

## PATHOTYPE PROFILE OF *Xanthomonas oryzae* pv *oryzae* ISOLATES FROM THE RICE ECOSYSTEM IN CUULONG REVER DELTA

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### ABSTRACT

*Bacterial leaf blight caused by Xanthomonas oryzae pv oryzae is one of the major diseases of rice in Mekong Delta. Diseased rice leave samples, collected from 13 provinces, were used for isolation and evaluated for the variation on pathogenicity. 41 isolates were used for inoculation on 10 differential rice varieties containing different single resistant genes, the results showed that the infection response were clearly compatible and incompatible reactions on differential rice varieties.*

*Gene xa 5 was highest effective against to disease, the next were Xa 7, Xa 21 and xa 13. Six pathogenic races were identified and their distribution was varied across the provinces in Mekong Delta. Race A, E and F were predominant, they occurred and infected on rice in 6 to 8 provinces, while race B, C and D in 3 to 5 provinces in Mekong Delta.*

**Key words:** differential, pathogenicity, pathogenic race, resistant gene.

### INTRODUCTION

Bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is the most important bacterial disease of rice in Asia (Mew 1987, Mew 1993) and most of the rice growing countries. The disease can affect rice plants at any plant growth stages (Suparyono 2004). Under the tropical condition such as in Mekong Delta, disease occurred in both dry and wet seasons. Some chemicals have been used in recent years, but they cannot control this disease under severe condition (Noda et al. 1999). The varietal resistance is the main control measure available, because there is no other control method economically effective for this disease (Yamamoto et al. 1977). The deployment of genes for resistance to *X. oryzae* pv. *oryzae* in commercial rice cultivars is recent. The introduction of these genes for resistance into rice is correlated with a change in the pathogenic diversity of *X. oryzae* pv. *oryzae* populations, that is, new races of the pathogen emerge and overcome deployed resistance (Mew 1992). Although bacterial leaf blight (BLB) in Mekong Delta as well as in Northern Part of Vietnam, the pathogenic variability of *Xoo* and varietal resistance have not yet been investigated in detail (Noda et al. 1999). At present, most of the existing commercial rice varieties are susceptible to the *Xoo* isolates collected in the south of Vietnam (E

et al. 1999). To solve such problems, the strategy of varietal resistance deployment needs to be established, and the information on the development and distribution of pathotype of *Xoo* is needed. In this study, therefore, we collected BLB samples in various region of MEKONG DELTA to survey the variation and distribution of races of BLB pathogen.

### MATERIALS AND METHODS

#### Collection of diseased leaves

Diseased rice leaves showing typical bacterial leaf blight symptom were collected from various rice fields over 13 provinces in the Mekong Delta, during the 2006 wet season 2006 and 2007 dry season.

Samples were collected based on the random sampling method, on rice plant at heading to approaching mature stages, as the disease usually develops well in these plants growth stages. Disease leaves were detached and put into the paper envelope. These envelopes were labeled explaining variety, location and sampling date, and put into the plastic boxes contained silicagel. The samples were taken in to the laboratory and kept in the refrigerator for further process.

#### Isolation of *Xanthomonas oryzae* pv. *oryzae*.

Diseased leaves were cleaned with tap water, and

air dried. These leaves were cut into small pieces about 5 to 7 cm and sterilized with 1 % sodium hypochloride solution, then washed in sterilized distilled water. These pieces were cut into smaller pieces about 5 x 5 mm in size and put into the test tube containing 1ml of sterilized distilled water for about 5 to 10 minutes, to allow the bacteria to ooze out from the leaf tissue. Using the sterilized loop needle with bacterial suspension streak onto petri dishes containing Wakimoto's medium (WF-P) (Ou 1985). The plates were incubated in room temperature (28-30°C) for 3 to 4 days. The single yellow, round and smooth margin, non flat, mucous colonies were selected and transferred into slant WF-P medium as pure culture. The single colony was selected as a representative strain for this study. These strains were maintained at 4°C up to 1 month and further evaluation. After isolation, all isolates were tested on pathogenicity by inoculation on the susceptible rice variety IR24 at 24 days old. If the BLB lesion appears, it means that this is surely *Xoo*.

### Pathotype evaluation

Seeds of the near isogenic lines and IR24 were obtained from IRRI, Philippines. These ten near isogenic lines with single resistance genes such as IRBB 1 (carring resistant gene *Xa-1*), IRBB 3 (*Xa-3*), IRBB 4 (*Xa-4*), IRBB 5 (*xa-5*), IRBB 7 (*Xa-7*), IRBB 10 (*Xa-10*), IRBB 11 (*Xa-11*), IRBB 13 (*xa-13*), IRBB 14 (*Xa-14*), and IRBB 21 (*Xa-21*) (Huang et al. 1997). Which were developed as international differential varieties of BLB isolates, and IR 24 used as the susceptible check. These varieties were first sown in plastic boxes, and 20 days later, seedling were transplanted into pots containing natural paddy soil of 10 liter in volume. Rice plants were grown under greenhouse condition. The disease inoculation was done when rice plants were 40 days old. The bacterial

suspension for inoculation were prepared using the 2 days old culture of each isolate in 20 ml of sterilized distilled water adjusted of 10<sup>8</sup> CFU/ml. To test the virulence of the strains, the fully expanded leaves were inoculated by the leaf-cutting method (Kauffman et al. 1973)

### Disease assessment

The lesion length from the cut leaf tip was measured in centimeters (cm) at 18 days after inoculation. Disease reactions were categorized according to lesion length. The lesion length was 0 to 6 cm classified as resistance (R) and more than 6 cm as susceptible (S) (Sanchez et al. 2000).

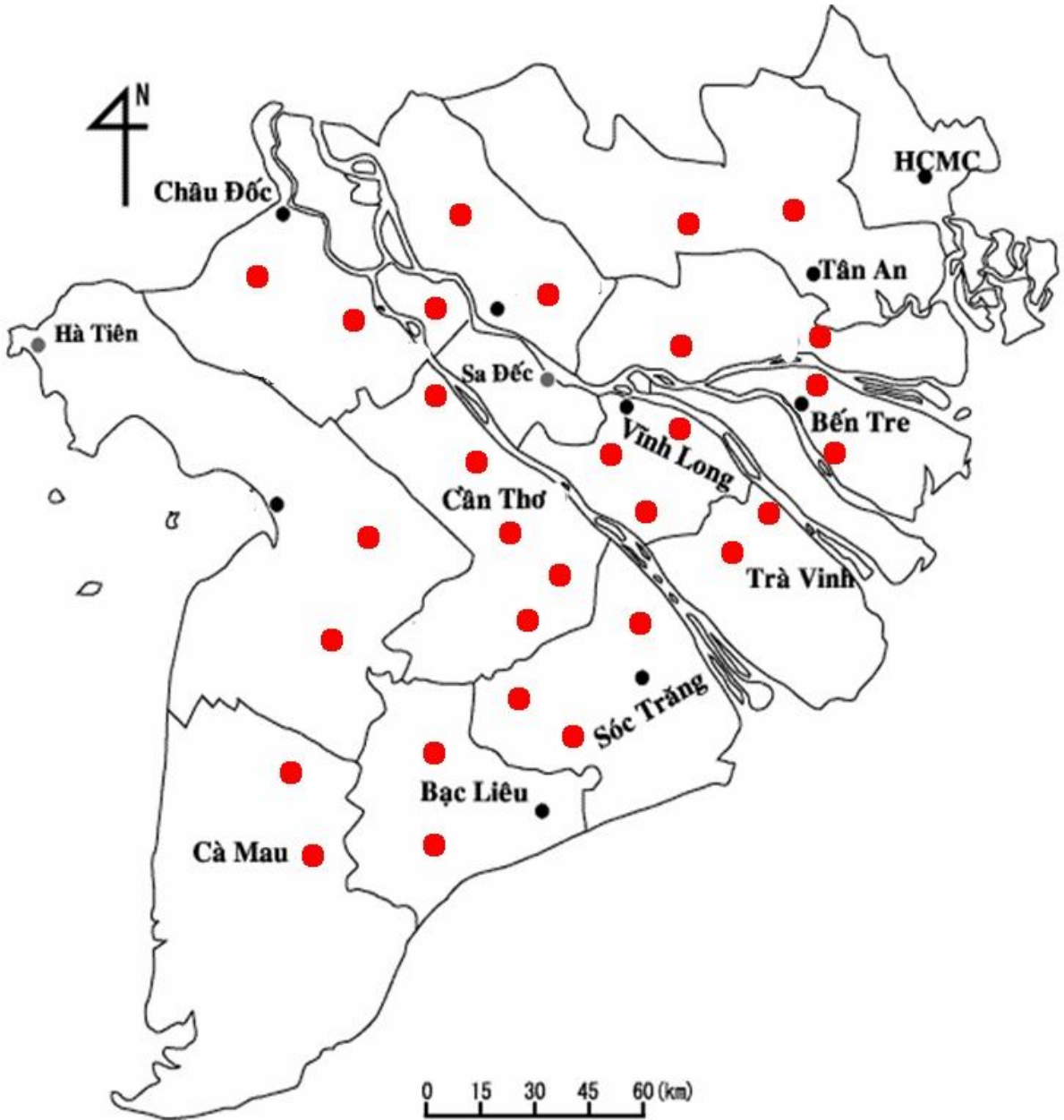
### Data management

The reactions of differential varieties were presented as the means of lesion length of each isolates (strains). All strains with the same reaction on the differential varieties were grouped in to a pathotype or pathogenic race.

## RESULTS

The diseased rice leaves were collected from 13 provinces at 30 sites (Fig.1). In 2006 wet season, the disease occurred in most of the provinces in Mekong Delta. To date, about 41 isolates were collected. All isolates formed yellow mucous colonies on WF-P medium and induced the typical disease symptom on susceptible check varieties IR24.

The detail of isolates collected was shown in table 1. Because of the samples were randomly collected on the farmer fields, some of which might be affected by the use of pesticides. Therefore, diseased rice leaves with the typical symptoms but none of BLB pathogen could be isolated from those samples.



**Fig. 1:** Collection sites of diseased rice leaves in Mekong Delta

**Table 1:** *Xanthomonas oryzae* pv. *oryzae* isolates collected from the rice cultivars grown at different locations in MEKONG DELTA in 2006 wet season.

No.	Isolates	Hosts	Provinces	No.	isolates	Hosts	Provinces
1	B 37	Nep	Long An	22	B 53	IR50404	Kien Giang
2	B 38	OM1490	Long An	23	B 54	OM2513	Kien Giang
3	B 39	OM2514	Long An	24	B 28	Jasmine 85	Can Tho
4	B 35	OM4498	Tien Giang	25	B 29	Jasmine 85	Can Tho
5	B 36	OM5930	Tien Giang	26	B 31	OM2395	Can Tho
6	B 55	Jasmine 85	Ben tre	27	B 40	IR50404	Can Tho
7	B 56	IR50404	Ben tre	28	B 61	OM5930	Hau Giang
8	B 58	OM3536	Ben tre	29	B 65	OMCS2000	Hau Giang
9	B 1	VD20	Dong Thap	30	B 66	OM2490	Hau Giang
10	B 2	Jasmine 85	Dong Thap	31	B 4	OM4495	Soc Trăng
11	B 73	IR50404	Dong Thap	32	B 9	OMCS2000	Soc Trăng
12	B 42	OM1490	Vinh long	33	B 12	OM576	Soc Trăng
13	B 43	OM2717	Vinh long	34	B 60	OM1490	Soc Trăng
14	B 47	OM576	Tra Vinh	35	B 10	HĐ1	Bac Lieu
15	B 48	OM4668	Tra Vinh	36	B 15	ĐS2006	Bac Lieu
16	B 51	OM4498	Tra Vinh	37	B 22	Jasmine 85	Bac Lieu
17	B 33	IR50404	An Giang	38	B 25	Jasmine 85	Bac Lieu
18	B 69	OM4495	An Giang	39	B 27	OM4668	Bac Lieu
19	B 70	Jasmine 85	An Giang	40	B 16	BTE 1	Ca Mau
20	B 71	OM2717	An Giang	41	B 17	OM576	Ca Mau
21	B 52	OM3536	Kien Giang				

- The bacterial leaf blight appeared in all the provinces of MEKONG DELTA. Based on the reaction of the isolates to the 10 differential rice varieties, 9 isolates were identified as pathotype A (Race A), and 4, 7, 8 and 9 isolates were pathotype B, C, D, E and F, respectively (Table 2).
- Race A was occurred on the 6 provinces such as Long An, Tien Giang, Dong Thap, Vinh Long, An Giang and Soc Trang. Race B was distributed in Soc Trang, Bac Lieu and Long An provinces. Race F was predominant and distributed in 8 provinces including An Giang, Can Tho, Dong Thap, Ben Tre, Bac Lieu, Soc Trang, Tra Vinh and Kien Giang.
- All races were virulent on the rice lines possessing single resistant genes *Xa -1*, *Xa - 3*, *Xa - 4*, *Xa - 10*, *Xa - 11* and *Xa - 14*, with most races producing the lesion lengths the same as on the susceptible check variety IR24 (Table 3).
- Gene *xa 5* was most effective against to races B, C, E and race F. Gen *Xa - 7* was effective against to race B, C and D.
- Race A, C, D and F of *Xanthomonas oryzae* pv. *Oryzae* were virulent on rice line containing resistance gene *Xa - 21*.
- Gene *xa -13* was only effective against race A of *Xanthomonas oryzae* pv. *oryzae* in Mekong Delta.

**Table 2:** Pathotype grouping of *Xanthomonas oryzae* pv. *oryzae* isolates based on their infection responses to 10 differential rice varieties.

No	Differential rice variety	Resistance gene	Pathotype					
			A	B	C	D	E	F
			B2, B29 B35, B36 B37, B42 B39, B60 B70	B 9 B 12 B 15 B 38	B 16 B17 B 31 B 71	B22, B27 B33, B43 B52, B53 B 65	B1, B25 B28, B51 B55, B56 B61, B66	B4, B10 B40, B47 B48, B54 B58, B69 B73
1	IRRB 1	<i>Xa -1</i>	S	S	S	S	S	S
2	IRRB 3	<i>Xa - 3</i>	S	S	S	S	S	S
3	IRRB 4	<i>Xa - 4</i>	S	S	S	S	S	S
4	IRRB 5	<i>xa - 5</i>	S	R	R	S	R	R
5	IRRB 7	<i>Xa - 7</i>	S	R	R	R	S	S
6	IRRB 10	<i>Xa - 10</i>	S	S	S	S	S	S
7	IRRB 11	<i>Xa - 11</i>	S	S	S	S	S	S
8	IRRB 13	<i>xa - 13</i>	R	S	S	S	S	S
9	IRRB 14	<i>Xa - 14</i>	S	S	S	S	S	S
10	IRRB 21	<i>Xa - 21</i>	S	R	S	S	R	S
11	IR 24	-						

**Table 3:** The lesion lengths of *Xanthomonas oryzae* pv. *oryzae* races on the leaves of differential rice varieties

No	Differential rice variety	Resistance gene	Pathotype (Race)					
			A	B	C	D	E	F
1	IRRB 1	<i>Xa -1</i>	28.14 a	10.61 b	23.88 bc	22.04 b	14.97 c	15.67 de
2	IRRB 3	<i>Xa - 3</i>	9.01 f	10.95 b	24.97 bc	24.69 a	17.02 c	20.44 bc
3	IRRB 4	<i>Xa - 4</i>	27.21 a	9.92 b	24.77 bc	20.65 b	16.72 c	27.09 a
4	IRRB 5	<i>xa - 5</i>	27.99 a	<b>5.66 c</b>	<b>3.51 e</b>	21.28 b	<b>3.66 e</b>	<b>5.05 g</b>
5	IRRB 7	<i>Xa - 7</i>	22.39 bc	<b>5.37 c</b>	<b>4.99 e</b>	<b>2.97 g</b>	9.24 d	13.05 ef
6	IRRB 10	<i>Xa - 10</i>	23.39 b	12.56 a	30.96 a	8.44 f	15.07 c	19.07 cd
7	IRRB 11	<i>Xa - 11</i>	14.58 e	12.75 a	22.40 c	13.93 cd	19.21 b	23.04 b
8	IRRB 13	<i>xa - 13</i>	<b>5.00 g</b>	10.78 b	25.87 b	13.10 de	21.60 a	19.50 c
9	IRRB 14	<i>Xa - 14</i>	19.30 d	10.67 b	25.17 bc	15.67 c	19.95 ab	22.42 bc
10	IRRB 21	<i>Xa - 21</i>	20.36 cd	<b>5.17 c</b>	12.83 d	20.53 b	<b>5.34 e</b>	10.38 f
11	IR 24	-	20.00 cd	13.28 a	27.09 b	21.06 b	20.13 ab	27.79 a

## DISCUSSION

Near isogenic rice lines having ten different major genes for resistance to *Xanthomonas oryzae* pv. *oryzae*, recently developed by IRRI were used to analyze virulence of 41 isolates of *Xanthomonas oryzae* pv. *oryzae* in Mekong Delta, and these infection responses were clear and easily classified into virulent or avirulent on the test rice lines possessing different genes for resistance (Adhikari 1999).

We identified six virulent groups based on infection responses elicited on rice lines containing single resistant genes. Pathogenic race of *Xanthomonas oryzae* pv. *oryzae* are defined by the ability to induce a combination of compatible (susceptible) and incompatible (resistant) reaction when tested on a standard set of differential host cultivars (Mew 1992). Indeed, ten near isogenic lines were effective for characterizing races of *Xanthomonas oryzae* pv. *oryzae*, because they all gave clearly compatible and incompatible reaction to the isolates or races studied.

The information of this study has significant implicating for regional gene deployment. In fact, some of the single resistant genes used can be expected to control bacterial leaf blight of rice in Mekong Delta.

## CONCLUSION

Six pathogenic races of *Xanthomonas oryzae* pv. *oryzae* i.e. A, B, C, D, E and F were observed in Mekong Delta during 2006 wet season and 2007 dry season. Their distribution dominance varied across provinces.

Race A, E and F were dominated and observed in most of provinces in Mekong Delta.

This addressed that bacterial leaf blight disease of rice is still a major thread to rice production in Mekong Delta.

## REFERENCES

Adhikari T B and RC Basnya. 1999. Virulence of *Xanthomonas oryzae* pv. *oryzae* on rice lines containing single resistance genes and gene combinations. In the American phytopathological society. Plant Disease,

vol.83 No.1, 46-50.

Huang N, ER Angeles, J Domingo, G Magpanty, S Singh, G Zhan, N Kumarvadevel, J Bennett and GS Khush. 1997. Pyramiding of bacterial leaf blight resistance genes in rice marker assisted selection using RFLP and PCR. Theor Appl Genet 95: 313-320.

Kauffman HE, APK Reddy, SPY Hsieh, SD Merca. 1973. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae* pv. *oryzae*. Plant Diseases. Rep.57: 537-541.

Lai Van E, Takahito Noda, Pham Van Du. 1999. Resistance assessment of rice cultivars to *Xanthomonas oryzae* pv. *oryzae* and pathogenicity testing of bacterial leaf blight isolates in Vietnam. OMonRice 7: 97-105.

Mew TW. 1987. Current status and future prospects of research on bacterial blight of rice. Annu. Rev. Phytopathol. 25:359-382

Mew TW, CM Vera Cruz, and ES Medalla. 1992. Changes in race frequency of *Xanthomonas oryzae* pv. *oryzae* in response to rice cultivars planted in the Philippines. Plant Dis. 76:1029-1032.

Mew TW, AM Alvarez, JE Leach, and J Swings. 1993. Focus on bacterial blight of rice. Plant Dis. 77:5-12.

Noda T, Pham van Du, Lai van E, Hoang Dinh Dinh, and H Kaku. 1999. Pathogenicity of *Xanthomonas oryzae* pv. *oryzae* strains in Vietnam. Annals of the Phytopathological Society of Japan: 65(3): 293-296.

Ou SH. 1985. Bacterial leaf blight. In Bacterial Diseases, Rice Disease 2<sup>nd</sup> edition: 61-96.

Sanchez AC, DS Brar, N Huang, Z Li, and GS Khush. 2000. Sequence tagged site marker-assisted selection for three bacterial blight resistance genes in rice. Crop Sci. 40: 792-797.

Suparyono Sudir and Suprihanto. 2004. Pathotype profile of *Xanthomonas oryzae* pv. *oryzae* isolates from the rice ecosystem in Java. Indonesian Journal of Agriculture Science:

5(2). 63-69.

Yamamoto T, HR Hifni, M Muchmud, T Nishizawa, and DM Tantera. 1977. Variation in pathogenicity of *Xanthomonas oryzae* pv

*oryzae* (Uyeda et Ishiyama) Dowson and resistance of rice varieties to the pathogen. Contr. Centr. Res. Ins. Agric. Bogo. No. 28: 22pp.

**Đánh giá sơ bộ kiểu kí sinh các mẫu li trích vi khuẩn *Xanthomonas oryzae* pv *oryzae* vùng Đồng Bằng Sông Cửu Long**

Bệnh bạc lá lúa do vi khuẩn *Xanthomonas oryzae* pv. *oryzae* là một trong các bệnh hại chính trên lúa. Các mẫu lá bệnh được thu thập ở 13 tỉnh vùng Đồng Bằng Sông Cửu Long (ĐBSCL) được sử dụng li trích, tách thuần vi khuẩn và đánh giá sự biến động độc tính của kí sinh gây bệnh bạc lá trong vùng. 41 mẫu vi khuẩn thuần cho phản ứng gây bệnh trên bộ chỉ thị 10 gồm dòng đấng gen chứa 10 gen kháng bệnh khác nhau. Kết quả phản ứng của các mẫu vi khuẩn này trên 10 gen kháng cho ra tính kháng nhiễm rất khác biệt. Gen *xa 5* có hiệu lực kháng bệnh cao nhất, kể đến là *Xa 7*, *Xa 21* và *xa 13*. Sáu nòi sinh lý đã được xác định và cho thấy chúng phân bố khác nhau trên các tỉnh vùng ĐBSCL. Nòi A, E và F phổ biến nhất và có xuất hiện gây hại ở từ 6 đến 8 tỉnh trong toàn vùng, trong khi nòi B, C và D xuất hiện ở từ 3 đến 5 tỉnh trong vùng.