Comparison of Virulence of *Xanthomonas campestris* pv. *oryzae* in Thailand and the Philippines

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ABSTRACT

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Isolates of *Xanthomonas campestris* pv. *oryzae* collected in Thailand and the Philippines were distinguished into groups of strains for virulence on rice (*Oryza sativa*) cultivars with different genes for resistance to the pathogen. All groups of this bacterial pathogen were avirulent on cultivar DV85 (recessive gene *xa-5* and dominant gene *Xa-7*). Group 0 was avirulent on all cultivars, including IR8 and RD9 that carry no genes for resistance to the pathogen. The Thai group I was comparable to the Philippine group I and was virulent only on IR8 and RD9. The Philippine group II was similar in virulence to the Thai group III but differed from it in that it was virulent on both RD7 (genes not analyzed) and IR20 (dominant gene *Xa-4*); the Philippine group II was virulent on RD7 but not on IR20. Only the Philippine group III isolates were virulent to IR1545-339 that has the recessive gene *xa-5* for resistance to the pathogen. Groups II and III were virulent on IR1695 (dominant gene *Xa-6*) and PI 231129 (recessive gene *xa-8*); both cultivars have adult plant resistance to *X. campestris* pv. *oryzae*. The data suggest that pathogen specialization has occurred and that the specificity of infection of these differential cultivars can be distinguished in the vegetative stage by lesion length or area.

Bacterial blight is one of the major rice (*Oryza sativa* L.) diseases in Asia. It is a serious disease threatening rice culture in Thailand, where it was first reported in 1963 in the Pathum Thani Province (7). The disease has become serious because many improved, high-yielding cultivars, when managed with high nitrogen levels and close spacing, have inadequate resistance to the pathogen. Lines developed at the International Rice Research Institute (IRRI) and other cultivars such as TKM 6 and IR22 (*Xa-4*), W1263 and DZ192 (*xa-5*), and Zenith (*Xa-6*) were resistant to the Philippine isolates of *Xanthomonas campestris* (Pam.) Dows. pv. *oryzae* (Ishiyama) Dye (= *X. oryzae* (Ishiyama) Dowson) (8).

Races of *X.c.* pv. *oryzae* were reported in Japan (1) and in the Philippines (5) on the basis of infection of rice cultivars with specific resistance at the reproductive and vegetative stages of plant growth. Later, the virulence of the pathotypes against a combined set of rice cultivars from Japan and IRRI was evaluated only at the reproductive stage of plant growth. Virulence of each pathotype differed among the rice differentials (3). The information suggested that other pathotypes of this bacterial pathogen might be present in the rice-growing countries in Asia.

The present project was initiated to compare the virulence of Philippine and Thai isolates of *X.c.* pv. *oryzae*.

MATERIALS AND METHODS

Rice cultivars. Nine rice cultivars (seven from IRRI, two from Thailand) were evaluated. The nine cultivars were evaluated in Thailand against the Thailand isolates and in the Philippines against the Philippine isolates. Cultivars from the same seed sources were used in both countries.

Cultivar IR8 was susceptible to all isolates in the Philippines, except those that apparently had lost their virulence. Cultivar RD9 was susceptible to all isolates in Thailand. The resistance of other rice cultivars varied (IR20, IR40, IR1545-339, PI 231129, DV85, and IR1695) depending on the gene or genes for resistance to the pathogen. Our results indicated that resistance in IR20 and IR40 is conditioned by a single dominant gene for resistance, *Xa-4*, and that IR1545-339 has a recessive gene, *xa-5*. Cultivar DV85 has one recessive gene similar to *xa-5* and one dominant gene, *Xa-7*. IR1695 has a dominant gene for adult plant resistance, *Xa-6*, and PI 231129 has a recessive gene, *xa-8*. Genes conveying resistance to the pathogen in RD7 have not been analyzed, but this cultivar is known to be resistant to *X.c.* pv. *oryzae* in Thailand.

Seeds of all the rice cultivars were sown first in seedboxes. At 21 days after seeding, uniform seedlings were transplanted into 15-cm clay pots, each containing three plants. Each pot received a basal fertilizer application of 90-60-60 kg of N-P-K/ha.

Bacterial isolates. Representative strains were chosen for three distinct groups of isolates found in the Philippines: strain PX061 of group I, PX079 of group II, and PX071 of group III. The bacterial pathogen in each group had a specific virulence reaction to certain differential rice cultivars (5). Strains PX085, PX052, PX07, and B31 of group I and PX088, PX087, PX063, and Isa 11 of group II were also tested.

The virulence of 37 Thai isolates was tested on these same cultivars. Most of the isolates were collected in 1978 from various localities in Thailand, including TB7803, TB7805, TB7807, TB7808, TB7810, TB7814, TB7821, TB7822, TB7831, TB7833, and TB7841; these were randomly selected and evaluated. However, some isolates collected in previous years and maintained as freeze-dried pure cultures also were tested and included in an initial study.

Inoculum and inoculation. The bacteria were cultured on peptone-sucrose agar medium slants for 48 hr at 28 C. Inoculum was prepared by suspending the bacterial mass in sterile distilled water (about 10^9 cells per milliliter).

Rice leaves were clipped 1 cm from the tip with a pair of scissors (sterilized in boiling water for 15 min) that had been dipped into the inoculum of each bacterial isolate.

Experimental design and disease management. The experimental plots were laid out on a split-plot design (2) with cultivars as the main plot and bacterial isolates as the subplots; each experiment had three replications. In greenhouse experiments, cultivars were inoculated with the Philippine isolates at IRRI and with the Thai isolates in Thailand.

Disease severity was rated by measuring the lesion length from the leaf tips 14 days after inoculation. The data were analyzed

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for the main effect and interaction effect between cultivars and bacterial isolates. Duncan's multiple range test was also used.

RESULTS

Length of lesions caused by Thai isolates. On each cultivar, lesions developed uniformly downward from the point of inoculation. The length from the leaf tip varied, however, among isolates. Mean lengths of lesions are given in Fig. 1. Ranges in length of lesions were 1.4–29.4 cm on IR8, 1.0–32.3 cm on RD9, 2.4–18.0 cm on IR20, 1.1–19.7 cm on RD7, 1.5–6.9 cm on IR1545-339, and 1.9–6.7 on DV85.

Virulence of bacterial isolates in Thailand. Nineteen isolates were selected as representative of four groups of strains differing in virulence from Thailand. Initial evaluation of virulence of the 19 isolates (collected in 1974, 1975, 1976, 1977, and 1978) on four differential rice cultivars with specific resistance or susceptibility to the pathogen indicated at least three groups of strains (Table 1). Later they were grouped according to their virulence against six rice cultivars (Table 2).

Group 0 strains were avirulent on all six rice cultivars and caused lesions with a mean length of less than 2 cm. Group I strains, however, were highly virulent on IR8 and RD9 and less virulent on the other four cultivars (IR20, IR1545-339, DV85, and RD7). Group II strains were virulent on IR8, RD9, and RD7. These strains caused longer lesions on IR20 than on IR1545-339 and DV85, but the lesions were considerably shorter than those on RD7. Group III strains were virulent on IR8, RD9, and IR20; the lesions were shorter on IR20 and RD7 than on IR8 and RD9 but were comparable to those caused by group II strains on RD7.

Virulence of Philippine isolates. The virulence of three representative strains of *X.c.* pv. *oryzae* on four differential rice cultivars was evaluated 30 days after sowing. All isolates were virulent on IR8 and less virulent on DV85 (Table 3). Strain PX079 in group II was more virulent than PX071 in group III on IR20, but PX071 was more virulent than the other two strains on IR1545-339. Strain PX061 in group I was virulent only on IR8.

The three strains were virulent on two additional rice cultivars (IR1695 and PI 231129) when they were inoculated at the booting stage (Table 4). The strains differed in their virulence on RD7. Strain PX079 was as virulent on RD7 as on IR20, but PX061 was not. Strain PX071 also was virulent on RD7, and its virulence on IR20 varied but was less than its virulence on IR1545-339.

Further evaluation of selected Philippine group I, II, and III isolates indicated that only the group II strains were virulent on RD7 and IR40 (Table 5). All isolates in the three groups were virulent on IR8 and IR1695, but only the group III strain was virulent on IR1545-339. Although isolates in each group caused lesions of different lengths, the disease reactions were similar.

DISCUSSION

Selected Thai isolates of *X.c.* pv. *oryzae* collected in 1974, 1975, 1976, and 1977 had comparable virulence to the Philippine isolates on the same set of differential rice cultivars. The overall virulence of the Thai isolates was considerably lower, however, possibly because of their continuous subculture.

All 37 isolates collected in Thailand in 1978 showed differences in virulence to the differential cultivars. Strains TB7807

Table 1. Virulence of selected strains collected in 1974 through 1977 in Thailand on four rice cultivars[y] with different genes for resistance to *Xanthomonas campestris* pv. *oryzae* strains

Strain	Virulence group	IR8 (0)	IR20 (*Xa-4*)	IR1545 (*xa-5*)	DV85 (*xa-5, Xa-7*)
		Lesion length from point of inoculation (cm)[z]			
TB7621	0	3.0 b	2.1 bc	1.8 b	2.4 a
TB7724	0	0.8 bc	0.9 c	1.3 b	1.2 a
Mean		1.9	1.5	1.5	1.8
TB7612	I	11.8 a	2.5 bc	1.4 b	1.1 a
TB7617	I	14.0 a	3.5 bc	2.0 b	2.1 a
Mean		12.9	3.0	1.6	1.6
TB7536	II	18.6 a	7.7 ab	2.3 b	2.6 a
TB7538	II	19.7 a	9.4 a	3.0 b	3.1 a
TB7410	II	19.1 a	11.7 a	5.4 ab	1.7 a
TB7615	II	20.4 a	10.4 a	5.0 ab	4.2 a
Mean		19.4	9.8	3.9	2.9

[y] 30 days after sowing.
[z] In a column, means followed by a common letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.

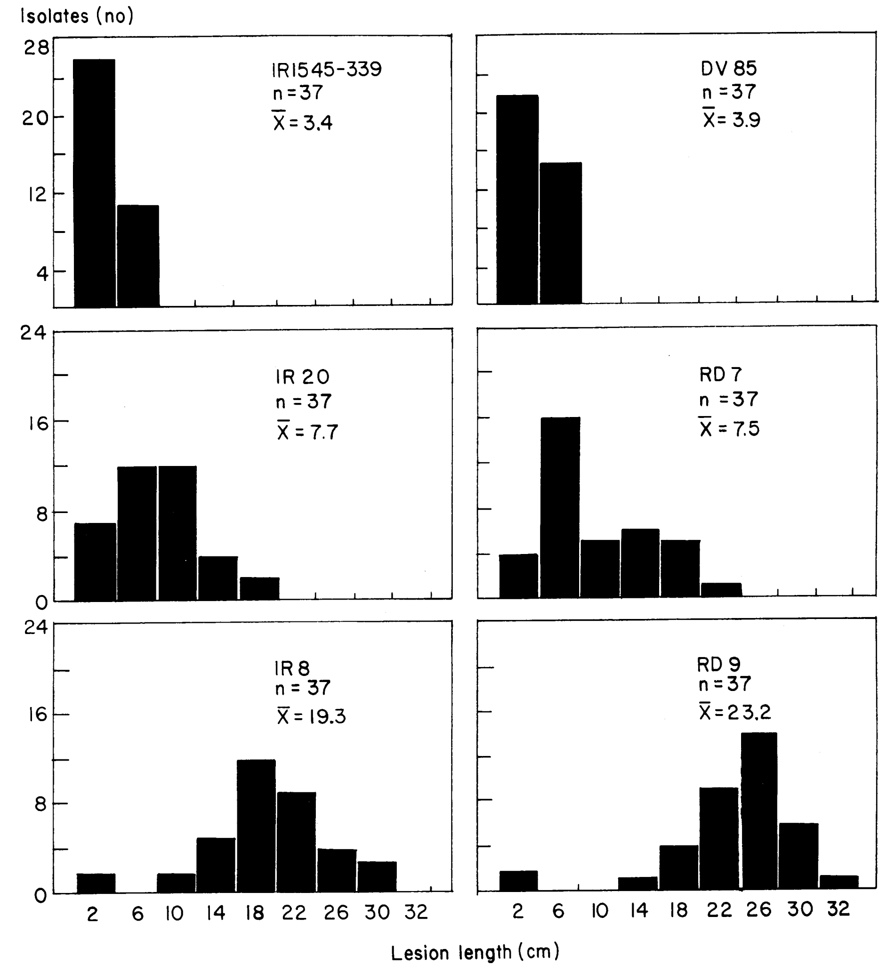

Fig. 1. Frequency distribution of lesions of uniform length and mean length caused by 37 isolates of *Xanthomonas campestris* pv. *oryzae* in Thailand on six rice cultivars differing in resistance to the pathogen.

Table 2. Virulence of selected strains collected in 1974 through 1977 in Thailand on six rice cultivars^y with different genes for resistance to *Xanthomonas campestris* pv. *oryzae*

Strain	Virulence group	IR8 (0)	IR20 (*Xa-4*)	IR1545 (*xa-5*)	DV85 (*xa-5, Xa-7*)	RD7 (unknown)	RD9 (0)
		Length of lesion from point of inoculation (cm)^z					
TB7807	0	1.4 d	1.4 f	2.1 bc	2.2 bc	1.1 d	1.0 d
TB7833	0	1.4 d	1.3 f	2.0 bc	1.7 c	2.6 cd	2.0 d
Mean		1.4	1.3	2.0	1.9	1.8	1.5
TB7808	I	30.2 a	4.5 de	2.7 bc	3.1 abc	5.6 c	25.6 b
TB7831	I	24.2 b	2.7 ef	1.9 bc	3.0 abc	4.7 c	28.7 a
TB7810	I	24.3 b	5.3 de	1.5 c	3.2 abc	5.6 c	30.0 a
Mean		26.2	4.2	2.0	3.1	5.3	27.9
TB7814	II	23.4 b	8.6 c	3.6 abc	4.8 abc	18.3 b	25.2 b
TB7822	II	24.8 b	8.6 c	4.3 ab	5.3 ab	19.0 b	32.3
TB7841	II	29.0 a	6.0 cd	3.6 abc	5.0 ab	23.3 a	30.4 a
Mean		25.7	7.7	3.8	5.0	20.2	29.3
TB7803	III	23.5 b	18.5 a	4.9 ab	5.3 ab	18.9 b	24.3 b
TB7821	III	20.8 b	16.3 b	3.0 abc	4.0 c	19.5 b	26.4 b
TB7805	III	16.0 c	15.0 b	6.6 a	6.2 a	18.9 b	20.5 c
Mean		20.1	16.5	4.6	5.2	19.1	23.7

^{y}30 days after sowing.
^{z}In a column, means followed by a common letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.

Table 3. Virulence of representative strains in the Philippines of *Xanthomonas campestris* pv. *oryzae* on four rice cultivars^y with specific genes for resistance

Strain	Virulence group	IR8 (0)	IR20 (*Xa-4*)	IR1545 (*xa-5*)	DV85 (*xa-5, Xa-7*)
		Lesion length/leaf length ratio^z			
PX061	I	0.75 a	0.17 b	0.10 b	0.04 c
PX079	II	0.85 a	0.77 a	0.16 b	0.02 c
PX071	III	0.80 a	0.23 b	0.62 a	0.21 b

^{y}30 days after sowing.
^{z}In each row, means followed by a common letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.

Table 4. Virulence of selected strains in the Philippines of *Xanthomonas campestris* pv. *oryzae* on seven rice cultivars^x with specific resistance

Strain	Virulence group	IR8 (0)	IR20 (*Xa-4*)	RD7 (unknown)	IR1545 (*xa-5*)	IR1695 (*Xa-6*)^y	PI 231129 (xa-8)^y	DV85 (*xa-5, Xa-7*)
		Lesion length from point of inoculation (cm)^z						
PX061	I	23.9 a	6.0 c	6.9 c	2.3 d	21.1 a	15.5 b	1.2 d
PX079	II	26.4 a	23.0 a	20.8 a	3.9 c	15.5 b	21.4 a	1.5 c
PX071	III	27.2 a	13.8 c	19.6 b	19.8 b	18.6 b	22.9 ab	6.1 d

^{x}40 days after sowing.
^{y}Function at adult plant stage (6).
^{z}In each row, means followed by a common letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.

Table 5. Virulence of selected strains in the Philippines of *Xanthomonas campestris* pv. *oryzae* on five rice cultivars^x with specific genes for resistance

Strain	Virulence group	IR8 (0)	IR1695 (*Xa-6*)^y	IR40 (*Xa-4*)	RD7 (unknown)	IR1545 (*xa-5*)
		Lesion length from point of inoculation (cm)^z				
PX052	I	18.7 abc	18.8 a	3.6 de	4.1 e	1.5 a
PX085	I	18.1 bcd	18.0 a	3.3 e	4.3 e	1.9 a
PX061	I	15.0 f	18.7 a	3.0 e	4.0 e	1.7 a
PX07	I	13.9 f	12.1 b	3.0 e	4.3 e	1.7 a
B31	I	9.6 g	11.0 bc	2.6 e	3.8 e	1.5 a
Mean		15.1	15.7	3.2	4.1	1.7
PX088	II	20.7 a	12.0 b	13.4 b	15.3 b	1.9 a
Isa 11	II	20.2 ab	20.1 a	16.8 a	16.3 ab	2.0 a
PX063	II	19.2 abc	12.5 b	15.1 ab	11.2 c	2.8 a
PX087	II	19.7 cd	13.1 b	16.0 a	18.1 a	1.9 a
PX079	II	16.2 de	9.2 c	11.0 c	10.4 c	1.9 a
Mean		19.2	13.4	14.5	14.3	2.1
PX071	III	19.0 abc	19.2 a	5.3 d	8.2 d	14.3 b

^{x}40 days after sowing.
^{y}Function at adult plant stage.
^{z}In each column, means followed by a common letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.

and TB7833 (Table 2) were avirulent on all the cultivars (including IR8 and RD9) that are very susceptible to *X.c.* pv. *oryzae* in tropical Asia. All of the other 35 strains were virulent on IR8 and RD9 and less virulent on IR1545-339 and DV85. Their virulence varied on cultivars RD7 and IR20, which has the *Xa-4* gene for resistance to the pathogen. Group I strains were less virulent on IR20 and RD7, but group II strains were more virulent on RD7 and less virulent on IR20. Group III strains were virulent on both IR20 and RD7.

The virulence of Thai group II and III strains was similar to that of Philippine group I and II strains. This was exemplified by strains PX061 and PX079, both capable of being avirulent or virulent to the resistance genes derived from the cultivar TKM 6 (6) and conditioned by the *Xa-4* gene in cultivars IR20 and IR40. But the virulence of the Thai group II strains differed from that of the Philippine group II strains. The Thai group II strains on IR20 were resistant and on RD7 were susceptible to the pathogen. Cultivars RD7 and IR20, however, reacted similarly to the bacterial blight pathogen in the Philippines (Tables 4 and 5).

The data suggest that pathogen specialization exists in *X.c.* pv. *oryzae*. The pathogen causing rice bacterial blight in tropical Asia was separated into at least four distinct strain groups based on the reaction of Philippine and Thai isolates on the differential rice cultivars IR20 (or IR40) with the *Xa-4* gene, IR1545 with the *xa-5* gene, DV85 with the *xa-5* and *Xa-7* genes, and adult plant resistance in IR1695 with the *Xa-6* gene and PI 231129 with the *xa-8* gene. Cultivars IR8 or RD9 were universally susceptible or had no genes for resistance to *X.c.* pv. *oryzae*. The specificity of infection of these differential rice cultivars in the vegetative stage could be distinguished by lesion length or lesion area based on total leaf area (4). Complete resistance to the pathogen at this growth stage was seldom observed, but differential interactions were demonstrated.

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