Changes in Race Frequency of *Xanthomonas oryzae* pv. *oryzae* in Response to Rice Cultivars Planted in the Philippines

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ABSTRACT

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In an extensive survey of rice-growing areas in the Philippines from 1972 to 1988, 960 strains of *Xanthomonas oryzae* pv. *oryzae* (the bacterial blight pathogen) were collected. These strains were separated into six races based on their interactions with a distinct set of five differential cultivars carrying different resistance genes. Race 1, which was predominant before improved modern rice cultivars highly responsive to nitrogen fertilizer and management become widely cultivated, was virulent to rice with *Xa-10*, *Xa-11*, and *Xa-14* genes. When cultivars with the *Xa-4* gene were introduced in early 1970s, race 1 declined but races 2 and 3 appeared throughout the country. By the late 1970s, race 2 constituted 80% of the pathogen population, which corresponded to the proportion of hectarage planted to rice cultivars possessing the *Xa-4* gene. This race composition has remained constant to the present time. Among the six races, four (races 1, 2, 3, and 5) have been detected often during the past 20 years. Strains of race 2, the most predominant race, appear to have a narrow spectrum of virulence among rice cultigens with new R-genes. Four races appear to have distinct geographical distributions. Race 1 continues to be detected in the lowlands, where traditional cultivars are planted, or in areas where cultivars with *Xa-4* have not been extensively cultivated. Race 3, once the most predominant in the southern Philippines, was found infrequently, as was race 4, which was found only in Palawan. Race 5 exists in the highlands and has dominated the bacterial population in Banaue, the mountain terraces more than 1,500 m above sea level. A very minute population of race 6 was also detected.

Bacterial blight of rice (*Oryza sativa* L.), caused by *Xanthomonas oryzae* pv. *oryzae* (ex Ishiyama 1922) Swings et al (10), has been reported in the Philippines since the 1950s. The disease is widely distributed in all rice-growing countries in Asia, Australia, the Sahelian region and other parts of Africa, and has recently appeared in the United States (Texas and Louisiana) (6,7). Bacterial blight became increasingly important in the 1960s when semidwarf, high-yielding cultivars were adopted. In the Philippines, the predominant strains isolated in the 1960s were those that could attack traditional cultivars (land races or farmer-selected cultivars) as well as the "new" high-yielding cultivars, such as IR8, which lacked resistance to the disease. The latter, developed by the International Rice Research Institute (IRRI) was the first modern rice cultivar adapted to tropical conditions. In contrast, cultivars TKM-6 and Sigadis were resistant to these strains (2). Consequently, incorporating resistance to bacterial blight into high-yielding backgrounds has been a major goal of national and international rice improvement programs (6).

Resistance to bacterial blight was combined with the semidwarf, high-yield characters in new cultivars. Widespread use of such cultivars has changed rice-growing conditions in the Philippines. In 1972, an outbreak of bacterial blight was found in IR20, a newly released resistant cultivar, in Isabela Province in the northern Philippines (6). Another cultivar with the same R-gene, IR30, was also found with the disease in the south. The IR20 type of resistance was due to a single dominant gene, *Xa-4*. Until recently, most of the improved rice cultivars planted on about 90% of the rice land in the Philippines have possessed the resistance gene *Xa-4* (C. David, *personal communication*).

Since changes in pathogen races as influenced by cultivars have long-term implications in disease management and varietal improvement, this study was conducted to determine the races of *X. o. oryzae* found in rice-growing areas and their distribution relative to the rice cultivars grown. Race distribution and frequency changes in selected areas were monitored over time. We also investigated the influence of modern cultivars on pathogen populations, race aggressiveness, and the epidemics occurring during the past 15 yr.

MATERIALS AND METHODS

Selection of collection sites. An annual systematic survey of fields with bacterial blight has been done during the wet season in rice fields of major rice-producing provinces of the Philippines since the late 1970s. Survey areas were selected on the basis of fields planted to rice, cultivars grown, and strains of the pathogen previously isolated and deposited in the Plant Pathology Division laboratory at IRRI. This plan allowed us to follow up race distribution in any particular area where the strains were first isolated. In each rice-growing area, collection sites, which may be in several localities in a particular province, were selected depending on the incidence of disease, and were not necessarily the same from year to year. When disease incidence was about 25–50% in given fields, three to five sampling sites were selected at about 10-m intervals along the road. Each site consisted of about 16 randomly selected hills, with 20-cm spacing between hills. When disease incidence was greater than 50%, or when the greatest severity was observed, three to five sampling areas at the borders of fields were selected randomly. In collection sites where rice was grown in smaller fields (less than 1 or 2 ha), samples were collected randomly from two or three sampling areas of nine hills each. The incidence of disease varies from year to year; thus, the number of samples differed from one collection time to another and from year to year.

Collection of infected leaves. Typical bacterial blight lesions were collected from traditional cultivars with unknown gene(s) and from improved cultivars, most of which contained the *Xa-4* gene. At each sampling area of at least 16 hills, about five to 10 infected leaves were randomly collected, whereas three to five leaves were taken in sampling areas with nine hills. Typical grayish, water-soaked lesions were chosen, but in the absence of such lesions, yellowish to orange necrotic lesions were also collected. Supplementary data, such as origin or location of collection, cultivar, date, and previous crop or cultivar planted were gathered when possible.

Bacteriological tests. We examined colony morphology of the strains on peptone sucrose agar (PSA) at four days after incubation at 28 C, cell morphology after Gram staining, growth characteristics, utilization of carbon and nitrogen sources (including L-alanine), acetoin production, resistance to 0.001% cupric nitrate, and growth on 0.2% vitamin-free casamino acids. The scheme of Vera Cruz et al (11) was used to differentiate *X. o. oryzae* from *X. o. oryzicola*. In all labor-

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atory tests, reference strain PDDCC 3125 of *X. o. oryzae* was included.

Isolation and grouping of strains. Single colonies on PSA plates that had been isolated from samples with typical bacterial blight lesions were streaked for clonal purity on Wakimoto's medium (WF-P). At least five single yellow and mucoid colonies with smooth margins similar to those of *Xanthomonas* were selected from each sample and maintained on modified WF-P at 4 C for up to 1 mo. Long-term storage of strains, based on pathogenicity tests, was either in 5% skim milk at −20 C or as lyophilized cultures. Cultures were revived in fresh slants of WF-P for subsequent tests.

Immediately after each survey trip, the strains were tested for aggressiveness on IR8 or IR24 and for race specificity on the five differential cultivars IR20 (*Xa-4*), Cas 209 (*Xa-10*), IR1545 (*xa-5*), DV85 (*xa-5, Xa-7*), and IR24 (susceptible) (6,8,9). A rating of resistant or susceptible was based on lesion length as described earlier (8,9) or by lesion area (6). Lesions were 1–3 cm long or covered less than 5% of the area of plants and were categorized as resistant, whereas lesions on susceptible cultivars were more than 15 cm long or covered more than 50% of the plant. Since all strains from all cultivars of each sample provided a similar pattern on the differential hosts at 2 wk after inoculation, only one culture per sample was reinoculated in subsequent confirmation tests. All tests were done in the greenhouse under controlled conditions.

RESULTS

Identity of the strains. To date, about 960 strains have been collected. All strains formed yellow mucoid colonies on PSA, and colony size ranged from 3 to 4 mm at 4 days after incubation. No strain formed soluble pigment on PSA. All other phenotypic features of the strains fit those described for *Xanthomonas* in *Bergey's Manual* (1) and those of *X. o. oryzae* sensu Vera Cruz et al (11).

Occurrence and frequency of races of *X. o. oryzae*. Six races were recognized based on virulence patterns among 960 strains that were inoculated to the differential hosts (Tables 1–3). In addition, races 1 and 2 appeared to increase in aggressiveness on rice cultivars IR24, and on IR24 and IR20, respectively (Fig. 1). Race 1 was virulent to cultivars with the *Xa-10* and *Xa-11* genes. The other five races caused lesions in cultivars with genes as follows: race 2 with *Xa-4* and *Xa-11*; race 3 with *Xa-4*, *Xa-10*, and *Xa-11*; race 4 with *xa-5*, *Xa-7*, *Xa-10*, and *Xa-11*; race 5 with *Xa-11*; and race 6 with *Xa-4*, *Xa-10*, *xa-5*, *Xa-7*, and *Xa-11*. The races varied in frequency and distribution over the years (Tables 1–3).

The strains were grouped by year of collection (1972–1974, 1975–1979, 1980–1984, 1985–1988) in relation to the adoption of modern cultivars by farmers. During the periods 1975–1979 and 1980–1984, the hectarage planted with modern cultivars increased by about 60–85% in both irrigated and rainfed areas. Most of these cultivars had the *Xa-4* gene. Other genes, except *Xa-11*, were incorporated in the breeding lines at IRRI, but until now no cultivars with these genes have been released in the Philippines. During 1985–1988, these cultivars accounted for 85–90% of the total rice production in the sampled areas.

During 1972–1974, only five races of *X. o. oryzae* were detected, with race 1 representing 82% of the collection. Most of the collections came from Laguna and Bulacan on Luzon Island (Table 1). During 1975–1979, races 2 and 3 at 43 and 26% of the total, respectively, were higher in frequency than race 1 (Table

Table 1. Frequency distribution of races of *Xanthomonas oryzae* pv. *oryzae* in the Philippines, 1972–1974[a]

Province	Number of strain				
	Race 1	Race 2	Race 3	Race 4	Race 5
Banaue	0	0	0	0	1
Ifugao	0	1	0	0	0
Isabela	1	3	2	0	0
Pangasinan	1	0	0	0	0
Pampanga	1	0	0	0	0
Bulacan	11	1	0	0	0
Laguna	29	0	0	0	0
Quezon	2	0	0	0	0
Palawan	3	0	0	3	0
Dipolog City	1	0	0	0	0
Total no.	49	5	2	3	1
Percent	82	8	3	5	2

[a]Based on reactions of differential cultivars IR20, Cas 209, IR1545, DV85, and IR24, inoculated by leaf clipping at maximum tillering in a greenhouse.

Table 2. Frequency distribution of races of *Xanthomonas oryzae* pv. *oryzae* in the Philippines, 1975–1979[a]

Region or province	Number of strain					
	Race 1	Race 2	Race 3	Race 4	Race 5	Race 6
Banaue	0	0	0	0	13	0
Isabela	1	4	0	0	0	0
Cagayan	1	0	0	0	0	0
Nueva Ecija	0	13	1	0	0	0
Laguna	3	3	0	0	0	8
Quezon	0	1	0	0	0	0
Bicol region	0	1	13	0	0	0
Palawan	4	0	0	0	0	0
Visayan region	0	11	0	0	0	0
Davao	0	9	12	0	0	0
Agusan del Sur	0	1	0	0	0	0
Total no.	9	43	26	0	13	8
Percent	9	43	26	0	13	8

[a]Based on reactions of differential cultivars IR20, Cas 209, IR1545, DV85, and IR24, inoculated by leaf clipping at maximum tillering in a greenhouse.

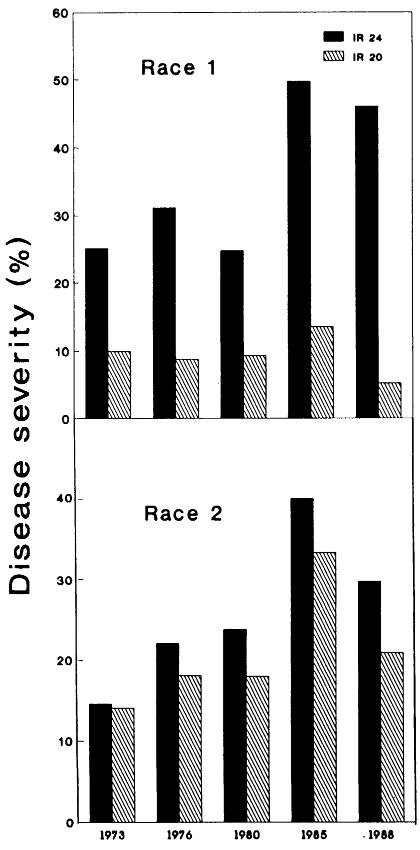

Fig. 1. Severity of bacterial blight, based on leaf area infection 14 days after inoculation, on rice cultivars IR24 and IR20 after inoculation with strains of *Xanthomonas oryzae* pv. *oryzae* collected in different years.

2). During 1982–1984, 83% of the strains belonged to race 2, whereas from 1985 to 1988, 64% were race 2 (Table 3).

Distribution. Race distribution across regions and provinces appeared specific to certain sites. During 1972–1974, race 1 was the most predominant race in the few sites sampled (Fig. 2); however, after 1979 race 2 gradually increased in frequency and was collected in many rice-growing areas where modern cultivars were grown (Fig. 2). Race 5 has been detected so far only in Banaue in northern Luzon, where traditional cultivars have been grown annually as single crops in terraces for the last 2,000 yr. This area has an elevation of about 1,525 m (5,000 ft) with an average temperature of 21 C. Other races were found only in the lowland rice fields, where the mean temperature is 29 C. Among the differentials, only IR24, with no gene for bacterial blight resistance, and IR8, carrying the *Xa-11* gene, were susceptible to race 5. Race 3 was also isolated from several provinces but at low frequency except in the Bicol region of Luzon Island and in Iloilo on Panay Island. Race 4 was found only in Palawan, another island to the southwest of Luzon, and race 6 was present in Laguna Province in southern Luzon.

Based on our survey data, we suggest that strains isolated from both traditional and modern cultivars that lack specific resistance to *X. o. oryzae* have broad virulence spectrums. Strains belonging to five races were found prior to the introduction of specific resistance genes that would select for these races. They could infect cultivars differing in resistance; in contrast, strains isolated from modern cultivars that possess resistance genes appear to be genotype-specific (Table 4). Based on chi-square tests, we concluded that occurrence of the strains was highly dependent on the cultivars of their isolation.

DISCUSSION

Bacterial blight of rice is commonly observed in the Philippines during the wet season from June to November. Typhoons (20–25/yr) and frequent rainstorms predispose plants to bacterial blight infection and disseminate the pathogen. Consequently, the disease has become endemic along typhoon paths, particularly among cultivars that are not resistant. Several severe epidemics occurred after the new high-yielding and nitrogen-responsive but susceptible cultivars were cultivated in the Philippines (2–6).

The frequency and severity of disease outbreaks among hectarages of improved rice cultivars have increased with the adoption of cultivars with the *Xa-4* gene since the early 1970s. Subsequently, the distribution and frequency of race 1 decreased whereas that of race 2 increased. Other resistance genes, such as *xa-5* and *Xa-7*, have been incorporated into improved breeding lines, but no cultivar with these genes has been planted in farm fields. Prior to the introduction of the cultivars with *Xa-4*, most (82%) strains collected were virulent on traditional cultivars (whose resistance to bacterial blight is unknown) that did not carry *Xa-4* and avirulent on cultivars with *Xa-4*. Gradually, the frequency of race 2, which is virulent to R-gene *Xa-4*, increased as modern cultivars with *Xa-4* became accepted. Race 2 dominated the bacterial populations in the areas planted to modern cultivars.

A more thorough sampling is now underway to explore further the variability among populations of each race. The sampling procedures we used until 1988 and reported in the present study

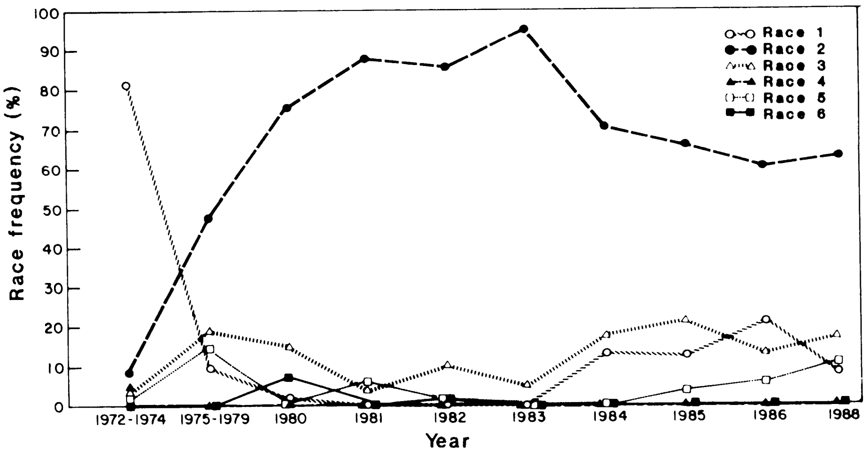

Fig. 2. Race composition of populations of *Xanthomonas oryzae* pv. *oryzae* collected in the Philippines from 1972 to 1988.

Table 3. Distribution of *Xanthomonas oryzae* pv. *oryzae* races by region and province in the Philippines, 1985, 1986, 1988[a]

Region Province	Number of strain Race 1	Race 2	Race 3	Race 4	Race 5	Race 6
Northern Luzon						
Ilocos Sur	5	1	0	0	0	0
Ilocos Norte	7	0	3	0	0	0
La Union	1	1	0	0	0	0
Nueva Viscaya	6	0	3	0	0	0
Cagayan	0	14	0	0	0	0
Isabela	0	26	0	0	0	0
Ifugao (Banaue)	0	0	0	0	32	0
Central Luzon						
Nueva Ecija	0	44	0	0	0	0
Tarlac	0	14	0	0	0	0
Bulacan	0	4	0	0	0	0
Pampanga	0	2	0	0	0	0
Southern Luzon						
Laguna	3	23	0	0	0	0
Palawan	19	13	0	0	0	0
Quezon	4	3	0	0	0	0
Bicol						
Camarines Sur	0	5	25	0	0	0
Albay	4	17	18	0	0	0
Sorsogon	0	2	9	0	0	0
Visayas						
Iloilo	0	4	20	0	0	0
Aklan	0	2	4	0	0	0
Capiz	0	5	1	0	0	0
Leyte	0	14	0	0	0	0
Mindanao						
Misamis Oriental	0	1	0	0	0	0
South Cotabato	0	58	2	0	0	0
North Cotabato	0	7	0	0	0	0
Davao	0	26	1	0	0	0
Sultan Kudarat	0	9	0	0	0	0
Maguindanao	0	1	0	0	0	0
Total no.	49	296	86	0	32	0
Percent	11	64	18	0	7	0

[a] Based on reactions of differential cultivars IR20, Cas 209, IR1545, DV85, and IR24, inoculated by leaf clipping at maximum tillering in a greenhouse.

Table 4. Virulence of *Xanthomonas oryzae* pv. *oryzae* isolated from various rice cultivars with genes for bacterial blight resistance to cultivars with *Xa* gene(s), 1972–1984[a]

R-gene Source of isolates	Percent virulence to *Xa* genes				
	Xa-11[b]	***Xa-4***[c]	***Xa-10***[d]	***xa-5***[e]	***xa-5, Xa-7***[f]
No R-gene					
IR cultivar	100 (107)[g]	30 (61)	90 (61)	10 (6)	10 (4)
Xa-4					
IR cultivar	100 (95)	100 (54)	14 (54)	0 (6)	0 (4)
Unknown R-gene					
IR line or cross	100 (93)	47 (52)	53 (52)	4 (5)	3 (4)
Other breeding line or cross	100 (101)	43 (58)	73 (57)	7 (6)	3 (4)
Native, local, or traditional cultivar	100 (102)	65 (58)	52 (58)	8 (6)	4 (4)

[a]Virulence was based on reactions of strains where resistance gene of source cultivar was obtained. Tests of independence between source of strain (R-gene) and virulence (%) to *Xa* genes were not significant based on the chi-square test: $\chi^2 = 130.1$, χ^2 computed = 32.0 at the 1% level of significance.
[b]Cultivar IR8.
[c]IR20.
[d]Cas 209.
[e]IR1545-339.
[f]DV85; *Xa-7* conveys resistance to races 2 and 3 at all rice growth stages.
[g]Figures in parentheses denote expected frequency based on the chi-square test.

may have been biased by the sample size collected from each site and bulked on a yearly basis. On the other hand, race 2 was widely distributed across regions and islands and over the years, at least in comparison with the other races. There was an apparent shift in frequency in race distribution of the bacterial population after wide cultivation of modern cultivars with the *Xa-4* gene.

Pathogen races compatible with cultivars possessing the *Xa-4* gene were detected before the wide cultivation of the modern or improved cultivars with uniform resistance. Other races may also have been present in all rice-growing regions but in numbers too few to be detected, as indicated by strain B56 detected in Davao in 1963 (8). Race 3, also virulent to cultivars with the *Xa-4* gene, was detected in Davao in the mid-1970s, but its frequency has also been low. In recent surveys, the predominance of race 2 in comparison with other races that are able to cause disease in cultivars with *Xa-4* may be caused by the greater compatibility of race 2 with currently grown cultivars possessing the *Xa-4* gene. In preliminary tests of breeding lines with *Xa-4* using inoculum prepared from strain PXO 86 of race 2, grayish water-soaked lesions, typical of blight, developed within 4 to 5 days after inoculation in the field (T. W. Mew, *unpublished*). Also, strains of race 2 isolated recently appeared to be more aggressive on IR24 and IR20 than those isolated previously. Yet, when a cultivar susceptible to both races 1 and 2 was inoculated in the field with a mixture of these races, race 1 was more frequently detected than race 2 during the crop growth (P. Roberts and T. W. Mew, *unpublished*). So, race 1 appeared to be better adapted to growth and survival than race 2 in cultivars carrying no *Xa-4* gene. With the adoption by farmers of cultivars with one or more specific R-genes, the race composition of the pathogen would initially be affected by the avirulence genes, but later other fitness factors, such as compatibility and competition, would have become involved. When the bacterial population stabilized, the predominant race(s) would possess not only specific virulence genes but also genes for high compatibility with the cultivars grown. A readjustment or rearrangement of the original population of races in response to cultivation of cultivars possessing the *Xa-4* gene thus happened. Studies with molecular markers of the pathogen and cultivars are being done to support the variation found using host differentials (J. Leach, *personal communication*). Whereas rice genotypes influence the race structure, continuous cultivation of a single host genotype may not reduce the genetic variability of *X. o. oryzae* (R. Nelson, *personal communication*).

Bacterial blight outbreaks have appeared frequently over the last few years but have not caused severe yield losses. Many IR cultivars grown in the Philippines are moderately susceptible to race 2. These cultivars appear to possess some level of horizontal resistance (T. W. Mew and C. M. Vera Cruz, *unpublished*), which appears to have made them durable despite continuous and widespread planting in the country. The level of horizontal resistance in the IR lines appeared to be enhanced by multiple crosses with traditional cultivars in a selection process where only highly susceptible plants were rogued from segregating populations (T. W. Mew and C. M. Vera Cruz, *unpublished*). Multiple but minor factors for resistance were probably carried over to the progenies. In initial tests, although many cultivars carry the *Xa-4* gene, disease severity based on lesion length or lesion area varied with strains of the races used in inoculation (T. W. Mew and C. M. Vera Cruz, *unpublished*). Genetic analyses of these cultivars are in progress.

Several forms of resistance have been identified in rice germ plasm. Most involve adult-stage resistance, where cultivars become more resistant to disease as the plants mature (12). However, seedling resistance, also known as overall resistance, also has been identified. The five differential cultivars used here possess overall resistance to specific races of *X. o. oryzae*.

No major or large-scale epidemics have occurred since the introduction of the *Xa-4* gene. Yet the frequent micro-scale epidemics have been testing the strength of resistance in the gene combinations in modern cultivars. Should these combinations be overcome by new, highly aggressive races of the pathogen, other genes for resistance such as *xa-5* and *Xa-7* have been incorporated in the IRRI breeding program, and advanced lines with these genes are being evaluated and readied for release. Thus, the potential for severe and widespread epidemics of bacterial blight appears limited despite favorable climatic conditions for disease development.

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