Sexually Fertile *Magnaporthe grisea* Rice Pathogens in Thailand

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ABSTRACT

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Sexual fertility and mating type distribution of *Magnaporthe grisea* field isolates collected in Thailand were analyzed from sites previously found to harbor diverse populations of the pathogen. Three hundred forty-one single conidium isolates of *M. grisea* collected from five sites in north, northeast, and central Thailand were evaluated for in vitro sexual fertility and mating type by pairing with strains of known mating type. Most isolates (67%) were infertile when crossed with the hermaphrodite tester strains; but fertile isolates of each mating type that yielded viable ascospores were detected in all sites from the northeastern and northern regions. MAT1-2 predominated over MAT1-1 in bioassay mating type. Male fertility (female sterility) predominated in fertile MAT1-1 (50 to 75%) and MAT1-2 (50 to 85%) isolates from all locations in Thailand; however, hermaphroditic and/or female fertile isolates were also detected in all but one site. Fertility, as determined by perithecia density, was low (<10 perithecia cm^{-2}) for most isolates, although a few produced in excess of 20 perithecia cm^{-2} .

Magnaporthe grisea is a hermaphroditic, heterothallic ascomycete (anamorph: *Pyricularia grisea*) pathogenic to a large number of gramineae species (9). Its greatest economic impact is as the causal agent of blast disease of rice, wheat, and millets worldwide. The rice blast pathogen is known for its high capacity for asexual reproduction and high degree of pathogenic variation in the field (24); however, until very recently, sexually fertile rice pathogens were considered to be extremely rare. This, combined with relatively simple population structures (5,17), as determined by DNA fingerprinting using the repetitive element MGR586 (6), suggested that *P. grisea* populations are generally composed of clonal lineages (16). Mutation and migration generally were considered to be the sources of observed variation (24), while more recently, evidence has been presented that parasexual recombination may contribute to genetic variation, even in putatively clonal populations (25).

As in other ascomycetes, mating type in *M. grisea* is controlled by a single locus, designated MAT1 (22), with fertile interactions only possible between individuals of opposite mating type (designated MAT1-1 and MAT1-2). However, the ca-

capacity of *M. grisea* isolates to produce perithecia (female fertility) is apparently controlled by genes at several loci, and these segregate independently of mating type and of pathogenicity on different hosts (12). High fertility is common in *M. grisea* strains from nonrice hosts (20). In fertility surveys of rice pathogens results were generally consistent in that female sterility was the norm, fertile isolates were typically male-fertile only, and mating type ratios were skewed (18,20). This rarity of female fertility was consistent with the reported abundance of transposable elements in *M. grisea* rice pathogens (23,24). Translocations and nonhomologous cross-over events can result in meiotic failure. Where the environment permits long-term persistence of well-adapted asexual lineages, mutations can accumulate at loci mediating fertility and result in a predominance of infertility in populations (11,23).

In the late 1980s, hermaphroditic (male- and female-fertile) rice pathogens were found and subsequently used as tester strains against field isolates as well as in back-crossing programs to develop fertile rice pathogens of both mating types (2). More recently, hermaphroditic isolates have been reported from southern China (4) and the Indian Himalayas (13), while a study from southern India reported no sexually fertile rice pathogens (5,19).

North and northeast Thailand are ancient rice-growing areas within the putative center of domestication of the crop (1). The approximately 50 MGR586 fingerprint groups identified in the rice blast fungus populations from this region (M. Poonsak,

unpublished data) represent a much greater genetic diversity than that reported from the Americas, Europe, or the Philippines (3,16,17). Hundreds of traditional land races and improved rice cultivars derived from selections within land races are grown in these regions on several million hectares under variable water regimes (8). In such an environment, a selective advantage for retention of female fertility in the fungus has been proposed (14). In this paper, we present results of a study to determine whether in these traditional rice-growing regions of Thailand sexual fertility is present in field populations of the rice blast pathogen.

MATERIALS AND METHODS

Collection and maintenance of isolates. Leaf blast lesions were collected during 1993 and 1994 from nurseries in five sites in Thailand: Khon Kaen Rice Experimental Station (KKN), Ubon Ratchathani Rice Research Center (UBN), Phrae Rice Research Center (PRE), Sanpatong Rice Experimental Station (SPT), and Ratchaburi Rice Experimental Station (RBN) (Fig. 1). Nurseries consisted of 35 local cultivars and land races plus 75 cultivars and lines originating from various regions of the world, deployed as part of a study to characterize the genetic diversity of the north and northeast Thailand *M. grisea* population (P. Mekwatanakarn, M. Levy, S. Sarkarung, W. Kositratana, and R. S. Zeigler, unpublished).

Sporulation was induced on leaves by overnight incubation in petri dishes at 25°C and 100% relative humidity. Single conidium isolates were generated by streaking conidia on 2% water agar (WA), then picking germinated single conidia after ~24 h and transferring to potato dextrose agar (PDA) for subsequent analyses. All mating type analyses were conducted on single conidium isolates. Long-term storage was on colonized sterile filter paper disks at -20°C.

Determination of mating type. Mating type was determined by pairing each unknown *P. grisea* isolate with two known mating type testers (7): TH12 (Mat1-2) and TH16 (Mat1-1) originally collected from rice in Thailand and generously provided by E. Roumen and J. L. Notteghem, CIRAD, Montpellier, France. (Strains should be requested from J. L. Notteghem, Ecole Nationale Supérieure Agronomique, 2,

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Fig. 1. Location of sites sampled for fertility in *Magnaporthe grisea* in Thailand. KKN: Khon Kaen Rice Experimental Station; UBN: Ubon Ratchathani Rice Research Center; PRE: Phrae Rice Research Center; SPT: Sanpatong Rice Experimental Station; RBN: Ratchaburi Rice Experimental Station.

Fig. 2. Perithecia (A) of *Magnaporthe grisea* produced between two tested isolates. Ascospore (B) of *M. grisea* from mature perithecia (C) of Thai isolate.

Place Pierre Viala, 34060, Montpellier, Cedex, France.) Field isolates were paired with the two standard testers on rice polish agar (RPA) in separate plates, and the plates were sealed with Parafilm and incubated at 30°C for 5 days. Formation of perithecia was induced by placing under continuous fluorescent light at 21°C. Observations were made after 30 days. Fertility was defined as formation of perithecia producing viable ascospores. Mating type of field isolates was designated in fertile crosses as the opposite of the tester with which perithecia were obtained. Definition of male, female, and hermaphrodite followed the nomenclature of Itoi (7): Male fertile field isolates were those for which perithecia formed only on the tester side of the colony merge zone (i.e., the field isolate did not produce perithecia). Female-fertile isolates were those that formed perithecia when paired with a tester, yet did not induce perithecia formation in the tester (i.e., did not function as males). Those field isolates for which pairings yielded perithecia on both sides of the merger line between the field isolate and the tester were designated as hermaphrodites. The density of perithecia (number of perithecia per cm²) was examined at the junction between the unknown isolate and the tester and calculated from three sampling areas of production of perithecia on RPA. Isolates were deemed fertile only if perithecia contained abundant ascospores. Viability of ascospores was tested by crushing mature perithecia (as indicated by septation in ascospores) in a drop of sterile distilled water under a coverslip on a glass slide. Ascospores were observed for germ tubes over 24 to 48 h. Government of Thailand Biosafety regulations prohibited the generation of single ascospore cultures. Plates with perithecia and slides with germinating ascospores were subsequently autoclaved.

Relative fertility of *M. grisea* isolates was determined as the abundance of perithecia and ascospores produced. The number of perithecia formed per cm² on RPA (15) was recorded in each cross, and isolates were classified into high fertility (>20 perithecia per cm²), intermediate fertility (10 to 19 perithecia per cm²), or low fertility (<10 perithecia per cm²) groups.

RESULTS

Three hundred forty-one single conidium isolates of *M. grisea* collected from five sites in Thailand (Fig. 1) were evaluated for mating type and fertility by pairing with testers of known mating type. Perithecia (Fig. 2A) containing ascospores (Fig. 2B) were repeatedly observed in these pairings. Most isolates (67%) were completely sterile (produced no perithecia) when crossed with the hermaphrodite tester strains TH12 and TH16; but isolates of each mating type were detected that

yielded at least some perithecia with viable ascospores. These fertile isolates were found in all sites from the northeast and northern regions, although Mat1-2 (24.6%) predominated over MAT1-1 (8.8%) (Fig. 3). In the central Thailand site (RBN), only MAT1-2 was detected. The highest frequencies of isolates that could be typed for MAT1-1 and MAT1-2 were detected in Ubon, a rice breeding station, where blast severity is typically high and where diversity for blast resistance in the host is also high. MAT1-1 and MAT1-2 hermaphrodites have been placed in the American Type Culture Collection (Accession numbers: ATCC 208985, 208986, and 208987).

Male fertility with female sterility predominated in both MAT1-1 and MAT1-2 mating types at all locations in Thailand, although male-fertility and female-fertility were more nearly balanced among MAT1-1 isolates. Female-fertile, male-sterile isolates were also detected within both mating types and at all sites. Hermaphroditic MAT1-1 or MAT1-2 isolates were rare but were detected in all sites. MAT1-1 hermaphrodites were not detected in PRE and SPT (Fig. 4). The degree of fertility in female-fertile and hermaphrodite isolates, as indicated by perithecia per cm², was generally low (Fig. 5), as was ascospore viability.

DISCUSSION

This report of fertile *M. grisea* strains in Thailand is consistent with other reports of sexual fertility in traditional rice-growing systems in the putative center of origin of the host (4,13). As in all other studies of fertility in rice-derived *M. grisea*, sterility was the norm, and where mating type could be determined via test crosses, mating type ratios were skewed. The overall mating type ratio reported here is similar to that reported by Yaegashi and Yamada (21) in a study of 130 isolates from six countries and by Notteghem and Silué (18) using 467 isolates from 34 countries. In this study, the mating type ratio varied with the geographical origin of the samples.

Male fertility with female sterility dominated among fertile isolates, while hermaphrodites were rare, and unlike most other reports from this species, female-fertile, male-sterile isolates were repeatedly observed. This is similar to reports of female fertility in isolates collected from *Setaria* and *Eleusine* (13). Loss, first of female fertility, then of male fertility, is expected in asexually reproducing ascomycetes where asexual, clonal reproduction is sufficient for season-to-season survival and where vegetative hyphae may serve as donors for male gametes (14). The repeated recovery of female-fertile, male-sterile isolates suggests that there may be a specialized male reproductive apparatus beyond vegetative hyphae (10).

Accumulation of mutations at fertility mediating loci can lead to sterility and

Fig. 3. Mating type distribution among 341 isolates of *Magnaporthe grisea* collected from five Thailand sites in 1993 and 1994. KKN: Khon Kaen Rice Experimental Station; UBN: Ubon Ratchathani Rice Research Center; PRE: Phrae Rice Research Center; SPT: Sanpatong Rice Experimental Station; RBN: Ratchaburi Rice Experimental Station.

Fig. 4. Percentage of males, females, and hermaphrodites among fertile MAT1-1 (A) and MAT1-2 (B) isolates from five sites in Thailand collected in 1993 to 1994. Mating type was determined by bioassay. KKN: Khon Kaen Rice Experimental Station; UBN: Ubon Ratchathani Rice Research Center; PRE: Phrae Rice Research Center; SPT: Sanpatong Rice Experimental Station; RBN: Ratchaburi Rice Experimental Station.

translocations, and chromosome length polymorphisms (CLPs) can lead to poor ascospore survival in otherwise fit lineages. The presence of female-fertile and hermaphroditic isolates in the Thailand populations suggests that while clonal reproduction apparently allows for the accumulation of fitness-neutral mutations leading to sterility, meiosis has been recent enough in some lineages to maintain the genetically complex female reproductive apparatus. The role of sexual recombination in determining population structure in the study regions cannot be determined from this study. However, the highly skewed mating type ratios, the rarity of female fertility, and the generally low fertility of most combinations

suggest that crossing is low in these populations.

Sexual fertility in this pathogen appears to be a population-specific phenomenon. The low fertility with the testers used in this study may reflect fertility of the field isolates or specific near-incompatibility between the tester isolates and the field populations, despite appropriate mating type match. CLPs and translocations that match within populations but differ among populations may determine between-population infertility yet allow within-population sexual reproduction. Earlier global studies of fertility in *M. grisea* tested few isolates from any one population against very few exotic tester strains. Thus, the low reported fertility may simply

reflect the low probability of encountering a fertile isolate within any population, compounded by possible gross genomic incompatibilities between tester isolates and field isolates from different populations. Earlier assessments of fertility in the species should be reexamined, as they may not accurately describe the fertility per se within sampled populations.

With our better understanding of the genomic organization of this fungus and new analytical tools, including more diverse testers, we can now better address the presence and distribution of fertility in *M. grisea* populations. Any global assessment of fertility should now be approached on a population by population basis. Initial mating type surveys of a target population may be cautiously done using PCR, with the caveat that inferences based on survey results be limited to presence or absence of mating type polymorphism. Populations showing evidence for both mating types may then be assayed for fertility using a range of tester isolates from different regions. As locally obtained hermaphroditic strains of both mating types become available from the initial assays, they should be used as testers for a systematic survey of local field isolates.

Fig. 5. Percentage of fertile isolates in each fertility class (perithecia density <10, 10 to 19, and ≥ 20 cm^{-2} for low, intermediate, and high classes, respectively) of *Magnaporthe grisea* within MAT1-1 (A) and MAT1-2 (B) from five Thailand sites in 1993 and 1994. KKN: Khon Kaen Rice Experimental Station; UBN: Ubon Ratchathani Rice Research Center; PRE: Phrae Rice Research Center; SPT: Sanpatong Rice Experimental Station; RBN: Ratchaburi Rice Experimental Station.

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