Pathogenic Variability of Monoconidial Isolates of *Pyricularia oryzae* in Korea and in the Philippines

J. M. BONMAN, Associate Plant Pathologist, T. I. VERGEL DE DIOS, Research Assistant, and J. M. BANDONG, Assistant Scientist, International Rice Research Institute, P.O. Box 933, Manila, Philippines, and E. J. LEE, Head, Department of Plant Pathology, Institute of Agricultural Sciences, Suweon, Korea

ABSTRACT

The pathogenic variation among monoconidial isolates of *Pyricularia oryzae* from single lesions and monospore cultures was examined with two differential cultivar sets. Tests in Korea and the Philippines indicated some variants but fewer than reported previously. Data support the hypothesis that *P. oryzae* is relatively stable pathogenically and that quantitative resistance to rice blast is not due to extreme pathogenic instability in the fungus.

Great pathogenic variation has been reported in *Pyricularia oryzae* Cav., the rice blast pathogen, both among monoconidial isolates (MI) from single lesions and among monoconidial subcultures from single-monospore cultures (5,18–20). New physiologic races were reported to appear with a frequency of about one new race for every four MI tested against both Philippine and international differential sets of rice cultivars (18,20). Because of their belief that so many races were appearing, these researchers suggested that if enough differential cultivars were used, possibly every conidium from a culture could be shown to represent a different race (16,18). These results generated the hypothesis that quantitative resistance in some rice cultivars, as indicated by fewer lesions per plant or per unit area of leaf, was due to the fungus constantly changing into numerous races (20). If a cultivar showed quantitative resistance to an isolate of *P. oryzae*, it was because only a few of the many races present in the conidial population could infect and cause lesions on the cultivar. Cultivars resistant to many races had a high level of quantitative resistance (1), and this was thought to be due to the pathogenic heterogeneity of *P. oryzae*.

In contrast to the results showing tremendous variability, other studies indicated the fungus is more patho-

©1987 The American Phytopathological Society

Accepted for publication 1 July 1986.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. § 1734 solely to indicate this fact.
genically stable. In tests of 600 monospore isolates from lesions and isolates, Latterell found that "changes in race pattern were rare" (10). Similarly, workers in Korea have found few variants among MI from single lesions or cultures (6,7). The hypothesis of extreme variation in pathogenicity has not found acceptance in Japan (4,9), suggesting a possible difference in the variability of *P. oryzae* in tropical versus temperate regions.

In this study, we examined the variability among MI of *P. oryzae* in Korea and in the Philippines.

**MATERIALS AND METHODS**

Experiments were conducted in 1983 and 1984 at the Institute of Agricultural Sciences (IAS), Rural Development Administration, in Suwon, Korea, and in 1983 to 1985 at the International Rice Research Institute (IRRI), Los Baños, Philippines. Experiments at IAS were done from June to October under conditions generally favorable for plant growth and infection by the blast pathogen. Experiments at IRRI were conducted throughout the year.

**Collection, isolation, and culture maintenance.** In the Philippines, seven leaf blast lesions from the IRRI blast nursery were collected in 1983 and 1983 lesions from leaves and panicles were collected from regions throughout the country in 1984. In Korea, two leaf blast lesions in 1983 and five leaf blast lesions in 1984 were collected from experimental fields at IAS.

Each lesion was placed in a moist petri dish and incubated at 25°C until sporulation. The sporulating lesion was gently tapped to dislodge conidia and allow them to fall onto a thin layer of water agar. Single conidia were identified with a stereomicroscope and aseptically transferred to agar slants. From some MI, subcultured MI were obtained by gently streaking a loopful of spore suspension from the parent MI onto water agar. Single conidia were picked aseptically and transferred to agar slants. One hundred seventy-six MI and 100 subcultured MI were obtained from Philippine lesions, and 87 MI and 30 subcultured MI, from Korean lesions.

 Cultures were maintained initially in prune agar slants (22) at IRRI and in potato-sucrose agar at IAS. For long-term storage, the culture slants at IAS were stored at 4°C; at IRRI, cultures were preserved using sorghum grains as a substrate. Grains were steeped, boiled, then autoclaved on two consecutive days. Sterile grains were inoculated with mycelial plugs and incubated at 25–28°C until covered with fungal growth. Colonized grains were dried at 40°C for 24 hr, transferred to a vial of silica gel,

### Table 1. Reactions of the Philippine differential cultivars to monoconidial isolates (MI) of *Pyricularia oryzae* from single lesions tested in Korea and in the Philippines

<table>
<thead>
<tr>
<th>Differential cultivar</th>
<th>M23-3</th>
<th>M23-2</th>
<th>IR-1</th>
<th>IR-2</th>
<th>IR-3</th>
<th>IM23-2</th>
<th>IM23-3</th>
<th>T-1</th>
<th>T-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Katakataka DA-2</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>CI 5309</td>
<td>R</td>
<td>R*</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Chokoto</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>CO25</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Wagwag</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Pai-kan-tao</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Peta</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Raminad Str. 3</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Taichung T.C.W.C.</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Lacrosse</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Shia-tao-tao</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Khao-tah-haeng 17</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
</tbody>
</table>

*No. of MI: 19 18 22 3 25 24 24 1 25 20 3 1 28

* Susceptible (S) reaction = >20% of plants with diamond-shaped lesions 1 x 3 mm or larger. In Philippine tests, all variants were retested.

* Korean experiments with isolates from Milyang 23 (M23) (three replicates).

* Philippine experiments with isolates from Milyang 23 (IM23), IR442-2-58 (IR), and Tetep (T) (three replicates).

* In one isolate, two replicates were classified as R reaction and one replicate was classified as S reaction. Not retested.

### Table 2. Reactions of the international differential cultivars to monoconidial isolates (MI) of *Pyricularia oryzae* from single lesions and monoconidial cultures tested in Korea (K) and in the Philippines (P)

<table>
<thead>
<tr>
<th>Differential cultivar</th>
<th>K1</th>
<th>K2</th>
<th>K3</th>
<th>K4</th>
<th>K5</th>
<th>K1-1</th>
<th>K2-1</th>
<th>K3-1</th>
<th>K4-1</th>
<th>K5-1</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P6</th>
<th>P7</th>
<th>P8</th>
<th>P9</th>
<th>P10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raminad Str. 3</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Zenith</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>NP 125</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Usen</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Dular</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Kanto 51</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Shia-tao-tao</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Caloro</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

*No. of isolates: 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 9 1

* Susceptible (S) reaction = >20% of plants with diamond-shaped lesions 1 x 3 mm or larger. At least two replicates tested at each inoculation; variant MI retested.

* Isolates from lesions on the cultivar Milyang 15.

* Korean cultures subcultured from isolates from Milyang 15; Philippine cultures from various rice cultivars, selected to represent diverse reaction patterns on the international differentials.
and stored at 4°C. The cultures from long-term storage were used as stock cultures for all restated isolates.

**Test cultivars.** Philippine (3) and international (2) differential sets were used. Seeds were soaked for 3 days, and 10 vigorous germinated seeds were selected and sown in uniformly fertilized soil. In the first experiments at IRRRI, seeds were sown in plastic trays 23 × 11 ×11 cm, one differential set to a tray. In later experiments, the differential cultivars were sown separately in plastic pots 9 cm in diameter. At IAS, seeds were sown in trays 15 × 7 × 8 cm, three differential cultivars to a tray. Before inoculation, seedlings were grown in a greenhouse until three- to four-leaf stage—14–21 days after sowing, depending on the time of year and location. Each experiment was replicated at least twice.

**Inoculum and inoculation.** In initial inoculations at IRRRI, sterile sorghum grain was used as substrate for increasing the inoculum. The fungus-colonized grains were washed with sterile water, blotted dry, and exposed under continuous fluorescent light for 3 days to induce sporulation. Spores were harvested by washing the grains with distilled water and filtering through cheesecloth. Subsequently at IAS and IRRRI, cultures were multiplied on either prune, oatmeal, or rice polish agar (22), depending on which medium gave the best sporulation. Sister MI were always inoculated on the same medium. Inoculum was produced from single mycelial patches, and test plants were inoculated as described previously (12). Inoculations were simultaneous for all MI derived from a lesion or parent MI. Inoculated seedlings were incubated in a dew chamber at 25°C for 24 hr, then transferred to a greenhouse.

**Disease evaluation.** Disease was scored 6–7 days after inoculation. Each seedling was examined and rated using a classification similar to one proposed by Ou (15), where 0 = no evidence of infection; 1 = brown specks smaller than 0.5 mm in diameter, no sporulation; 2 = brown specks about 0.5–1 mm in diameter, no sporulation; 3 = roundish to elliptical lesions about 1–3 mm in diameter with gray center surrounded by brown margin. As typical spindle-shaped blast lesions 3 mm or longer with necrotic gray centers and water-soaked or reddish brown margins, little or no coalescence of lesions; and 5 = lesions as in 4 but the upper portions of one or two leaves killed by coalescence of lesions. Scores of 4 and 5 were considered susceptible reactions. Similar to previous studies (18), a cultivar was considered susceptible when more than 20% of the seedlings showed reactions of class 4 or 5. At IRRRI, isolate-cultivar combinations were restaged when classification was not consistent, such as combinations showing differences between replicates, and for isolates showing deviations from the predominant reaction pattern of their sister MI.

**RESULTS AND DISCUSSION**

Among MI taken from single lesions and tested on the Philippine differential cultivars, only a few differed from the predominant reaction pattern of the lesion (Table 1). Of the 213 MI tested in Korea and the Philippines, only eight variant isolates were identified. These eight variants originated from lesions IR-1, IM-23-2, and T-1 and represent only four races (Table 1). Similarly, when the international differentials were used to test MI from lesions and cultures, only one variant was found among 180 MI tested (Table 2). The reactions of the variants at IRRRI were confirmed by restesting. Sometimes, MI reacted inconsistently in the first test, especially at IRRRI against the differential cultivars CO25, Usen, and Zenith, but upon restearing they were the same as their sister MI. These cultivars were poor differentials for the Philippine *P. oryzae* isolates because their response often tended to be intermediate, with predominantly type 3 lesions, rather than completely resistant, without sporulating lesions, or highly susceptible, with many type 4 lesions.

Without replication and repetition, erroneous conclusions could have been drawn from these experiments. For example, by applying Ou and Ayad's (18) classification method, 32% of the cultivars with intermediate reactions were classified as susceptible in one replicate but resistant in another. Clearly, such variation is inherent in the host response and the method of assessing this response, not in the pathogen. In previous work (18–20), no mention was made of treatment replication or restesting variant MI, and inoculations of MI from one source were apparently not all done at the same time. Thus, it is possible that the variation encountered was not due to differences between MI but to differences in environment and plant condition. Rice blast is notoriously sensitive to environment and physiologic condition of the host. Nitrogen level, temperature, plant water stress, post-inoculation leaf wetness, plant age, and leaf age (8,14,17) can all alter blast reaction, and some of these factors can influence cultivars differentially. Also, disease escape is common with the spray inoculation method, even with susceptible cultivars (21). To decrease environment and plant effects, we inoculated all MI from one source on the same day.

Neither the Philippine nor the international differential sets are ideal, especially since several of these cultivars apparently have some level of quantitative resistance. Efforts are under way to develop near-isogenic lines, each with a different single blast resistance gene, using a highly susceptible recurrent parent (13). Such a set of lines could provide a better means of describing isolates of *P. oryzae*.

In both Korea and the Philippines, we found little variation among MI from a single source. These data support the findings of Latterell (10,11) that *P. oryzae* is relatively stable pathogenically. If so, then quantitative resistance to blast in rice cultivars is not due to extreme pathogenic instability in *P. oryzae*.

**LITERATURE CITED**

single lesions and monoconidial cultures. Phytopathology 58:179-182.


