Distribution of the Mating Type Alleles in *Magnaporthe grisea* Populations Pathogenic on Rice

J. L. Notteghem and D. Silué

Institut de Recherches en Agronomie Tropicale, Avenue du Val de Montferrand, Laboratoire de Phytopathologie, 34032 Montpellier CEDEX, France.

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ABSTRACT

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Four hundred sixty-seven *Magnaporthe grisea* isolates were collected from rice in 34 countries. Each isolate was tested for mating type with two MAT1-1 and two MAT1-2 fertile (hermaphroditic) standard isolates. Thirty-two percent of these isolates are MAT1-1, and 20% are MAT1-2. The remaining 48% do not produce perithecia with any of the four fertile testers. In most locations, only one mating type was present. In two locations (one in Ivory Coast and one in Cameroon), isolates of both mating types were found, but they were intersterile. Almost all the isolates had very low fertility, because they were, in general, female sterile and sometimes also male sterile. Few crosses were fertile enough to permit the isolation of germinating ascospores. Crosses between the hermaphroditic isolate GUY11 and three other male isolates gave ascospore progeny with mating type that segregated 1:1 among the progeny. This suggests that these isolates could be used for the genetic analysis of pathogenicity.

Additional keywords: *Pyricularia grisea*, *Pyricularia oryzae*.

Magnaporthe grisea (T. T. Hebert) Yaegashi & Udagawa (1) is a hermaphroditic heterothallic ascomycete (4). One gene with two alleles, designated MAT1-1 and MAT1-2 according to the genetic nomenclature of Yoder et al (29), controls its mating type. The anamorphs of *M. grisea* are currently classified as *Pyricularia oryzae* for isolates pathogenic to rice and *Pyricularia grisea* for isolates pathogenic for other graminae. Both anamorphs have the same sexual stage and are interfertile (5,7,9–11,20,22,25,27,28). These criteria, with others, have led to the proposition of grouping both anamorphs into *P. grisea* (16), the same name used in this paper.

Few crosses are possible between *P. grisea* isolates pathogenic to rice, because most of them are female sterile (6,10,14). Furthermore, when perithecia are produced, ascospore viability is very low (10,15,27). There are two possible ways of resolving this problem. One is to cross isolates that are pathogenic to rice and have a low fertility with fertile isolates pathogenic to graminae other than rice. Unfortunately, progeny obtained from such crosses have a low pathogenicity on rice (13,14,21–23), and backcrosses are necessary to recover progeny fully pathogenic on rice (21,23). Besides avirulence genes, pathogenicity on rice and fertility have been shown to be under polygenic control (13,23). Thus, it might be difficult to obtain fertile progeny as pathogenic to rice as field isolates are. The second way is to look for isolates pathogenic on rice that have a better fertility than those so far collected. The earlier results suggest that many isolates have to be screened to achieve this goal. Kato and Yamaguchi (10) and Yaegashi and Yamada (27) reported the screening of a total of 926 isolates, and none were sufficiently fertile to carry out genetic studies. However, an opportunity to screen many isolates for their fertility in crosses was presented by a new collection of rice isolates set up as a part of a European Community project on the variability of the rice blast fungus in the CIRAD (Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement) laboratory of Montpellier, France. Therefore, we have tested 467 rice isolates for their ability to form perithecia when mated with two MAT1-1 and two MAT1-2 standard isolates.

Because sexual reproduction produces progeny of the two mating types in equal proportions, analysis of mating type ratios in *M. grisea* populations may indicate occurrence of sexual reproduction in nature. The mating type gene distribution could also provide an evaluation of genetic diversity of *M. grisea* populations.

MATERIALS AND METHODS

Collection of isolates. Four hundred sixty-seven *Pyricularia grisea* isolates were collected from rice between 1976 and 1990. This collection was composed of isolates with wide diversity in geographic source and virulence. This was achieved by collecting isolates from several regions within 34 countries representative of almost all the rice-growing areas and from diverse host (rice) genotypes. Among the 467 isolates, 25 were obtained from other laboratories, and 442 were isolated from diseased rice leaves or panicles collected in fields. In all cases, a monoconidial isolation was made. Conidia contain three nuclei that originate from one nucleus in the conidiophore (3). Therefore, monoconidial isolates are genetically homogenous.

Storage of isolates. Each isolate was stored just after monoconidial isolation. Two methods were used simultaneously. In the first one, the fungus was grown on rice culms in small tubes for 10 days, then dried at 30 C, and stored at −20 C. In the second method, similar to that described by Valent et al (22), the cultures were grown on filter paper overlaying an agar medium. The colonized filter paper was then dried at 30 C, cut into small

pieces, and distributed into several sterile paper bags. Each paper bag was then placed in a plastic bag, which was sealed under vaccum and stored at −20 C. This second method allowed the multiplication of initial stock of each isolate.

Cultivation media. Complete rice flour media (rice flour, 14 g; yeast extract, 2.5 g; agar, 15 g; water, 1 L; and speciline 500,000 IU, added after autoclaving) were used for conidial production and for crosses.

Standard isolates for mating type determination. For mating type identification, each isolate was tested with four standard fertile isolates. Three of the testers were choosen as standard hermaphroditic isolates among 19 fertile isolates provided by H. Kato, (13 from finger millet, *Eleusine coracana*, and six from rice). Two of the three isolates were of mating type MAT1-1 (OG2,IN1) and the other of mating type MAT1-2 (OG5) (Table 1). All three were isolated from finger millet and were not pathogenic to rice, but they produced a high number of perithecia with most isolates having the opposite mating type. The fourth tester, GUY11, has already been described (15). GUY11 was isolated in 1979 from diseased rice leaves collected in French Guiana. This isolate was shown to be hermaphroditic, and it can be used as a MAT1-2 tester. Therefore, all the rice isolates of our collection were mated with GUY11, the only hermaphroditic rice pathogen available at this time, and with three hermaphroditic isolates from finger millet.

The use of additional testers chosen among the 19 fertile isolates received from H. Kato or among fertile progeny obtained from the cross GUY11 × ML25 (17) did not significantly improve mating type identification. Therefore, additional testers were not retained in this study.

Crossing method. Petri dishes containing rice flour agar were inoculated with two standard isolates of the same mating type and one isolate to be tested, as already described (23). After three days of growth at 28 C, the petri dishes were placed under fluorescent lights at 20 C. They were observed 15 and 21 days after inoculation, and the number of perithecia was recorded. When perithecia were observed in crosses between GUY11 and other rice isolates, ascus production and ascospore morphology were observed under the microscope.

Ascospore isolation. Mature perithecia were transferred to 4% water agar and then opened with a sharp scalpel under a stereomicroscope (×64). Asci were scatterred on agar using a

TABLE 1. Origins and references of fertile strains of *Magnaporthe grisea*

Code number of IRAT laboratory	Code number of original laboratory	Mating type	Country of origin	Original host	References
OG2	UG77-15-1-1	MAT1-1	Uganda	*Eleusine coracana*	H. Kato
IN1	IN77-24-1-1	MAT1-1	India	*E. coracana*	H. Kato
OG5	UG77-14-1-1	MAT1-2	Uganda	*E. coracana*	H. Kato
JP16	KEN73-0-1	MAT1-1	Japan	*Oryza sativa*	H. Kato
IN2	IN77-51-3	MAT1-1	India	*O. sativa*	Kato and Yamaguchi 1982
JP4	INA72	MAT1-1	Japan	*O. sativa*	Kiyosawa 1967
JP9	INA168	MAT1-1	Japan	*O. sativa*	Kiyosawa 1967
CH2	CH40-1	MAT1-1	China	*O. sativa*	Kolmer and Ellingboe 1988
CD128	...	MAT1-1	Ivory Coast	*O. sativa*	
ML25	...	MAT1-1	Mali	*O. sativa*	
GUY11	...	MAT1-2	French Guiana	*O. sativa*	Leung et al 1988

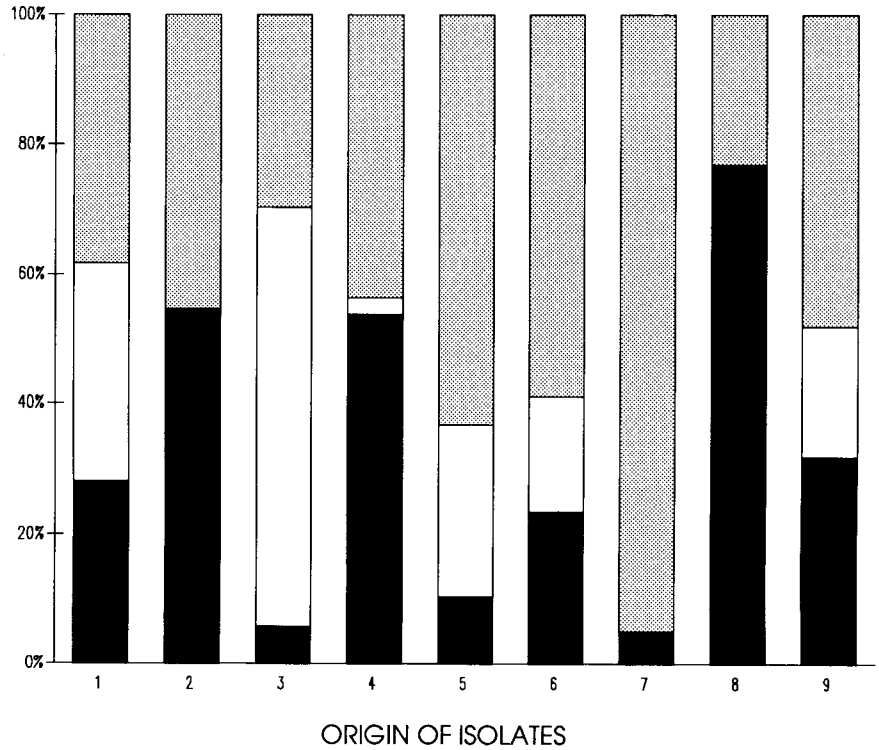

Fig. 1. Comparison of mating type distributions for isolates of *Magnaporthe grisea* from eight regions. Origins of the isolates are: 1, Western Africa (Ivory Coast, Mali, Burkina Faso), 110 isolates; 2, central Africa (Burundi, Rwanda, Zaire), 53 isolates; 3, Madagascar, 17 isolates; 4, northern Asia (China, S. Korea, Taiwan), 35 isolates; 5, Philippines, 38 isolates; 6, northern South America (Surinam, Guyana, French Guiana), 17 isolates; 7, Brazil, 39 isolates; 8, Europe (France, Italy, Spain), 48 isolates; 9, average of 34 countries, 467 isolates. Lightly shaded areas = unknown; darkly shaded areas = MAT1-1; and white areas = MAT1-2.

glass microroll pin. After half an hour, the spontaneous lysis of ascus walls allowed the dispersal of the ascospores using a glass roll pin. Ascospore germination was observed 6–8 hr after dissection at 28 C. Germinated ascospores were then transferred onto the rice flour agar.

Pathogenicity assay. *P. grisea* isolates were cultivated on rice flour agar at 28 C under fluorescent lights. After 10 days of subculture, the mycelium was scraped and flooded with water. After agitation, this suspension was filtered through a cheesecloth yielding essentially a mycelium-free spore suspension, which was ajusted to 25,000 conidia per milliliter. Inoculation was performed by injecting the spore suspension between leaf sheaths of 3-wk-old rice plants (four-leaf stage). Symptoms were recorded 7 days later. The susceptible rice cultivar Maratelli was used in all experiments to check the pathogenicity of isolates or progeny on rice.

RESULTS

The mating type of 243 isolates (52% of the collection) was identified. The remaining 48% did not mate with any testers. Differences in the number of isolates from each country made it difficult to compare these samples. It appears more interesting to compare samples from wide geographical areas as shown in Figure 1. In three areas, West Africa, Philippines, and northern South America, we found both mating types in roughly equal abundance. In four regions, one mating type was dominant. Only MAT1-1 isolates were found in Europe and central Africa, and MAT1-1 isolates were predominant in northern Asia. MAT1-2 isolates predominated in Madagascar. The fertility of isolates from Brazil was particularly low.

It is of interest to know the distribution of mating type at the 14 locations where at least four isolates were collected at the same time (Table 2). At five locations, all isolates were sterile. At eight sites, only one mating type, either MAT1-1 (two locations) or MAT1-2 (six locations), was found. At two African sites (Ndock in Cameroon and Farako in Ivory Coast), isolates of both mating types were found. Attempts to mate these isolates of opposite mating type were unsuccessful, indicating that they were intersterile.

The efficiency of the four fertile standard isolates in crosses with unknown isolates was similar (Table 3), but they had some specific compatibilities. The use of four standard isolates instead of two allowed the identification of the mating type of almost 13% more tested isolates than it would have been possible if only isolates OG5 and IN1 had been used as testers.

None of the 467 *P. grisea* isolates tested were female fertile. GUY11 mated with approximately 30% of isolates of opposite mating type. Only seven of all these crosses were fertile enough to obtain viable ascospores.

All crosses between GUY11 and isolates pathogenic on rice were further investigated by testing ascospores for viability and rice pathogenicity. Germination of ascospores isolated from the cross GUY11 × INA168 was too low to get more than one ascospore per perithecia. Progeny from the crosses GUY11 × CH40-1 and GUY11 × IN77-51-3 were not as pathogenic on rice as field isolates. Progeny from the cross GUY11 × KEN73-0-1 did not segregate 1:1 for mating type, probably due to problems during meiosis. In the three best crosses so far identified (GUY11 × JP4, GUY11 × CD128, GUY11 × ML25), we were able to obtain random ascospore progeny pathogenic on rice, among which mating type segregated 1:1. Ascospore germination was too low (around 5%) to allow tetrad isolation. We were able to back-cross the progeny of these crosses to GUY11. In two cases, we were able to make back-crosses up to BC7, but after BC3 no increase of fertility was observed (18). Analysis of the segregation of virulence among those progeny is in progress (19).

DISCUSSION

Determination of the frequency of each mating type in natural populations of *M. grisea* requires large numbers of field isolates and highly fertile standard tester strains of known mating type. Results from Kato and Yamaguchi (10) and from Yaegashi and Yamada (28) are similar to those reported here. In Japan, only MAT1-1 isolates were identified by Kato and Yamaguchi (10). But Yaegashi (24) and Yaegashi and Yamada (28) found, respectively, that 23 and 18% of isolates were MAT1-2. The low percentage of MAT1-2 isolates, identified by Kato and Yamaguchi (10), among 451 isolates from Japan (0%) as well as among 267 isolates from 18 countries (6.7%) could be partially due to a low fertility of the MAT1-2 testers they used. Yaegashi and Yamada (28) observed the following mating type distribution among 130 isolates from six countries: MAT1-1, 20%; MAT1-2, 32%; and unknown, 48%. This result is close to the ratio we observed among

TABLE 2. Mating type of sets of four or more strains of *Magnaporthe grisea* isolated at the same time and location

Continent	Country	Location	Data of isolation	MAT1-1	MAT1-2	?[a]	Total
Africa	Burkina Faso	Bobo Dioulasso	10/84	0	0	4	4
		Banfora	10/84	0	3	1	4
	Benin	Moussourou	9/85	0	8	0	8
	Cameroon	Karewa	10/85	3	0	1	4
		Ndock[b]	10/85	1	2	2	5
	Ivory Coast	Bouaké	10/84	0	4	6	10
		Odienné	9/84	0	3	1	4
		Farako[b]	10/84	3	1	1	5
	Senegal	Djibelor	10/86	0	4	0	4
South America	Brazil	Goiania	3/85	0	0	13	13
	Columbia	Santa Rosa	4/86	0	4	6	10
	Panama	David	8/86	1	0	5	6
Asia	Japan	Fukushima	9/86	0	0	4	4
	China	Tari	10/86	0	0	4	4
	Philippines	Cavinte	9/86	0	0	7	7
Europe	France	Camargue	10/86	7	0	0	7

[a]Numbers under the heading "?" indicate the number of isolates that failed to mate with either tester.
[b]Places where isolates of both mating types were collected.

TABLE 3. Efficiency of four fertile isolates of *Magnaporthe grisea* in mating type identification

Percentage of MAT1-1 isolates			Percentage of MAT1-2 isolates		
With OG5 and GUY11	With OG5	With GUY11	With OG2 and IN1	With IN1	With OG2
40	49	11	42	42	16

467 isolates from 34 countries: MAT1-1, 32%; MAT1-2, 20%; and unknown, 48%. Because mating type ratio varies with the geographical origin of the samples, the ratio is greatly influenced by sampling.

This study confirmed the low fertility of *M. grisea* isolates pathogenic on rice, as shown by the female sterility of almost all the isolates and the low viability among the ascospores obtained in most successful crosses. Nevertheless, we were able to identify fertile crosses by testing a large number of isolates. Some crosses were fertile enough to carry out a genetic analysis of pathogenicity (19). This progress was mainly due to the isolate GUY11, which is female fertile and transmits this characteristic to its progeny. Leung et al (15) and Ellingboe et al (2) have also taken advantage of the hermaphroditic fertile isolate GUY11 to conduct genetic analysis of avirulence.

In nature, only the anamorph of *M. grisea* was observed. In laboratory conditions, perithecia were obtained by co-inoculation on rice of compatible laboratory isolates improved for fertility (17). If sexual reproduction of *M. grisea* occurs on rice, it is probably infrequent and not necessary for the disease cycle. It should be noted that isolates pathogenic to some gramineae other than rice, as those pathogenic to *E. coracana* (finger millet), are highly fertile (8,26). Isolates pathogenic to rice seemed to have lost their ability for sexual reproduction. This process is not yet complete, and a few isolates are still fertile.

One consequence of the absence of sexual reproduction would be that we could consider that isolates with different mating types are clones that have been genetically isolated for a long time. This was observed in the two locations where three mating classes (MAT1-1, MAT1-2, unknown) were identified (Table 2). Because these isolates were intersterile, such polymorphism indicates that the epidemics within a single rice field could be due to different clonal populations.

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