldwide distributed isolates [2, 23]. Particularly, in a survey of 467 rice pathogens from 34 countries of Europe, Asia, Northern South America and Central Africa, MAT1.1 was found in Europe, Central Africa and in Northern Asia [13]. Also we have shown that the level of fertility among the Argentinean isolates was very low (7.2%). Similar results have been reported for isolates from other countries [10, 12, 13]. The low fertility of the *M. grisea* isolates as indicated by female sterility, suggest that the capacity for expressing the female characteristic might have been lost. Hermaphroditic isolates of *M. grisea* are

known to behave as male fertile (female sterile) due to degeneration from sexuality to unisexuality [17]. Leslie and Klein have developed a model to explain the occurrence of sterility in Ascomycetes [24]. They propose that many different single mutations could result in female sterility and that maleness should be less prone to loss by random mutation. Studies on the fertility of the rice blast pathogen have shown a consistent pattern, where isolates were typically male fertile only [13, 16, 25].

Only perithecia without asci and ascospores were observed in crosses involving the 125 rice isolates as it was reported by Kato and Yamaguchi [11], Yaegashi [26], and Tanaka et al. [27]. This may be due to genetic differences in compatibility, since other isolates produced ascospores under the same conditions. A variety of genetic abnormalities, including mutations, chromosomal duplications and heterogeneity, and formation of lagging chromosomes during nuclear divisions at the time of ascus formation, are possible reasons for resulting in barren perithecia [27].

The population structure of isolates pathogenic to rice appears to be clonal, as evidenced by the absence of female fertility among the rice isolates in most rice growing regions. The presence of the same multilocus haplotypes [28, 29] and the predominance of a single mating type in most rice growing regions [13] also support this conclusion. Thus, the sexual cycle does not seem to be a source of variation in nature for isolates of *M. grisea* that infect rice in Argentina. Probably, other evolutionary forces, such as mutation, gene flow and recombination through parasexual cycle are alternative sources of genotypic variation that must be considered [30].

We conclude that the sample populations of *M. grisea* from rice in Argentina are similar to populations from rice in other countries with respect to mating-type distribution and fertility status [13, 22, 23, 25, 31–34].

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