Mating Type Distribution and Fertility Status of *Magnaporthe grisea* Populations from Various Hosts in India

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ABSTRACT

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Production of perithecia, asci, and ascospores by Indian isolates of *Magnaporthe grisea* is rare and has not been found among the Southern Indian isolates of the blast pathogen. From among 138 monoconidial isolates that infect rice and other hosts, we now report the distribution of mating types (MAT1-1 and MAT1-2) of *M. grisea* in finger millet and paragrass (*Brachiaria mutica*)-infecting isolates. Twenty-eight of the 96 finger millet isolates, 5 of the 16 paragrass isolates, and none of the 26 rice isolates produced fertile perithecia in laboratory matings with fertile testers. Backcrosses of ascospore progenies to the parental *M. grisea* isolate but not to the tester strain resulted in fertile perithecial formation, and a further backcrossing scheme indicated definite fertility patterns of Mendelian inheritance in *M. grisea*.

Additional keywords: blast, finger millet, perithecia

The perfect state of *Pyricularia grisea* (Cooke) Sacc. was first discovered by Hebert (3), who demonstrated heterothallism in this fungus. Since then, efforts have been made to produce the perfect state of *P. grisea*, the causal organism of blast in many economic crops such as rice, wheat, and finger millet around the world. The formation of perithecia in crosses of isolates from grass, millets, and cereals (8,14,17,23) has been reported outside India.

The ability of *Magnaporthe grisea* (Hebert) Barr (teleomorph of *P. grisea*) isolates to produce perithecia indicates a complex phenotype (19), and mating ability is an excellent criterion for examining the relationship among isolates of the fungus. The genetic relationship between female fertility and pathogenicity to rice in *M. grisea* was found to segregate randomly. Female fertility segregated in a 1:1 ratio independent of mating type (9). Itoi et al. (4) distinguished Japanese isolates of *P. grisea* as mating type "A" or "a" depending on whether the perithecia were black-beaked or white-beaked. One gene with two alleles, designated MAT1-1 and MAT1-2 according to the genomic nomen-

clature of Yoder et al. (26), controls its mating type (10,12,13). The mating type genes have been isolated using the genomic subtraction technique for cloning mating type idiomorphs from *M. grisea* (5).

Extensive mating experiments have shown differences in the degree of sexual compatibility between *Magnaporthe* isolates from various hosts in Japan (8,22). Fertility in *M. grisea* field isolates ranges from total sterility (inability to mate with any other strain), through female sterility (ability to mate only as a male), to full fertility (ability to mate either as male or female; 18). According to Valent et al. (19), at least one parent must be female fertile, with the capacity to produce perithecia, and two parents must be able to produce asci with viable ascospores. In general, crosses between isolates of *M. grisea* from goosegrass (*Eleusine indica* (L.) Gaertn.) and weeping lovegrass (*Eragrostis curvula* (Schrader) Nees) had higher fertility (6,13,23).

The sexual stage is regarded as advantageous because of the variety of genotypes that can arise due to recombination. An understanding of the distribution of mating types could indicate the chances of sexual reproduction, and can be important for the control of blast through resistance breeding programs. Since there has been no report on the production of the sexual state in Southern India (1), we initiated a careful search for the trait (20) when we observed that a rice isolate of *M. grisea* from Kerala produced ascospores in the laboratory (*unpublished results*). The objectives were to test the mating type and sexual compatibility of the South Indian isolates, which

included 96 *M. grisea* isolates from finger millet, 26 from rice, and 16 from nonrice hosts. Standard testers of mating types MAT1-1 and MAT1-2 were used for identification of the degree of sexual compatibility between the *Magnaporthe* isolates from the different hosts.

MATERIALS AND METHODS

Collection of samples. Plants showing typical blast symptoms on leaf, neck, and fingers (panicle) were collected at different periods during 1993 to 1995. Collections comprising 265 samples were assembled from the finger-millet-growing South Indian states of Tamil Nadu, Andhra Pradesh, Karnataka, and Kerala (site of a seedbed experiment; Fig. 1A; Table 1). Blast-infected samples were also collected from rice from Nepal and Tamil Nadu, and paragrass (*Brachiaria mutica* (Forssk.) Stapf.), a grass species that grows next to rice in field bunds, from Kerala and Tamil Nadu.

Isolation and maintenance of *M. grisea*. Infected samples with single and discrete blast lesions were washed in running water for about 30 min, surface-sterilized in 0.01% mercuric chloride solution, and placed on moist filter paper in petri dishes. The plates were incubated at 25°C for inducing sporulation. The sporulating lesion was gently tapped to dislodge the conidia onto a potato dextrose agar (PDA) plate. Monoconidial isolates were picked up aseptically using a long capillary tube, grown separately as pure cultures, and stored for further use. Long-term storage was in sterile filter paper disks at -20°C.

Determination of mating type. Mating type was determined by pairing each unknown *M. grisea* isolate with four standard hermaphroditic testers (supplied by J. L. Notteghem, CIRAD, France). Two of the standard testers, KA-3 and KA-7, were mating type MAT1-1, and the other two, KA-9 and GUY-11, were MAT1-2. The testers KA-3, KA-7, and KA-9 (not pathogenic to rice) were originally isolated from finger millet and produced numerous perithecia with isolates of the opposite mating type (13). The fourth tester, GUY-11, was isolated by J. L. Notteghem from diseased rice leaves collected in French Guyana (11,12).

Crossing method and ascospore isolation. Crosses were made by pairing agar blocks, cut from the margins of growing colonies, about 5 cm apart on oat meal agar

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(OMA) plates. The agar plates were sealed and incubated at 27°C for a week, and then placed under continuous white fluorescent light at 22 to 24°C. After 30 days of incubation, the junctions between the mated individuals were examined for perithecial formation.

Individual perithecia were removed from the agar surface, rinsed in sterile water to remove the adhering conidia, crushed, and dragged along the agar surface of PDA plates to produce a trail of asci on the agar surface (2). Individual colonies were picked with a bent glass needle, transferred to PDA plates, and stored on sterile filter paper disks.

Mating scheme. Pairwise matings were made between the fertile finger millet isolate Pb2.1.1 and tester KA-3. The resulting progenies were backcrossed again to determine if higher fertility is introgressed into laboratory isolates that infect finger millet. The progenies were observed for fertility. The flow chart for the mating scheme is shown in Figure 2.

Assessing fertility of *M. grisea* isolates. Compatibility between the *M. grisea* isolates of finger millet, rice, and paragrass was determined by the capacity to form perithecia, asci, and ascospores. The number of perithecia formed per 5 mm² agar surface was recorded by a measure of fertility in each cross. Based on a modified method of Leung and Williams (11,12), isolates were classified as high (more than 20), intermediate (10 to 19), and low (less than 10 perithecia/5 mm²) fertility groups. Isolates that did not produce perithecia were classed infertile. All isolates that produced perithecia were assessed again for fertility. Perithecia were also scored for the presence or absence of asci and ascospores.

RESULTS

Distribution of mating type. A total of 96 monoconidial isolates from finger millet, 26 from rice, and 16 from paragrass (*Brachiaria mutica*) were isolated from the blast samples. Their mating behavior was identified using tester strains. All four testers were effective for mating type identification. Mature perithecia were observed about a month after isolates of different mating types were crossed.

The distribution of mating types of *M. grisea* isolates is shown in Table 2. Of the 96 finger millet isolates, 28 showed perithecial formation with 15 isolates of MAT1-1 type and 13 of MAT1-2 (1:1) type. Finger millet isolates from both locations in Andhra Pradesh (Chittoor and Gudipala) were of MAT1-1 type, while both mating types were observed in the collection of isolates from Tamil Nadu, Kerala, and Karnataka. However, one of the two mating types was predominant in these areas of sampling. While MAT1-2 type was dominant in Pattambi (Kerala) and Paiyur (Tamil Nadu), MAT1-1 type

was dominant in Thiruvallur (Tamil Nadu) and Mandhya (Karnataka). Monoconidial isolates derived from a blast lesion were of the same mating type. The examples are, PA 83.1.1 and PA83.1.3, AP313.1.1 and AP313.1.2, AP314.1.1 and AP314.1.2, PB1.1.2 and PB1.2.1, PB2.1.2 and PB2.2.1, PB3.1.2, PB3.2.1 and PB3.2.2, PG2.1.1 and PG2.1.2 (Table 3). All isolates collected from Gummidipoondi (Tamil Nadu) were uniformly sterile.

Among the 16 *M. grisea* isolates from paragrass, production of perithecia was observed in 5, and all of them belonged to mating type MAT1-1. None of the rice isolates produced perithecia; hence, mating type was not determined. In crosses be-

tween the rice tester GUY-11 and the 96 finger millet isolates, perithecial formation was observed in two combinations but without the production of asci and ascospores (Table 3).

Fertility of *M. grisea* isolates. Table 3 lists the 33 isolates of *M. grisea* (finger millet and paragrass) that were fertile. Using perithecial production as an indicator of fertility, the fertility of progenies derived from intermatings were classified as high, intermediate, or low. Of the 28 finger millet isolates, 13 (13.5%) were highly fertile (more than 20 perithecia/5 mm²), 12 (12.5%) were intermediately fertile (10-19 perithecia/5mm²), 3 (3.1%) were low in fertility (less than 10 perithe-

Fig. 1. (A) Map showing the sampling locations in Southern India where *Magnaporthe grisea* from different hosts were collected. (B) Mature perithecium produced in the laboratory when a *M. grisea* isolate pathogenic to finger millet was mated with a fertile tester, KA-3 (supplied by J. L. Notteghem, France) (×100). (C) Ascospores of *M. grisea* (×200).

Table 1. *Magnaporthe grisea* isolates from finger millet, rice, and paragrass (*Brachiaria mutica*) obtained from the different states of India

Sample no.	State	No. of leaf blast leaves and lesions		Monoconidial isolates
Finger millet				
1.	Tamil Nadu	58	149	33
2.	Andhra Pradesh	23	49	26
3.	Kerala	6	21	19
4.	Karnataka	39	46	18
Rice				
1.	Nepal	5	12	9
2.	Tamil Nadu	8	24	17
Paragrass				
1.	Kerala	8	16	6
2.	Tamil Nadu	7	25	7

Finger millet and paragrass isolates were most fruitful in frequency and degree of fertility when crossed with testers, while crosses with rice isolates were uniformly sterile.

1C) and progenies A1.1 to A1.8 were isolated (Fig. 2). When these first-generation progenies were crossed back to the parental strains, none of the crosses to the tester strain KA-3 produced perithecia, while 5 out of 8 crosses to parental isolate Pb2.1.1 produced perithecia with ascospores. Crosses with A1.1, A1.5, A1.7, and A1.8 produced a single row of perithecia (indicating female sterility and male fertility), while with A1.6 a double row of perithecia (indicating male and female fertility) was produced. Hundreds of perithecia produced on the side nearer to A1.6 were buff-colored (pigment-defec-

To further examine the inheritance of fertility, ascospores B1.9, B1.10, and B1.11 were isolated from the cross Pb2.1.1 and Asc A.1.1. From the buff-colored perithecia produced in the cross between Pb2.1.1 and Asc A1.6, ascospores Xa, Xb, Xc, Xd, and Xe; and Ya, Yb, Yc, Yd, and Ye were isolated. Ascospores Za, Zb, Zc, and Zd were isolated from the darkly pigmented perithecia. When these second-generation progenies were crossed back to KA-3 and Pb 2.1.1, it was observed that the crosses Ya and B1.9 with KA-3 could produce perithecia with the production of asci and ascospores. Xa and Xd produced perithecia with the parental strain Pb2.1.1. The other combinations, however did not form perithecia (Fig. 2).

similar studies with 130 rice isolates tested by Yaegashi and Yamada (25), 20% were MAT1-1, 32% were MAT1-2, and 48% were not fertile. Results presented in Table 3 show also that *Eleusine* isolates were more compatible with each other and with the *Brachiaria* isolates than with the rice isolates. Our results indicate that the mating type varies with the geographic region. That an isolate obtained from rice in Kerala produced ascospores (Brindha and Gnanamanickam, unpublished results) shows, perhaps, that rice isolates from regions of cooler temperatures may influence perithecial formation. This needs to be verified.

Variation in genetic traits conditioning sexual reproduction among the isolates was observed. *M. grisea* isolates from finger millet and paragrass were highly fertile in terms of perithecial production when compared to rice isolates. Similar results have been reported by Kato et al. (8) and Kato and Yamaguchi (7). Host association had been described as an important factor conditioning the fertility of *M. grisea* field isolates (9,12), and genetic distinctiveness of rice and nonrice isolates in terms of their fertility observed among the Indian isolates in the present study confirms this. The hermaphrodites that are commonly

found among finger millet isolates may account for their high fertility. The rice isolates used in this study behaved only as males, suggesting that these might have been hermaphroditic, and may have lost the female characteristic due to the degeneration of sex to unisexuality. However, the availability of isolates such as GUY-11 shows that this loss is not yet complete. Studies of Yaegashi and Yamada (24), Leung (10), and Notteghem and Silue (13) confirm the low fertility of *M. grisea* isolates pathogenic on rice, as shown by the low viability of ascospores in the most successful crosses.

Two crosses involving finger millet isolates and tester GUY-11 produced perithecia but were devoid of asci and ascospores. Reports of Yaegashi (22), Tanaka et al. (16), and Kato and Yamaguchi (7) have shown that crosses involving rice isolates result in barren perithecia. This may be due to the genetic differences in compatibility, as other isolates produced ascospores under the same conditions. A variety of genetic abnormalities, including mutations, chromosomal duplications, and heterogeneity are known to result in barren perithecia (16). The use of limited number of *M. grisea* isolates of rice evaluated, and the fewer locations from which they were

collected, may have contributed to the non-production of perithecia or the production of barren perithecia in crosses involving these rice isolates. Therefore, our search is being extended to involve a larger number of rice isolates collected from the different rice-growing regions of India.

The population structure of isolates pathogenic to rice appear to be clonal, as evidenced by the near absence of female fertility among the rice isolates. The presence of the same multilocus haplotypes (15,21), and the predominance of a single mating type in most rice growing regions (13), also support this. Thus, the sexual cycle does not seem to be a source of variation in nature for isolates of *M. grisea* that infect rice in India. Itoi et al. (4) and Valent et al. (18) have also reported similar results. If sexual reproduction occurs, it is probably infrequent and might not be significant for the rice blast epidemiology.

Backcrossing of the progenies to parental strains resulted in the enhancement of fertility in subsequent generations. Such backcrossing schemes confirm their usefulness in bridging the infertility barrier among *P. grisea* isolates.

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Table 3. Mating type and fertility of Indian isolates of *Magnaporthe* when crossed with standard testers

Isolate no.	Host of origin	Tester	Mating type	Sex organs produced	Perithecia ^a
Tamil Nadu					
TOP71.1.1	Finger millet	KA-9	MAT1-1	Perithecia	26
PA83.1.1	Finger millet	KA-7	MAT1-2	Perithecia, asci, and ascospores	7
PA83.1.3	Finger millet	KA-7	MAT1-2	Perithecia	15
TPO52.1.2	Finger millet	KA-9	MAT1-1	Perithecia	26
TPO53.1.1	Finger millet	KA-9	MAT1-1	Perithecia, asci, and ascospores	20
PA84.1.1	Finger millet	KA-7	MAT1-2	Perithecia, asci, and ascospores	12
TPO75.1.1	Finger millet	KA-7	MAT1-2	Perithecia	20
PA82.1.2	Finger millet	KA-3	MAT1-2	Perithecia, asci, and ascospores	12
TG44.1.1	<i>Brachiaria mutica</i>	KA-9	MAT1-1	Perithecia	20
TG43.1.2	<i>B. mutica</i>	KA-9	MAT1-1	Perithecia, asci, and ascospores	18
Andhra Pradesh					
AP313.1.1	Finger millet	KA-9	MAT1-1	Perithecia	18
AP313.1.2	Finger millet	KA-9	MAT1-1	Perithecia, asci, and ascospores	17
AP314.1.1	Finger millet	KA-9	MAT1-1	Perithecia, asci, and ascospores	23
AP314.1.2	Finger millet	GUY-11	MAT1-1	Perithecia	8
AP311.1.2	Finger millet	KA-9	MAT1-1	Perithecia, asci, and ascospores	35
AP315.1.1	Finger millet	KA-9	MAT1-1	Perithecia, asci, and ascospores	15
AP328.1.1	Finger millet	KA-9	MAT1-1	Perithecia	15
Karnataka					
K348.1.1	Finger millet	KA-9	MAT1-1	Perithecia	25
K349.1.1	Finger millet	KA-9	MAT1-1	Perithecia, asci, and ascospores	24
K381.1.2	Finger millet	KA-9	MAT1-1	Perithecia, asci, and ascospores	5
K385.1.2	Finger millet	KA-3	MAT1-2	Perithecia, asci, and ascospores	21
Kerala					
Pb1.1.2	Finger millet	KA-7	MAT1-2	Perithecia, asci, and ascospores	19
Pb2.1.1	Finger millet	KA-3	MAT1-2	Perithecia, asci, and ascospores	21
Pb2.1.2	Finger millet	KA-9	MAT1-1	Perithecia, asci, and ascospores	38
Pb2.2.1	Finger millet	GUY-11	MAT1-1	Perithecia	18
Pb3.1.2	Finger millet	KA-7	MAT1-2	Perithecia, asci, and ascospores	12
Pb3.2.1	Finger millet	KA-3	MAT1-2	Perithecia, asci, and ascospores	22
Pb3.2.2	Finger millet	KA-7	MAT1-2	Perithecia, asci, and ascospores	28
Pb5.1.2	Finger millet	KA-3	MAT1-2	Perithecia	18
Pb8.1.2	Finger millet	KA-3	MAT1-2	Perithecia, asci, and ascospores	19
Pg2.1.1	<i>B. mutica</i>	KA-9	MAT1-1	Perithecia, asci, and ascospores	19
Pg2.1.2	<i>B. mutica</i>	KA-9	MAT1-1	Perithecia	26
Pg1.3	<i>B. mutica</i>	KA-9	MAT1-1	Perithecia, asci, and ascospores	28

^a Number of perithecia in 5-mm² agar surface.

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