Host Range, Mating Type, and Fertility of Pyricularia grisea from Wheat in Brazil

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ABSTRACT

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Isolates of *Pyricularia grisea* collected from wheat in regions of Brazil were inoculated on various gramineous plants to determine their host range. A set of 42 different gramineous plants belonging to nine tribes was analyzed by plant reactions and sporulation potential. The strain from wheat infected gramineous plants of five tribes: Hordeae, Festuceae, Aveneae, Chlorideae, and Agrosteae, but not Japanese differential or Brazilian lowland and upland rice cultivars. Mating types were analyzed by crossing wheat and finger millet strains belonging to two mating types. Fourteen wheat isolates out of 16 were classified as *MATI-1*, and one was *MATI-2*. Fertility was determined by crossing the wheat strain with strains of other gramineous plants. Mature ascospores were produced when the wheat strain crossed with strains from *Eleusine coracana*, *Brachiaria plantaginea*, *Setaria italica*, and different wheat isolates. Although a rice strain was able to infect wheat seedlings and panicles experimentally, the main wheat strain was suggested to be different from the rice strain and similar to the *Eleusine* strain.

Wheat blast disease caused by *Pyricularia grisea* (Cooke) Sacc. (25) was first identified in 1985 in seven northern coun-

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ties of the state of Paraná, Brazil (8). In 1986 the disease spread to 27 counties of northern and western Paraná, where severe losses were documented (8), and to 14 counties of southern Mato Grosso do Sul (6) and São Paulo (8). The disease that arose in a small region spread fast and was finally identified in the state of

Rio Grande do Sul in 1988 (24). Currently, wheat blast is present in all wheat crop regions of Brazil, which covered approximately 3,000,000 ha in 1991. Although the disease severity varies greatly with the region and weather, the yield loss of wheat caused by the pathogen was estimated to be 10-11% in Mato Grosso do Sul (5).

The fungus *P. grisea* has been documented to cause disease in many crops of cash value around the world, including corn (2), millets (17,26), and barley (23). Although studies have confirmed the specialization of *P. grisea* on various host plants, rice is the main staple crop on which the fungus causes major disease losses. Presently, however, the wheat blast fungus has become a limiting factor affecting wheat production in Brazil.

When this disease was first reported in wheat, the similarity between wheat blast and rice blast suggested that the same agent was attacking both wheat and rice grown in the same area (9). This hy-

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pothesis was reinforced by the fact that the wheat strain of *Pyricularia* provoked a different reaction across rice genotypes represented by Japanese differential cultivars (8).

Many researchers have focused on determining the host range of the fungus Pyricularia. The rice blast fungus has shown a broad host spectrum that varies, possibly according to genetic and environmental differences, in each research work. The contradictory and variable results demonstrate the difficulty of pinpointing a precise host range. As for the host range of wheat blast, some researchers have observed the capacity of the rice blast fungus to infect wheat plants (1,10,22), but cross-inoculation was not mentioned in any of these studies because there had been no natural occurrence of the disease on wheat. Igarashi (9), reporting wheat blast for the first time in Brazil, mentioned barley, oat, rye, and corn as hosts. A precise knowledge of the host range may be important to elucidate the role of grasses, weeds, and other crops surrounding wheat fields as potential sources of inocula and to identify the relationships among strains from various hosts.

In Brazil, most attempts to control wheat blast have been by foliar application or seed treatment with fungicides (3,4,18,20,21). The insufficient control achieved by such methods can be explained, partially, by the lack of infor-

mation concerning epidemiological aspects of the causal organism. Wheat blast has also attracted the attention of other scientists abroad. Restriction fragment length polymorphism was used to determine the relationship among rice, corn, finger millet, and Brazilian wheat strains (19). In a group of *Magnaporthe grisea*, repeated sequences were investigated to separate the wheat and rice blast fungi (28,29).

Production of the perfect stage of *P. grisea* in culture was first reported by Hebert (7), and then other scientists successfully produced the sexual stage from grass, millets, and rice (15,16,27,31). There is no information, to our knowledge, concerning the perfect stage of the wheat blast fungus. Mating ability and behavior of nuclei during ascospore formation are excellent criteria for examining the relationship among different strains of the fungus.

The objectives of this study were to provide information about the host range and mating type of the wheat blast fungus and the degree of sexual compatibility between the wheat strain and other *Pyricularia* strains.

MATERIALS AND METHODS

Nomenclature. We define a strain as all the isolates originating from the same host species. An isolate is the monoconidial culture obtained from an individual plant.

Table 1. Isolate designation, host, and geographical origin of isolates of *Pyricularia grisea* in Brazil

| Isolate | Host | Town | State |
|---------|------------------------|------------------|--------------------|
| 1-3 | Wheat | Londrina | Paraná |
| 4-7 | Wheat | Jaguapitã | Paraná |
| 8 | Wheat | B.V. Paraíso | Paraná |
| 39 | Wheat | Londrina | Paraná |
| 45 | Wheat | Florínea | São Paulo |
| 46 | Wheat | Rio Brilhante | Mato Grosso do Sul |
| 48 | Wheat | Itaporã | Mato Grosso do Sul |
| 49 | Wheat | Dourados | Mato Grosso do Sul |
| 50 | Wheat | Rio Brilhante | Mato Grosso do Sul |
| 52 | Wheat | Dourados | Mato Grosso do Sul |
| 53 | Wheat | Candido Mota | São Paulo |
| 10-11 | Rice | Paulo Frontin | Paraná |
| 12 | Rice | Cambará | Paraná |
| 13-14 | Rice | Campo Mourão | Paraná |
| 15 | Rice | Tupãssi | Paraná |
| 16-18 | Rice | Campo Mourão | Paraná |
| 19-24 | Rice | Ponta Grossa | Paraná |
| 25 | Rice | Londrina | Paraná |
| 26 | Rice | Irati | Paraná |
| 42 | Rice | Riolandia | São Paulo |
| 27 | Digitaria horizontalis | Tupãssi | Paraná |
| 28 | D. horizontalis | Tupãssi | Paraná |
| 29 | D. horizontalis | Pedrinhas | São Paulo |
| 30 | D. horizontalis | Londrina | Paraná |
| 31 | D. horizontalis | Primeiro de Maio | Paraná |
| 32 | D. horizontalis | Alvorado do Sul | Paraná |
| 33 | D. horizontalis | Irati | Paraná |
| 34 | Brachiaria plantaginea | Tupãssi | Paraná |
| 35 | B. plantaginea | Vilĥa Velha | Paraná |
| 54 | B. plantaginea | Londrina | Paraná |
| 55 | B. plantaginea | Londrina | Paraná |
| 36 | Cenchrus echinatus | Alvorada do Sul | Paraná |
| 37 | Setaria geniculata | Londrina | Paraná |
| 38 | Echinocloa colonum | Primeiro de Maio | Paraná |

Collection and isolation. Wheat plants infected with blast fungi were collected from various regions of Brazil (Table 1). Infected plants of rice and other hosts neighboring wheat fields were also gathered. Infected tissues of diseased plants were cut into small pieces and placed on moist filter paper in petri dishes, then incubated under light for 24 hr at 25 C. After monoconidial isolation, the fungus was grown on potato-dextrose agar slants at 25 C for short-term use. For long-term storage, pure cultures were grown on sterilized barley seeds in a vial, then dried thoroughly at 25 C and kept in a case with silica gel at 5 C. Approximately 50 isolates were collected from the states of Paraná, São Paulo, and Mato Grosso do Sul, where the disease has been serious. Besides wheat and rice, the following diseased grass weeds were gathered: Digitaria horizontalis Willd., Brachiaria plantaginea (Link) Hitchc., Cenchrus echinatus L., Setaria geniculata (Lam.) Beauv., and Echinocloa colonum Link.

Host range. A set of seedlings of 42 gramineous plants belonging to nine tribes was inoculated to determine the host range of the blast fungus (Table 2). Seeds of different species were obtained from the following sources: Triticum aestivum 'Anahuac' and Brachiaria plantaginea from the University of Londrina, Londrina, Brazil; Hordeum vulgare 'Kashima-mugi', *Panicum miliaceum* 'Yamanashi-kei 1', and *Setaria italica* 'Aka-awa', from the National Agriculture Research Center, Japan; Dactylis glomerata 'Kitamidori' and Festuca arundinacea 'Hokuryo' from Hokkaido Agricultural Experimental Station, Japan; Lolium perenne 'Kiyosato' from Takii Seed Co., Japan; and Secale cereale 'Haru-ichiban', Poa pratensis 'Toroy', Lolium multiflorum 'Mammoth Italian B', Festuca rubra 'Futsu-kei', F. pratensis 'First', Chloris gayana 'Katanbora', Avena sativa 'Taiho', Phalaris arundinacea 'Venture', Phleum pratense 'Hokuou', Agrostis tenuis 'High Land', Setaria faberii, S. viridis, Digitaria sanguinalis, Sorghum sudanense 'High Sudan', S. vulgare 'Sweet sorghum', Zea mays 'Yumi-113SF', Eragrostis curvula, Agrostis alba, Panicum coloratum, Paspalum notatum, P. umbellatum, Penisetum glaucum, Coix lacrima-jobi, and Euchlaena mexicana from Yukijirushi Seed Co., Japan. Anthoxanthemum odoratum, Echinocloa crusgallii, Digitaria ciliaris, D. horizontalis, D. ischaemum, Penisetum clandestinum, Cenchrus ciliaris, and Brachiaria platyphylla were purchased from Herbseed Co., United Kingdom. For Eleusine coracana. a set of seven different lines was obtained: Shiramine (Japan), Manigaon (Nepal, obtained from the National Agriculture Research Center, Japan), INDAF 6 (provided by R. Basavaraju, Agricultural Research Institute, Bangalore, India),

India 57 (collected in Mysore, India, and reproduced), Purna (Nepal, from the National Agriculture Research Center, Japan), Iiya-Zairai (Japan, from the National Agriculture Research Center), and UG 14 (Uganda, provided by J. Mukiibi, Mukerele University). Eleusine africana was represented by UG 99 (Uganda, from Mukerele University). The Japanese rice differentials (Table 3); Brazilian upland rice cultivars IAC 25, IAC 47, and IAC 165; lowland rice cultivars IAC 238, IAC 242, IAC 4440, and CICA 9; and the weeds, red and black rice (Oryza sativa L.), were included. Plants for the inoculations were grown in a greenhouse at 25 C in seedling cases measuring 5.5×15 cm. The number of seeds varied from three for large seeds (corn and Coix lacrimajobi) to seven for medium seeds (rice, oat, wheat, and barley), to 20 or more for small seeds (Phleum pratense, Digitaria, Agrostis, etc.).

Inoculation. Spores of each fungus were harvested from 14-day-old colonies growing on oatmeal agar petri plates. After 10-11 days, when the entire oatmeal agar surface was covered, aerial mycelia were washed off by gentle rubbing with a paintbrush after flooding with sterilized water. The colony was

then exposed to near-ultraviolet light (360 nm, 40 W) at 22 C for 3 days to induce sporulation. The conidial and mycelial suspension was filtered through two layers of cheesecloth and adjusted to a concentration of 1×10^5 spores per milliliter. Tween 20 at a concentration of 10⁻⁴ was added. Inoculation was done by spraying 20 ml of the spore suspension onto the abaxial and adaxial leaf blade with an air compressor. Plants were at the three- to four-leaf stage, and each inoculation was repeated at least four times per isolate. Soon after, plants were placed in a dew chamber in darkness at 25 C for 15 hr and then transferred to

Table 2. Infection type of plants inoculated with Pyricularia strains isolated from Triticum aestivum, Brachiaria plantaginea, and Oryza sativa

| | Host origin and isolate number | | | | | | | | | | | | | | | | | | |
|--|--------------------------------|---|--------|--------|---|---|----|----|------------------------|----|----|-----|----|----|----|----|----|----|---|
| Tribe | Wheat B. po | | | | | | | | <i>plantaginea</i> Ric | | | ice | | | | | | | |
| Host | 1 | 2 | 3 | 4 | 7 | 8 | 39 | 45 | 46 | 48 | 49 | 50 | 52 | 53 | 34 | 35 | 54 | 13 | 1 |
| Hordeae | | | | | | | | | | | | | | | | | | | |
| Hordeum vulgare L. | 4 a | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 3 | 4 | 3 | 3 | 3 | 3 |
| Triticum aestivum L. | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 3 | 4 | 4 | 3 | 4 | 3 | 3 | 0 | 0 | 0 | 0 | 3 |
| Secale cereale L. | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 4 | 4 | 3 | 3 | 3 | 4 | 3 | 0 | 0 | 0 | 4 |
| Festuceae | | | • | • | • | - | - | | | | - | _ | - | | _ | - | | | |
| Dactylis glomerata L. | 4 | 4 | 3 | 4 | 4 | 3 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 3 | 3 | 3 | 4 |
| Poa pratenses L. | 3 | 4 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 0 | 0 | 0 | 4 | 0 |
| Lolium multiflorum Lam. | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 0 | 3 | 3 | 4 |
| Lolium perenne L. | 4 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 4 | 4 | 3 | 4 | 4 | 3 | 0 | 4 | 3 | 0 | 4 |
| Festuca arundinacea Schreb. | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 |
| Festuca rubra L. | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 3 | 4 | 4 | 3 | 3 | 3 | 0 | 4 | 4 | 3 | 3 |
| Festuca pratensis Huds. | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 3 | 4 | 4 | 3 | ó | 4 | 0 | 0 |
| Chlorideae | • | • | • | • | • | • | • | • | | • | • | | • | • | | · | • | • | · |
| Eragrostis curvula (Schrad.)Ness | 3 | 4 | 0 | 0 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 0 | 0 |
| Eleusine coracana (L.) Gaertn. | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Eleusine africana KennO'Byrne | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Chloris gayana Kunth | 4 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 4 | 3 | 4 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Orvzeae | 7 | 2 | 3 | 3 | 3 | 3 | , | 3 | 2 | 7 | 3 | 7 | 2 | v | U | v | U | U | v |
| Oryza sativa L. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 4 |
| Aveneae | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | 3 | 7 |
| Avena sativa L. | 4 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 0 |
| Phalaris arundiancea L. | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 4 | 3 | 4 | 3 | 3 | 3 | 3 | 3 | 3 |
| Anthoxanthemum odoratum L. | 3 4 | 4 | 3 | 4 | 4 | 4 | 3 | 3 | 3 | 3 | 3 | 4 | 4 | 3 | 4 | 4 | 4 | 3 | 4 |
| Phleum pratense L. | 4 | 4 | 3 4 | 4 | 4 | 4 | 3 | 3 | 3 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 0 |
| Agrosteae | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 3 | 3 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | U |
| Agrostis tenuis Sibth. | 4 | 4 | 0 | 2 | 2 | 3 | 2 | 3 | 0 | 3 | 2 | 3 | 0 | 3 | 0 | 0 | 0 | 2 | 0 |
| Agrostis alba L. | 3 | 3 | 0 | 3 4 | 3 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 |
| Paniceae | 3 | 3 | U | 4 | 3 | 3 | 3 | 3 | U | 3 | 3 | 3 | U | U | U | U | U | 3 | U |
| Panicum coloratum L. | 0 | 0 | ^ | 2 | ^ | • | ^ | 0 | 4 | 2 | 0 | ^ | ^ | Λ | ۸ | Λ | 0 | ^ | r |
| Panicum miliaceum L. | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 4 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Echinocloa crusgalli Beauv. | 2 | 0 | 0 | 0 | 2 | 2 | 3 | 2 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 3 | 0 | 1 | 0 |
| Setaria italica (L.) Beauv. | 0 | 0 | 0 | 3 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| Setaria italica (L.) Beauv. Setaria fabérii Herm. | 1 | 0 | 0 | 3 | 2 | 3 | 4 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| • | 3 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 2 |
| Setaria viridis (L.) Beauv. | 3 | 1 | 3 | 1 | 0 | 0 | 1 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Paspalum notatum Flügge | 3 | 4 | 3 | 2 | 2 | 4 | 4 | 3 | 2 | 3 | 3 | 3 | 4 | 3 | 3 | 3 | 3 | 3 | 0 |
| Paspalum umbellatum (Retz.) Koeler | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| Digitaria ciliaris (Retz.) Koch | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Digitaria horizontalis Willd. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Digitaria ischaemum (Schreb.) Schreb. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Digitaria sanguinalis (L.) Scop. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Penisetum clandestinum Hochst. | 4 | 3 | 3 | 4 | 3 | 3 | 4 | 4 | 4 | 4 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 0 |
| Penisetum glaucum L. | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| Cenchrus ciliaris L. | 0 | 0 | 4 | 0 | 3 | 3 | 0 | 3 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 2 | 0 | 0 | 0 |
| Brachiaria plantaginea (Link) Hitchc. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 4 | 4 | 0 | 0 |
| Brachiaria platyphylla (Griseb.) Nash. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 4 | 3 | 0 | 0 |
| Andropogoneae | | | | | | | | | | | | | | | | | | | |
| Sorghum sudanense (Piper) Stapf. | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Sorghum vulgare Pers. | 1 | 0 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 1 | 1 |
| Maydeae | | | | | | | | | | | | | | | | | | | |
| Coix lacrima-jobi L. | 1 | 0 | 2 | 2 | 2 | 1 | 3 | 3 | 2 | 2 | 2 | 3 | 2 | 0 | 0 | 0 | 0 | 3 | 3 |
| Zea mays L. | 1 | 2 | 1 | 3 | 2 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 1 | 0 |
| Euchlaena mexicana Schrad. | 1 | 0 | 1 | 1 | 1 | 4 | 1 | 3 | 3 | 1 | 3 | 4 | 3 | 0 | 3 | 3 | 3 | 0 | 0 |

^a Infection scores: 0 = no infection; 1 = minute, pinhead-sized spots; 2 = small brown to dark brown lesions with no distinguishable centers; 3 = small eyespot-shaped lesions with gray centers; 4 = typical blast lesions, eliptical with gray centers, usually coalescing.

a growth chamber at 25 C with alternate light and dark periods of 12 hr.

Disease evaluation. Plants were examined for symptoms 6 days after inoculation. Infection types were classified into five categories: 0 = no visible reaction; 1 = minute, pinhead-sized spots; 2 = small brown to dark brown lesions with no distinguishable centers; 3 = small eyespot-shaped lesions with gray centers; and 4 = typical blast lesions, elliptical with gray centers. Lesion types 0, 1, and 2 were regarded as resistant, with no

sporulation within lesions after incubation for 15 hr in the moist chamber at 25 C. Lesion types 3 and 4 were considered susceptible, since spores were recovered from lesions after incubation under the same conditions. The variable sizes of plants, and consequently of their leaves, did not allow us to use lesion size as a parameter to score disease. As a population, plants were regarded as susceptible if they exhibited susceptible symptoms in more than 50% of the plants in each inoculation and if the same reaction

Table 3. Infection type of rice inoculated with Pyricularia strains isolated from Triticum aestivum, Brachiaria plantaginea, and Oryza sativa

| | Host origin and isolate number ^a | | | | | | | | |
|-----------------------------|---|----------------|------|----|--|--|--|--|--|
| | | | Rice | | | | | | |
| Rice cultivars | Wheat | B. plantaginea | 13 | 15 | | | | | |
| Japanese differentials | | | | | | | | | |
| and resistance genes | | | | | | | | | |
| Shin 2 (Pi-k ^s) | О р | 0 | 0 | 4 | | | | | |
| Aichi Asahi (Pi-a) | 0 | 0 | 4 | 4 | | | | | |
| Ishikarishiroke (Pi-i) | 0 | 0 | 0 | 3 | | | | | |
| Kanto 51 (Pi-k) | 0 | 0 | 0 | 0 | | | | | |
| Tsuyuake $(Pi-k^m)$ | 0 | 0 | 0 | 0 | | | | | |
| Fukunishiki (Pi-z) | 0 | 0 | 0 | 0 | | | | | |
| Yashiromochi (Pi-ta) | 0 | 0 | 3 | 3 | | | | | |
| Pi no. 4 (Pi - ta^2) | 0 | 0 | 0 | 0 | | | | | |
| Toride $1(Pi-z^t)$ | 0 | 0 | 0 | 3 | | | | | |
| Bl-1 $(Pi-b)$ | 0 | 0 | 3 | 3 | | | | | |
| K-59(Pi-t) | 0 | 0 | 4 | 4 | | | | | |
| Brazilian upland | | | | | | | | | |
| IAC 25 | 0 | 0 | 3 | 0 | | | | | |
| IAC 47 | 0 | 0 | 3 | 0 | | | | | |
| IAC 165 | 0 | 0 | 3 | 0 | | | | | |
| Brazilian lowland | | | | | | | | | |
| IAC 238 | 0 | 0 | 0 | 4 | | | | | |
| IAC 242 | 0 | 0 | 0 | 4 | | | | | |
| IAC 4440 | 0 | 0 | 0 | 0 | | | | | |
| CICA 9 | 0 | 0 | 0 | 4 | | | | | |
| Weeds | | | | | | | | | |
| Red rice | 0 | 0 | 4 | 4 | | | | | |
| Black rice | 0 | 0 | 4 | 0 | | | | | |

^a Isolates from wheat: 1-4, 7, 8, 39, 45, 46, 48-50, 52, 53. Isolates from *B. plantaginea*: 34, 35, 54.

Table 4. Infection type of Brazilian wheat inoculated with Pyricularia isolates from rice

| Isolate | Wheat cultivar | | | | | | | | | |
|---------|----------------|----------|----------|----------|--|--|--|--|--|--|
| no. | Anahuac | IAPAR-28 | IAPAR-17 | OCEPAR-7 | | | | | | |
| 10 | 3 a | 3 | 3 | 4 | | | | | | |
| 18 | 3 | 4 | 4 | 4 | | | | | | |
| 19 | 3 | 3 | 3 | 3 | | | | | | |
| 22 | 3 | 3 | 3 | 3 | | | | | | |
| 26 | 3 | 3 | 4 | 3 | | | | | | |
| 13 | 0 | 0 | 3 | 4 | | | | | | |
| 12 | 0 | 0 | 0 | 3 | | | | | | |
| 20 | 0 | 3 | 0 | 0 | | | | | | |
| 24 | 0 | 0 | 3 | 2 | | | | | | |
| 15 | 3 | 0 | 2 | 0 | | | | | | |
| 11 | 0 | 0 | 0 | 0 | | | | | | |
| 14 | 0 | 0 | 0 | 0 | | | | | | |
| 16 | 0 | 0 | 0 | 0 | | | | | | |
| 17 | 0 | 0 | 0 | 0 | | | | | | |
| 21 | 0 | 0 | 0 | 0 | | | | | | |
| 25 | 0 | 0 | 0 | 0 | | | | | | |

^a Infection scores: 0 = no infection; 1 = minute, pinhead-sized spots; 2 = small brown to dark brown lesions with no distinguishable centers; 3 = small eyespot-shaped lesions with gray centers; 4 = typical blast lesions, eliptical with gray centers, usually coalescing.

was verified in three out of four replications.

Mating type. Mating compatibility of wheat blast isolates was determined by pairing the unknown isolate with fertile testers of MAT1-1 (formerly A; G 10-1) and MAT1-2 (formerly a; Z 2-1) of isolates from finger millet (Eleusine coracana). These isolates were the most fruitful in frequency and degree of fertility (12,17,30,32). The three-point culture method (11) was employed. Mycelial fragments of the tester and wheat isolates were placed about 4 cm apart at each point of a triangle on oatmeal agar in a petri dish. These crosses were kept at 25 C for 5 days in an incubator in darkness and then on a laboratory bench at 20 C under continuous fluorescent illumination. The formation of perithecia occurred at the intersection of mycelial growth of the isolates about 1 mo later if the cross between isolates was compatible. The presence of asci and ascospores was verified with the aid of a mi-

Compatibility. The compatibility of the wheat blast fungus with other strains of *Pyricularia* was also examined by measuring the capacity to form perithecia, asci, and ascospores of paired strains. The wheat strain was paired with *Pyricularia* strains isolated from *Brachiaria* plantaginea, Digitaria horizontalis, Cenchrus echinatus, Setaria geniculata, Echinocloa colonum, and rice from Brazil and Eleusine coracana, Setaria italica, and Panicum miliaceum from Japan. All crosses were replicated at least three times.

RESULTS

The pathogenic reactions of the set of gramineous plants to the wheat blast strain are presented in Table 2. Plants belonging to the tribes Hordeae, Festuceae, Oryzeae, and Aveneae reacted uniformly to all wheat isolates. Other tribes, Chlorideae, Agrosteae, Paniceae, Andropogoneae, and Maydeae, showed different responses in each species against different isolates. Among them, seven lines of *Eleusine coracana* and one line of E. africana (Chlorideae) and Penisetum clandestinum (Paniceae) showed susceptible reactions to all 14 isolates of wheat. All wheat isolates were pathogenic to the wheat cultivar Anahuac, satisfying Koch's postulates and substantiating that this cultivar is one of the most susceptible to wheat blast in the field.

In Panicum miliaceum, sometimes many marginal lesions occurred on the leaves. It seems that the pathogen penetrated through the water pores at the leaf margin, and colonization was limited to the surrounding tissues. Because typical cuticular infection was absent, these symptoms as a whole were classified as lesion type 2, and P. miliaceum was regarded as resistant, even though a few

b Infection scores: 0 = no infection; 1 = minute, pinhead-sized spots; 2 = small brown to dark brown lesions with no distinguishable centers; 3 = small eyespot-shaped lesions with gray centers; 4 = typical blast lesions, elliptical with gray centers, usually coalescing.

spores were formed.

Among plants of the Paniceae tribe, only Brachiaria plantaginea and Penisetum clandestinum showed uniform susceptibility to Pyricularia isolated from B. plantaginea. Hordeum vulgare, seven lines of E. coracana, one line of E. africana, and some plants of the Festuceae and Chlorideae tribes were also infected. All species of the Aveneae tribe were susceptible; they were the most susceptible to the blast fungus from Brachiaria.

At least one isolate of the rice strain infected three species of the Hordeae tribe, six species of Festuceae, and four species of Aveneae. In Oryzeae, only one species, Oryza sativa, was infected by two isolates from rice.

The reaction of Japanese differentials and some Brazilian rice cultivars to the wheat strain of *Pyricularia* is shown in Table 3. None of the isolates from wheat were virulent on any of the 20 rice cultivars tested.

Conversely, Table 4 shows that some isolates of the rice blast fungus were capable of infecting wheat. Five isolates out of 16 caused susceptible reactions in four Brazilian wheat cultivars. Two isolates infected two cultivars, and three others infected one cultivar. Consequently, nine isolates out of 16 rice blast fungi employed were pathogenic to at least one wheat cultivar, and six isolates had no pathogenicity.

The mating behavior of wheat blast isolates was determined with tester lines from finger millet. Table 5 presents the mating characteristics of the strains in this study. Among 16 wheat isolates only one was MAT1-2, 14 were MAT1-1, and one was not identified. In five isolates, perithecia were formed in double rows on the intersection, indicating hermaphroditic characteristics. Twelve isolates from rice did not form any perithecia; therefore, their mating type was undetermined. Among the four isolates mated, one isolate was MATI-1 and three were MAT1-2. Two isolates from Brachiaria plantaginea were MAT1-1 and two were undetermined. Three out of seven isolates of Digitaria horizontalis were MAT1-2, and the remaining isolates did not form perithecia. The mating types of Pyricularia isolates from Cenchrus echinatus, Setaria geniculata, and Echinocloa colonum were undetermined due to the lack of perithecium formation. Eleven isolates of wheat were completely compatible with the finger millet strain and formed perithecia, asci, and ascospores. Four isolates formed only perithecia without asci. When paired with the rice isolates, only a few perithecia were observed.

Table 6 indicates the compatibility of the wheat blast strain with other *Pyricularia* strains. The wheat isolates were compatible with each other, *Brachiaria* plantaginea, *Eleusine coracana*, and *Setaria italica* strains. When mated with Panicum miliaceum and Digitaria horizontalis strains, a few perithecia formed without asci. The crosses between wheat and rice, Cenchrus echinatus, Setaria geniculata, and Echinocloa colonum strains produced no perithecia.

DISCUSSION

The wheat blast strain collected at random from different regions of Brazil did not produce any symptoms on any rice cultivars (Table 3). Japanese differential cultivars were employed because their genetic constitution was best known. The rice cultivars IAC 25 and IAC 47, the most popular rice cultivars in the states of São Paulo, Paraná, and Mato Grosso do Sul, where the wheat blast is prevalent, and CICA 9, the cultivar grown in northern Paraná, where the disease was first reported, were not infected by any wheat isolates. Red and black weed rice, known as sources of inocula of P. grisea in rice, were resistant. Previous work in Brazil (8) identified five races of wheat blast fungi with Japanese differential rice cultivars and with tests for pathogenicity to CICA 9.

The striking difference between that work (8) and our data may indicate the existence of two distinct entities causing wheat blast in Brazil—one originating from the rice blast strain, since the strain employed in our study caused symptoms on seedlings and panicles in a number of Brazilian commercial wheat cultivars that were also employed in the previous work. Many workers (1,10,22) have already reported the possibility that wheat is a host of the rice blast strain.

Another completely different strain, found over a large area in 1991 and 1992 (Table 1), could be involved in the wheat blast disease. The strain used in our study could neither cause disease in any rice plants tested (Table 3) nor produce any perithecia when mated with the rice strain (Table 6). Moreover, the pathogenic pattern of this wheat strain in various gramineous species of nine tribes confirmed its difference from the rice strain. These facts coincide with those suggested on the basis of molecular research (19,29).

A series of inoculation tests on barley and rye (tribe Hordeae) and oat (Ave-

Table 5. Mating type and fertility of Brazilian isolates of *Pyricularia* from various hosts crossed with the finger millet strain

| Isolate | e | Mating | | |
|---------|------------------------|--------|----------------|----------------------------------|
| no. | Host | type | Sex | Sex organs produced |
| 1 | Wheat | MATI-I | Male | perithecia, asci, and ascospores |
| 2 | Wheat | MAT1-1 | Male | perithecia |
| 4 | Wheat | MAT1-1 | Hermaphroditic | perithecia, asci, and ascospores |
| 5 | Wheat | MAT1-1 | Hermaphroditic | perithecia, asci, and ascospores |
| 6 | Wheat | MAT1-1 | Male | perithecia |
| 7 | Wheat | MAT1-2 | Male | perithecia, asci, and ascospores |
| 8 | Wheat | MAT1-1 | Hermaphroditic | perithecia, asci, and ascospores |
| 39 | Wheat | MAT1-1 | Female | perithecia, asci, and ascospores |
| 45 | Wheat | MAT1-1 | Female | perithecia |
| 46 | Wheat | MAT1-1 | Hermaphroditic | perithecia, asci, and ascospores |
| 48 | Wheat | MAT1-1 | Female | perithecia, asci, and ascospores |
| 49 | Wheat | MAT1-1 | Hermaphroditic | perithecia, asci, and ascospores |
| 50 | Wheat | MATI-I | Female | perithecia, asci, and ascospores |
| 52 | Wheat | MATI-I | Female | perithecia, asci, and ascospores |
| 53 | Wheat | MATI-I | Female | perithecia |
| 12 | Rice | MAT1-2 | Male | perithecia |
| 13 | Rice | MAT1-1 | Male | perithecia |
| 15 | Rice | MAT1-2 | Male | perithecia |
| 42 | Rice | MAT1-2 | Male | perithecia |
| 29 | Digitaria horizontalis | MAT1-2 | Male | perithecia |
| 30 | D. horizontalis | MAT1-2 | Male | perithecia |
| 33 | D. horizontalis | MAT1-2 | Male | perithecia |
| 34 | Brachiaria plantaginea | MAT1-2 | Male | perithecia, asci, and ascospores |
| 35 | B. plantaginea | MAT1-2 | Male | perithecia, asci, and ascospores |

Table 6. Compatibility among Pyricularia strains

| Host (isolate number) | Sex organs produced |
|--|--|
| Triticum aestivum (8) \times T. aestivum (7) T. aestivum L. (48) \times Brachiaria plantaginea (35) T. aestivum (46) \times Eleusine coracana (Z 2-1) T. aestivum (8) \times Setaria italica (YNSI 1-1-5) T. aestivum (8) \times Panicum miliaceum (YNPM 1-1-1) T. aestivum (8) \times Digitaria horizontalis (33) T. aestivum (7) \times Oryza sativa (13, 15) T. aestivum (4) \times O. sativa (12) T. aestivum (7,8) \times Cenchrus echinatus (36) | perithecia, asci, and ascospores perithecia, asci, and ascospores perithecia, asci, and ascospores perithecia, asci, and ascospores perithecia perithecia none none none |
| T. aestivum $(7,8) \times$ Setaria geniculata (37) | none |
| T. aestivum $(7.8) \times$ Echinocloa colonum (38) | none |

neae) confirmed the pathogenicity of all wheat isolates sampled in different regions of Brazil. The high susceptibility of these cereals, which are commonly grown near wheat fields, indicates their role as potential sources of inoculum.

The susceptibility of wheat to the Eleusine blast strain was reported previously in Japan (13). In this study all wheat isolates caused serious damage in seven lines of finger millet from different countries and one line of E. africana from Uganda. The pathogenic pattern of the wheat strain in various gramineous plants showed a similarity to the pattern of the strain from E. africana (14). They may be closely related. Since the tester lines for mating type were obtained from finger millet (E. coracana), the fertility also indicated the similarity between wheat and finger millet strains; therefore, our wheat strain is more similar to the Eleusine strain than any others analyzed.

The existence of pathogenic variation among wheat isolates in relation to some plants including wheat is interpreted to be an example of pathogenic specialization or physiological race such as *Pyricularia grisea* in rice. An earlier study (8) already emphasized the breakdown of wheat cultivars that had previously shown resistance.

The Brachiaria strain of blast caused no infection in wheat plants, and the wheat strain also was unable to cause pathogenicity on plants of the Brachiaria genus, which are widely distributed as both pasture and weeds. In addition, this strain had a different infection range from the wheat strain, although they could mate. The Pyricularia strain from Brachiaria caused no infection in Japanese or Brazilian rice cultivars, denoting the same inability as the wheat strain. These results show that the wheat blast strain was not derived from the blast pathogen on Brachiaria plantaginea. Blast disease on grasses in pasture, which encompasses a large area in Brazil, should be monitored more accurately during the summer season.

Table 5 shows the mating type of the isolates studied. Ability to mate was demonstrated in more than 90% of wheat isolates, 42% of isolates from *Digitaria horizontalis*, and 17% of those from rice. Mating types *MATI-1* and *MATI-2* were found in the wheat blast strain. The sexual stage has not yet been found in nature.

There seemed to be a variation in genetic traits conditioning sexual reproduction among the isolates. Despite the high percentage of mating in wheat isolates crossed with finger millet isolates, some formed only perithecia without asci or ascospores. These results probably occur

because of genetic differences in compatibility of isolates rather than unsuitable conditions for ascus formation, since other isolates produced ascospores under the same conditions. A variation in fertility was also observed in strains from different hosts that were paired with the wheat strain the (Table 6). The most fruitful crosses were obtained when the wheat strain was intermated or mated with strains from Eleusine coracana. Brachiaria plantaginea, and Setaria italica. When the wheat strain was crossed with Panicum miliaceum and Digitaria horizontalis strains, perithecia formed but no asci or ascospores were observed. This indicates only partial similarity between them but enough to determine the mating characteristics.

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