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Phylogenetic and pathotypic analysis of rice bacterial blight Race 3

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Abstract

Eight Philippine races of *Xanthomonas oryzae* pv. *oryzae* have been identified based on virulence phenotypes observed on a set of five differential varieties. One of these, Race 3, was found to consist of two phylogenetically distinct lineages based on DNA fingerprinting analysis. To determine, if the two lineages could be differentiated based on host-specificity, 186 strains of Race 3 were analyzed with additional fingerprints and 76 selected isolates with additional differential rice varieties. The strains were separated into 36 haplotypes clustering in three groups (*IS1113*-B, -C, and -G) at the 75% similarity level. Isolates varied in their reaction to a rice line carrying the resistance gene *Xa7*, however, the variability was not consistent within lineage. Aggressiveness of isolates belonging to lineage *IS1113*-B and -G was significantly greater when tested during the dry season than when tested during the wet season. However, no such differences were evident for isolates from lineage *IS1113*-C, indicating that environmental effects presumably light and temperature are genotype-specific.

Introduction

Since differential host–pathogen interactions were first demonstrated between the wheat stem rust pathogen *Puccinia graminis* and different wheat cultivars (Stakman and Levine, 1922), 'races' have been defined for many host–pathogen systems. A race is a composite virulence phenotype on a number of standard host cultivars carrying different resistance genes. The number of races defined is a function of the number of differential hosts used and races are frequently considered to be genetically distinct groups (Caten, 1987).

Although several authors have pointed out that race typing may not be the most useful way to characterize pathogen populations (e.g., Wolfe and Schwarzbach, 1975), the race concept is still widely used in plant pathology. In recent years, DNA fingerprinting has been utilized extensively for the characterization of pathogen populations. This approach can help in clarifying phylogenetic relationships within and among populations (Leung et al., 1993). Phylogenetic lineages have become a key tool for understanding the population biology of pathogens such as the blast fungus *Pyricularia grisea* (Zeigler et al., 1995) and the potato late blight pathogen *Phytophthora infestans* (Fry and Goodwin, 1997).

Both race-typing and DNA fingerprinting have been applied to Philippine populations of the rice bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae*. Eight Philippine races of *X. oryzae* pv. *oryzae* were identified based on the virulence combinations observed on a set of five differential varieties (Nelson et al., 1994). Among the more than 4,000 strains of *X. oryzae* pv. *oryzae* collected in the Philippines between 1972 and

1992 and maintained at the International Rice Research Institute (IRRI), 186 were classified as Race 3 based on their reaction on a standard set of differential cultivars. Until 1992, strain IRN135 (PXO79) was used as the 'type strain' to represent Race 3 for genetic studies, resistance screening, and breeding. Because DNA fingerprinting analysis indicated that strains identified as Race 3 consisted of two phylogenetically distinct lineages (Nelson et al., 1994), a strain (IRN254 = PXO87) belonging to the second lineage was later added as an additional 'type strain'.

Although the two 'Race 3' groups were not consistently differentiated based on pathogenicity tests (Nelson et al., 1994), preliminary data suggested that the two 'Race 3' lineages may show quantitative differences in lesion lengths (i.e. aggressivity) on several differentials used. In particular, isolates varied in reaction to the differential possessing *Xa-7*, (IR-BB7). The present study was undertaken to further explore the phylogenetic and pathotypic differences among strains of *X. oryzae* pv. *oryzae* classified as Race 3. In particular, we tested the hypothesis that the two known Race 3 lineages constitute distinct races, to identify resistance gene(s) that could be used to differentiate these two lineages within Race 3, and to assess the spatial and temporal patterns of variation within Race 3 in the Philippines.

Materials and methods

Bacterial strains, culture and maintenance

The bacterial strains selected for study were collected between 1972 and 1992 in the Philippines and maintained as skim milk stocks or as lyophilized cultures at the IRRI. Strains were revived and grown on modified Wakimoto's medium (WF-P, Karganilla and Natural, 1973) prior to DNA isolation. One hundred and eighty-six strains were selected based on their recorded reactions to a standard set of differential cultivars which indicated that the strains were of Race 3 (Table 1). In addition, 28 strains from a 1992 collection from Calauan near IRRI that were Race 2 (like Race 3 but *R* for IR-BB10, see Table 1) were included because unreplicated pathogenicity tests had indicated a variable reaction to IR-BB7 (*Xa-7*). Also, the commonly used type strain for Race 2, IRN253 (= PXO86) and the strain IRN35 (= PXO35; Race 1), which was the only known representative of RFLP probe IS1113 group F were included. IRN35 was included for comparison after the detection of a new, very different haplotype among the race 3 isolates.

Before the initial differential tests were performed, the aggressiveness of the cultures revived from long-term storage was verified. Bacteria for inoculum were prepared as described by Mew et al. (1982). All strains were clip inoculated (Kauffman et al., 1973) on 4–7-week-old plants of the susceptible cultivar IR24. Leaves with long water-soaked lesions were harvested 10–14 days after inoculation and stored at 4 °C until isolation of bacteria. Leaf sections including about 5 mm of the advancing border of the lesion and 5 mm of adjacent healthy tissue were surface sterilized in 100% ethanol for 10 s and rinsed twice in sterile distilled water. Bacteria were allowed to ooze into 0.5 ml sterile distilled water for 15 min or longer and the ooze was streaked onto WF-P plates. After four days incubation at 30 °C, bacterial growth was transferred to WF-P slants. Cultures were maintained on agar slants at 4 °C for 2–4 weeks and at −20 °C in skim milk for longer-term storage.

Table 1. Reaction of near isogenic differential rice lines to *Xanthomonas oryzae* pv. *oryzae* Races 1, 2, and 3

Line Gene	IR24 *none*[a]	IR-BB4 *Xa-4*	IR-BB5 *xa-5*	IR-BB10 *Xa-10*	IR-BB14 *Xa-14*	IR-BB7 *Xa-7*
Race 1[b]	S	R	R	S	S	R
Race 2	S	S	R	R	S	R
Race 3	S	S	R	S	S	V

[a]IR24 is known to carry *Xa-18*, a gene that is ineffective against all known Philippine strains. IR24 was used as the susceptible recurrent parent for the development of the near-isogenic lines (NILs) used in this study. The NILs presumably carry *Xa-18*.

[b]Race designations are based on the reactions to all differentials except IR-BB7.

S = susceptible reaction, R = resistant reaction, V = variable reaction.

RFLP analysis

DNA isolation, probes and labeling. Genomic DNA was extracted from 20 ml of overnight nutrient broth cultures of each strain following the CTAB method as modified by Raymundo et al. (1990). The DNA pellet was dissolved in 100 µl TE.

IS1113, a transposable element on a plasmid (Nelson et al., 1994) and plasmid pBS*avrXa10* were used as probes. pBS*avrXa10* contains a 3.1 kb *Bam*H1 fragment internal to the *avrXa10* gene of *X. oryzae* pv. *oryzae* (Hopkins et al., 1992). Both probes were produced as described elsewhere (Nelson et al., 1994). The probes were labeled with digoxigenin by random priming using Boehringer-Mannheim DNA labeling reagents according to the manufacturer's recommendations.

For RFLP analysis, genomic DNA was digested at 37 °C with *Eco*R1 or with *Bam*H1 (Boehringer-Mannheim, Germany or Quadrant) as described by the manufacturer. DNA fragments were separated by electrophoresis through 0.7% agarose in 0.5× TBE buffer at 20 – 40 V for 16 – 48 h. Alkali transfer to Hybond N or Hybond N^+ (Amersham, U.K.), prehybridization, hybridization, and posthybridization washes were done according to the membrane manufacturer's instructions. Hybridized bands were visualized by an alkaline phosphatase-conjugated antibody against digoxigenin, followed by NBT-X-Phosphate color reaction according to the manufacturer's procedure (Boehringer-Mannheim) or by chemiluminography on Du Pont Cronex X-ray film using AMPPD (3-[2′-spiroadamatane]-4-methoxy-4-[3″-phosphoryloxy]-phenyl-1,2-dioxetane) as chemoilluminescent substrate for alkaline phosphatase (Tropix, Inc.). DNA of each strain was electrophoresed at least twice to confirm banding patterns.

IS1113 patterns were determined for all 214 strains and combined patterns based on all three probe/enzyme combinations were determined for 149 selected strains. The selection was based on IS1113 type and time and place of the strains' collection (data not shown).

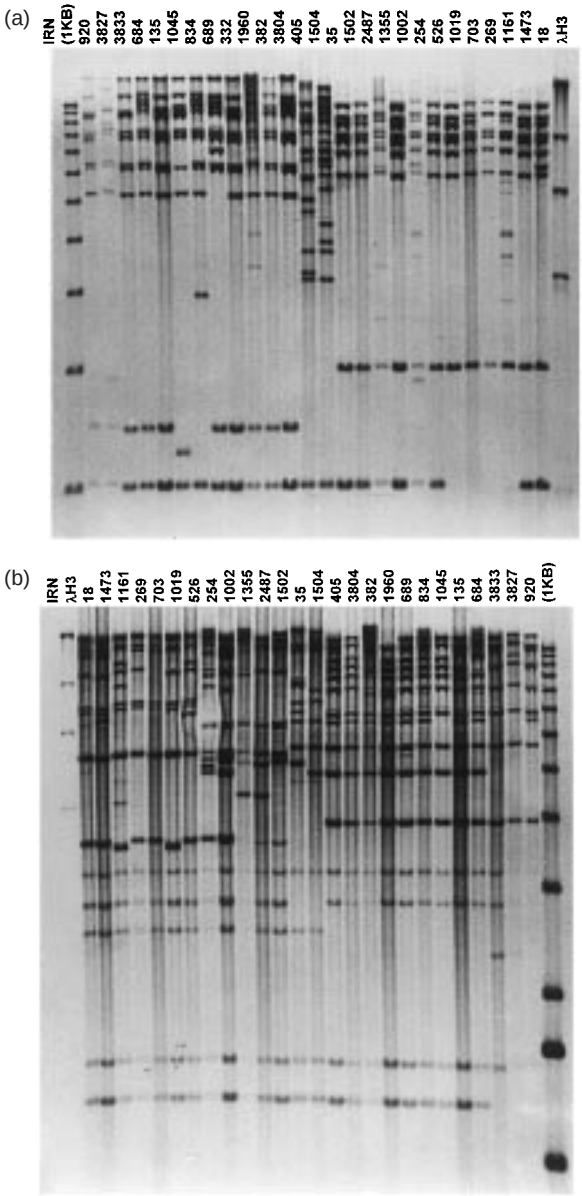

Figure 1. Blots of *Eco*R1 digested DNA from *Xanthomonas oryzae* pv. *oryzae* probed with probes *IS1113* (a) and *AvrXa10* (b). Strains are identified with strain record numbers at the top of each lane.

Numerical analysis of RFLP data. Strains with unique banding patterns ('haplotypes') were electrophoresed on the same gel (Figure 1) to confirm band identities and differences. RFLP patterns were compared visually to previously recorded patterns (Nelson et al., 1994) and by electrophoresing strains side by side and named accordingly. Patterns were converted into binary data, i.e., the presence and absence of bands at different positions were recorded as 1 and 0, respectively. If a band was present in all strains it was omitted from the analysis. Dice's coefficient of similarity $(2m_{xy}/[m_x + m_y]$, where m_{xy} is the number of bands

in common between two haplotypes x and y, and $[m_x + m_y]$ is the total number of bands of the two haplotypes; Rohlf, 1992) was calculated for all possible pairs of strains for the three probings separately and for the combined data. The unweighted pair-group method, arithmetic average (UPGMA, Sneath and Sokal, 1973) was used to generate dendrograms based on the similarity matrix. For the separate RFLP analyses, haplotypes that clustered together usually at 75% similarity were assigned to groups named with capital letters. Different haplotypes within clusters were given numerical designations. The robustness of the dendrograms was assessed with bootstrap analysis running 2000 iterations (Felsenstein, 1985, 1988; Nelson et al., 1994; Yap and Nelson, 1996).

Pathogenicity tests

Differential cultivars. Seeds of differential cultivars were soaked for 24 h, incubated for 48 h, and planted as a dense stand. Three 17–19 day old plants were transplanted into black plastic pots approximately 12 cm in diameter and 20 cm high. To increase organic matter content, ground rice straw was routinely added to the soil at approximately 0.01% of the soil dry weight. Plants were fertilized with 1 g ammonium sulfate per pot one week after transplanting and again 18–20 days later. Insecticides were applied when needed. All plants were 54–56-days-old at inoculation.

The differential cultivars used for the first and second test and their resistance genes are listed in Table 3. Seeds were obtained from the Division of Plant Breeding Genetics and Biochemistry at IRRI. All cultivars except Tetep were near-isogenic to the cultivar IR24 (Ogawa et al., 1991). IR24, and probably the isolines, possess the resistance gene *Xa-18*, which is ineffective against all known strains of *X. oryzae* pv. *oryzae* in the Philippines.

Inoculations and assessments. For the first differential test, refrigerated cultures on WF-P slants (cultures had been re-isolated recently) were used to produce inoculum. For the second test, cultures were revived from skim milk stocks and cultured for four to five days before sub-culturing for inoculum production.

For each cultivar/strain combination, three to four young but fully expanded leaves per plant per replicate were clip inoculated. If the youngest fully expanded leaf of a tiller was damaged, the older leaf next to it was inoculated instead. Each test was replicated three times. Because of the large number of strains to be tested, inoculations had to be conducted over periods of several weeks (3 Feb. 93 to 1 Mar. 93, and 1–30 Sept. 93). Therefore, two control strains that are routinely used at IRRI as representatives of the two major *IS1113* groups (IRN135 = PXO79, group B and IRN254 = PXO87, group C) were inoculated on a set of differentials at each inoculation date to assess possible effects of inoculation date. One person measured the lesion length 14 days after inoculation.

Differences in pathogenicity and aggressiveness among RFLP groups on the differential host cultivars and effects of inoculation period were analyzed with t-tests (SAS, 1988).

Results

RFLP analysis

Between 7 and 21 bands were observed for *IS1113/Eco*RI, 12–18 for *avrXa10/Eco*R1 and 5–9 for *avrXa10/Bam*H1 (Figure 1). A total of 99 band positions were scored for the composite data-set of the three enzyme/probe combinations. Thirteen *IS1113* haplotypes were identified for the Race 3 strains, clustering into three groups at the 75% similarity level. The more common haplotypes were found in most of the five regions in the Philippines. Three strains originating from three different fields from the island Palawan on the same day in 1989 clustered in a previously unknown group G (Figure 2). The same three clusters of Race 3 strains were formed based on the *avrXa10/EcoRI* and *avrXa10/BamHI* data-sets, though the bootstrap values were lower (data not shown).

Based on the combined data from 149 strains, 36 haplotypes were defined for the Race 3 strains, clustering into the same groups as based on *IS1113* in a very robust phenogram (Figure 2). Strains that had identical haplotypes when analyzed with one probe alone differentiated into up to 13 different haplotypes when using additional probe/enzyme combinations. One haplotype comprised strains of both Races 2 and 3 (IRN1342 [Race 2], IRN1361 [Race 3], IRN2463 [Race 3]). Four haplotypes were represented only by Race 2 (IRN3804, 3805, 3833, and 3841), and one additional haplotype was defined for the single Race 1 strain included. IRN689 had a unique haplotype but could not be assigned a race as it has lost virulence

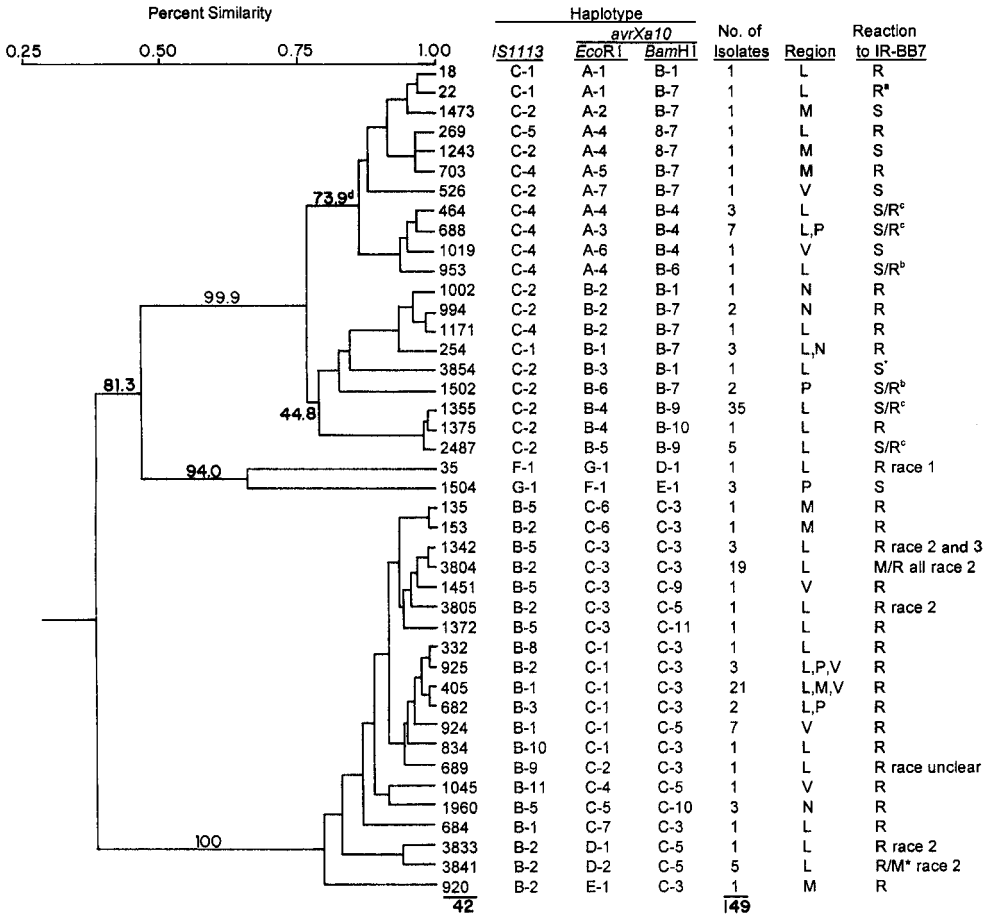

Figure 2. Phylogenetic tree based on the similarity (unweighted pair-group method, arithmetic average, UPGMA) among DNA fingerprints for strains of bacterial blight (*Xanthomonas oryzae* pv. *oryzae*). The tree was constructed based on the combined fingerprints from *Eco*RI digested DNA probed with probe *IS113* or probe pBS*avrXa10* and *Bam*H1 digested DNA probed with pBS*avrXa10*. Type strains are identified by strain record number (IRN). Haplotypes based on either of the three probes alone, the number of strains found per haplotype and the regions where the haplotypes were found are noted to the right of the type strain number. Regions were lowlands, Luzon island (L), highlands, Luzon island (N), Mindanao (M), Palawan (P), and Visayas (V). Unless indicated otherwise, strains were Race 3. The numbers on the branches of the tree indicate the percentage of times the strains to the right of the branching point were grouped together during bootstrapping analysis. Numbers close to 100 indicate a robust pattern.

during storage. Fourteen haplotypes were represented by more than one strain, and four of these haplotypes were found on more than one island. IRN1355 represented the most common haplotype, which included 35 strains collected in the lowlands of Luzon between 1989 and 1992. Twenty-one strains shared their haplotype with IRN405; this group was collected from three distinct regions of the Philippine archipelago (the Visayas, Luzon, and Mindanao) between 1981 and 1989.

Pathogenicity tests

A total of 92 strains were subjected to virulence analysis: 45 strains were tested on nine differentials in February/March 1993, and 80 were tested on eight differentials in September/October 1993. The second test included 33 strains from the first test. Eighty-one strains from the historical collection that had been recorded as Race 3 were tested. Four strains (IRN1057, IRN1468, IRN1451, and IRN1342) were found to be avirulent

on IR-BB10, and were therefore reclassified as Race 2. IRN689 had lost its virulence during storage and no race could be assigned. These strains and all other strains of Races 2 and 1 were not included in the statistical comparisons discussed below.

Seeds of IR-BB5 were contaminated, at a low frequency, with seeds of an unknown but susceptible genotype. Long lesions that were produced on the supposed IR-BB5 plants were not used for the means' comparisons because they were not repeatable. There was no reason to suspect that any of the other seed sources was contaminated, as the results were very similar between replications.

The two control strains, IRN135 and IRN254, exhibited considerable variation in lesion length throughout the two periods of inoculation (Figure 3). Differences between the two strains were small and not repeatable over time. For example, IRN254 produced longer lesions than IRN135 on IR-BB4 and IR-BB10 during the first inoculations (differences were 4.3 and 4.4 cm, respectively, $P < 0.05$). The differences were much smaller and non-significant during the second period. There was also no substantial effect of the two test periods on the performance of the two strains, although sometimes differences of 1–4 cm were statistically significant at $P < 0.05$.

In both tests, strains belonging to *IS1113* group B consistently produced necrotic, resistant-type lesions on IR-BB7. Twenty-seven out of 50 group C strains and all 3 strains of group G, produced water-soaked lesions on IR-BB7. These lesions were substantially longer than those induced on IR-BB5 and were sometimes over half the length of the lesions produced on IR24 (Table 2). However, other strains from group C-002 and C-004 produced very short, resistant-type lesions. Fifteen of the group B strains, 14 of the group C, and all 3 group G strains were included in the second test during September 1993. Strains that had been avirulent and virulent to IR-BB7 in the first test behaved similarly in the second test except for two cases where previously moderately virulent strains were avirulent in the second test. Overall, lesion lengths on IR-BB7 were significantly greater for strains of group C than for strains from group B (Table 3). No statistical comparison with group G was made because that group contained only 3 strains.

The differences between groups B and C interacted with testing period. In addition to the differences in reaction to IR-BB7, the group B strains produced significantly shorter lesions than the group C strains on all differentials except on IR-BB5 during the second test (Table 3). While group C strains produced almost identical lesion lengths during both test periods, the group B strains produced significantly shorter lesions during the second test as compared to the first test (Table 4). The three strains from group G were affected like the

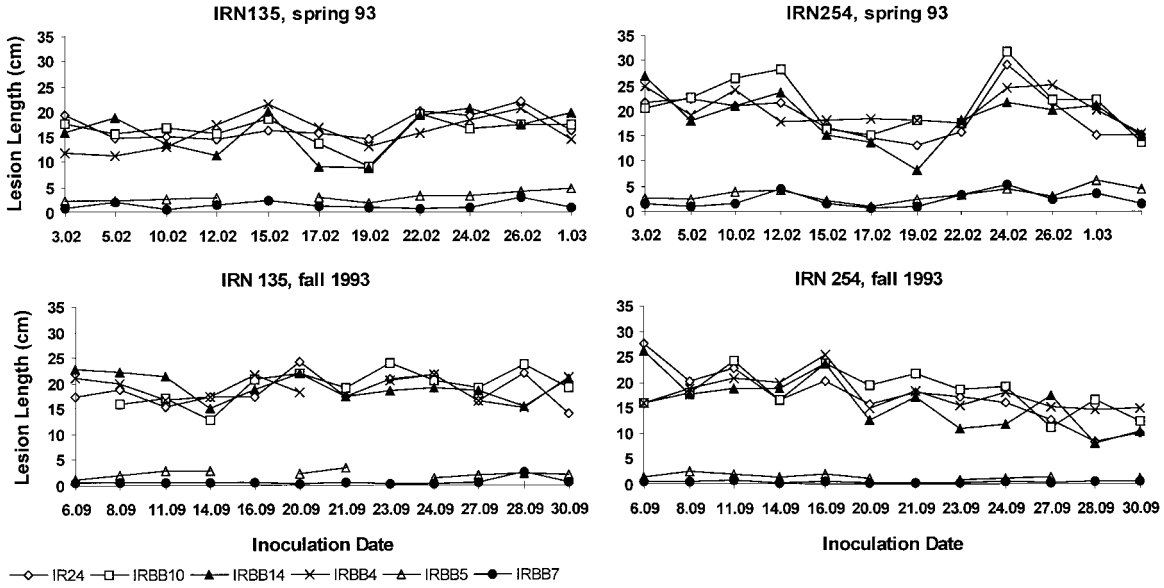

Figure 3. Reaction of six differential rice lines to two strains during two testing periods with 12 inoculations each. Testing periods were February/March and September 1993.

Table 2. Differential reactions of rice line IR-BB7 carrying bacterial blight resistance gene *Xa-7* to 76 rice bacterial blight Race 3 isolates belonging to different haplotypes as defined by probe *IS1113*. Reactions were considered susceptible (S) when the lesion length was more than 50% of that observed on the susceptible control IR24; intermediate (M), when the lesion length was between 20% and 50% of that observed on IR24 and otherwise resistant (R)

IS1113 group	Number of strains reacting to IR-BB7			Total
	S	M	R	
B-001	0	0	9	9
B-002	0	0	7	7
B-003	0	0	2	2
B-005	0	0	4	4
B-008	0	0	1	1
Subtotal	0	0	23	
C-001	0	2	2	4
C-002	3	13	15	31
C-004	4	5	5	14
C-005	0	0	1	1
Subtotal	7	18	23	
G-001	2	1	0	3
Total	9	21	46	76

group B strains by greenhouse conditions. However, the t-tests were only marginally significant ($P < 0.1$) because of the small sample size.

Discussion

Race 3 of *X. oryzae* pv. *oryzae* was shown to consist of three distinct lineages, which showed inter- and intra-lineage variation for virulence on the resistance gene *Xa-7*. Inter-lineage differences in aggressiveness on several near-isogenic lines were sensitive to environmental effects. In previous studies, two lineages were detected for Race 3, which has been defined by virulence to rice varieties/lines carrying *Xa-4*, *Xa-10*, and *Xa-14* and avirulence on those carrying *xa-5* (Leach et al., 1992; Mew et al., 1992; Nelson et al., 1994). In the present study, the polyphyletic origin of Race 3 was confirmed and extended, using a much larger sample of isolates from the historical collection of strains collected from 1972 to 1992. Using RFLP markers, three distinct groups were defined with a within-group similarity of at least 75% based on DNA band similarity. While strains belonging to cluster *IS1113*-B were consistently avirulent to *Xa-7*, about half of the strains from group C and all three group G strains were virulent to *Xa-7*.[1]

The *Xa-7* gene has been known to give variable reactions (T. Mew, IRRI, personal comm.), and therefore IR-BB7 has not been used as a differential host for race typing. Our results indicate that at least part of that variability might be due to qualitative differences among pathogen strains that had been assigned to the same race but that are genetically very different. Although the markers used allowed us to define a group of strains that is consistently avirulent on *Xa*-7, the markers did not discriminate between virulent and avirulent genotypes in general.

All Race 2 strains included in the study including the strains that were reclassified as Race 2 belonged to group B confirming results by Nelson et al. (1994). Like the group B strains from Race 3 they were all avirulent to IR-BB7.

Susceptible-type lesions on IR-BB7 were often shorter than on the other differentials and, on a few occasions, repeated tests were inconsistent, suggesting that these specific host–pathogen interactions are highly sensitive to environmental conditions. Plant and leaf age may also play a role, because differences between plants and leaves that differed only a few days in age appeared greater than in the other differentials (personal observation).

Group G consisted of only one haplotype and was very different from all other haplotype groups that were identified by Nelson et al. (1994). The strain with the most similar haplotype to group G was IRN35 (= PXO35; Race 1, *IS1113* group F, Figure 2), which has been noted in previous studies for its unique and very distinct haplotype (Leach et al., 1992; Nelson et al., 1994) and which had been included as a reference for that reason in this study. IRN35 was collected in 1972 near IRRI in the island of Luzon, while the three group G strains were all collected in Palawan on the same day in 1989 from three different but nearby fields and no surveys have been made there ever since.

Group C strains were detected in the Philippines since the earliest collections made in 1972. Two Group B strains (IRN153 and IRN682) were collected in Mindanao, the southern-most island of the Philippines, in 1975 and 1976. By the 1980s, Race 3 strains of

[1]Information on virulence and RFLP phenotypes of all the strains tested in this study can be obtained from the first author Dr. Maria Finckh.

Table 3. Effects of IS1113 RFLP genotype on the aggressiveness of bacterial blight Race 3 strains on differential rice cultivars during two test periods in February/March (Test 1) and in September 1993 (Test 2). Each test was replicated three times during each period

Test	Host	Gene	Lesion length (cm)						*P*-value
			IS1113 group B			*IS1113* group C			
			Mean	(Min	Max)	Mean	(Min	Max)	
			($n = 17$)			($n = 18$)			
1	IR24	*Xa-18*[a]	21.1	(3.5	30.2)	21.7	(3.2	31.4)	0.8423
1	IR-BB1	*Xa-1*	18.8	(4.6	27.7)	20.3	(3.1	31.2)	0.5681
1	IR-BB2	*Xa-2*	22.7	(4.4	31.8)	22.9	(4.3	36.3)	0.9458
1	IR-BB4	*Xa-4*	19	(4.0	28.1)	18.8	(1.5	25.7)	0.9302
1	IR-BB5	*xa-5*	2.7	(1.1	4.5)	2.6	(0.9	5.5)	0.9011
1	IR-BB7	*Xa-7*	1.8	(0.7	4.1)	6.1	(0.6	12.8)	0.0016**
1	IR-BB10	*Xa-10*	20.1	(3.4	27.0)	20.1	(3.7	30.2)	0.9777
1	IR-BB14	*Xa14*	18.5	(3.3	26.5)	18.8	(2.0	27.6)	0.9259
1	Tetep	*Xa-1,2,12*	22.9	(6.5	33.6)	22.1	(5.9	30.2)	0.7212
	Mean		16.4			17			
			($n = 18$)			($n = 44$)			
2	IR24	*Xa-18*	13.6	(2.5	28.0)	21.8	(3.5	36.3)	0.0019**
2	IR-BB3	*Xa-3*	9.3	(1.9	20.4)	14.9	(2.0	23.5)	0.0036**
2	IR-BB4	*Xa-4*	13.2	(2.8	27.1)	20.9	(2.9	32.1)	0.0025**
2	IR-BB5	*xa-5*	1.7	(0.4	2.8)	2.6	(0.9	6.5)	0.2997
2	IR-BB7	*Xa-7*	0.6	(0.3	1.4)	5.7	(0.4	23.7)	0.0001**
2	IR-BB8	*xa-8*	5.3	(0.6	10.9)	13.5	(2.3	24.9)	0.0001**
2	IR-BB10	*Xa-10*	13.4	(0.8	27.1)	22.2	(3.7	33.4)	0.0011**
2	IR-BB14	*Xa-14*	12.7	(2.2	27.4)	20.8	(2.0	32.6)	0.0009**
	Mean		8.8			15.3			

[a]*Xa-18* does not confer resistance to any known strain of bacterial blight in the Philippines. Thus, all IR-BB lines (near-isogenic to IR24) could have the gene.

Group B were collected from all regions in the Philippines. Group G strains have not been detected in Luzon, although extensive collections have been carried out in recent years (Ardales et al.,1996; George et al., 1997).

The first Race 3 strain virulent to *Xa-7* was collected in 1982 (IRN526). Only eight Race 3 strains were collected between 1972 and 1982, however, reflecting the low frequency of the pathotype at the time (Mew et al., 1992). It appears very likely that commercial deployment of rice varieties carrying *Xa-7* would lead to the rapid selection of a new race from Race 3, Group C with virulence to *Xa-7*.

We have provided evidence on interactions between environmental conditions and host–strain interactions. The most apparent difference in climatic conditions between February/March and September is the amount of solar radiation. Radiation is markedly reduced during the monsoon season (September), when most days are overcast, while early in the year, sunshine is nearly continuous. Concurrently, day-time temperatures in the greenhouse are higher and more variable early in the year than in September. The environmental conditions greatly affected the host–pathogen interactions, reducing the lesion length caused by strains belonging to groups B and G, but not by the strains belonging to group C. We have observed that lesion lengths caused by bacterial blight isolates in another greenhouse at IRRI, where light was partially excluded due to insect screens were also substantially lower than in an unscreened part of the greenhouse. To confirm light and temperature effects, experiments would have to be repeated preferably under more controlled conditions.

'Type' strains of the known races of *X. oryzae* pv. *oryzae* have been used for years for screening and characterization of the resistance of rice varieties. Initially, a single strain of Race 3 was used. Based on the DNA fingerprinting results of Nelson et al. (1994), an additional type strain of Race 3 was added in 1994. By chance, however, the two Race 3 type strains chosen (IRN135 = *IS1113* B-005 and IRN254 = *IS1113* C-001) both belong to relatively rare *IS1113* groups

Table 4. Effect of testing date on lesion sizes caused by bacterial blight Race 3 strains belonging to different RFLP groups as defined by probe IS1113. Testing periods were February/March (Test 1) and September 1993 (Test 2). Each test was replicated three times during each period

IS1113 group	Differential host	*n*	Lesion length (cm)		*P*-value
			Test 1	Test 2	
B	IR-BB10	15	21.2	13.3	0.002**
B	IR-BB14	15	19.8	11.8	0.001**
B	IR-BB4	15	20.0	12.4	0.004**
B	IR-BB5	15	2.7	2.1	0.304
B	IR-BB7	15	1.9	0.6	0.000**
B	IR24	15	22.4	12.5	0.000**
C	IR-BB10	14	22.9	24.3	0.545
C	IR-BB14	14	21.7	23.3	0.501
C	IR-BB4	14	21.6	23.4	0.478
C	IR-BB5	14	2.8	3.1	0.565
C	IR-BB7	14	7.1	6.1	0.669
C	IR24	14	25.1	24.8	0.922
G	IR-BB10	3	25.8	17.0	0.083
G	IR-BB14	3	23.0	12.8	0.054
G	IR-BB4	3	20.0	13.7	0.187
G	IR-BB5	3	2.6	1.4	0.075
G	IR-BB7	3	17.2	7.7	0.055
G	IR24	3	24.8	12.9	0.023*

and are avirulent to *Xa-7*. They thus neither represent the phylogenetic nor the pathotypic diversity within Race 3. The inclusion of a Race 3 strain virulent on *Xa-7* in routine tests will widen the range of possible resistance gene combinations that can be detected in tested varieties.

References

Ardales EY, Leung H, Vera Cruz CM, Mew TW, Leach JE and Nelson RJ (1996) Hierarchical analysis of spatial variation of the rice bacterial blight pathogen across diverse agroecosystems in the Philippines. Phytopathology 86: 241–252

Caten CE (1987) The concept of race in plant pathology. In: Wolfe MS and Caten CE (eds) Populations of Plant Pathogens: Their Dynamics and Genetics (pp 21–37) Blackwell Scientific Publ., Oxford

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791

Felsenstein J (1988) Phylogenies from molecular sequences: Inference and reliability. Annual Review of Genetics 22: 521–565

Fry WE and Goodwin SB (1997) Resurgence of the Irish potato famine fungus. Bioscience 47: 363–371

George MLC, Bustamam M, Cruz WT, Leach JE and Nelson RJ (1997) Movement of *Xanthomonas oryzae* pv. *oryzae* in Southeast Asia detected using PCR-based DNA fingerprinting. Phytopathology 87: 302–309

Hopkins CM, White FF, Choi SH and Guo AL (1992) Identification of a family of airvalence genes from *Xanthomonas oryzae* pv. *oryzae*. Mol. Plant–Microbe Interactions, 5: 451–459

Karganilla AD and Natural MP (1973) A comparative study of culture media for *Xanthomonas oryzae*. Philipp Agric 57: 141–152

Kauffman HE, Reddy APK, Hsieh SPY and Merca SD (1973) An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. Plant Disease Reporter 57: 537–541

Leach JE, Rhoads ML, Vera Cruz CM, White FF, Mew TW and Leung H (1992) Assessment of genetic diversity and population structure of *Xanthomonas oryzae* pv. *oryzae* using a repetitive DNA element. Applied and Environmental Microbiology 58: 2188–2195

Leung H, Nelson RJ and Leach JE (1993) Population structure of plant pathogenic fungi and bacteria. Advances in Plant Pathology 10: 157–205

Mew TW, Vera Cruz CM and Medalla ES (1992) Changes in race frequencies of *Xanthomonas oryzae* pv. *oryzae* in response to the planting of rice cultivars. Plant Disease 76: 1029–1032

Mew TW, Vera Cruz CM and Reyes RC (1982) Interaction of *Xanthomonas campestris* pv. *oryzae* and a resistant rice cultivar. Phytopathology 72: 786–789

Nelson RJ, Baraoidan MR, Cruz CMV, Yap IV, Leach JE, Mew TW and Leung H (1994) Relationship between phylogeny and pathotype for the bacterial blight pathogen of rice. Applied and Environmental Microbiology 60: 3275–3283

Ogawa T, Yamamoto T, Khush GS and Mew TW (1991) Breeding of near-isogenic lines of rice with single genes for resistance to bacterial blight Pathogen (*Xanthomonas campestris* pv. *oryzae*). Jap J Breed 41: 523–529

Raymundo AK, Nelson RJ, Ardales EY, Baraoidan MR and Mew TW (1990) A simple method for detecting genetic variation in *Xanthomonas campestris* pathovar *oryzae* (Xco) by restriction fragment length polymorphism (RFLP). Int Rice Res Newsl 15: 8–9

Rohlf FJ (1992) NTSYS-pc., version 1.7. Numerical Taxonomy and Multivariate Analysis System. Setauket, N.Y., Exeter Publishing

Sneath PHA and Sokal RR (1973) Numerical Taxonomy. W.H. Frieeman and Co., San Francisco

Stakman EC and Levine MN (1922) The determination of biologic forms of *Puccinia graminis* on *Triticum* spp. Tech Bull Minn Agric Exp Stn 8: 3–10

SAS (1988) Statistical Analysis Systems. SAS/STAT user's guide. Cary, NC, SAS Institute Inc.

Wolfe MS and Schwarzbach E (1975) The use of virulence analysis in cereal mildews. Phytopathologische Zeitschrift 82: 297–307

Yap IV and Nelson RJ (1996) WinBoot: A program for performing bootstrap analysis of binary data to determine the confidence limits of UPGMA-based dendrograms. In: Anonymous (ed) IRRI Discussion Paper Series no. 14. P.O. Box 933, Manila Philippines, International Rice Research Institute

Zeigler RS, Cuoc LX, Scott RP, Bernardo MA, Chen DH, Valent B and Nelson RJ (1995) The relationship between lineage and virulence in *Pyricularia grisea* in the Philippines. Phytopathology 85: 443–451