Variability of Xanthomonas oryzae: Specificity in Infection of Rice Differentials

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ABSTRACT

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Rice cultivars with specific resistance to bacterial blight showed distinct differential reactions to isolates of *Xanthomonas oryzae*. Lesion length caused by 80 isolates collected from different regions in the Philippines was determined by the different genes for resistance of the cultivars. Specificity in infection of the different rices suggested three different virulence groups or strains. A major group included those that infected IR8 or rice that carries no genes for resistance to bacterial blight. Specificity of the isolates in differential infection of the rice also was shown in infection of rice of different ages planted in the greenhouse or in the growth room under conditions most favorable for plant development.

Additional key words: *Oryza sativa*, virulence.

Xanthomonas oryzae (Uyeda et Ishiyama) Dowson, the causal agent of bacterial blight of rice, is widely distributed in Asia (10) and the northern part of Australia (1) and was recently reported in the western hemisphere (7). Virulence of the pathogen varies (5,11,14), but most observers fail to recognize the distinct pathogenic strains or pathotypes sensu van der Plank (19).

In Japan, three genes for resistance to *X. oryzae* have been identified, one or two in each group of rice cultivars (16). Based on these differentials, three distinct pathotypes (races) were reported (3). Pathotype I infects only rice of the Kinmaze group, which carries no genes for resistance. Pathotype II infects cultivars in both the Kinmaze and Kogyoku groups which bear *xa1* for resistance. Pathotype II does not attack cultivars in the Rantaj-emas group, which carries three genes, including *xa1* and *xa2*, for resistance. All three rice groups are susceptible to Pathotype III, and Wase Aikoku, which bears a dominant gene *Xa3*, is resistant to all the pathotypes. A clear distinction of differential interactions between isolates of the bacterium and rice cultivars has not been demonstrated in tropical Asia where differences in virulence of isolates are not sufficiently great or clear-cut to identify them as races or pathotypes. This implies that variation in virulence of *X. oryzae* is merely a continuum of "slight genetic differences" (2). If a cultivar is resistant to the most virulent isolate, it probably is resistant to other isolates throughout the region. Australian isolates of the bacterium are considered to be exceptions (2).

In 1972 an isolate from the northern part of the Philippines, designated "Isabela strain," was found to break down resistance controlled by the dominant gene *Xa4* (4). And in 1975, the International Rice Research Institute (IRRI) cultivars carrying the same dominant gene for resistance became susceptible to bacterial blight in Iloilo on Panay Island and in Davao on Mindanao Island, Philippines. Analysis of pathogenicity indicated that these isolates were similar to the Isabela strain or isolate PXO63 (Table 1). Also, isolate B56 (Table 1) was collected in 1963 in Davao and caused a disease reaction similar to that of PXO63.

This paper reports our studies on the specificity of Philippine isolates of *X. oryzae* and their differential interactions on a set of rice cultivars with either no functional genes or different genes for bacterial blight resistance identified at IRRI. It was possible to classify 80 isolates in four virulence groups or strains.

MATERIALS AND METHODS

Bacterial isolates. Of the 80 isolates of *X. oryzae* used, some were collected in 1963 and others after 1970 from different locations in the Philippines. They were maintained at −10 C on agar slants of Wakimoto's medium (10). As a routine procedure, all isolates were

TABLE 1. Virulence of new isolates of *Xanthomonas oryzae* from Davao and Iloilo, Philippines, compared with those of other Philippine isolates identified by the International Rice Research Institute by 1975

Cultivar designation	Gene for resistance	Reaction to inoculation with isolates:[a]									
		PXO78[b]		PXO79[b]		B56[c]		PXO63		PXO61	
		Lesion (cm)	Reaction[d]	Lesion (cm)	Reaction	Lesion (cm)	Reaction	Lesion (cm)	Reaction	Lesion (cm)	Reaction
IR20	*Xa4*	14.3 a	S	16.5 a	S	10.5 a	S	13.3 a	S	4.1 a	MR
IR30	*Xa4*	17.8 a	S	14.8 a	S	11.1 a	S	15.4 a	S	6.4 a	MR
IR1545-339	*xa5*	2.4 b	R	2.7 b	R	3.3 b	MR	2.4 b	R	2.4 b	R
BJ1	*xa5*	3.4 b	MR	2.5 b	R	3.3 b	MR	2.6 b	R	1.5 b	R
TN1	0	33.2 c	S	24.5 c	S	25.3 c	S	25.3 c	S	34.3 c	S
IR8	0	29.5 c	S	25.1 c	S	20.7 c	S	22.4 c	S	25.8 c	S

[a] Means in the same column followed by a common letter are not significantly different $P = 0.05$ by Duncan's multiple range test.
[b] PXO78 from Iloilo and PXO79 from Davao on IR30, 1975.
[c] Isolate from Davao collected in 1963.
[d] R = resistant (lesion length 1–3 cm); MR = moderately resistant (lesion length 3–6 cm); S = susceptible (lesion length > 9.0 cm)

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subcultured periodically. In this study all isolates were tested for pathogenicity on TN1 or IR8, and cultures developed from reisolations from infected tissues were used in all experiments.

Rice differentials. Based on preliminary tests of 500 rice cultivars, IR20, IR1545-339 (IR1545), and Cempo selak were selected as differentials. Resistance had been evaluated previously for IR20 and IR1545 (9,13,17). Later RP291-2O (RP291), DV85, and ARC5756 were included based on the analysis of genes for resistance to the disease.

Cultivar IR8, which has no major functional gene for resistance to the Philippine isolates, was used as a susceptible check. Cultivars with the dominant gene *Xa4*, IR40 or IR20 and occasionally IR30, were used. The resistance of IR1545 is controlled by a recessive gene *xa5*. All these cultivars are semidwarf and of an improved plant type. Cultivars DV85 and ARC5756 are tall, traditional cultivars of Bangladesh and India and have two genes, one recessive, which is similar to that of IR1545 (ie, *xa5*), and the other dominant, *Xa7* (18). Cultivar RP291 is from India and derives its gene (*xa5*) for resistance from BJ1.

Plants of each cultivar were grown in pots (10 plants per pot of each cultivar) in the greenhouse or the growth room. In the growth room, the maximum and the minimum temperatures were 29 and 21 C, respectively, and the relative humidity was 75%.

Inoculation procedures and disease measurements. Each iso-late was incubated on PSA agar slants for 72–96 hr at 28 C. The inoculum was prepared by suspending bacteria in sterile distilled water to an OD of 0.7–1.0 (about 10^9 cells/ml) at 600 nm as determined with a Spectronic 20 colorimeter (Bausch and Lomb, Rochester, NY).

Inoculations were done by clipping the tips of the leaves of 40–50 day old plants with scissors that had been dipped in the inoculum suspension (6).

Because bacterial blight infection from the artificial inoculation was initiated from the cut end of the leaves, the amount of disease for each isolate-cultivar combination was rated by measuring the lesion length 14 days after inoculation. Specificity of isolates was determined by differential infection based on the length of lesion development of a given rice cultivar. A split-plot design with three replications of each treatment with cultivars as the main plot and isolates as the subplot was used. The data then were analyzed for the interaction effect of the cultivars and the bacterial isolates.

RESULTS

Range of lesion size on the differentials. The normal distribution of lesion length caused by the 80 isolates suggested that variation in lesion development was conditioned by the interactions between isolates and cultivars (Fig. 1). For IR8, the range varied from 1.0 to 36.0 cm with a median of 21.5 cm. For IR40 and for Cempo selak, lesion length varied from 1.0 to 24.0 cm, and the median was skewed to 8.8 and 12.2 cm, respectively. On IR1545, about 60 (75%) of the isolates caused lesion length of less than 3.0 cm, with a median length of 2.1 cm.

About 4% of the isolates caused lesions shorter than 6.0 cm on IR8, the susceptible check, indicating a loss of virulence; these isolates also caused shorter lesions on other rice cultivars (Table 2). Among IR40, IR1545, and Cempo selak, the lesions varied in length according to isolate-cultivar combinations. Among the three cultivars, 36 isolates (45%) caused lesions longer than 9.0 cm on IR40, and 62 isolates (77.5%) caused lesions longer than 9.0 cm on Cempo selak. Only three isolates caused distinctly susceptible reactions (lesions longer than 9.0 cm) on IR1545; otherwise the cultivar was resistant (1.0–3.0 cm) or moderately resistant (3.0–6.0 cm) to the other 77 isolates.

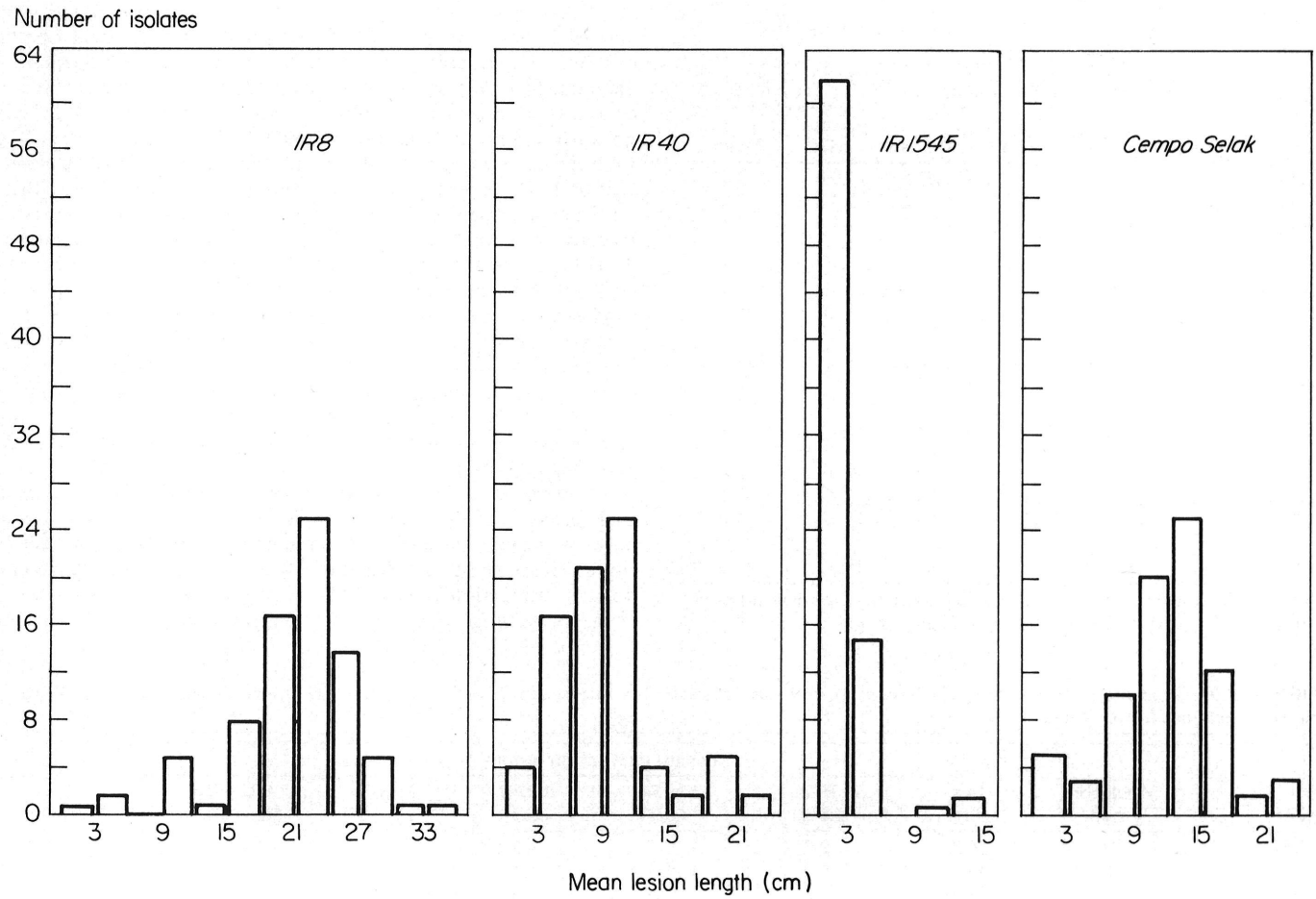

Fig. 1. Frequency distribution of mean lesion length caused by the Philippine isolates of *Xanthomonas oryzae* on susceptible (IR8) and different resistant rice cultivars.

Grouping of the isolates. Based on the lesion caused on each rice cultivar, the isolates can be placed in four virulence groups (Table 2). Group 0 caused shorter lesions on all the cultivars. Group I caused longer lesions on IR8 and on Cempo selak than on IR40 or IR1545. Group II caused susceptible reactions on IR8 and IR40 or Cempo selak but resistant reactions on IR1545. The IR40 and Cempo selak cultivars were susceptible to group II isolates, but lesions on the former were considerably longer than on the latter. Group III isolates attacked all the rice cultivars tested except DV85 but caused shorter lesions on IR40, related lines, or named cultivars bearing the same dominant gene *Xa4* for resistance than on IR1545 or Cempo selak.

Specificity of infection. Six rice cultivars were used to evaluate specificity of three selected isolates, one each from groups I, II, and III. The plants were grown in the growth room. We used cultivars DV85 and ARC5756, which carry two genes for resistance and are resistant to all the isolates collected from different geographic regions in the Philippines. The other four cultivars included IR8, IR20, IR1545, and RP291, the resistance of which appears similar to that of IR1545.

In the growth room, the lesions caused by the isolates representing the three groups on all the rice cultivars tested varied from those in greenhouse experiments (length was reduced considerably), but the relative disease reactions were consistent. Although lesion length varied on IR20, IR1545, and RP291, it was related to infection with specific isolates (Table 3). Isolate PXO79 caused longer lesions on IR20 than on all others, except IR8. Likewise, isolate PXO71 caused longer lesions on IR1545 and RP291 than on IR20 and therefore was more virulent on IR1545 and RP291. Isolate PXO61, which for many years was used as an isolate representative of the common population of the bacterium and for which resistant genes have been identified at IRRI, attacked only IR8. The infection of PXO61 was more severe than or equal to that of the other groups (Table 3). The order of virulence determined by the mean lesion length on all the cultivars indicated that PXO71 was the most virulent. ,

Analysis of variance indicated that the interaction effect (V × I) between the rice differentials (V) and the isolates (I) was highly significant (Table 3).

Age of differential cultivars and infection.. Young rice plants generally are more susceptible than old plants to bacterial blight. However, specificity in infection may not be related to plant age. Plants of four rice differentials (IR8, IR20, IR1545, and DV85) were tested at three different ages against three distinct isolates (PXO61, PXO79, and PXO71) in the greenhouse and in the growth room. Plants that were 55 and 30 days old and grown in the greenhouse were inoculated on the same day. Forty-day-old plants were tested in the growth room.

Except for the susceptible (IR8) and resistant (DV85) checks, specificity, expressed as lesion length, was exhibited in isolate-cultivar combinations but was not related to the age of plants tested (Fig. 2). Isolate PXO79, which was specific to cultivars bearing a dominant gene for resistance such as IR20, caused longer lesions on this cultivar at 30 days and 55 days in the greenhouse and at 40 days in the growth room. A similar phenomenon was observed on IR1545 inoculated with isolate PXO71. Because IR20 and IR1545 are resistant to PX061, this isolate caused shorter lesions than the other two isolates on all plants of these two cultivars.

TABLE 2. Lesion development of selected isolates on four rice differentials of different resistance to bacterial blight

Strain	Virulence group	Length (cm) of lesions on leaves of rice cultivars:[a] IR8	IR40	IR1545	Cempo selak	Mean
PXO40		4.4 a	2.1 a	1.2 a	2.9 a	2.9
PXO46	0	2.7 a	2.9 a	1.6 a	2.3 a	2.4
B19		3.6	2.3 a	2.0 a	1.2 a	2.4
B18		11.7 b	3.4 a	1.1 a	9.1 b	6.3
B31	I	21.2 c	6.3 a	4.0 a	13.1 c	11.1
PXO61		22.4 c	6.2 a	1.9 a	15.0 c	10.9
B56		15.4 b	11.0 b	2.8 a	9.5 b	9.5
PXO63		20.8 c	18.1 c	2.5 a	9.7 b	12.7
PXO73	II	26.0 d	21.1 d	3.5 a	14.6 c	16.3
PXO79		20.2 c	18.3 c	3.6 a	15.7 c	14.4
PXO69		22.2 c	11.2 b	13.9 b	18.6 c	16.8
PXO70	III	27.4 d	11.0 b	16.4 b	13.2 c	17.5
PXO71		29.1 d	8.7 b	17.9 b	15.6 c	17.8
Mean		18.0	10.1	5.4	11.5	11.3

[a]Means in the same column followed by a common letter are not significantly different $P = 0.05$ by Duncan's multiple range test.

DISCUSSION

The specificity of the *X. oryzae* isolates in the Philippines was demonstrated by the lesions produced on a set of rice cultivars differing in genes for resistance. Specificity was confined to a particular isolate-cultivar combination and showed a vertical relationship between the virulence of the isolates and resistance of the differentials. Virulence is an attribute of isolates of a pathogen and therefore is relative (8). Based on the present data on the differential interactions, four virulence groups of the isolates were distinguished. Group 0 isolates appeared to be weakly virulent and caused short lesions on all cultivars tested. Cultivar IR8, which has no known functional genes for bacterial blight resistance in the Philippines, was susceptible to all the isolates from virulence groups I, II, and III, except for those that may have lost their virulence. Cultivars DV85 and ARC5756 each have two genes, one recessive similar to that of IR1545 and the other dominant but different from the gene present in IR20 and IR40. Both were resistant to all the isolates tested. Group III is composed of three isolates that produced symptoms on IR1545 and RP291, the two varieties that appear to carry the gene *xa5* for resistance. Group III isolates were more virulent on these cultivars than on those carrying the dominant resistance gene *Xa4*. Likewise, group II isolates, which are exemplified by PXO79, seemed to be more specific on rice carrying the *Xa4* gene for resistance, such as IR20 and related lines.

The mean lesion length of the test cultivars caused by isolates in each group was not highly variable, but a difference was noted between groups. Parlevliet (12) commented that data based on 24 rice cultivars and 50 isolates published by Ou et al (11) showed no distinct interactions because the isolates displayed a much wider range of pathogenicity on some cultivars than on others. Our

TABLE 3. Differential interactions[a] in the growth room between selected strains in virulence groups of the Philippine *Xanthomonas oryzae* and rice cultivars with different genes for resistance

Strain	Virulence group	Length (cm) of leaf lesions of rice cultivars: IR8 (0)	IR20 (*Xa4*)	IR1545 (*xa5*)	RP291 (*xa5*)	DV85 (*xa5*, *Xa7*)	ARC5756 (*xa5*, *Xa7*)	Mean
PXO61	I	20.8	2.3	1.7	1.5	1.0	1.4	4.8
PXO79	II	14.8	9.0	2.1	1.2	1.3	1.5	5.0
PXO71	III	19.3	3.3	10.8	7.0	1.5	2.6	7.4
Mean		18.3	4.9	4.9	3.2	1.3	1.8	5.7

[a]The interaction effect variety times isolate (V × I): F = 34.9, $P = 0.01$.

results (Tables 2 and 3) demonstrated a definite ranking of virulence with the use of differentials. Ou et al (11) stated that no cultivars were resistant to one isolate but susceptible to all others. However, using a different set of resistant cultivars identified at IRRI and tested against isolates from different Asian countries, Reddy and Ou demonstrated differential interactions (15).

Workers in Japan, using differentials possessing known genes for resistance, have identified races or pathotypes of *X. oryzae* (3). These genes for resistance have not, however, been functional against bacterial blight in the tropics. Also, the pin-prick method of inoculation was used in Japan, whereas we used the clipping method, and virulence of indigenous isolates was probably different in the two countries. A collaborative effort is underway between IRRI and several other national rice research programs in Asia to develop an international set of differentials on a functional gene basis.

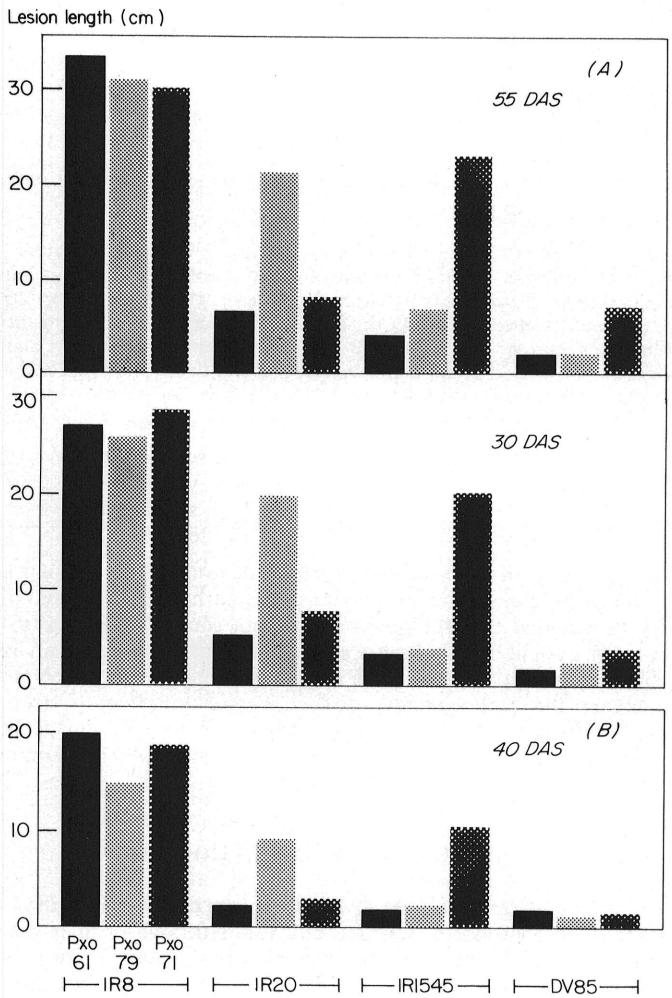

Fig. 2. Interactions between selected isolates of *Xanthomonas oryzae* and rice cultivars of different ages grown in **A)** the greenhouse, at 55 and 30 days after sowing (DAS), and **B)** the growth room, at 40 days after sowing.

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