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Diversity in Virulence of *Xanthomonas oryzae* pv. *oryzae* from Northern Vietnam

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Abstract

A survey of the geographical distribution of pathogenic races of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) causing bacterial blight (BB) of rice in northern Vietnam was conducted. We characterized strains of *Xoo* collected from nine regions in this part of Vietnam in 2001 and 2002 using polymerase chain reaction (PCR)-based DNA fingerprinting and virulence analysis. The pathogenicity of Vietnamese 84 strains was tested for their virulence to BB near-isogenic lines with a single resistance gene. Based on virulence analysis using 11 differential lines (IR24, IRBB1, IRBB2, IRBB3, IRBB4, IRBB5, IRBB 7, IRBB10, IRBB11, IRBB21 and Taichung Native 1), each harboring a single resistance gene, four pathotypes (tentatively designated as races G1 to G4) were identified. All strains were virulent to the resistance genes *Xa1, Xa2, Xa10*, *Xa11*, *Xa14* and *Xa18,* and *xa5* and *Xa21* were effective against a major portion of the Vietnamese *Xoo* population; followed by *Xa7.* Most of the Vietnamese hybrid and improved varieties cultivated in northern Vietnam were susceptible to all races and no Vietnamese variety was resistant to all races. Our study revealed that strains from northern Vietnam comprised two major races (G2 and G3) and two minor races (G1 and G4), which were widely distributed and detected at lower frequencies, respectively, in northern Vietnam. Seventy six strains of *Xoo* were analyzed by DNA fingerprinting using two PCR-based assays. Two groups, designated as clusters I and II, were respectively identified, in which cluster I contained race G4 strains and cluster II race G1 strains. Combining DNA and pathotype analysis, we hereby present the situation of *Xoo* recently isolated in northern Vietnam.

Discipline: Plant disease
Additional Key words: bacterial blight of rice, near-isogenic line(s), race, resistance gene

Introduction

Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a widespread and destructive disease affecting rice in irrigated and rainfed environments. Bacterial blight emerged as a serious rice disease Vietnam following the introduction of semidwarf, high yield, and susceptible rice cultivars from China. The use of resistant rice cultivars is an inexpensive and environmentally-friendly approach to protecting rice against the disease. Because some resistance genes are only effective against particular pathogen subpopulations (races), it is important to understand the structure of the pathogen population to determine the optimal resistance strategy. Information on the pathogen population structure would increase the knowledge of pathogen diversity in time and space.

Geographically, northern Vietnam is of interest because rice in this region is grown under diverse climatic conditions. Accordingly, pathogenic or genetic diversity of the pathogen is expected. Northern Vietnam is one region where distribution of the pathogenic race has not previously been studied in detail, although the pathogenicity of *Xoo* in southern Vietnam was analyzed by Noda et al.[10].

Although over 30 resistance genes have been genetically defined in rice cultivars and germplasm, the effec-

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tiveness of resistance genes varies across locations due to the geographical structure of the pathogen population. The evolution of new pathogenic races remains a constant potential threat, as indicated by the case of breakdown of resistance in the Japanese cultivar Asakaze in 1957[7]. To prevent such breakdown of resistance, several breeding strategies have been proposed, the success of which depends on careful race prediction of pathogens and detailed genetic information on the resistance of rice cultivars.

This study is part of a regional effort to apply knowledge of the pathogen population structure to the deployment of resistance genes. Moreover, DNA fingerprinting using rep-PCR was used to characterize Vietnamese *Xoo* strains. This research was undertaken to provide information on the current population structure of the pathogen in northern Vietnam.

Materials and methods

1. Collection of infected leaf samples

Bacterial blight-infected leaf samples were collected in farmers' fields from 11 different locations during the period 2001-2002. The locations of the sampling sites were several kilometers apart. At most sites, several rice fields showed symptoms of bacterial blight. Diseased samples were collected from randomly selected points in each field. The distances between the sampling fields in a site varied depending on the cultivars grown and the incidence and severity of the bacterial blight there.

2. Bacterial strains

The diseased leaf samples were cut into small pieces, about 1 cm in length, including the margin of typical lesions, and sterilized with 70 % ethyl alcohol and 1 % sodium hypochlorite solution. Each sample was then homogenized with 1 ml of sterile distilled water. The resulting suspension was diluted with sterile distilled water, appropriately diluted samples were streaked on potato semi-synthetic agar medium (PSA)[14] and incubated at 30ºC for 4 days. The resulting viscous, yellow bacterial colonies were transferred to PSA slants and cultured at 30ºC for 2 days, whereupon single colonies were selected from each leaf sample. PCR using specific primers was also implemented for rapid *Xoo* identification. Specific primers to amplify the internally transcribed spacer region of ribosomal DNA, XOR-F (5'-GCATGACGTCATCGTCCTGT-3') and XOR-R2 (5'-CTCGGAGCTATATGCCGTGC-3') designed by Adachi & Oku[1] were used. The DNA template for PCR was extracted from bacterial cells (approximately 10^8 cfu/ml) by the following method. One hundred µl of each bacterial suspension was maintained at 95 ºC for 8 min and centrifuged at 10,000 rpm for 3 min. The supernatant was then used directly as a DNA template for the PCR. Optimal conditions for PCR are as follows, a 25-µl reaction volume containing 2.5 µl of 10 × *Taq* buffer (100 mM Tris-HCl, pH 9.0, 500 mM KCl and 1% Triton X-100), 1.25 U of *Taq* polymerase, 2.5 mM $MgCl_2$, 200 µM dNTPs, 2.0 µl of DNA template solution and 0.2 µM of each primer. Each reaction mixture was overlaid with 15 µl of mineral oil. PCR was performed with a thermal cycler using the following protocol: initial denaturation at 94ºC for 30 sec, followed by 30 cycles comprising denaturation at 94ºC for 1 min, annealing at 60ºC for 30 sec, extension at 72ºC for 1 min and an additional extension step at 72ºC for 7 min. PCR products were resolved in a 2% agarose gel in a Tris-borate EDTA (TBE) buffer (pH 8.0) and stained with ethidium bromide. A single 470-bp fragment was amplified from the DNA template solution of *Xoo*.

For long-term preservation, the purified strains were maintained in 10% skim milk containing 0.05% L-glutamic acid at –40ºC and as lyophilized cultures. The stored strains were then revived on a PSA medium for the pathogenicity test. Information on bacterial strains, geographic locations, and host cultivars was as previously described[5]. All the isolates collected were inoculated onto the susceptible rice cultivar IR24 to confirm their pathogenicity.

3. Pathogenicity analysis

The plant materials included near-isogenic lines harboring specific resistance genes for bacterial blight (BBNILs): IRBB1 (*xa1*), IRBB2 (*Xa1, Xa2*), IRBB3 (*Xa3*), IRBB4 (*Xa4*), IRBB5 (*xa5*), IRBB7 (*Xa7*), IRBB10 (*Xa10*), IRBB11 (*Xa11*), IRBB14 (*Xa14*) and IRBB21 (*Xa21*) along with a set of standard check cultivars IR24, and Taichung Native 1. Each near-isogenic line carries a defined resistance gene backcrossed into susceptible cv. IR24[11]. Nine Vietnamese hybrid varieties and 12 Vietnamese varieties improved by conventional breeding were also evaluated for their resistance to the representative strains of each race.

Plants were grown under natural photoperiodic conditions. The plants were then transplanted approximately 30 days after sowing into concrete beds at the Hanoi Agricultural University in Vietnam. The soil was fertilized with N-P-K at 9-6-6 g/m^2 before transplanting, and additional nitrogen was applied at 3g/m^2 before inoculation.

Strains of *Xoo* were transferred to PSA slants and incubated at 28ºC for 72h. Inoculum was prepared by suspending the bacteria, which had been grown on PSA for 3 days at 28ºC, in sterile distilled water. The inoculum density was adjusted to approx. 10^9 cfu/ml using a spectrum

photometer. Plant inoculation was carried out by clipping the tip (about 1 to 2 cm) of the fully expanded uppermost leaf with scissors that had been dipped into the inoculum[8]. For each cultivar-strain combination, twenty leaves per cultivar were inoculated per replicate, and each test was replicated more than three times. The lesion lengths (cm) on 10 inoculated leaves were measured 18 days after inoculation.

Disease reactions were classified according to the mean lesion length (LL) as follows: resistant (R), LL < 5 cm; moderately susceptible to moderately resistant (M), LL = 5 to 15 cm; and susceptible (S), LL > 15 cm.

4. IS-Polymerase chain reaction (PCR) analysis

Genomic diversity was evaluated by a PCR assay based on the amplification of sequences between copies of the endogenous *Xoo* insertion sequence (IS) elements, IS*1112* and IS*1113*[2,3,6,12,13]. The repetitive primer sequences corresponding to JEL1 (5'-CTCAGGTCAGGTCGCC-3') and JEL2 (5'-GCTCTACAATCGTCCGC-3'), and J3 (5'-GCTCAGGTCAGGTCGCCTGG-3') were used to determine whether they could reveal polymorphism in *Xoo* isolates in northern Vietnam. The DNA template for PCR was extracted from cells of *Xoo* (approximately 10^8 cfu/ml) using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) according to suppliers' protocols. All amplifications were carried out in a final volume of 25µl and performed in a programmable thermal cycler (MyCycler, BIO-RAD). The reaction mixtures for PCR contained (as a final concentration) 50 pmol of primer, 50 ng of DNA template, 312.5 µM of each deoxynucleoside triphosphate (dNTP) (Sigma Chemical Co.,), two units of *Taq* polymerase (Promega Corp.), and 10 % (vol/vol) dimethyl sulfoxide (DMSO). The 5 × reaction buffer stock solution contained 10 mM Tris-HCl (pH8.3), 25 mM KCl, 3.5 mM $MgCl_2$, and 160 ng of bovine serum albumin per ml. Conditions for the PCR using JEL1 and JEL2 primers were as follows: initially denatured for 1 min at 94ºC, and then subjected to 30 cycles of PCR (10 s of denaturation at 94ºC, 1 min of annealing at 62ºC, and 8 min of extension at 65ºC). The amplification with the J3 primer began with a denaturation phase at 95ºC for 7 min followed by 30 cycles of denaturation at 94ºC for 1 min and annealing and extension at 68ºC for 3 min each. The final extension cycle was at 65ºC for 15 min, followed by incubation at 4ºC for 20 h. After completion of PCR, samples were stored at 4ºC until gel electrophoresis. A 10-µl portion of each amplified PCR product was resolved on a gel containing a mixture of 0.75% agarose in 0.5 × Tris-borate-EDTA buffer (89 mM Tris, 89 mM boric acid, and 0.5 M EDTA, pH 8.0), stained with ethidium bromide, and photographed on an UV transilluminator. DNA from 76 strains of *Xoo* was amplified with the IS-PCR primer. Experiments were repeated three times to confirm the DNA band identities and differences.

5. Data analysis

To determine the genetic relationships among PCR-RFLP types, the presence or absence of bands at 31 different positions was converted into binary data; i.e. the presence or absence of a band, which was coded as 1 or 0, respectively. The density of the bands was considered to be constant. Each strain was tested on at least three PCR runs performed at different times to confirm the differences. Similarity coefficients were estimated using the Dice coefficient[4], and the dendrogram was constructed from similarity coefficient data, with bootstrap analysis performed as previously reported[15].

Results

1. Selected isolates

A total of 127 bacterial isolates were collected from 11 different locations in northern Vietnam during the period 2001-2002. All isolates formed yellow mucoid colonies on PSA, with colony sizes ranging from 3 to 4 mm 4 days after incubation. No isolate formed a soluble pigment on PSA. In PCR, with the specific primers XOR-F and XOR-R2 for *Xoo*[1], a single 470-bp fragment was amplified from the lysed cells of all isolates (data not shown). One hundred and twenty-seven isolates were tested in a preliminary experiment for virulence to the standard susceptible variety IR24. The length of lesions varied from 0.7-33.6 cm. Isolates with lesion length of 5 cm or less were determined as avirulent strains, whereas those with lengths exceeding 15 cm were deemed virulent. Among 127 isolates, 84 (Table 1) were virulent (lesion length exceeding 15 cm) to IR 24 and selected for further experiments.

2. Virulence analysis of isolates

Using a differential set comprising 10 near-isogenic lines and the susceptible control cultivar IR24, 84 isolates were classified mainly into four virulence groups G1 to G4, designating as race due to the significant interaction between G1 and G2 vs. *Xa*3 and *Xa*4 in Table2. All isolates tested were virulent to IR24, IRBB1, IRBB2, IRBB10, IRBB11 and TN 1. In terms of the response of IRBB3, IRBB5, IRBB7 and IRBB21 to the isolates, there were two types showing a typical resistance reaction (lesion length less than 5 cm) and a moderately susceptible to moderately resistant reaction (5-15 cm). The moderately susceptible to moderately resistant reaction of IRBB3,

Table 1. *Xanthomonas oryzae* pv. *oryzae* strains collected from different locations in northern Vietnam

Strain	Race	Rice cultivar	Location (Commune, District, Province)	Date (year/month/date)
HAU01008-1	G4	Te do (Improved variety)	Hai Duong	2001/9/26
HAU01016	G4	C-70 (Improved variety)	Hai Duong	2001/9/25
HAU01027-1	G4	Nhi uu-838 (Hybrid variety)	Luong Son, Hoa Binh	2001/9/25
HAU01027-3	G3	Nhi uu-838 (Hybrid variety)	Luong Son, Hoa Binh	2001/9/25
HAU01030-1	G4	Khang dan (Improved variety)	Dong Anh, Ha Noi	2001/9/26
HAU01030-3	G3	Khang dan (Improved variety)	Dong Anh, Ha Noi	2001/9/26
HAU01041	G4	Tao phong hai (Hybrid variety)	Ha Tay	2001/9/25
HAU01043	G1	TN13-4 (Improved variety)	Ha Noi	2001/9/26
HAU02001	G4	Tap giao-1 (Hybrid variety)	Gia Lam, Ha Noi	2002/5/26
HAU02008-1	G3	Nhi uu-838 (Hybrid variety)	Quynh Giang, Quynh Luu, Nghe An	2002/8/29
HAU02008-3	G3	Nhi uu-838 (Hybrid variety)	Quynh Giang, Quynh Luu, Nghe An	2002/8/29
HAU02008-4	G3	Nhi uu-838 (Hybrid variety)	Quynh Giang, Quynh Luu, Nghe An	2002/8/29
HAU02009-1	G2	Tap giao-1 (Hybrid variety)	Quynh Giang, Quynh Luu, Nghe An	2002/8/29
HAU02009-2	G2	Tap giao-1 (Hybrid variety)	Quynh Giang, Quynh Luu, Nghe An	2002/8/29
HAU02009-3	G2	Tap giao-1 (Hybrid variety)	Quynh Giang, Quynh Luu, Nghe An	2002/8/29
HAU02009-4	G2	Tap giao-1 (Hybrid variety)	Quynh Giang, Quynh Luu, Nghe An	2002/8/29
HAU02010-1	G2	Nhi uu-838 (Hybrid variety)	Quynh Giang, Quynh Luu, Nghe An	2002/8/29
HAU02010-2	G2	Nhi uu-838 (Hybrid variety)	Quynh Giang, Quynh Luu, Nghe An	2002/8/29
HAU02010-3	G2	Nhi uu-838 (Hybrid variety)	Quynh Giang, Quynh Luu, Nghe An	2002/8/29
HAU02010-4	G2	Nhi uu-838 (Hybrid variety)	Quynh Giang, Quynh Luu, Nghe An	2002/8/29
HAU02012-1	G2	Nhi uu-838 (Hybrid variety)	Dien Hong, Dien Chau, Nghe An	2002/8/28
HAU02012-2	G2	Nhi uu-838 (Hybrid variety)	Dien Hong, Dien Chau, Nghe An	2002/8/28
HAU02012-3	G2	Nhi uu-838 (Hybrid variety)	Dien Hong, Dien Chau, Nghe An	2002/8/28
HAU02012-4	G2	Nhi uu-838 (Hybrid variety)	Dien Hong, Dien Chau, Nghe An	2002/8/28
HAU02013-1	G2	Khang dan (Improved variety)	Dien Ky, Dien Chau, Nghe An	2002/8/28
HAU02013-2	G2	Khang dan (Improved variety)	Dien Ky, Dien Chau, Nghe An	2002/8/28
HAU02013-3	G2	Khang dan (Improved variety)	Dien Ky, Dien Chau, Nghe An	2002/8/28
HAU02013-4	G2	Khang dan (Improved variety)	Dien Ky, Dien Chau, Nghe An	2002/8/28
HAU02014-1	G3	Nhi uu-838 (Hybrid variety)	Dien Ky, Dien Chau, Nghe An	2002/8/28
HAU02014-2	G3	Nhi uu-838 (Hybrid variety)	Dien Ky, Dien Chau, Nghe An	2002/8/28
HAU02014-3	G3	Nhi uu-838 (Hybrid variety)	Dien Ky, Dien Chau, Nghe An	2002/8/28
HAU02014-4	G3	Nhi uu-838 (Hybrid variety)	Dien Ky, Dien Chau, Nghe An	2002/8/28
HAU02017	G2	Hybrid-903 (Hybrid variety)	Ha Bi, Kim Boi, Hoa Binh	2002/10/12
HAU02018	G3	Q-5 (Improved variety)	Vinh Hong, Binh Giang, Hai Duong	2002/10/2
HAU02019-1	G1	Q-5 (Improved variety)	Vinh Hong, Binh Giang, Hai Duong	2002/10/2
HAU02019-2	G1	Q-5 (Improved variety)	Vinh Hong, Binh Giang, Hai Duong	2002/10/2
HAU02019-4	G1	Q-5 (Improved variety)	Vinh Hong, Binh Giang, Hai Duong	2002/10/2
HAU02020-1	G2	Nep thom (Improved variety)	Vinh Hong, Binh Giang, Hai Duong	2002/10/2
HAU02020-2	G2	Nep thom (Improved variety)	Vinh Hong, Binh Giang, Hai Duong	2002/10/2
HAU02020-3	G2	Nep thom (Improved variety)	Vinh Hong, Binh Giang, Hai Duong	2002/10/2
HAU02021-1	G3	Nep thom (Improved variety)	Vinh Hong, Binh Giang, Hai Duong	2002/10/2
HAU02021-2	G3	Nep thom (Improved variety)	Vinh Hong, Binh Giang, Hai Duong	2002/10/2
HAU02022-2	G3	Te do (Hybrid variety)	Vinh Hong, Binh Giang, Hai Duong	2002/10/2
HAU02024-1	G3	Khang dan (Improved variety)	Xuan Quan, Van Giang, Hung Yen	2002/10/2
HAU02024-2	G3	Khang dan (Improved variety)	Xuan Quan, Van Giang, Hung Yen	2002/10/2
HAU02024-6	G4	Khang dan (Improved variety)	Xuan Quan, Van Giang, Hung Yen	2002/10/2
HAU02024-7	G4	Khang dan (Improved variety)	Xuan Quan, Van Giang, Hung Yen	2002/10/2
HAU02026-2	G3	Boi tap son thanh (Hybrid variety)	An Dao, Phu Ninh, Phu Tho	2002/9/20
HAU02026-4	G3	Boi tap son thanh (Hybrid variety)	An Dao, Phu Ninh, Phu Tho	2002/9/20
HAU02026-5	G3	Boi tap son thanh (Hybrid variety)	An Dao, Phu Ninh, Phu Tho	2002/9/20
HAU02028-2	G2	Hybrid variety	Tuy Loc, Yen Bai	2002/9/18
HAU02028-3	G3	Hybrid variety	Tuy Loc, Yen Bai	2002/9/18
HAU02028-4	G3	Hybrid variety	Tuy Loc, Yen Bai	2002/9/18
HAU02028-5	G3	Hybrid variety	Tuy Loc, Yen Bai	2002/9/18

Table 1. (continued)

Strain	Race	Rice cultivar	Location (Commune, District, Province)	Date (year/month/date)
HAU02028-6	G3	Hybrid variety	Tuy Loc, Yen Bai	2002/9/18
HAU02028-8	G3	Hybrid variety	Tuy Loc, Yen Bai	2002/9/18
HAU02032-1	G2	Hybrid variety	Tuy Loc, Yen Bai	2002/9/18
HAU02032-2	G2	Hybrid variety	Thinh Hung, Yen Binh, Yen Bai	2002/9/18
HAU02032-3	G2	Hybrid variety	Thinh Hung, Yen Binh, Yen Bai	2002/9/18
HAU02034-1	G3	Nhi uu-838 (Hybrid variety)	Thinh Hung, Yen Binh, Yen Bai	2002/9/18
HAU02034-2	G3	Nhi uu-838 (Hybrid variety)	Cuong Thinh, Yen Bai	2002/9/17
HAU02034-3	G3	Nhi uu-838 (Hybrid variety)	Cuong Thinh, Yen Bai	2002/9/17
HAU02034-4	G3	Nhi uu-838 (Hybrid variety)	Cuong Thinh, Yen Bai	2002/9/17
HAU02034-5	G3	Nhi uu-838 (Hybrid variety)	Cuong Thinh, Yen Bai	2002/9/17
HAU02034-6	G3	Nhi uu-838 (Hybrid variety)	Cuong Thinh, Yen Bai	2002/9/17
HAU02034-7	G3	Nhi uu-838 (Hybrid variety)	Cuong Thinh, Yen Bai	2002/9/17
HAU02035-1	G2	Hybrid variety	Cuong Thinh, Yen Bai	2002/9/17
HAU02035-2	G2	Hybrid variety	Thuan Chau, Son La	2002/10/11
HAU02035-3	G2	Hybrid variety	Thuan Chau, Son La	2002/10/11
HAU02035-4	G2	Hybrid variety	Thuan Chau, Son La	2002/10/11
HAU02036-1	G2	Nep tan (Improved variety)	Thuan Chau, Son La	2002/10/11
HAU02036-2	G2	Nep tan (Improved variety)	Thuan Chau, Son La	2002/10/11
HAU02036-3	G2	Nep tan (Improved variety)	Thuan Chau, Son La	2002/10/11
HAU02036-4	G2	Nep tan (Improved variety)	Thuan Chau, Son La	2002/10/11
HAU02038-1	G2	San uu-63 (Hybrid variety)	Thuan Chau, Son La	2002/10/11
HAU02038-2	G2	San uu-63 (Hybrid variety)	Thuan Chau, Son La	2002/10/11
HAU02038-3	G2	San uu-63 (Hybrid variety)	Thuan Chau, Son La	2002/10/11
HAU02040-1	G2	San uu-63 (Hybrid variety)	Moc Chau- Son La	2002/10/10
HAU02041-2	G2	Nep tan (Improved variety)	Moc Chau- Son La	2002/10/10
HAU02041-3	G2	Nep tan (Improved variety)	Moc Chau- Son La	2002/10/10
HAU02042-1	G3	San uu-63 (Hybrid variety)	Moc Chau- Son La	2002/10/10
HAU02042-2	G3	San uu-63 (Hybrid variety)	Moc Chau- Son La	2002/10/10
HAU02042-3	G2	San uu-63 (Hybrid variety)	Moc Chau- Son La	2002/10/10
HAU02042-4	G3	San uu-63 (Hybrid variety)	Moc Chau- Son La	2002/10/10

Table 2. Pathogenicity analysis of the 84 strains of *Xanthomonas oryzae* pv. *oryzae* on the 11 differential varieties containing a single gene for resistance

Differentials variety	Gene involved	Reaction[1)] to race								
		G1		G2			G3			G4
IR24	*Xa16, Xa18*	S	S	S	S	S	S	S	S	S
IRBB1	*Xa1*	S	S	S	S	S	S	S	S	S
IRBB2	*Xa1, Xa2*	S	S	S	S	S	S	S	S	S
IRBB3	*Xa3*	R	M	S	S	S	S	S	S	S
IRBB4	*Xa4*	S	S	R	R	R	S	S	S	S
IRBB5	*xa5*	R	R	R	R	R	R	R	M	R
IRBB7	*Xa7*	R	R	R	R	M	R	R	R	S
IRBB10	*Xa10*	S	S	S	S	S	S	S	S	S
IRBB11	*Xa11*	S	S	S	S	S	S	S	S	S
IRBB21	*Xa21*	R	M	M	R	R	M	R	M	R
Taichung Native 1	*Xa14*	S	S	S	S	S	S	S	S	S
No. of strain		1	3	15	22	2	4	28	1	8

[1)] S: susceptible (lesion length > 15 cm; M: moderately susceptible to moderately resistant (lesion length between 5 and 15 cm); R: resistant (lesion length < 5 cm).

IRBB5, IRBB7 and IRBB21 was shown in 3, 1, 2 and 23 isolates, respectively. The isolates of *Xoo* showed varying virulence on the five near-isogenic lines with resistance genes *Xa3, Xa4, xa5, Xa7* and *Xa21.* Eighty six percent of the isolates were classified into two major groups G2 and G3. Seven isolates were selected as representative strains of four races.

3. Race distribution

As indicated in Tables 1 and 2, races G2 and G3, dominated and were distributed throughout northern Vietnam. Race G1, which was the prominent race throughout Vietnam as reported by Noda et al.[10], showed limited distribution and was only found in Hanoi and Hai Duong provinces in this study. Eight strains virulent to IRBB7 and belonging to G4 were distributed in Hai Duong, Hanoi, Hoa Binh, Ha Tay and Hung Yen provinces.

4. Resistance of Vietnamese hybrid varieties

Nine major hybrid varieties cultivated around Hanoi province were evaluated for their resistance to seven representative strains belonging to four *Xoo* races. As shown in Table 3, most of these varieties except Boi ta tap 49 to race G2 (HAU02009-2) and Boi tap son thanh to race G2 (strains HAU02009-2 and HAU02035-1) were susceptible or moderately susceptible to moderately resistant to all Vietnamese races. Representative strain HAU02024-6 belonging to race G4 was virulent to all hybrid varieties.

5. Resistance of Vietnamese improved varieties

Twelve Vietnamese improved varieties were examined for their resistance to Vietnamese races. As shown in Table 4, the improved varieties Cr-203 and Q5 were resistant to the predominant race G2. Of the 12 varieties, two, eight and six varieties stood out in terms of resistance to race G2 strains HAU02036-1, HAU02009-2 and HAU02035-1, respectively. However, all the improved varieties cultivated in northern Vietnam were susceptible or moderately susceptible to moderately resistant to race G3 and G4.

6. PCR analysis using IS repetitive gene primers

Seventy-six strains were examined for DNA fingerprinting analysis using the IS*1112* and IS*1113* repetitive elements as primers (J3 and JEL1/JEL2). Representative DNA fingerprints of different races by strains collected from different geographical locations in northern Vietnam were shown in Figs. 1 and 2. DNA fingerprinting of 76 strains showed distinct banding patterns, while DNA fragments comprising 15 to 30 bands were generated with IS*1112*- and IS*1113*-based PCR primers. There were 42 band positions scored in the PCR-based DNA fingerprints. The genetic relationships among strains and banding patterns were analyzed by cluster analysis, with two major genetic clusters detected (Ⅰ and Ⅱ, Fig. 3) and a weak correlation between cluster and geographic population observed (Fig. 4). For example, strains of G3 and cluster Ⅱ predominated in Yen Bai, while strains belong-

Table 3. Reaction of Vietnamese hybrid varieties to strains of *Xanthomonas oryzae* pv. *oryzae* collected in northern Vietnam

Hybrid variety	Reaction[1)] to race (representative strain)						
	G1	G2			G3		G4
	(HAU01043)	(HAU02036-1)	(HAU02009-2)	(HAU02035-1)	(HAU02021-2)	(HAU02034-6)	(HAU02024-6)
Nhi uu-63	M (8.4)	S (37.9)	S (30.2)	S (32.1)	S (24.1)	S (34.0)	S (27.1)
Nhi uu-838	M (13.3)	S (34.1)	S (33.4)	S (32.7)	S (33.7)	S (32.2)	S (27.8)
Nhi uu-32A	S (25.5)	S (32.3)	S (29.7)	S (29.8)	S (23.5)	S (23.5)	S (22.0)
Bac uu-903	M (5.8)	M (11.2)	M (8.5)	M (8.3)	S (20.1)	S (20.0)	S (20.2)
San uu-63	M (9.4)	S (33.0)	S (34.5)	S (35.4)	S (29.4)	S (27.7)	S (30.3)
Boi ta tap 49	S (29.1)	M (8.1)	R (3.8)	M (5.7)	S (20.8)	S (22.9)	S (33.1)
Boi tap son thanh	S (24.4)	M (7.2)	R (4.7)	R (4.0)	M (11.5)	M (9.9)	S (25.2)
D-uu 527	M (9.6)	S (33.9)	S (35.3)	S (35.7)	S (29.5)	S (29.8)	S (36.7)
Nong uu;28	S (19.1)	S (17.2)	M (9.1)	S (20.4)	S (16.1)	S (20.8)	S (28.4)
IR24 (Susceptibility check)	S (21.6)	S (29.2)	S (29.2)	S (32.4)	S (25.9)	S (25.9)	S (29.8)

[1)] S: susceptible; M: moderately susceptible to moderately resistant; R: resistant.
Numbers in parentheses indicate lesion length (cm).

Table 4. Reaction of Vietnamese improved varieties by conventional breeding to strains of *Xanthomonas oryzae* pv. *oryzae* collected in northern Vietnam

Improved variety	Reaction[1)] to race (representative strain)						
	G1	G2			G3		G4
	(HAU01043)	(HAU02036-1)	(HAU02009-2)	(HAU02035-1)	(HAU02021-2)	(HAU02034-6)	(HAU02024-6)
C-70	S (26.7)	M (13.8)	R (4.9)	S (15.9)	S (26.8)	S (20.8)	S (29.8)
C-71	S (28.9)	M (7.7)	M (5.8)	M (9.0)	S (21.4)	S (18.8)	S (32.6)
Xi-23	S (21.9)	M (5.4)	R (3.1)	R (4.4)	S (15.3)	S (18.9)	S (22.7)
Cr-203	S (16.8)	R (4.3)	R (2.1)	R (2.6)	M (11.8)	M (12.9)	S (17.5)
Q5	S (17.0)	R (3.7)	R (2.0)	R (1.7)	M (5.2)	M (6.1)	S (18.1)
Khang dan	M (11.0)	M (6.8)	R (3.5)	R (3.4)	M (11.4)	M (9.6)	S (23.4)
TN 13-5	S (28.4)	M (11.3)	R (4.8)	M (6.6)	S (20.0)	S (22.9)	S (31.6)
Huong thom 1	S (34.1)	M (8.3)	R (2.7)	R (4.4)	S (19.0)	S (18.0)	S (28.5)
DT10	S (20.5)	M (9.3)	R (2.1)	R (3.5)	S (16.8)	M (12.8)	S (20.0)
TK-90	M (12.4)	S (21.9)	S (20.6)	S (18.5)	S (16.7)	S (15.9)	M (13.2)
Nep cai hoa vang	R (2.8)	S (17.0)	S (16.0)	M (14.3)	M (13.3)	S (19.1)	M (13.7)
Nep hoa vang	R (3.8)	S (28.6)	S (26.3)	S (19.9)	S (18.9)	S (18.3)	S (22.5)
IR24 (Susceptibility check)	S (29.7)	S (32.7)	S (28.9)	S (26.0)	S (31.8)	S (28.8)	S (36.5)

[1)] S: susceptible; M: moderately susceptible to moderately resistant; R: resistant.
Numbers in parentheses indicate lesion length (cm).

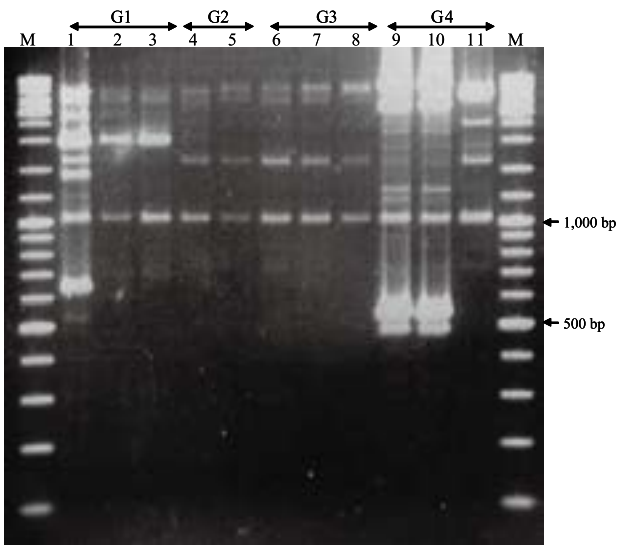

Fig. 1. DNA fingerprints of *Xanthomonas oryzae* pv. *oryzae* strains using polymerase chain reaction with IS primer J3

Lane 1 to 11; HAU01043, HAU02019-1, HAU02019-2, HAU02009-1, HAU02028-2, HAU02021-2, HAU01030-3, HAU02026-5, HAU01008-1, HAU01016, HAU01027-1. The DNA molecular marker (100 bp ladd er) are on the lane labeled M.

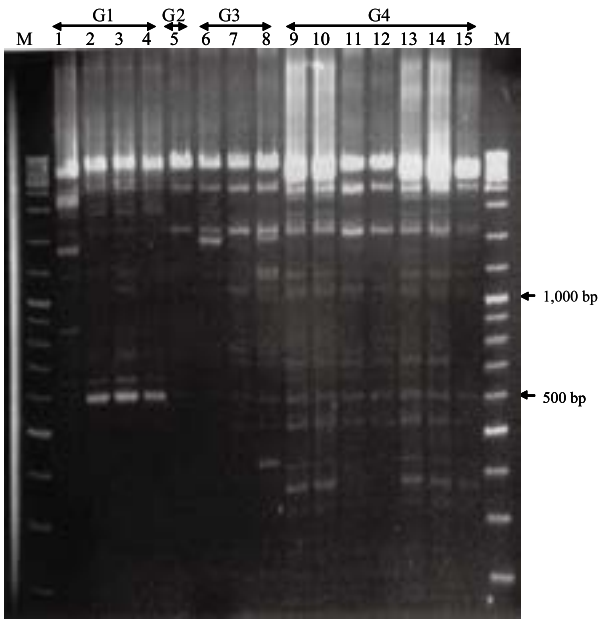

Fig. 2. DNA fingerprints of *Xanthomonas oryzae* pv. *oryzae* strains using polymerase chain reaction with IS primers JEL1 and JEL2

Lane 1 to 15; HAU01043, HAU02019-1, HAU02019-2, HAU02019-4, HAU02028-2, HAU02026-5, HAU02021-2, HAU01030-3, HAU01008-1, HAU01016, HAU01027-1, HAU01030-1, HAU02001, HAU02024-6, HAU02024-7. The DNA molecular marker (100 bp ladder) are on the lane labeled M.

ing to G2 and cluster I predominated in Son La and Nghe An.

Discussion

We evaluated the pathogenicity in the *Xoo* population in northern Vietnam as an initial step to understanding the pathogen population structure. Vietnamese strains were divided into 4 races based on their virulence to six different varieties, IR24, IRBB3, IRBB4, IRBB5, IRBB7 and IRBB21. It was revealed that the northern Vietnam strains comprised two major races (G2 and G3). In this study, we observed that some differential varieties, IRBB5 (*xa5*), IRBB 7(*Xa7*) and IRBB21 (*Xa21*) were re-

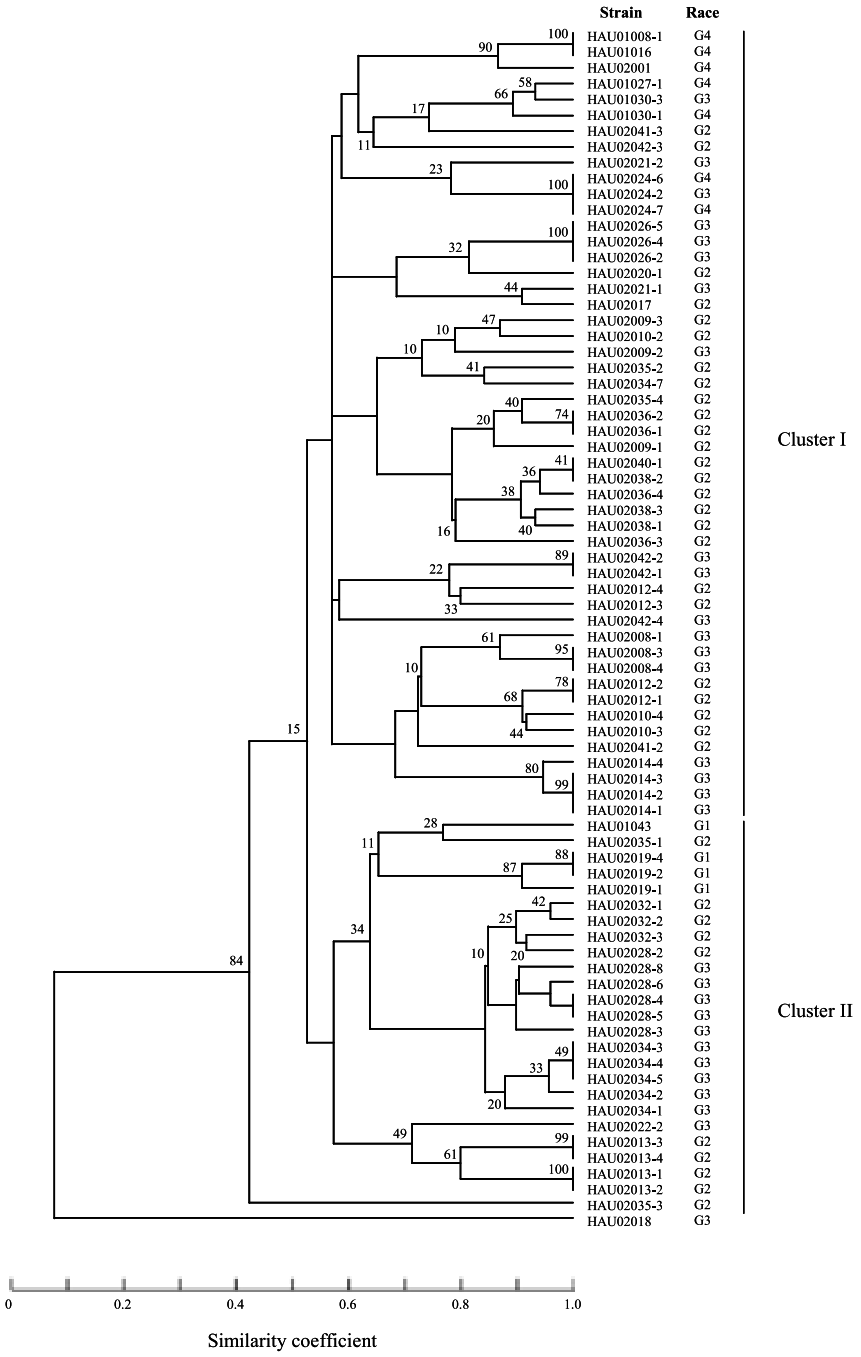

Fig. 3. Genetic diversity of Vietnamese strains (races) of *Xanthomonas oryzae* pv. *oryzae* on the basis of PCR amplification with IS-PCR primers J3 and JEL1/JEL2

A similarity coefficient was calculated based on DNA fingerprints and using the Dice coefficient[4]. A dendogram was constructed using the unweighted pair group method, with arithmetic average clustering. Values on the branches represent the results of bootstrap analysis with 1,000 iterations. Strains, races and cluster are indicated on the right.

sistant to most of the strains tested. Therefore, a breeding program should be initiated to transfer these resistant genes from differential varieties to high quality varieties of Vietnamese rice to control the disease effectively. Lee et al.[9] and Adhikari et al.[2] showed that strains virulent to *Xa21* gene were widely distributed in Korea and Nepal. Several strains moderately virulent to *Xa21* (of lesion length between 10 and 15 cm) were detected in our limited sample range from northern Vietnam. If *Xa21* becomes useful in the breeding program, a better understanding of the diversity of the *Xoo* population will be required.

Xoo strains isolated mainly from the Mekong delta (southern Vietnam) were evaluated for virulence on a series of 11 near-isogenic lines and seven additional varieties, with 6 races identified by Noda et al.[10]. Our results showed that predominant races between northern and southern Vietnam represented distinct populations of *Xoo*, indicating substantial geographical differentiation between the pathogen populations in different agroecosystems. The pathotype diversity was influenced by the collection site and cultivars sampled. Moreover, the diversity in Vietnam may reflect the relatively broad range of environmental conditions under which rice is cultivated and the wide use of diverse traditional rice cultivars. Furthermore, the improved varieties showed diverse reactions to strains of race G2, suggesting the diverse genetic background of these varieties widely grown in Vietnam. It has been suggested that cultivar differences exerted a strong influence on the *Xoo* pathogen population structure. Systematic sampling of *Xoo* populations within the various regions should be required to determine whether or not the differences in pathogen diversity suggested by these results reflect real differences in diversity among regions.

As PCR analysis using IS*1112* and IS*1113* primers reveals numerous bands per strain, it provides useful information to assess genetic relationships in the *Xoo* population. Based on cluster analysis derived from DNA fragments by PCR-based assays, the Vietnamese strains broadly comprised two different subpopulations, and a partial association was found between phylogenic groups and pathotypes. Adhikari et al.[3] observed a partial relationship between the groups determined by the PCR-based assays and virulence, but molecular polymorphism was largely independent of virulence. The transposable element used in these studies was assumed to be phenotypically neutral and suitable for phylogenetic analysis. Adhikari et al.[2,3] and George et al.[6] also found evidence of the geographic migration of *Xoo* using multicopy genetic markers. They suggested that the distribution might be through germplasm exchange. This assumption is reasonable, especially in the case of the Vietnamese race G4, which was distributed within a limited area of northern Vietnam and all G4 strains were grouped into cluster I. To understand the evolution and differentiation of pathogens, further research is necessary.

In conclusion, our study revealed the existence of a

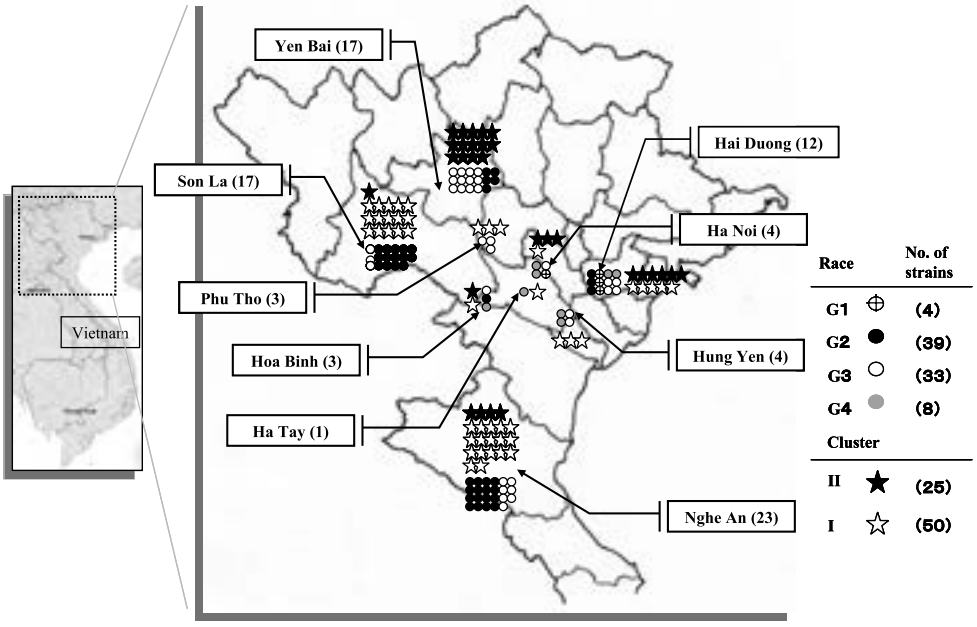

Fig. 4. Relationship between geographical distribution of race and genetic cluster of *Xanthomonas oryzae* pv. *oryzae* in northern Vietnam

Number in parentheses indicate the number of *X. oryzae* pv. *oryzae* strains.

diverse population of *Xoo* in northern Vietnam. It appears that genes may exist in improved varieties that differ from BBNILs. It also provides the foundation to monitor predominant strains in different target regions for the strategic deployment of resistance genes utilized in the breeding program. The identification of useful resistance genes through virulence analysis will support a gene deployment approach to managing the disease using resistant cultivars.

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