Variability of Pathogenicity in Races of *Xanthomonas campestris* pv. oryzae in Japan

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Introduction

Bacterial leaf blight of rice caused by *Xanthomonas campestris* pv. *oryzae* is one of the most serious diseases of rice in Asian rice-growing countries. Some chemicals have been developed in Japan to control this disease, but none of them have fully been effective in case where the disease incidence was very severe. Therefore, use of resistant varieties is recognized to be the most effective and economical countermeasure against this disease under the present conditions. Since pathogenic specialization in the causal bacterium of rice bacterial leaf blight was first reported in Japan by Kuhara et al.[11], a number of reports[2,9,14,16] have been published on the variability of pathogenicity in the bacterium and the resistance of rice varieties.

In Japan, the isolates of *X. campestris* pv. *oryzae* have been divided into five races according to their patterns of virulence to differential rice varieties, while the rice varieties have been classified into seven groups based on their response patterns to bacterial races[15]. Horino and Hartini[2], employing the differential system developed in Japan for differentiating Indonesian isolates of *X. campestris* pv. *oryzae*, found a new bacterial race and designated it as race VI. With regard to the differential rice varieties, Ogawa[13] reported that each of the present races I and II could be divided into two groups and that the rice variety IR 8 should be added to the Japanese differentials.

The present study was conducted to investigate the geographical distribution of pathogenic races of *X. campestris* pv. *oryzae* in Japan during the period 1973 to 1987. This paper reports the variability found in the pathogenicity of the causal bacterium collected in Japan.

Materials and methods

1) *Isolation and identification of causal bacterium*

Rice leaves affected by bacterial leaf blight were collected from various districts of Japan during the period 1973 to 1987. Leaf segments including the marginal portions of fresh lesions were surface-sterilized by the usual method and then homogenized in 10 m*l* of sterile distilled water and appropriate dilutions were mixed with melted nutrient agar medium kept at 50°C in a water-bath. The mixture was poured into a petri dish and the plates were incubated at 25°C for 4 days. The viscous and yellow bacterial colonies developed were transferred to PSA medium and cultured at 25°C for two days. On the basis

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of bacteriological and pathological tests[10], they were identified as *X. campestris* pv. *oryzae*. In testing their pathogenicity to many differential rice varieties, the bacterial cells suspended in 10% skim-milk containing 0.05% L-glutamic acid were lyophilized. Inocula were prepared from cultures grown on PSA medium at 25°C for 2 days, and each suspension was prepared in sterile distilled water to adjust its concentration to 10^8–10^9 cells/*ml*.

2) Pathogenicity test

Five rice varieties representing each of the Japanese differential groups except Elwee and Heen Dikwee groups, Kinmaze, Kogyoku, Tetep, Chugoku 45 and Java 14, and a number of other varieties were used. The test plants were grown in seedling boxes in an upland nursery bed, and transplanted to the experimental paddy fields. Fertilizers were applied at the standard dosage. Inoculation was carried out on the central parts of the topmost fully developed leaves of mature plants by the five-needle pricking method. Three weeks later, the inoculated plants were examined for determining the disease index for each isolate, with quantified scores of 0 (most resistant) through 7 (most susceptible) according to the standard classification proposed by Ezuka and Horino[1]. The degree of resistance was evaluated on the basis of the mean value of disease indices of five leaves, and graded as resistant(R) when the value was 2.0 or below, or otherwise as susceptible(S)[4].

Results

1) Yearly distribution of races of X. campestris pv. oryzae in Japan during 1973 to 1987

The isolates collected from various locations of Japan during 1973 to 1987 were examined in regard to their qualitative virulence to five differential rice varieties[3,5,12] (Table 1 & Fig. 1). The isolates of predominant races I and II distributed in almost all locations of Japan under study. The isolates of race III were found in the central and western parts of Japan with exceptions in 1983 and 1985 when no isolate was found. In the Kyushu and Okinawa districts of southern Japan, all

Table 1. Classification and incidence of *Xanthomonas campestris* pv. *oryzae* in Japan during 1973 to 1987

Year	No. of isolates	Race						
		I	II	III	IV	V	VI	VII
1973	128	73 (57.0)*	44 (34.4)	11 (8.6)	0 (0)	0 (0)	0 (0)	0 (0)
1975	299	186 (62.2)	84 (28.1)	25 (8.4)	3 (1.0)	1 (0.3)	0 (0)	0 (0)
1977	265	157 (59.3)	78 (29.4)	26 (9.8)	4 (1.5)	0 (0)	0 (0)	0 (0)
1979	188	109 (58.0)	63 (33.5)	15 (8.0)	1 (0.5)	0 (0)	0 (0)	0 (0)
1983	122	74 (60.7)	48 (39.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1985	162	100 (61.7)	60 (37.0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.3)
1987	125	65 (52.0)	55 (44.0)	5 (4.0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	1,289	764 (59.27)	432 (33.51)	82 (6.36)	8 (0.62)	1 (0.08)	0 (0)	2 (0.16)

* Indicating percentages in parentheses.

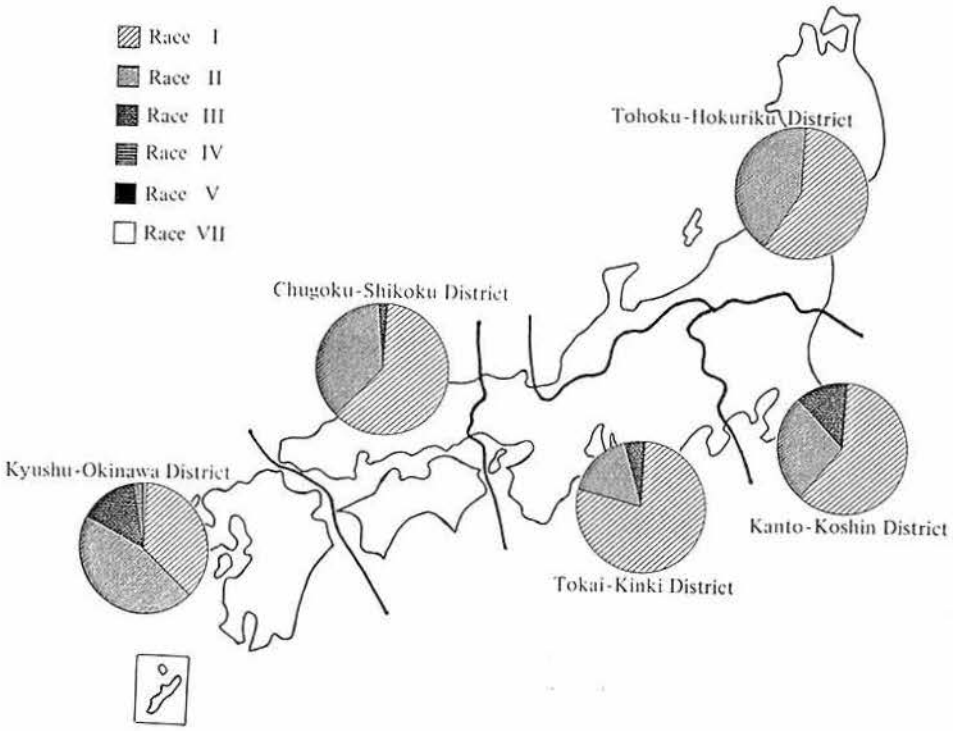

Fig. 1. Geographical distribution of pathogenic races of *Xanthomonas campestris* pv. *oryzae* from Japan in 1973 to 1985

of six races except for race VI, which was differentiated from Indonesian isolates, were distributed, and race II isolates were most predominant. The high incidence of race II may have been caused by predominant cultivation of Kogyoku group varieties, which could be infected by race II. However, the finding that race II which could attack Kogyoku group varieties was also found in the northern area of Japan where Kinmaze group varieties have been cultivated extensively, suggests that some other factors might be associated with the distribution of bacterial races.

2) *Identification of a new pathogenic race VII*

One hundred and sixty-two isolates collected in 1985 were tested in a preliminary experiment to determine their virulence to the Japanese differential rice varieties, including Kinmaze, Kogyoku, Te-tep, Chugoku 45 and Java 14. Most of these isolates were identified as already known races, but two isolates, H8581 and H8584 were virulent to Kinmaze, Kogyoku, Chugoku 45 and Java 14, while avirulent to Te-tep. These two isolates were subjected to the repeated tests in this experiment to identify their virulence to various rice varieties of each differential group. The results obtained are presented in Fig. 2. These isolates were virulent to Kinmaze group rice varieties except for IR 24 and Milyang 23 as well as to Wase Aikoku and Java group varieties. On the other hand, these isolates were avirulent to IR 24 and Milyang 23 of the Kinmaze group, Rantai Emas groups, IRRI varieties and other Kogyoku group varieties. From these results, it was concluded that these two isolates showed a new virulence pattern which was different from that of any of the already known races and proposed that this new pathogenic group should be hereafter designated as race VII.

Table 2. Relationship between rice varietal groups and races of *Xanthomonas campestris* pv. *oryzae* in Japan

Varietal group or variety	Representative varieties	Reaction to races*								
		I A	I B	II	III A	III B	IV	V	VI	VII
Kinmaze group	Kinmaze, Koshihikari / Milyang 23, IR 24	S	S	S	S	S	S	S	S	S/R
Kogyoku group	Kogyoku, Tokai 12 / Daiyoshi, Calorina	R	R	S	S	S	S	R	R	S/R
Rantai Emas group	Te-tep, Rantai Emas 2	R	R	R	S	S	S	R	S	R
Wase Aikoku group	Wase Aikoku 3, Hosokara	R	R	R	R	R	S	S	R	S
Java group	Java 14, Jamica	R	R	R	R	R	S	R	—	S
Elwee group	Elwee, Dickwee-1	S	—	R	R	—	S	R	—	—
Heen Dikwee group	Heen Dikwee-1, M 104	S	—	R	R	—	S	S	—	—
IR 8	IR 8	S	R	R	R	S	S	R	—	R

* R : Resistant, S : Susceptible, — : Not tested.

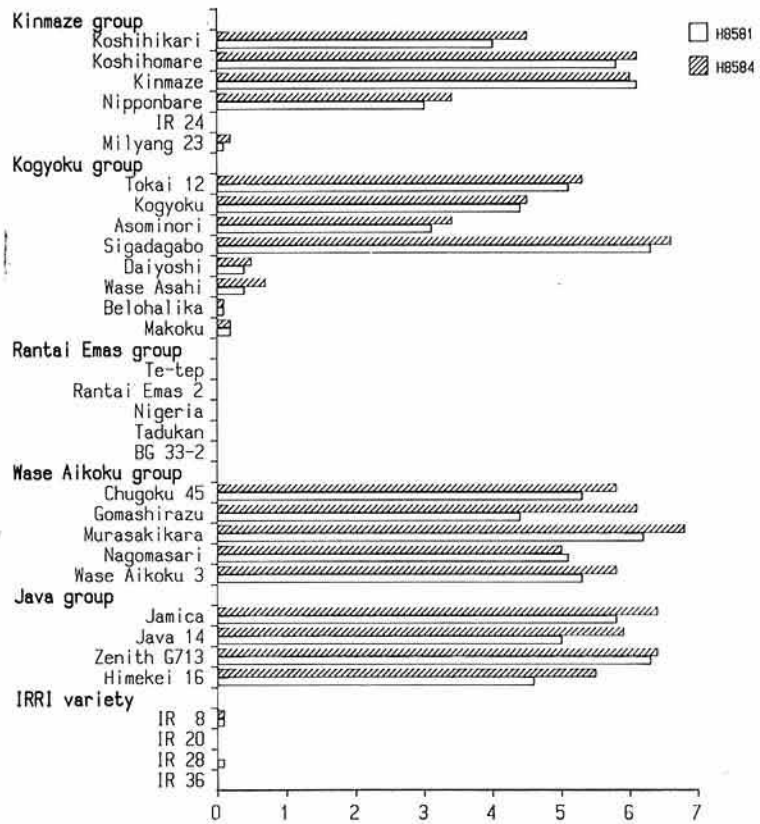

Fig. 2. Virulence of *Xanthomonas campestris* pv. *oryzae* isolates, H 8581 and H 8584 belonging to race VII, to 32 rice varieties of 5 differential groups and IRRI varieties

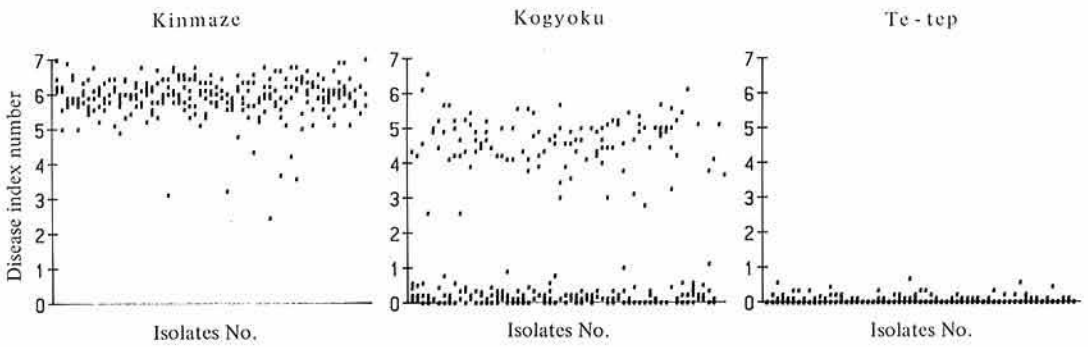

Fig. 3. Virulence of 282 isolates of races I and II which were collected from various districts of Japan in 1983 and 1985, to three differential varieties

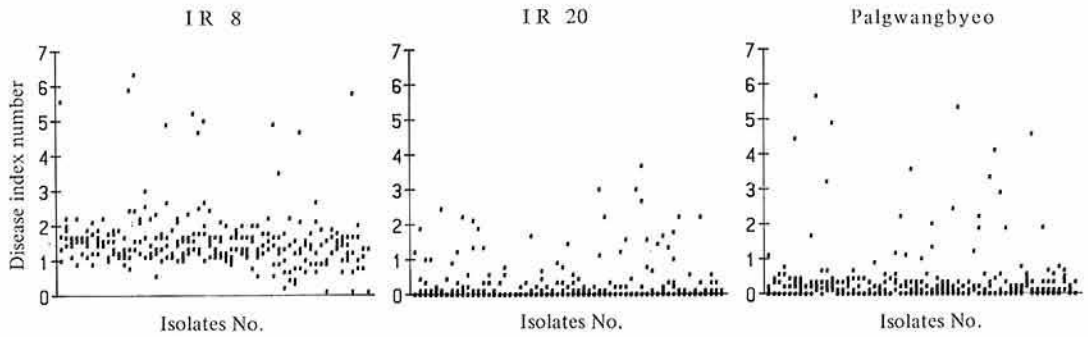

Fig. 4. Virulence of 282 isolates of races I and II which were collected from various districts of Japan in 1983 and 1985, to the IRRI and Korean rice varieties

The reactions of various races, including the newly discovered race, to the differential rice varieties are summarized in Table 2.

3) Pathogenic specialization in races, I, II and III

(1) Test 1

Ten rice varieties, including IRRI and Korean rice varieties, were used to differentiate the virulence of 282 isolates of *X. campestris* pv. *oryzae* collected in Japan in 1983 and 1985. The reactions of these isolates to the five differential Japanese varieties were clearly identified as to whether they were virulent or avirulent except for a few isolates indicating weak virulence, and therefore they could easily be classified as races I and II in the Japanese differential system (Fig. 3). However, the reactions of IRRI and Korean rice varieties to the same isolates were not always clearly differentiated (Fig. 4). Since the reactions of those varieties, including IR 8, IR 20, Tongil, Palgwangbyeo (Suweon 284) and Milyang 23, to these isolates showed consecutive disease indices, the above mentioned system for resistance evaluation could not be applicable to these varieties. In order to differentiate the virulences of these isolates to the IRRI and Korean rice varieties, the critical level of disease index number for distinguishing resistance and susceptibility was tentatively shifted from 2.0 to 3.0. In this connection, it is proposed that the present bacterial races I and II be divided into four and five sub-groups, respectively, according to their differences in virulence to IRRI

Table 3. Subdivision of isolates of race I according to pathogenicity to four IRRI and Korean rice varieties

Race*	Differential varieties				No. of isolates
	IR 8	Tongil	Milyang 23	Palgwangbyeo	
I—A	S**	R	R	R	6
I—B	R	R	R	R	162
I—C	S	S	R	R	0
I—D	S	R	S	R	4
I—E	S	R	S	S	1

* Tentative name. ** R : Resistant, S : Susceptible.

Table 4. Subdivision of isolates of race II according to pathogenicity to three IRRI and Korean rice varieties

Race*	Differential varieties			No. of isolates
	Tongil	IR20	Palgwangbyeo	
II—A	R**	R	R	95
II—B	S	R	R	4
II—C	R	S	R	1
II—D	R	S	S	2
II—E	R	R	S	7

* Tentative name. ** R : Resistant, S : Susceptible.

and Korean varieties (Tables 3 & 4).

(2) Test 2

One hundred and twenty-five isolates collected in 1987 were subjected to test to identify their pathogenicity to 18 varieties, including the 10 varieties used in the above-stated test. The clipping method developed by Kauffman et al.[8] was used in this test. The degree of resistance in each variety-isolate interaction was evaluated on the basis of the average value of lesion length as follows: R (resistant); 4 cm and below, S (susceptible); over 4 cm. The reaction patterns of these isolates to the differential varieties are shown in Table 5. The present bacterial races I, II and III could be further divided into a number of sub-groups, i.e., 10 sub-groups for race I, 17 sub-groups for race II and 3 sub-groups for race III. Five varieties such as Chugoku 45, IR 20, Tongil, Milyang 42 and DV 85 were resistant to all the isolates under testing.

Discussion

The above results indicate that the variation of pathogenicity in Japanese races of *X. campestris* pv. *oryzae* is very complex. Such complexities have also been reported in other Asian countries[6,7]. The genetics of resistance to bacterial leaf blight have been extensively studied in Japan and at the IRRI, where a number of dominant and recessive genes for resistance to isolates from several Asian countries were identified[11]. From these results, it is suggested that further studies be required to more clearly understand the host-parasite relationship in bacterial leaf blight.

It is also proposed that a genetic and pathological approach to the use of horizontal resistance should be considered to reduce damages caused by breakdown of vertical resistance resulted from variation in the pathogenicity of the causal bacterium. It would also be important to investigate the physiological and ultrastructural aspects of the host-parasite relationship.

Many thanks go to the staff members of the Agricultural Experiment Stations and Plant Protection Offices throughout Japan, who have assisted the authors in obtaining leaves affected by bacterial leaf blight for this study.

Table 5. Subdivisions of isolates of races I, II and III according to pathogenicity to 18 rice varieties from several Asian countries including the Japanese differentials

Race*	Reaction of differential rice varieties to bacterial isolates**																	
	Kin-maze	Ko-gyoku	Te-tep	Chugoku 45	Java 14	IR 8	Palgwa-ngbyeo	Sigada-gabo	Asomi-nori	IR 36	Nan Jing 11	IR 24	Jian Yang Ai	IR 30	Xie Zuo 12	Raek-yeong	Dena	Bhulu
I A—1	S	—	—	—	—	S	—	—	—	—	S	S	S	—	S	—	S	S
I A—2	S	—	—	—	—	S	—	—	—	—	S	S	S	—	S	—	—	—
I A—3	S	—	—	—	—	S	—	—	—	—	S	S	S	—	S	—	—	S
I A—4	S	—	—	—	—	S	—	—	—	—	S	S	S	—	S	—	S	—
I B—1	S	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
I B—2	S	—	—	—	—	—	—	—	—	—	S	—	—	—	—	—	—	—
I B—3	S	—	—	—	—	—	—	—	—	—	S	—	S	—	—	—	—	—
I B—4	S	—	—	—	—	—	—	—	—	—	S	S	S	—	—	—	—	—
I B—5	S	—	—	—	—	—	—	—	—	—	S	S	S	—	S	—	—	—
I B—6	S	—	—	—	—	—	—	—	—	S	S	S	S	—	—	—	—	—
II— 1	S	S	—	—	—	—	—	—	—	—	S	S	S	—	—	—	—	—
II— 2	S	S	—	—	—	—	—	S	—	—	—	—	—	—	—	—	—	—
II— 3	S	S	—	—	—	—	—	S	—	—	S	—	S	—	—	—	—	—
II— 4	S	S	—	—	—	—	—	S	S	—	S	—	S	—	—	S	—	—
II— 5	S	S	—	—	—	—	—	S	—	—	S	S	S	—	—	—	—	—
II— 6	S	S	—	—	—	—	—	S	—	S	S	S	S	—	—	—	—	—
II— 7	S	S	—	—	—	—	—	S	—	—	S	S	S	—	S	—	—	—
II— 8	S	S	—	—	—	—	—	S	—	—	S	S	S	—	S	—	S	—
II— 9	S	S	—	—	—	—	—	S	—	—	S	S	S	—	S	S	—	—
II—10	S	S	—	—	—	—	—	S	S	S	S	S	S	—	—	S	—	—
II—11	S	S	—	—	—	—	—	S	S	—	S	S	S	—	S	S	—	—
II—12	S	S	—	—	—	—	S	S	S	—	S	S	S	—	S	S	—	—
II—13	S	S	—	—	—	—	S	S	S	—	S	S	S	—	S	S	—	S
II—14	S	S	—	—	—	—	S	S	S	S	S	S	S	S	S	S	—	—
II—15	S	S	—	—	—	—	S	S	S	—	S	S	S	—	—	S	—	—
II—16	S	S	—	—	—	—	S	S	S	S	S	S	S	—	S	S	—	—
II—17	S	S	—	—	—	—	S	S	S	—	S	S	S	—	—	—	—	—
III A—1	S	S	S	—	—	—	—	S	S	—	S	S	S	—	S	S	—	—
III A—2	S	S	S	—	—	—	—	S	S	—	S	S	S	—	S	S	S	—
III A—3	S	S	S	—	—	—	S	S	S	—	S	S	S	—	S	S	—	—

* Tentative name. ** S : Susceptible, — : Resistant.

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