Physiologic Specialization of *Pyricularia oryzae* in the Philippines

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ABSTRACT

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The rice blast pathogen, *Pyricularia oryzae*, was collected from various regions in the Philippines and tested on widely grown Philippine cultivars and the International, Japanese, and Korean differential cultivar sets. Isolates with virulence to Philippine IR cultivars were common in samples from lowland areas, where such cultivars are widely grown, but virulence was rare among isolates from Zamboanga del Sur, where upland rice is cultivated. Virulence to C22 was most frequent among Zamboanga del Sur isolates. Virulence to IR56, UPLRi3, and UPLRi5 was rare. Virulence to the newly released cultivars IR58, IR60, and IR62 was present in the fungus population. IR60 and IR62 showed a higher frequency of intermediate reaction types than other IR cultivars. None of the three differential sets could adequately differentiate agriculturally important isolates. Differential sets using established cultivars grown within a geographic region are recommended for practical use in describing the virulence of a blast pathogen population.

In the past, widespread rice blast epidemics have occurred in the Philippines (13). Recently, the disease has not been a production problem in the country as a whole, but it has caused serious losses at some sites. A blast epidemic occurred in the upland rice crop in the multiple cropping production program of the Philippine Ministry of Agriculture in Zamboanga del Sur Province during 1982, resulting in a 50% yield decrease compared with the previous 3 yr (7). The cultivar IR54, which was bred for lowland irrigated environments, was most heavily damaged. The upland-adapted cultivar C22 also suffered losses. In 1983, blast was severe in several lowland rice-growing areas of South Cotabato Province sown to a wide range of cultivars. In the large rice-growing region of Central Luzon, blast has occurred on IR50 but rarely on IR36 and IR42. Similarly, the disease has occurred on IR50 in the Visayas region and on the island of Mindanao (9).

During 1983 and 1984, we assayed the population of the causal agent *Pyricularia oryzae* Cavara from several regions of the Philippines. Our purpose was 1) to test various *P. oryzae* populations for differences in virulence frequencies to Philippine rice cultivars, 2) to see if virulence to newly released rice cultivars was present in the fungus population, and 3) to test several blast differential sets for their ability to differentiate agriculturally important virulences in the pathogen population.

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MATERIALS AND METHODS

Collection, isolation, and culture maintenance. Rice leaves and panicles with typical blast lesions were collected during the wet season (May to November) in 1983 and 1984. In both years, specimens were collected from farmers' fields in the provinces of Nueva Ecija, South Cotabato, and Zamboanga del Sur by personnel from the International Rice Research Institute (IRRI), Los Baños, Laguna, where all subsequent work was conducted. In 1984, collections were made from nurseries planted with diverse germ plasm in Batangas, Ifugao, and Laguna provinces and from farmers' fields in Bulacan, Leyte, and Tarlac provinces. Specimens were dried and kept in a desiccator before isolation.

Single lesions were placed on glass rods in petri dishes with wet filter paper and incubated at 25 C until sporulation. For mass isolation, the sporulating lesion was examined under a stereomicroscope, and a group of conidia was aseptically transferred with a pointed capillary tube to a prune agar slant (15). To obtain single-conidial isolates, conidia were streaked onto a thin layer of water agar. Individual conidia were identified with a stereomicroscope and aseptically transferred and cultured. Seventy-three mass-conidial cultures from single lesions were obtained in 1983, and 97 monoconidial cultures, each from a single lesion, were isolated in 1984. Previous work under our test conditions indicated that single-conidial cultures derived from a single lesion are generally all of one race (5). Thus, mass- and single-conidial isolates from single lesions were considered equivalent for the purposes of this study.

All cultures were maintained initially in prune agar slants. For long-term storage, sorghum grains were used as a substrate. The grains were steeped, boiled, and autoclaved on two consecutive days. The grains were seeded with the fungus and incubated at 25–28 C until all grains were colonized. The colonized grains were dried at 40 C for 24 hr, transferred to a vial of silica gel, and stored at 4 C. These colonized grains were used as stock cultures for all retested isolates.

Test cultivars. In 1983, 13 cultivars currently grown in the Philippines (Table 1) and the Japanese differential set (19) were tested against the isolates obtained that year. A plastic tray (37 × 26 × 11 cm) was divided equally into 22 rows, and 15 seeds of each test cultivar were sown to a row. Two replicates were prepared for each isolate.

In 1984, three additional Philippine cultivars, IR62, UPLRi 7, and Mal-os, were tested, and the Korean (10) and International (1) differential sets were included. C039 was used as a susceptible check cultivar. The set of test cultivars was sown randomly in three plastic trays (23 × 11 × 11 cm), and C039 was included in each tray. The tray was divided into 12 rows, and at least 10 seeds of each test cultivar were sown to a row. For each isolate, two replicates were tested, each on a different date.

Among the Philippine cultivars tested, the IR cultivars were adapted to lowland rice culture and all others were adapted to upland rice culture. The cultivars IR58, IR60, and IR62 were recently released by the Philippine Seed Board.

Inoculation and disease evaluation. To produce inoculum, isolates were multiplied on either prune agar, oatmeal agar (15), or rice polish agar (15) depending on which medium gave the best sporulation in initial tests with the three media. About 3 ml of conidia and hyphal suspension from an agar slant was seeded to the medium in a petri plate. The inoculated plates were incubated at 25–28 C for 5–7 days until the entire agar surface was covered with mycelial growth. The growth was scraped with a sterilized rubber spatula, and the plate was exposed to fluorescent light for 3 days to induce heavy sporulation. The culture was flooded with distilled water mixed with 0.02% Tween 20 or 0.5%

gelatin, and the conidia were dislodged by scraping. The suspension was filtered through cheesecloth, and the concentration of conidia was estimated with a hemacytometer, then standardized to 5 $\times 10^4$ conidia per milliliter.

Plants were inoculated 18–20 days after sowing, using an electric sprayer to apply 30 ml of conidial suspension per tray of seedlings. The trays were rotated slowly during inoculation to ensure uniform distribution of inoculum. Inoculated seedlings were incubated in a dew chamber at 25 C for 24 hr, then transferred to a greenhouse.

In 1983, replicates were inoculated simultaneously for each isolate, and five to 10 isolates were used during each inoculation. In 1984 tests, only one replicate of an isolate was inoculated at a time, and the second replicate was tested after all isolates had been tested once.

Disease was scored 6–7 days after inoculation. Each seedling was examined and rated using a classification similar to one proposed by Ou (11), 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 margins, lesions capable of sporulation; 4 = typical spindle-shaped blast lesions capable of sporulation, 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 about half of one or two leaf blades killed by coalescence of lesions. Scores of 0–3 were considered resistant reactions, and scores of 4 and 5 were considered susceptible reactions. Generally, plant-to-plant reactions within a cultivar were consistent, with most plants scored either as 0–3 or as 4–5. In a few cases, however, the reaction differed within a cultivar by replicate or between replicates. These cultivars and isolates were retested until consistent results were obtained.

RESULTS AND DISCUSSION

Virulence of *P. oryzae* populations. The frequency of virulence to lowland cultivars IR36, IR42, IR50, IR52, IR54, and IR58 was high in *P. oryzae* samples taken from areas such as Laguna, Nueva Ecija, South Cotabato, Bulacan, Leyte, and Tarlac, where IR cultivars are grown (Table 1). In the upland rice area of Zamboanga del Sur, however, virulence to the IR cultivars was relatively rare and the fungus population instead had a high frequency of virulence to upland cultivars such as C22, UPLRi7, and Denorado, which are grown in this area.

The host from which the isolate originated was important. For example, two isolates collected from Zamboanga del Sur in 1984 were from IR cultivars, and these isolates showed virulence patterns similar to those of isolates from areas where IR cultivars predominate. The 1984 sample from South Cotabato consisted of three isolates taken from the traditional cultivar, Barao. These three isolates showed a pattern unlike those of the 1983 isolates from this province, being avirulent on IR cultivars and virulent on C22 and UPLRi7. Pathogen samples from Batangas, Ifugao, and Laguna were collected mostly from nurseries and seed-increase plots planted to many cultivars. This diversity of host germ plasm seems to result in a broader spectrum of virulence in the *P. oryzae* isolates taken from these areas. For example, in 1984, only five isolates were compatible with both C22 and one or more IR cultivars, and all of these originated from Batangas, Ifugao, and Laguna. Similarly, in 1983, very few of these isolates were found, and they all came from Zamboanga del Sur, where both IR54 and C22 have been cultivated. In general, differences between *P. oryzae* populations from various geographic locations in the Philippines probably reflect differences in resistance genotypes of the cultivars grown in these areas. Similar conclusions were reached in Japanese (8) and international (12) studies.

Virulence to IR56 was rare in both years, even in areas where virulence to IR cultivars was common (Table 1). The only exception was the sample from South Cotabato in 1983, and the isolates from this area that were virulent on IR56 were originally isolated from IR56. All isolates virulent to IR56 could attack the other tested IR cultivars. Unlike the other IR cultivars tested, IR56, IR60, and IR62 have the cultivar PTB-33 in their pedigree (4). Perhaps IR56 carries a resistance gene from PTB-33 that is not present in the other IR cultivars.

The overall frequency of virulence to IR36, IR42, and IR50 was similar (Table 1). Consequently, the more common occurrence of blast observed on IR50 in farmers' fields is presumably due to other factors, such as the relative partial resistance of the cultivars (6) rather than to a higher frequency of virulence to IR50 in the fungus population.

Among the seven Philippine upland cultivars tested, virulence to Mal-os and Kinandang Patong was frequent in samples from all locations. Mal-os is known to be one of the more susceptible traditional upland rice cultivars in Zamboanga del Sur, so its susceptibility to many of the isolates tested is not surprising. Kinandang Patong, however, is grown widely by upland rice farmers in Batangas and rarely shows disease. Seedling tests may be inadequate to describe the resistance of cultivars such as Kinandang Patong, and the possible reasons for the low disease on Kinandang Patong under field conditions, such as partial or adult plant resistance, deserve further study. Virulence to UPLRi3 and UPLRi5 was rare among the tested isolates but was highest in samples from Batangas and Zamboanga del Sur. These two upland cultivars have shown complete resistance in experimental plots and blast nursery tests at IRRI. Thus, the

Table 1. Percentage of isolates collected from various Philippine sites in 1983 and 1984 virulent to Philippine rice cultivars[a,b]

	Isolates (%) virulent to Philippine rice cultivars							
	Predominantly upland		Predominantly lowland					
Cultivar	Batangas (n = 18)	Zamboanga del Sur (n = 47)	Ifugao (n = 15)	Laguna (n = 15)	Nueva Ecija (n = 34)	South Cotabato (n = 31)	Other[c] (n = 10)	Mean (n = 170)
IR36	28	6	60	93	97	90	100	60
IR42	61	17	60	93	100	90	100	67
IR50	28	4	60	93	100	90	100	60
IR52	28	4	60	93	100	90	100	60
IR54	28	15	53	73	100	90	90	60
IR56	0	4	0	7	0	45	0	10
IR58	22	9	60	87	100	87	100	59
IR60	17	4	20	27	76	74	90	41
IR62[d]	11	11	7	7	39	0	50	19
UPLRi3	28	15	7	0	0	0	0	8
UPLRi5	22	9	13	7	3	0	0	6
UPLRi7[d]	6	67	33	13	0	100[e]	0	24
C22	11	81	47	20	0	10	0	31
Kinandang Patong	89	91	60	60	76	71	60	77
Denorado	33	43	13	0	0	0	0	16
Mal-os[d]	89	78	73	67	67	67	40	71

[a] All isolates were tested at least twice, the number of isolates (n) was 73 in 1983 and 97 in 1984. Only reactions 4–5 considered virulent.
[b] The check cultivar, C039, was susceptible to all isolates in 1984.
[c] Isolates from Bulacan, Laguna, Leyte, and Tarlac provinces.
[d] Not tested in 1983.
[e] For 1984 South Cotabato sample, n = 3.

isolates virulent to UPLRi3 and UPLRi5 will be useful for resistance screening work and as tools for assessing the level of partial resistance in these two cultivars.

Virulence to newly released cultivars. IR62 was released in 1984, and IR60 and IR58 were released in 1983. Although virulent isolates were less frequent for IR62 and IR60 than for IR58, virulence to these cultivars was present in the pathogen population (Table 1). Virulence to IR36, IR42, IR50, IR52, IR54, and IR58 was strongly associated. If an isolate was virulent on one of these cultivars, it was nearly always virulent to all of them. Isolates capable of attacking these IR cultivars showed either type 3 or type 4–5 reactions on IR60 and IR62. Type 3 reactions were more common on IR60 and IR62 than on IR58 (Fig. 1). Against many of the isolates, IR60 and IR62 apparently show incomplete resistance as defined by Parlevliet (14), because they produce lesions capable of sporulation (reaction type 3), but these lesions are not typical of a fully susceptible reaction.

Utility of differential sets. Agriculturally important isolates that differed from one another, such as those virulent or avirulent on the widely grown IR36, were often classified as the same race with the International, Japanese, and Korean differentials. Thus, data from these differential sets did not provide an adequate description of the pathogen population in the Philippines. The use of conventional differential sets and the expression of results as race numbers may not yield practical information (3,18) and, as pointed out by Chin (2), could lead to erroneous conclusions about local or regional changes in the structure of the pathogen population with time. For example, using the International differential set,

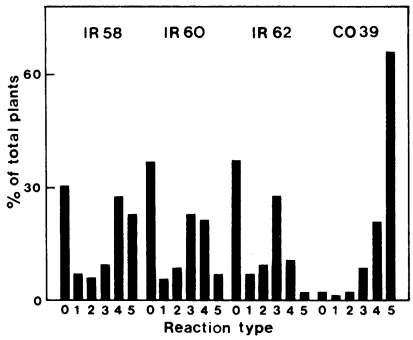

Fig. 1. Frequency distribution of reaction types for three newly released Philippine rice cultivars and the susceptible check C039 inoculated with isolates of *Pyricularia oryzae* collected in 1984. Data are from about 1,600 plants per cultivar.

Veeraraghavan and Dath (16,17) found only one reaction pattern when they tested isolates from throughout India. Perhaps they would have encountered important differences between isolates if they had used widely grown Indian cultivars. Such cultivars are preferable to conventional differential sets for practical use in describing the virulence of the *P. oryzae* populations within the rice-producing region or country.

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