Interaction of *Xanthomonas campestris* pv. *oryzae* and a Resistant Rice Cultivar

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ABSTRACT

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A rice cultivar, Cas 209 (IRRI Accession 15793), responded differentially to strains of virulence group (VG) II of *Xanthomonas campestris* pv. *oryzae* in the Philippines indicating that VG II contains a mixture of strains that are either virulent (VG IIa) or avirulent (VG IIb) to Cas 209. Strains of VG I, VG IIa, VG IIb, and VG III can be distinguished by their differential virulence on the rice differential cultivars IR8, IR20, Cas 209, IR1545-339, and DV85. The differential reactions were consistent for two inoculation methods and for inoculations at both early tillering and maximum tillering growth stages of rice. The results confirm the presence of pathogenic races of the rice bacterial blight pathogen in the Philippines.

Results of previous studies by Mew and Vera Cruz (10) who used the leaf-clipping inoculation method demonstrated that *Xanthomonas campestris* pv. *oryzae* from the Philippines showed some degree of pathogenic specialization on a set of differential cultivars of rice. Bacterial strains were classified in virulence groups (VG) based on the lengths of lesions induced by the strains on specific cultivars. The differential response of the rice cultivars to strains of different virulence groups was significant.

Horino et al (8) carried out a more comprehensive study on the virulence of strains of *X. campestris* pv. *oryzae* from Japan and the Philippines to the set of rice differentials used in Japan and that developed at The International Rice Research Institute (IRRI). On the basis of infection of flag leaves following inoculation by the double-needle pin-pricking method, Horino et al (8) found nine groups of bacterial strains with distinct patterns of pathogenicity. That indicated the existence of races of the rice bacterial blight pathogen similar to those in fungal pathogens. The cultivar Cas 209 from Senegal was first identified and used as a differential cultivar by Mew and Vera Cruz (11), and was also included in the studies by Horino et al (8).

In IRRI's Bacterial Blight Screening Nursery Cas 209 responded differentially to two bacterial strains, PXO 83 and PXO 79 previously designated under VG II. Cas 209 was resistant to PXO 83 and susceptible to PXO 79 regardless of whether fully unfolded leaves at vegetative stage or flag leaves at reproductive stage were inoculated.

In this article, we report the results of our studies of the response of Cas 209 to strains of VG II and compare the results of two methods of inoculation in evaluating cultivar × strain interactions.

MATERIALS AND METHODS

Rice cultivars. In addition to Cas 209 (IRRI Accession 15793), four rice differentials with known genes for bacterial blight resistance (10)—IR8 has no genes for resistance, IR20 has *Xa*-4, IR1545-339 has *xa*-5, and DV85 has *xa*-5 and *Xa*-7—were used in the study. IR36 and Pelita I/1 both of which have *Xa*-4 were also included in the experiments to evaluate selected strains of VG II. Seeds were sown in seedboxes and transplanted to 20.3-cm-diameter clay pots 10 days after sowing (DS) at three plants per pot. The soil was fertilized with N-P-K at 90-60-60 kg/ha mixed with the soil before transplanting. The pots were kept flooded with tap water. The plants were usually inoculated at 40 DS.

Bacterial strains. Strains previously designated as VG II were used in experiment I in addition to representative strains PXO 61 (from VG I), and PXO 71 (from VG III). Strains of VG I are virulent on cultivars with no genes for resistance; those of VG II are virulent also on cultivars with *Xa*-4; and strains of VG III are virulent also on cultivars with *xa*-5, but induce a variable reaction in cultivars with *Xa*-4. Plants of cultivar DV85 are resistant to strains of all the three virulence groups.

The stock cultures were maintained on slants of peptone sucrose agar (PSA) at −10 C.

Inoculum and inoculation. Cultures from several 72-hr slants on PSA were suspended in 5 ml of sterile distilled water, and the suspensions were bulked and shaken vigorously for 5 min. Dilutions of the bulked suspension were prepared and the concentrations of viable cells were determined by dilution plate counts. Before a series of dilutions from the initial suspension was prepared, two of the most suitable dilutions were plated for each strain to determine the actual number of colony-forming units on Suwa's medium (15). This medium, instead of PSA, was used because it yielded a higher colony count. Inoculum of each strain was prepared and adjusted to a series of dilutions ranging from 10^6 to 10^1 cells per milliliter.

Both the leaf-clipping method and the double-needle pin-pricking method of inoculation were used for evaluating the reaction of Cas 209 and other differentials against the 16 VG II strains and representatives of VG I and III strains. The double-needle pin-pricking method was used to determine the dose-response of Cas 209 to representative strains of each virulence group. The order of inoculation was done from lowest inoculum concentration of each strain to the highest. In the double-needle pin-pricking method, fully expanded leaves of 40-day-old plants were gently pricked at the central portion with needles infested with inoculum. In the leaf-clipping method, scissors were dipped in inoculum before clipping the tips of leaves. The needles or scissors were sterilized before being used for different strains or different inocula.

Experimental design. To test the virulence of strains on different cultivars, a split plot design was used with cultivars as the main plot and isolates as subplots. In the test of effects of inoculum dose and plant age, a split-split plot design was followed, where growth stage of the plants was assigned to the main plot, strains as subplot, and inoculum density as sub-subplots (7). All experiments had four replications. For each replication, at least 25 leaves were inoculated.

Assessment of infection and disease severity. We had previously found that visible symptoms of infection on a rice leaf blade artificially inoculated with both the leaf-clipping method and the double-needle pin-pricking method at an inoculum concentration

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of 10^6 cells per milliliter could be noted after about 3 days. For a cultivar susceptible to bacterial blight, the progressing lesion usually reached the leaf sheath at 14 days after inoculation (DI).

In the present study, leaves inoculated by the pin-pricking method were evaluated first at 3 DI and then at 7 DI, and finally at 14 DI. A negative reaction indicated no visible response to a dose of inoculum from the inoculated point of leaf blades at 7 DI. Lesion development on individual leaves at 14 DI was rated according to the Standard Evaluation Systems for Rice (9), in which a rating of 1 means less than 1% of the leaf blade area affected; 3, 1–5%; 5, 6–25%; 7, 26–50%; and 9, >50%.

Finney's (6) procedure of probit analysis was used to analyze the dose-response experiments and to calculate values of ED_{50}, median-effective dose for successful infection. The IRRI Statistics Department assisted in the data analysis.

RESULTS

Resistance of Cas 209 and other rice differentials. Blight lesions appeared and progressed on IR8, IR20, IR36, and Perlita I/1 when they were inoculated by the leaf-clipping method with all strains of VG II at an inoculum concentration of 10^9 cells per milliliter (Table 1). Cas 209 was resistant to all VG II strains except strain PXO 79. PXO 61, the common strain of VG I, was as virulent as to Cas 209 as PXO 79 (Table 1).

In subsequent experiments, the virulence of more VG II strains and other representative strains previously designated as VG I and III (10) were evaluated using the leaf-clipping method. All 18 strains of VG II were virulent to IR8 and IR20; the blight lesions affected more than 25% of the leaf blades (a score of 7) or more than 50% of the leaf area (a score of 9) of these cultivars. Six of the VG II strains were virulent to Cas 209, but 12 of them were avirulent to Cas 209 (Table 2). The virulent strains caused lesions ranging in length from 24 to 30 cm, while those avirulent to Cas 209 caused lesions ranging from 1 to 2 cm in actual measurements. PXO 61 of VG I was less virulent to Cas 209 and IR20 than the strains of VG IIa while PXO 71 of VG III was highly virulent to Cas 209 but inconsistently virulent to IR20.

Dose-response of Cas 209 to infection by strains of different virulence groups. Dose-response experiments were conducted on strains PXO 79 of VG IIa, PXO 86 of VG IIb, PXO 61 of VG I, and PXO 71 of VG III. The results of inoculations by the pin-pricking method at two vegetative growth stages, early tillering and maximum tillering, showed that increasing concentrations of inoculum of the four strains caused increasing degrees of infection except that there was no infection by PXO 86 at the lower inoculum concentrations (Table 3 and Fig. 1). The median effective dosages (ED_{50}) of the inocula as numbers of bacterial cells in suspensions required to induce visible infection were not significantly different among PXO 79, PXO 61, and PXO 71 at the early tillering stage, but concentrations more than 1,000-fold greater were required with PXO 86 (Table 4). At the maximum tillering stage, the ED_{50} values for PXO 61, PXO 79, and PXO 71 were higher than those at the early tillering stage; and for PXO 86, the ED_{50} was 10^{19} times higher than that of the other strains (Table 4).

TABLE 1. Response of rice cultivars IR8, Cas 209, and selected cultivars that have gene *Xa*-4 for bacterial blight resistance to two virulence groups (VG) of *Xanthomonas campestris* pv. *oryzae* in the Philippines

	Length of lesions (cm)[a]						
	VG II						VG I
Cultivar	PXO 63	PXO 79	PXO 82	PXO 86	PXO 78	PXO 83	PXO 61
IR8	27.5	27.0	29.5	24.3	27.6	27.3	28.2
IR20	25.2	25.7	22.9	21.7	27.9	26.6	5.9
IR36	25.7	24.4	25.0	19.6	26.1	27.3	7.8
Perlita I/1	25.7	25.8	25.8	21.3	25.4	26.3	6.2
Cas 209	2.5	36.4	3.2	1.5	2.6	2.6	35.1

[a] Mean of three replications of three plants each replication inoculated by the leaf-clipping inoculation method with 10^9 cells per milliliter. Lesions were measured 14 days after inoculation.

TABLE 2. Differential response of rice cultivar Cas 209 to virulence group II strains of *Xanthomonas campestris* pv. *oryzae*[a]

	Disease index[c] (1–9)		
Strain[b]	IR8	IR20	Cas 209
PXO 18 (IIa)	9	7	9
PXO 79 (IIa)	9	7	9
PXO 81-L (IIa)	9	7	9
PXO 87 (IIa)	9	7	9
PXO 88 (IIa)	9	7	9
PXO 22 (IIa)	9	7	9
PXO 47 (IIb)	9	7	1
PXO 63 (IIb)	9	7	1
PXO 63-6 (IIb)	9	7	1
PXO 64 (IIb)	9	7	1
PXO 65B (IIb)	9	7	1
PXO 73 (IIb)	9	7	1
PXO 78 (IIb)	9	7	1
PXO 82 (IIb)	9	7	1
PXO 83 (IIb)	9	7	1
PXO 86 (IIb)	9	7	1
PXO 61 (I)	7	3	7
PXO 71 (III)	9	[d]	9

[a] Plants were inoculated by the leaf-clipping method with 10^9 cells per milliliter.
[b] Virulence groups of isolates indicated in parentheses.
[c] 1 = less than 1% leaf area affected, 3 = 1–5%, 5 = 6–25%, 7 = 26–50%, 9 = >50%.
[d] Variable, ranging from 3 to 7.

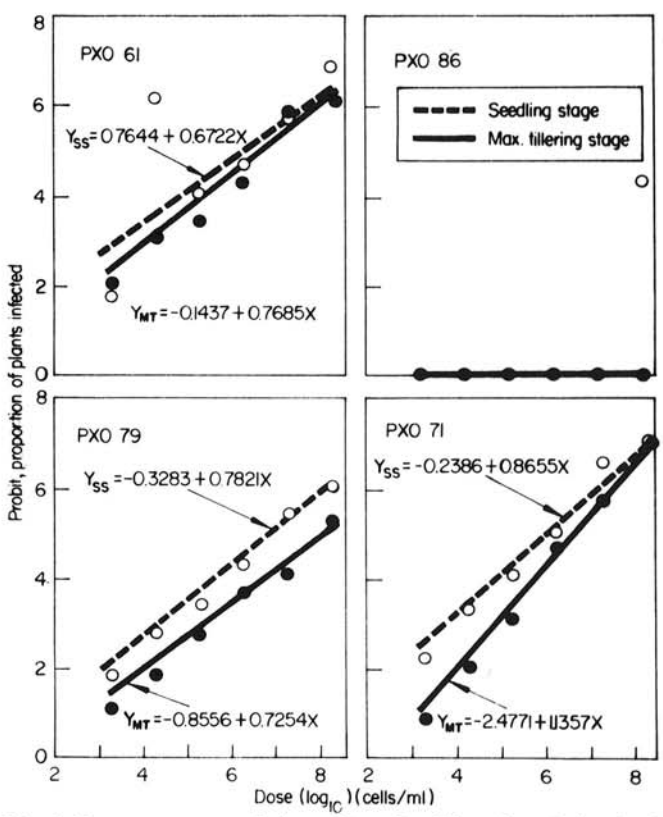

Fig. 1. Dosage response of rice cultivar Cas 209 to inoculation by the pin-pricking method with cell suspensions of strains PXO 61, PXO 71, PXO 79 and PXO 86 of *Xanthomonas campestris* pv. *oryzae*. Plants were inoculated at the early tillering and maximum tillering stages and scored 7 days later.

The percentages of inoculation sites that developed infection induced by strains virulent and avirulent to Cas 209 were well correlated with bacterial blight severity readings from those inoculations (Figs. 2 and 3). For PXO 86, the linear correlation coefficient of severity to percentage infection by pin-pricking was highly significant even though it was expressed over a fairly narrow range of severity because Cas 209 is resistant to PXO 86. Percentages of infection by the three strains virulent to Cas 209 at different inoculum concentrations were significantly correlated to the disease severity index from 1 to 9 at both early tillering and maximum tillering stages.

Pathogenic specialization of *Xanthomonas campestris* pv. *oryzae* on rice differentials. Forty-five days after sowing, at the vegetative stage, IR20, Cas 209, and IR1545 responded differentially to PXO 61, PXO 71, PXO 79, and PXO 86 strains of *X. campestris* pv. *oryzae* when inoculated by leaf clipping at an inoculum concentration of $\sim 10^9$ cells per milliliter (Table 5). IR8 was susceptible, with a disease severity index of 7 to 9 to all strains except PXO 40 (VG 0), which apparently had lost its virulence. DV85, with a disease score of 1 to 3, was resistant to all strains. IR20 was resistant to strain PXO 61, susceptible to PXO 86 and PXO 79, and varied in response to PXO 71. The resistance of IR20 to PXO 61 was, however, incomplete at this stage of plant growth. Cas 209 was resistant to PXO 86, and IR1545-339 to PXO 61, PXO 79, and PXO 86. The complete resistance of Cas 209 to PXO 86 at this stage confirmed results as shown in previous section.

TABLE 3. Mean disease rating of rice plants of cv. Cas 209 at maximum tillering stage inoculated with four strains of *Xanthomonas campestris* pv. *oryzae* at six inoculum concentrations with the pin-pricking method of inoculation.

	Rating[a] (1–9) at inoculum density[b] (cells/ml) of					
Strain	$\times 10^3$	$\times 10^4$	$\times 10^5$	$\times 10^6$	$\times 10^7$	$\times 10^8$
PXO 61	0.1	1.9	2.7	8.3	8.2	8.7
PXO 71	0.1	1.2	3.3	6.7	8.5	8.6
PXO 79	0.2	2.6	1.9	4.4	8.0	7.9
PXO 86	0	0	0	0.5	0.7	1.3

[a] Mean of three replications
[b] Inoculum density × 1.92 for PXO 61, × 1.82 for PXO 71, × 1.93 for PXO 79, and × 1.51 for PXO 61.

TABLE 4. Median effective dosages (ED_{50}) of four strains of *Xanthomonas campestris* pv. *oryzae* that induce infection in rice cultivar Cas 209 at early and maximum tillering stages, 7 and 14 days after inoculation (DI).

	ED_{50} (cells/ml)			
	Early tillering stage		Maximum tillering stage	
Strain	7 DI	14 DI	7 DI	14 DI
PXO 61	1.9×10^6	2.7×10^5	6.1×10^6	5.1×10^6
PXO 71	1.1×10^6	1.9×10^5	4.0×10^6	1.6×10^5
PXO 79	8.7×10^6	2.9×10^5	9.8×10^7	6.1×10^5
PXO 86	...[a]	6.5×10^8	...[a]	6.7×10^{24}

[a] No infection.

TABLE 5. Disease reaction of respresentative strains of *Xanthomonas campestris* pv. *oryzae* from virulence groups 0, I, II, and III on rice cultivars that have diverse genes for resistance

	Reaction[a]				
Differential[b]	PXO 40	PXO 61	PXO 86[c]	PXO 79[c]	PXO 71
IR8 (0)	R	S	S	S	S
IR20 (*Xa*-4)	R	R	S	S	R/S
Cas 209 (?)	R	S	R	S	S
IR1545-339 (*xa*-5)	R	R	R	R	S
DV85 (*Xa*-1, *xa*-5)	R	R	R	R	R

[a] R = resistant, S = susceptible.
[b] Genes for resistance are in parentheses.
[c] Strains PXO 86 and PXO 79 were previously included in the same virulence group, VG II (10).

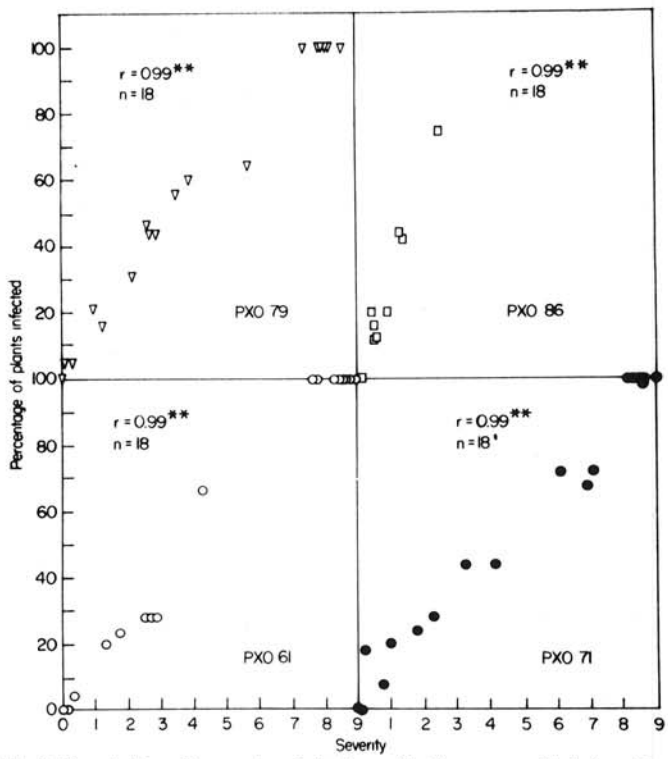

Fig. 2. Correlation of percentage infection with disease severity induced by strains PXO 61, PXO 71, PXO 79, and PXO 86 of *Xanthomonas campestris* pv. *oryzae* at the early tillering stage. The first three are virulent and the fourth avirulent to Cas 209.

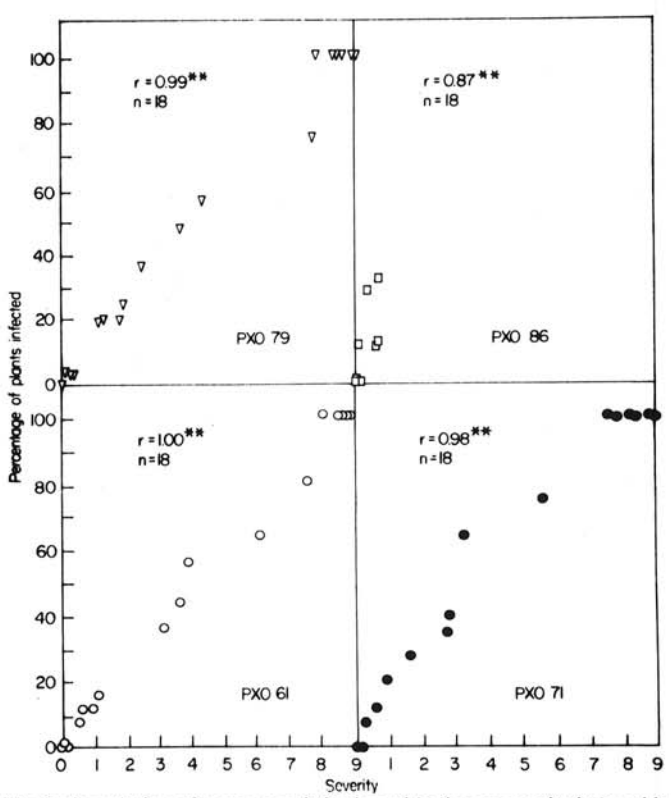

Fig. 3. Correlation of percentage infection with disease severity induced by strains PXO 61, PXO 71, PXO 79, and PXO 86 of *Xanthomonas campestris* pv. *oryzae* at the maximum tillering stage. The first three were virulent and the fourth avirulent to Cas 209.

DISCUSSION

Cas 209 was highly resistant to some strains previously designated as VG II (10) but susceptible to other strains of VG II as well as to strains of VG I and VG III when inoculated by using both the leaf-clipping inoculation and the double-needle pin-pricking inoculation methods. The response of Cas 209 to VG II strains clearly indicated that the group was a mixture of strains. With cultivars Cas 209, IR8, IR20, IR1545-339, and DV85, the bacterial population of *X. campestris* pv. *oryzae* in the Philippines can be differentiated into four distinct groups, confirming that pathogenic races of *X. campestris* pv. *oryzae* occur in tropical Asia.

Dose-response of Cas 209 to the four races of *X. campestris* pv. *oryzae* indicated that the ED_{50} values for visible infection at the early tillering and maximum tillering stages of the rice development adequately separated PXO 86 from the three virulent strains PXO 61, PXO 71, and PXO 79. At early tillering stage, the ED_{50} values of all the three strains virulent to Cas 209 were similar but at maximum tillering, the ED_{50} for PXO 71 was lower than PXO 61 and PXO 79. Whether this indicates that the susceptibility of Cas 209 to virulent strains was variable is under study. It appears, however, that the dose-response measured as ED_{50} values may provide a useful method of evaluating not only the host resistance as indicated by Ercolani (3) but also the heterogeneity of a pathogen population.

The phenomenon of differential interactions between rice cultivars and strains of *X. campestris* pv. *oryzae*, has been demonstrated previously (1,2,4,10,12,13). Some scientists claimed a vertical host-parasite relationship (4,10,14) and others indicated a gradual increase in virulence of the bacteria from eastern to southeastern and southern Asia on a few test cultivars (2,12,13). In the latter studies, the difference in virulence was too small to show a significant degree of specialization among strains in pathogenicity on the test rice cultivars. On the other hand, strains of the bacteria in Japan were highly virulent to various rice cultivars with specific resistance (4). When Asakaze, a resistant cultivar, was introduced to the farmers, it was severely affected by a virulent strain in a few years (5). Subsequently, bacterial strains with distinct pathogenicity were identified on a set of differential rice cultivars that had different genes for resistance. The set of rice cultivars used in the studies of the weak interaction between strains and cultivars at IRRI (10) was different from that used in Japan. In the studies at IRRI, a significant interaction between rice differentials and bacterial strains was found, but no complete reversal of reaction occurred with any pair of resistant cultivars and virulent strains. With the addition of Cas 209 as a differential, such a reversal occurs. Cas 209 is susceptible to PXO 61 but resistant to PXO 86 whereas IR20 was resistant to PXO 61 but susceptible to PXO 86.

Response of the cultivars to the strains was not affected by the inoculation methods using a high inoculum concentration in the studies conducted. It has been demonstrated that there was a strong correlation between the leaf-clipping inoculation method and the double-needle pin-pricking inoculation using the same inoculum concentration of the same strains on the same rice cultivars (8).

The early work in Japan (4) and the results by the authors and others (10,16) are the first steps in the exploration of the presence of races within the population of *X. campestris* pv. *oryzae* in rice-growing countries in Asia. Detailed information on host-parasite interactions from all countries is urgently needed in view of the extensive international collaboration on rice breeding and dissemination of genetic materials. The work by Horino et al (8) has suggested that races in Japan, a temperate region, differ from those in the Philippines. There is no indication from their study that races in the tropics are more virulent than those in temperate zones. It can be expected that there are still other races in other tropical Asian countries. Initial results of collaborative research among scientists in Asia, have shown that there are races but they have different virulence in South Asia (A. P. K. Reddy and S. A. Miah, *personal communication*; IRRI, 1980). Identification and development of a set of differential cultivars differing in functional genes for resistance are needed to characterize the virulence of those races. The set of differentials used by Horino et al (8) to combine those from Japan and IRRI could serve as basis for further development and evaluation.

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