Cross-Incompatibility Among Indian Isolates of *Pyricularia grisea*

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ABSTRACT

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There have been few investigations into the occurrence of the perfect state of *Pyricularia grisea* in India. We collected isolates of the fungus from several native cereal and grass hosts. We made 756 crosses among 172 monoconidial isolates from these collections of *P. grisea.* None of the matings produced typical perithecia with asci and ascospores. However, 76 crosses produced perithecia-like structures devoid of asci and ascospores. The sexual stage may be rare in India, but this does not seem to interfere with the ability of the pathogen to cause severe epidemics.

Blast disease is the most important disease of rice. There is a great deal of controversy regarding the taxonomy and nomenclature of *Pyricularia.* The form occurring on rice has been called *Pyricularia oryzae* by Cavara. The type species of the genus *Pyricularia* is *Pyricularia grisea* (Cooke) Sacc. The teleomorph of both anamorphs (*P. oryzae* and *P. grisea*) is *Magnaporthe grisea* (T. T. Hebert) Yaegashi & Udagawa (18). Some workers have reported that *P. oryzae* and *P. grisea* are morphologically indistinguishable and interfertile (24,30,36). Most of the taxa known from India are morphologically indistinguishable and several are considered varieties or formae of the species *Pyricularia grisea, Pyricularia oryzae* or *Pyricularia higginsii* (22). In spite of the confusion encountered in the classification and nomenclature of *Pyricularia, Pyricularia grisea* has been considered to be the correct name for the rice blast disease fungus (18), and the same name is used in this report. We regard *M. grisea* as the teleomorph for both *P. oryzae* and *P. grisea.*

The perfect state of *Pyricularia* from cultivated cereals and wild grasses was first reported by Hebert in 1971. He also demonstrated that the fungus was heterothallic. Following Hebert's report, successful production of the perfect state in the genus *Pyricularia* has been reported by many investigators from Japan (6,9,10,24, 26,27,33–40). Both mating types can be found in the same field at the same time, and the failure to observe the perfect state in nature may not be due to geographical isolation (33). Hermophrodites have also been found among isolates from ragi, *Eleusine coracana* (7). Oatmeal agar or potato-sucrose agar is ideal for formation of abundant perithecia. Addition of zinc, methionine, and parts of various plants to modified Sachs's medium increases fruit body formation (5,26). Through mating among lab-constructed isolates of *P. oryzae* from rice, finger millet, and weeping lovegrass, the inheritance of electrophoretic variants of six enzymes was demonstrated to be under single-gene control in each case, as determined by tetrad and random spore analysis (14). Single-gene differences likewise determine the ability of *Pyricularia* species to infect different species of host grasses (29). The avirulence of *P. grisea* to different rice cultivars is controlled by single genes (2,13,21,31). *Pyricularia grisea* has features that commend it as a model system for plant pathology (28). Although *P. grisea* and *P. oryzae* strains are interfertile (24,30,36), the majority of field isolates are infertile (1,30). Random segregation of mating type, female fertility, and pathogenicity to rice in *Magnaporthe grisea* were studied in detail (11).

In the genome of *M. grisea,* repeated (MGR) sequences can be used to study the pathogenicity and fertility of isolates of the fungus (31). The repeated sequences serve as useful genetic markers in cloning genes for specific characteristics and also in the study of the epidemiology of rice blast disease and evolution of the pathogen (4). Major pathotypes of the rice blast pathogen populations in the United States were identified by the recently developed MGR-DNA fingerprinting technique (16). Characterization of resistance genes strongly depends on the choice of isolates and, therefore, genetic analysis of avirulence in isolates is important for such studies (21). A number of *M. grisea* isolates were tested for mating type (17). The studies 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. Clonal populations of *M. grisea* were analyzed by pulsed-field gel electrophoresis (PFGE) to identify karyotypic variability in the blast fungus (23). MGR-DNA fingerprinting analysis provides the genetic lineage of the pathotypes for evaluating pathogen variation and the dynamics of the pathogen (15). MGR-DNA genetic markers have also been used to examine the genetic diversity of the pathogen on a microgeographic scale (32). The mating-type genes of the rice blast fungus, *M. grisea,* have been recently isolated using the genomic subtraction technique for cloning mating-type idiomorphs from *M. grisea* (8).

Sexual fertility in the blast fungus is important for blast disease management, especially since some new resistance-breeding strategies for control of the blast fungus pathogen of rice are based on the apparent clonality of the pathogen. Since no published reports have discussed the occurrence of the sexual state of the blast fungi in India, an attempt was made to study sexual compatibility among Indian isolates of *Pyricularia.*

MATERIALS AND METHODS

***Pyricularia* cultures.** Forty-six isolates of *Pyricularia* were made from diseased specimens representing 9 host species collected from various places in India. Eleven isolates of *Pyricularia* from *Oryza sativa* (rice), 17 from *Eleusine coracana* (ragi), one from *Setaria italica* (thenai), two from *Pennisetum typhoideum* (cumbu), one from *Zingiber officinale* (ginger), one from *Curcuma longa* (turmeric), eight from *Panicum repens* (wild grass), four from *Brachiaria mutica* (wild grass), and one from *Leersia hexandra* (wild grass) were made. From each of these isolates, not less than five monoconidial isolates were prepared and all the isolates were maintained on oatmeal agar medium (25) throughout the study. The isolates were mated in duplicate in petri dishes on rice-straw or barley grains partially embedded on a variety of media such as modified Sachs's agar (5), water agar (26), malt extract agar (26), Czapek-Dox agar without

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sucrose (26), rice bran (rice bran, 20 g; agar, 20 g; water, 1 liter), and oatmeal with rice bran agar (oatmeal agar supplemented with 10 g rice bran per liter). The other 19 media are not listed here as the results were not encouraging.

Techniques and procedures followed in mating experiments. The success of sexual matings was evaluated by two methods: mycelial agar block (5) and mycelial suspension (5,10). Three other methods are not described here because the results were consistently negative. Substrates used in mating experiments were autoclaved straw pieces or powdered rice, ragi and wheat straw; autoclaved fresh stems or leaves of ragi, *Panicum repens*, or *Brachiaria mutica*; sterilized barley grains, corn husk, filter paper disks; or surface-sterilized scenesced leaves of *Panicum repens* and *Brachiaria mutica*. Incubation conditions tried in mating experiments included room temperature and natural day light, 25°C with continuous illumination from 40W fluorescent cool day lamps, 4°C in a refrigerator, 20°C under diffuse light conditions, or room temperature under NUV light (F 40-BLB/Sylvania). After inoculation, the matings were periodically examined over a period of 2 to 3 months. for formation of perithecia.

RESULTS

Matings with parent cultures. Of the 72 crosses involving five different isolates from rice, 11 from ragi, four from *Panicum repens*, two from *Brachiaria mutica*, one from *Setaria italica*, two from *Pennisetum typhoideum*, and one each from ginger and turmeric, only seven crosses produced perithecia-like structures on Sachs's, rice bran, and water agar. Of the seven crosses, perithecia-like structures were observed in matings within an isolate from ragi. Such structures were also produced when isolates from *Panicum repens* were mated with isolates from ragi, *Panicum repens, Brachiaria mutica*, and ginger. Although seven of the 72 crosses produced perithecia-like structures, neither asci nor ascospores were found in the structures. Perithecia-like structures were scattered around the inoculum on these media. These structures were produced at the junction of the two isolates and were partially embedded in the medium. Mature perithecia-like structures were brown to dark brown and globose, consisted of peridium with a diameter of 473 µm (range 240 to 720 µm) and a beak that averaged 235 µm (range 156 to 480 µm) in width and 460 µm (range 264 to 960 µm) in length. The peridial wall consisted of almost angular cells measuring 30.2 × 23.1 µm.

Matings with monoconidial isolates. The details of the crosses made between different monoconidial isolates of *Pyricularia* from 9 hosts are summarized in Table 1. Of the 756 crosses tried, 76 matings produced perithecia-like structures as described above. Such structures were formed mostly on oatmeal supplemented with rice bran, Sachs's, Czapek-Dox, malt extract, and water agar. Of the 76 matings that produced perithecia-like structures, 33 matings produced such structures on oatmeal agar supplemented with rice bran. It is evident that the crosses between rice isolates did not produce perithecia-like structures, but such structures were produced in matings between ragi isolates. Although perithecia-like structures were found in some of the matings, they were devoid of asci and ascospores. Also, the production of such structures varied with the media used.

DISCUSSION

Attempts to produce the perfect state of *Pyricularia* (*Magnaporthe grisea* (T. T. Hebert) Yaegashi & Udagawa) in the present study with 172 monoconidial isolates from nine different hosts in 756 combinations were fruitless although other workers have demonstrated limited production of the perfect state with matings between isolates from cereals and grasses. However, certain matings yielded perithecia-like structures devoid of asci and ascospores. The production of such structures has been reported earlier in the case of an isolate of *Pyricularia* from *Eleusine indica* (40). It is evident from the results that perithecia-like structures were produced only by about 10% of the crosses. It is further evident from the data that crosses between isolates from rice, ragi, or *Panicum repens* and other isolates produced such structures to the extent of 5, 14, and 6%, respectively. Such structures were more frequently observed in crosses between isolates from ragi or crosses involving ragi isolates. The mating types of the cultures could not, however, be verified with established mating types owing to quarantine restrictions that prohibit import of pathogenic cultures into the country. Nonetheless, it seems pertinent to point out here that it is not uncommon to find perithecia devoid of asci (5,10,33). Kato and Yamaguchi (9) and Yaegashi and Yamada (39) also reported the screening of a total of 926 isolates and none were sufficiently fertile to carry out genetic studies. The factor that limits fertility in *M. grisea* crosses may be differences in genome arrangement between strains (23).

It was reported that field isolates do not often mate in vitro (2,13) and also a vast majority of field isolates are infertile (1,30). Only a few crosses are possible between *P. grisea* isolates pathogenic to rice, because most of them are female sterile (6,9,12). When perithecia are produced, ascospore viability is very low (9,13,39). Most of the field isolates behave as male in the mating process (6,7,11,30). Complexity of fertility in *M. grisea* is partially responsible for incompatibility (19, 20). If sexual reproduction of *M. grisea* occurs on rice, it is probably infrequent and not necessary for the disease cycle (17). Notteghem and Silué (17) observed three mating classes (MAT1-1, MAT1-2, and unknown) in two locations (Cameroon and Ivory Coast) and these isolates were

Table 1. Attempted crosses between pairs of monoconidial isolates of *Pyricularia grisea* from different hosts

Isolates from host plants	Rice	Ragi	*Panicum repens*	*Bracharia mutica*	Thenai	Cumbu	Ginger	*Leersia hexandra*	Turmeric (*Curcuma longa*)	Total
O. sativa	11	133	35	36	2	8	8	7	1	241
(Rice)		(9)[a]	(1)	(1)			(1)			(12)
E. coracana		250	68	12	15	2	7	12	1	367
(Ragi)		(37)	(8)	(1)	(3)		(1)		(1)	(51)
P. repens			3	92	9	4	6	8	2	124
				(5)		(1)		(1)		(7)
B. mutica					1	1	9	1	1	13
S. italica						2	1	1	1	5
(Thenai)							(1)			(1)
P. typhoideum							1	1	1	3
(Cumbu)							(1)	(1)	(1)	(3)
Z. officinale								1	1	2
(Ginger)								(1)	(1)	(2)
L. hexandra									1	1
Total no. of crosses										756
										(76)

[a] Numbers in parenthesis show the number of crosses producing perithecia-like structures.

also intersterile. It was further suggested that such polymorphism indicates that the epidemics within a single rice field could be due to different clonal populations (17). In the present study, the nonproduction of mature perithecia with asci and ascospores in the isolates may be due to the genetic background and incompatibility of the isolates. It is further evident that the potential for sexual reproduction in these isolates is extremely limited. Results in the present study suggest that the sexual stage is rare in India and that *Pyricularia* species in India appear to be largely asexual, but this does not seem to interfere with the ability of these fungi to cause severe epidemics. The sexual stage is also rare elsewhere in the world where rice blast is a problem. The sexual cycle appears to be an unlikely source of variation in nature for *M. grisea* strains that infect rice (1). Contradictory results in studies on *Pyricularia* are not surprising in view of the immense variability of the fungus. In the absence of the sexual stage, the parasexual cycle in *M. grisea* may be a possible mechanism for variation in the pathogen in nature (1,3). Such variability might arise due to heterokaryon formation between the strains and subsequent mitotic recombination that might further lead to a wide host range of the pathogen.

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