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Pathogenicity of Rice Blast (*Pyricularia oryzae* Cavara) Isolates from Mekong River Delta, Vietnam

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Abstract

A total of 94 blast isolates were collected from five provinces in the Mekong River Delta in southern Vietnam. The pathogenicities of these isolates were evaluated using 25 international differential varieties (DVs) covering 23 resistance genes and a susceptible Chinese cultivar, Lijiangxintuanheigu (LTH). Based on the reaction patterns of the DVs, the isolates were classified into two clusters (I and II). Isolates virulent towards the DVs for *Pit*, *Piz-t*, *Pi19*(t), and *Pita* were more frequent in cluster I than in cluster II. Isolates virulent towards the DVs for *Pi3*, *Pi5*(t), and *Pita-2* were more frequent in cluster II than in cluster I. Differences were also found in their geographical distributions, with isolates of cluster I dominant in the southern region of the Mekong River Delta and those of cluster II dominant in the northern region. Finally, the blast isolates were classified into 67 races. Based on these results and information on donor rice cultivars, the relationships between blast races and cultivated rice varieties were discussed. This information will be useful for understanding the variation of blast races distributed along the Mekong River in the countries of Southeast Asia.

Discipline: Agricultural Environment
Additional key words: variation, race, rice (*Oryza sativa* L.), differential system

Introduction

Rice blast caused by the pathogen *Pyricularia oryzae* Cavara is one of the most serious diseases affecting rice (*Oryza sativa* L.) worldwide (Zeigler et al. 1994). The use of resistant cultivars is the most economical method of controlling blast disease in rice. However, using such cultivars is effective for only a limited time due to the breakdown of resistance genes as more virulent blast races occur. The interaction between host resistance and blast fungus virulence can be explained by the gene-for-gene theory: every resistance gene in the host corresponds to an avirulence gene in the pathogen (Flor 1971, Silue et al. 1992). Based on this theory, differential varieties (DVs) have been developed that can distinguish pathotypes (races) by the patterns of reaction to each strain of pathogen, and which can be used to identify blast pathogen population structures.

The Mekong River Delta is a major agricultural region in Vietnam, and rice production in this region has increased rapidly due to the development of new cultural practices and the expansion of irrigation systems (Sang et al. 1996). More than 70% of the rice cultivars presently grown in Vietnam are new high-yielding, short-duration

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cultivars introduced by the International Rice Research Institute (IRRI), or are cultivars with genes sourced from the IRRI gene bank. However, the intensive application of high-density, direct seeding and the high input of nitrogen fertilizers have provided favorable conditions for rice diseases. After the introduction of IRRI cultivars, blast has subsequently become a major rice disease in the Mekong River Delta, affecting an increasingly large area, and since the early 1980s has often caused severe damage to rice production (Sang et al. 1996).

Don et al. (1999) reported that the blast races between the Red River Delta in northern Vietnam and the Mekong River Delta in southern Vietnam were different, and that the genetic variation of the Mekong River Delta was limited compared with that of the Red River Delta, based on the lineage and pathogenicity analyses using nine Japanese DVs by Yamada et al. (1976). Blast races of the Mekong River Delta showed high frequencies of virulence to DVs for *Pik-s*, *Pia*, and *Pita*. Noda et al. (1999) clarified the pathogenicities of 129 blast isolates from rice collected mainly in the Mekong River Delta in 1995 and 1996 by using 12 Japanese DVs selected by Yamada et al. (1976) and Kiyosawa (1984). Two DVs—Aichi-asahi for resistance gene *Pia* and K59 for *Pit*—were susceptible to 93.8% and 86.0% of the isolates, respectively. Those results indicate that blast isolates from the Mekong River Delta area in southern Vietnam were mainly virulent to *Pia*, *Pit*, *Pik-s*, and *Pita*. Conversely, none of the isolates were found to be virulent to the DVs, Shin 2, (harboring *Pik-s* and *Pish*), Kusabue (*Pik*, *Pish*), Fukunishiki (*Piz*, *Pish*), Pi No. 4 (*Pita-2*, *Pish*), BL1 (*Pib*, *Pish*), Toride 1 (*Piz-t*), or K60 (*Pik-p*). The reference rice line AA/S2-3 that carries only one resistance gene (*Pish*) was also found to be resistant to all of the isolates, but another line (AA/S2-75 carrying only *Pik-s*) was susceptible to 95.3% of the isolates. Therefore, the reactions of resistance genes other than *Pish* in Shin 2, Kusabue, Fukunishiki, Pi No. 4, and BL1 were masked by *Pish*. Those results suggest that it was difficult to classify in detail the pathogenic specialization of blast isolates from the Mekong River Delta area by using the Japanese DVs, and that the analyses by Don et al. (1999) and Noda et al. (1999) were incomplete.

Tsunematsu et al. (2000) and Kobayashi et al. (2007) developed 25 monogenic lines as a set of international DVs targeting 23 kinds of resistance genes: *Pish*, *Pib*, *Pit*, *Pia*, *Pii*, *Pi3*, *Pi5*(t), *Pik-s*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, *Pi7*(t), *Pi9*(t), *Piz*, *Piz-5*, *Piz-t*, *Pita-2*, *Pita*, *Pi12*(t), *Pi19*(t), and *Pi20*(t). Those monogenic lines were produced by introducing single resistance genes into the genetic background of a Chinese Japonica Group rice cultivar—LTH. Telebanco-Yanoria et al. (2010) bred near-isogenic lines of LTH (LTH NILs) as advanced DVs targeting 11 resistance genes: *Pib*, *Piz-5*, *Pi9*(t), *Pi3*, *Pia*, *Pik-s*, *Pik*, *Pik-h*, *Pi7*(t), *Pita*, and *Pita-2*. The monogenic lines and LTH NILs are minimally influenced by genetic background, and are the most effective materials for use as international DVs. Hayashi & Fukuta (2009) proposed a new international system for the designation of virulent blast isolates using monogenic lines. This system designates the blast isolates by the reaction patterns of LTH and 25 monogenic lines targeting the 23 resistance genes. Using this set of common international DVs and the new designation system, the pathogenicities of blast isolates have been evaluated and the differentiation of blast races clarified in West Africa (Odjo et al. 2014), Cambodia (Fukuta et al. 2014), Japan (Kawasaki-Tanaka et al. 2016), and Bangladesh (Khan et al. 2016). Evaluation using the set of common DVs and the common system of characterization of blast isolates allows a comparison of information from different countries or regions.

In the current study, the pathogenicity of newly collected blast isolates from the Mekong River Delta, southern Vietnam, was analyzed by using the new set of international DVs developed by Tsunematsu et al. (2000), Kobayashi et al. (2007), and Telebanco-Yanoria et al. (2010), as well as the designation system by Hayashi & Fukuta (2009). Differentiations of blast races in this region are also discussed.

Materials and methods

1. Sampling of infection rice plants and single spore isolation

A total of 94 blast isolates (*Pyricularia oryzae*) were collected in 2007 and 2013 from the infected leaves and panicles of rice (*Oryza sativa*) cultivars. Sampling was conducted in irrigated lowland fields in five provinces in the Mekong River Delta area: Dong Thap, Long An (and Ho Chi Minh City), Vinh Long, Tra Vinh, and Bac Lieu (Supplemental Table 1). One infected rice plant was sampled from each paddy field. Rice is cultivated three times per year: in the early wet season from March to June, the late wet season from July to October, and the dry season from November to February. Two rice cultivations with intercrops such as soybean or mung bean are also shown. In this case, rice is cultivated from October to January and from May to November, and the intercrop is from January to March. Single crop cultivation from August to February is only carried out using landraces in a saline area. This means that rice is cultivated in the Mekong River Delta throughout the

year, except in the saline area. Farmers used 10-15 rice cultivars per season, such as IR 50404, Jasmine 85, OM 576, OM 2514, OM 2517, OM 2518, OM 4900, OM 5451, OM 5629, OM 6162, OM 7347, OM 8108, OM Cs2000, RVT, and VND95-20, which are improved and short-duration types developed using or including IRRI cultivars as the parents. The landraces in the saline area are Mot Bui Do, Tai Nguyen, Nep ba Bong, Doc Phung, and Trang Chum. The blast samplings were mainly conducted in the dry seasons of 2007 and 2010, and in the early wet season of 2013.

Single spores were isolated from the infected leaves or panicles incubated on moist filter paper in a petri dish at room temperature for 24 h, as per the protocols of Hayashi et al. (2009). Colonies from single conidia were grown on water agar for a period of five to seven days, and then two to three plugs were cut and transferred to the center of a fresh rice straw agar plate with sterile filter paper on its surface. Finally, the fungus was grown on top of the filter paper and after any necessary aseptically drying, the filter paper was stored at −20°C for repeated access to the original isolates. The isolate can be conserved semi-permanently in this condition.

2. Inoculation

To produce inocula, stock isolates stored on the filter paper discs were cultured on oatmeal agar medium. The inoculated plates were incubated at 25°C to 28°C for 12 to 14 days. The culture was scraped with a sterilized toothbrush, and the plates were exposed to continuous light for four to five days to induce heavy sporulation. Conidia were dislodged from the incubated plates into sterilized distilled water containing 0.01% Tween 20 by gently rubbing with a paintbrush. Spore suspensions were filtered through four layers of gauze mesh and the concentration was adjusted to 1×10^5 conidia per ml by using a hemocytometer (Hayashi et al. 1998).

Inoculation was carried out using three rice plants following the methods of Bonman et al. (1986) and Hayashi et al. (2009). Seedling cell trays (with cells 16 mm in diameter × 25-mm deep, in a 5×7 array) containing 20- to 21-day-old seedlings (approximately 4- to 5-leaf stage) of the 25 DVs and LTH were placed on a rotating plate, and the seedlings were sprayed uniformly with 10 ml of spore suspension per tray by using an electric atomizer (0.3-mm spray nozzle; Airtex, Japan) at 0.1 MPa. Inoculated seedlings were incubated in a dew chamber at 25°C for 20 h, and then transferred to a greenhouse maintained at a temperature of 25 ± 1°C and relative humidity of 70% to 80%.

3. Estimation of infection degree and race designation

At seven days after inoculation, the degree of infection of each DV and LTH by each blast isolate was evaluated by using a six-point rating scale (from 0 to 5), where 0 = no evidence of infection; 1 = brown specks smaller than 0.5 mm in diameter, and no sporulation; 2 = brown specks about 0.5 to 1 mm in diameter, and no sporulation; 3 = roundish to elliptical lesions about 1 to 3 mm in diameter with a gray center surrounded by brown margins; lesions capable of sporulation; 4 = typical spindle-shaped blast lesions capable of sporulation, 3 mm or longer with necrotic gray centers and water-soaked or reddish brown margins, and little or no coalescence of lesions; and 5 = lesions as in 4, but with about half of one or two leaf blades killed by the coalescence of lesions. The reactions of the plant were further categorized, with plants showing infection scores of 0 to 2 as being resistant, and plants showing infection scores of 3 to 5 as being susceptible in most cases (Hayashi & Fukuta 2009). The only exceptions were DV IRBL5-M, with an infection score between 2 to 5 being categorized as susceptible, and DVs IRBLsh-B and IRBLta2-Pi, with infection scores from 4 to 5 being categorized as susceptible, according to the method of Hayashi & Fukuta (2009). This is because the ranges of resistant reactions in IRBL5-M, IRBLsh-B, and IRBLta2-Pi are changed and different from the other DVs. The blast isolate was categorized into virulence or avirulence based on the score in each DV and LTH, and the frequency of virulence among all isolates was calculated. Two duplications were performed to evaluate the degree of infection in a differential variety, and the mean was used as the representative value in each.

The designation of blast isolate into race was determined as per the new international designation system proposed by Hayashi & Fukuta (2009) using the reaction patterns of the 25 DVs, including monogenic lines by Tsunematsu et al. (2000), LTH NILs by Telebanco-Yanoria et al. (2010), and LTH. These DVs plus LTH were divided into five groups (“U”, “i”, “k”, “z”, and “ta”) (Table 2). The “U” group includes the four DVs for *Pish*, *Pib*, *Pit*, and *Pia*, and LTH; “i” includes the three DVs for *Pii*, *Pi3*, and *Pi5*(t) in the *Pii* locus on chromosome 9; “k” includes the seven DVs for *Pik-s*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, and *Pi7*(t) with the *Pik* region on chromosome 11; “z” includes the four DVs for *Pi9*(t), *Piz*, *Piz-5*, and *Piz-t* in the *Piz* region on chromosome 6; and “ta” includes the seven DVs for *Pita-2* (two lines), *Pi12*(t), *Pita*(two lines), *Pi19*(t), and *Pi20*(t) in the *Pita* region on chromosome 12.

Each of the four DV groups "i", "k", "z", and "ta" includes multiple alleles or resistance genes located in the same chromosome regions. The other four resistance genes (*Pia* on chromosome 11, *Pish* and *Pit* on chromosome 1, and *Pib* on chromosome 2) located independently in different chromosome regions and LTH as the susceptible control are gathered in group "U". In this study, the two monogenic lines (IRBLkh-K3 and IRBLk-Ka) in Hayashi & Fukuta (2009) were replaced by two LTH NILs (IRBLkh-K3[LT] and IRBLk-Ka[LT]), respectively. Each DV group consisted of some unit(s) (1-3), and up to three DVs (genes) were allocated in each unit. We applied codes 1, 2, and 4 to the susceptible reactions of each of the three respective varieties to the blast isolates. Blast races were designated by the combined sum of codes representing the reactions of the three varieties in each unit, using Gilmour's method (Gilmour 1973). Isolates classified this way were designated as "reaction type" within each cultivar unit and as "races" using the set of all five reaction types.

4. Classification of blast isolates and genetic diversity

Cluster analysis was performed using Ward's hierarchical method (Ward 1963) based on the data of infection scores of the 25 DVs and LTH by blast isolates, using JMP 11.2 (JMP 11.2 for Windows, 2014; SAS Institute, Inc., Cary, NC, USA). The relationships between the pathogenicity of the cluster groups, and the ecosystems and geographical distributions were evaluated.

The diversity of the pathogens was calculated using Simpson's diversity index (Simpson 1949). The index value varies from 0 to 1, where 0 denotes no diversity and 1 denotes maximum diversity.

Results

1. Variation of virulent blast isolates

The pathogenicities of 94 blast isolates that were collected from the Mekong River Delta in southern Vietnam were investigated. These were sampled from five provinces: Dong Thap (three blast isolates), Long An (and Ho Chi Minh City) (38), Vinh Long (16), Tra Vinh (11), and Bac Lieu (13). A total of 63 and nine blast isolates were collected in the dry seasons of 2007 and 2010, respectively, and 18 were collected in the early wet season of 2013. There were no records on the sampling dates for the other four isolates. These pathogenicities were investigated based on the reactions of 25 DVs, including 23 monogenic lines (Tsunematsu et al. 2000), two LTH NILs (Telebanco-Yanoria et al. 2010), and the susceptible control cultivar LTH (Fig. 1).

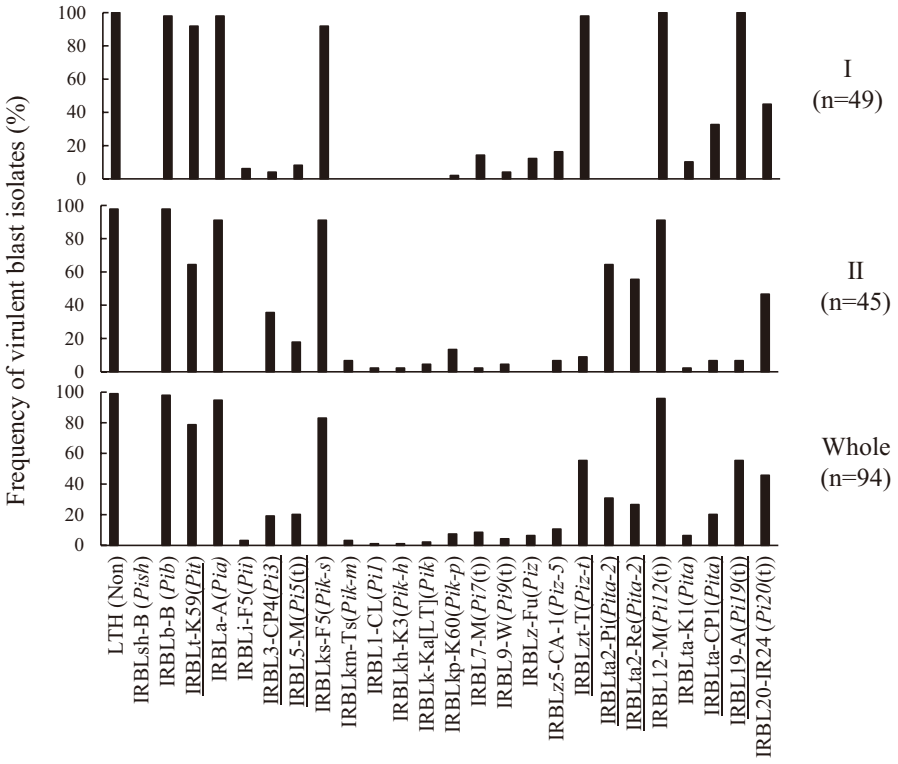

Fig. 1. Frequencies of virulent blast isolates to differential varieties and susceptible variety, LTH

A wide variation was found in the pathogenicities of the blast isolates. Around 70% of the blast isolates were virulent to five DVs for *Pib*, *Pit*, *Pia*, *Pik-s*, and *Pi12* (t), and fewer than 20% of the isolates were virulent to 13 DVs for *Pish*, *Pii*, *Pi3*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, *Pi7*(t), *Pi9*(t), *Piz*, *Piz-5*, and *Pita* (IRBLta-K1). The other seven DVs for *Pi5*(t), *Piz-t*, *Pita-2* (two lines), *Pita* (IRBLta-CP1), *Pi19*(t), and *Pi20*(t) varied from 20% to 70% (Fig. 1).

2. Classification of blast isolates based on the reaction patterns of DVs

Blast isolates were classified by cluster analysis with Ward's hierarchical method into two groups (cluster I and cluster II), based on the reaction patterns of DVs. Cluster I included 49 isolates (52.1% of the total), and cluster II included 45 isolates (47.9%) (Table 1 and Supplemental Fig. 1).

The frequency of isolates virulent towards eight DVs for *Pit*, *Pi3*, *Pi5*(t), *Piz-t*, *Pi19*(t), *Pita* (IRBLta-CP1), and *Pita-2* (two lines) differed remarkably between clusters I and II (Fig. 1). The frequency of isolates virulent towards DVs for *Pit, Piz-t*, *Pi19*(t), and *Pita* were higher in cluster I than in cluster II. In contrast, the frequencies of isolates virulent towards *Pi3*, *Pi5*(t), and *Pita-2* (two lines) were lower in cluster I than in cluster II.

These frequencies of blast isolates in clusters I and II were always around 50% in the three samplings of 2007, 2010, and 2013, and there was no marked difference (Supplemental Table 1). These results suggest that the variations of blast races did not change dramatically over time.

3. Geographical distribution of blast isolates in cluster groups

The frequencies of blast isolates in cluster groups varied among the five provinces in the Mekong River Delta (Don Thap, Long An (and Ho Chi Minh City), Vinh Long, Tra Vinh, and Bac Lieu) where we collected the blast samples. The blast isolates of cluster I were distributed dominantly in the two provinces of Tra Vinh and Bac Lieu in the southern part of the delta, and the blast isolates of cluster II were distributed dominantly in the three provinces of Dong Thap, Long An (and Ho Chi Minh City), and Vinh Long in the northern part of the delta (Fig. 2).

4. Dominant reaction types of blast isolates

Differences in the frequencies of reaction types, categorized based on the virulence to subsets of DVs, were found between clusters I and II (Table 2).

In the "U" group, seven reaction types (U03, U21, U23, U43, U60, U61, and U63) were found among the 94 blast isolates. Among these reaction types, two were dominant—U23 and U63. Few blast isolates were categorized into the other five reaction types. Specifically, U23 was virulent to the two DVs for *Pib* and *Pa*, and to LTH, and U63 was virulent to the three

Table 1. Blast isolates collected from southern Vietnam and the classification thereof

Cluster group	No. of blast isolates (%)											
	Rice variety name as the host plant											Total
	IR 50404	Jasmine 85	OM 2514	OM 576	VN 95-20	VND 95-20	OM 2517	OM 2717	OM 2718	OM 3242	Unknown	
I	6 (6.4)	0 (0.0)	2 (2.1)	9 (9.6)	2 (2.1)	3 (3.2)	0 (0.0)	1 (1.1)	1 (1.1)	2 (2.1)	23 (24.5)	49 (52.1)
II	10 (10.6)	1 (1.1)	1 (1.1)	3 (3.2)	5 (5.3)	8 (8.5)	1 (1.1)	2 (2.1)	0 (0.0)	0 (0.0)	14 (14.9)	45 (47.9)
Total	16 (17.0)	1 (1.1)	3 (3.2)	12 (12.8)	7 (7.4)	11 (11.7)	1 (1.1)	3 (3.2)	1 (1.1)	2 (2.1)	37 (39.4)	94 (100.0)

A total of 94 blast isolates were collected from 11 rice varieties in five provinces in southern Vietnam.
Blast isolates were classified into cluster groups I and II, based on the reaction patterns of monogenic lines (Tsunematsu et al. 2000) and near-isogenic lines of LTH (Telebanco-Yanoria et al. 2010) as the differential varieties for blast resistance genes.
Cluster analysis was conducted using Ward's hierarchical clustering method.
Each blast sample was collected from a different paddy field.

DVs for *Pib*, *Pit*, and *Pia*, and to LTH. The frequency of blast isolates for U23 in cluster II was higher than that in cluster I, and the frequency of U63 in cluster I was higher than that in cluster II. These results indicate that the reaction of the DV for *Pit* differentiated cluster I from cluster II. The values of Simpson's diversity index (Simpson 1949) for cluster I, cluster II, and the entire group were 0.22, 0.57, and 0.43, respectively.

In the "i" group, six reaction types (i0, i2, i4, i5, i6, and i7) were found. A total of 73 isolates were categorized into reaction type i0, avirulent to three DVs for *Pii*, *Pi3*, and *Pi5*(t), with 46 isolates found in cluster I and 27 isolates found in cluster II. The blast isolates of reaction type i0 were mainly included in cluster I. In cluster II, seven isolates of i2 virulent to the DV for *Pi3* and eight isolates of i6 virulent to the DVs for *Pi3* and *Pi5*(t) were also included. The remaining six blast isolates were categorized into the other three reaction types (i4, i5, and i7). The values of Simpson's diversity index for cluster I, cluster II, and the entire group were 0.12, 0.58, and 0.38, respectively. These results indicate that reactions in DVs for *Pi3* and *Pi5*(t) were divided between clusters I and II.

In the "k" group, eight reaction types (k000, k004, k010, k100, k102, k104, k111, and k177) were found. A total of 16 blast isolates were categorized into k000 and 63 were categorized into k100 as the dominant reaction types. The frequency of k000 was higher in cluster II than in cluster I. Each of the other reaction types had less than six isolates. The values of Simpson's diversity index for cluster I, cluster II, and the entire group of blast isolates were 0.41, 0.59, and 0.52, respectively. These results indicate that many blast isolates were virulent only to *Pik-s*, and there was no major difference between clusters I and II in the virulence genes of blast isolates to the *Pik* allele resistance genes.

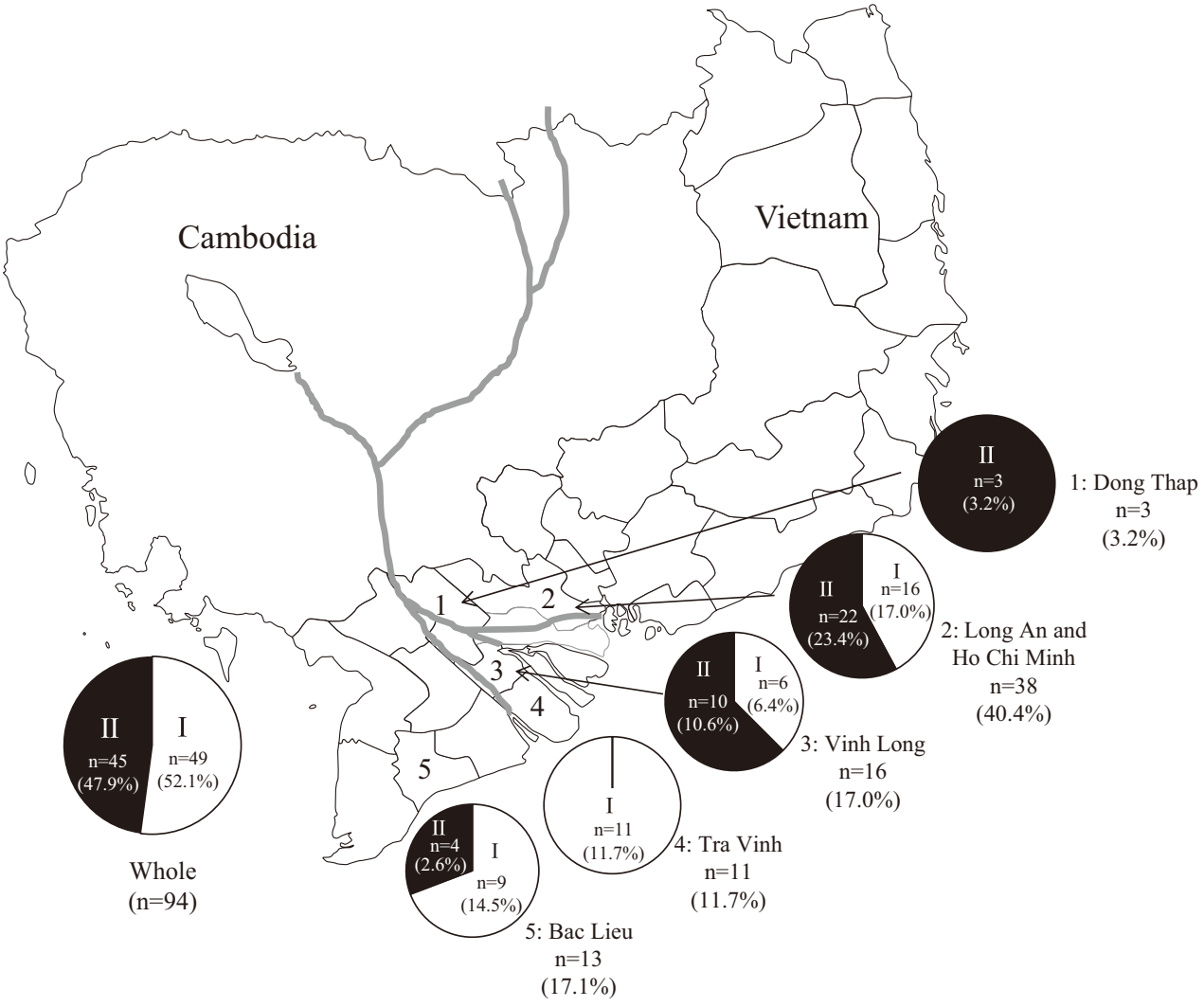

Fig. 2. Distributions of blast isolates classified into two cluster groups in southern Vietnam

Table 2. Number of blast isolates in each reaction type categorized based on the reactions of 25 differential varieties and LTH

Reactions to DV										No. of virulent isolates (%)		
Resistance gene in DV										Cluster		
Reaction type in "U" group	*Pish*	*Pib*	*Pit*	LTH	*Pia*	-	-	-	-	I	II	Total
U23	a	v	a	v	v	-	-	-	-	3 (17.6)	14 (82.4)	17 (100.0)
U63	a	v	v	v	v	-	-	-	-	43 (62.3)	26 (37.7)	69 (100.0)
Othres	(U03,U21,U43,U60,U61)									3 (37.5)	5 (62.5)	8 (100.0)
Total										49 (52.1)	45 (47.9)	94 (100.0)
Diversity index										0.22	0.57	0.43

Reaction type in "I" group	*Pii*	*Pi3*	*Pi5*(t)	-	-	-	-	-	-	I	II	Total
i0	a	a	a	-	-	-	-	-	-	46 (63.0)	27 (37.0)	73 (100.0)
i2	a	v	a	-	-	-	-	-	-	0 (0.0)	7 (100.0)	7 (100.0)
i6	a	v	v	-	-	-	-	-	-	0 (0.0)	8 (100.0)	8 (100.0)
Others	(i4,i5,i7)									3 (50.0)	3 (50.0)	6 (100.0)
Total										49 (52.1)	45 (47.9)	94 (100.0)
Diversity index										0.12	0.58	0.38

Reaction type in "k" group	*Pik-s*	-	-	*Pik-m*	*Pi1*	*Pik-h*	*Pik*	*Pik-p*	*Pi7*(t)	I	II	Total
k000	a	-	-	a	a	a	a	a	a	4 (25.0)	12 (75.0)	16 (100.0)
k100	v	-	-	a	a	a	a	a	a	37 (58.7)	26 (41.3)	63 (100.0)
Others	(k004,k010,k102,k104,k111,k177)									8 (53.3)	7 (46.7)	15 (100.0)
Total										49 (52.1)	45 (47.9)	94 (100.0)
Diversity index										0.41	0.59	0.52

Reaction type in "z" group	*Pi9*(t)	-	-	*Piz*	*Piz-5*	*Piz-t*	-	-	-	I	II	Total
z00	a	-	-	a	a	a	-	-	-	1 (2.6)	38 (97.4)	39 (100.0)
z04	a	-	-	a	a	v	-	-	-	38 (90.5)	4 (9.5)	42 (100.0)
Others	(z02, z05,z06, z07, z12, z14)									10 (76.9)	3 (23.1)	13 (100.0)
Total										49 (52.1)	45 (47.9)	94 (100.0)
Diversity index										0.39	0.28	0.62

Reaction type in "ta" group	*Pita-2* (Re)	*Pita-2* (Pi)	*Pi12*(t)	*Pita* K1	*Pita* CP1	-	*Pi19*(t)	*Pi20*(t)	-	I	II	Total
ta401	a	a	v	a	a	-	v	a	-	12 (92.3)	1 (7.7)	13 (100.0)
ta403	a	a	v	a	a	-	v	v	-	20 (100.0)	0 (0.0)	20 (100.0)
ta421	a	a	v	a	v	-	v	a	-	13 (81.3)	3 (18.7)	16 (100.0)
ta700	v	v	v	a	a	-	a	a	-	0 (0.0)	12 (100.0)	12 (100.0)
ta702	v	v	v	a	a	-	a	v	-	0 (0.0)	10 (100.0)	10 (100.0)
Others	(ta000,ta002,ta402,ta431,ta500,ta502,ta600,ta602,ta703)									4 (17.4)	19 (82.6)	23 (100.0)
Total										49 (52.1)	45 (47.9)	94 (100.0)
Diversity index										0.70	0.84	0.87

a: avirulant; v: virulant.
Diversity index was calculated by the method of Simpson (1949).

In the "z" group, eight reaction types (z00, z02, z04, z05, z06, z07, z12, and z14) were found. Reaction types z00 and z04 were dominant, and the numbers of isolates differed between clusters I and II. Only one isolate in cluster I was found to be of reaction type z00, avirulent to all four DVs, and the remaining 38 isolates were in cluster II. In contrast, reaction type z04 virulent to DV for *Piz-t* was found for 38 blast isolates in cluster I and four in cluster II. Each of the other six reaction types had fewer than five isolates. The values of Simpson's diversity index for cluster I, cluster II, and the entire group of isolates were 0.39, 0.28, and 0.62, respectively. These results indicate that blast isolates were differentiated between clusters I and II by the reaction of DV for *Piz-t*.

In the "ta" group, 14 reaction types (ta000, ta002, ta401, ta402, ta403, ta421, ta431, ta500, ta502, ta600, ta602, ta700, ta702, and ta703) were found. Five reaction types (ta401, ta403, ta421, ta700, and ta702) had more than nine blast isolates in each and were dominant. Three reaction types (ta401, ta403, and ta421) that were virulent to DVs for *Pi12*(t) and *Pi19*(t), had higher frequencies of blast isolates in cluster I than in cluster II. The remaining two reaction types (ta701 and ta702), which were all virulent to DVs for *Pita-2*(two lines), and *Pi12*(t), were found only in cluster II. Each of the other six reaction types included fewer than seven blast isolates. These results indicate that clusters I and II were divided mainly by the reactions of DVs for *Pita-2*. The values of Simpson's diversity index for cluster I, cluster II, and the entire group of isolates were 0.70, 0.84, and 0.87, respectively.

5. Race designation

The 94 blast isolates from the Mekong Delta were finally categorized into 67 races based on the reaction types of the five groups of DVs (Supplemental Table 1).

Among these blast isolates, several were categorized into 10 races: U63-i0-k100-z04-ta401 (cluster I: three isolates), U63-i0-k100-z04-ta403 (II: four), U63-i0-k100-z04-ta406 (I: three), U63-i0-k100-z04-ta421 (I: three), U63-i0-k100-z06-ta403 (I: two), U23-i0-k100-z00-ta702 (II: four), U63-i0-k100-z00-ta406 (II: three), U63-i0-k100-z00-ta702 (II: six), U63-i2-k100-z00-ta700 (II: four), and U63-i6-k100-z00-ta700 (II: four). Each of the other 57 races only had one isolate.

6. Relationship between rice variety and blast isolates

A total of 57 of the 94 blast isolates were collected from only four rice cultivars (IR 50404, OM 576, VN95-29, and VND95-29) according to interviews with farmers, and the blast isolates from each rice cultivar showed similar reaction types and race designations (Table 3 and Supplemental Table 1).

A total of 16 blast isolates from IR 50404 showed reaction types U23, U43, and U63 in the "U" group, and ta401, ta421, ta431, ta500, ta602, ta700, and ta702 in the "ta" group. These isolates shared a common virulence to DVs for *Pib* and *Pia* in the "U" group, and for *Pi12*(t) in the "ta" group. These results suggest that IR 50404 harbors the resistance genes *Pa*, *Pib*, and *Pi12*(t) in its genetic background. There were 12 blast isolates from OM 576 that also showed a common virulent reaction in DVs for *Pib* and *Pi12*(t). Seven blast isolates from VN95-20 also had common virulent reactions in DVs for *Pib, Pia*, and *Pi12*(t). A total of 11 isolates from VND95-20 showed common virulent reactions in DVs for *Pib* and *Pia*.

The common reactions among blast isolates originating from different rice varieties suggest that the virulence of reaction types corresponded with resistance genes in the genetic background of the DV(s).

Discussion

The 94 blast isolates collected from the five provinces in the Mekong River Delta, southern Vietnam, showed a wide variation in the frequencies of virulence to DVs and LTH (Fig.1). These blast isolates were classified into cluster groups I and II, based on the reaction patterns of DVs and LTH (Supplemental Fig. 1). The isolates in cluster I showed higher frequencies of virulence to the DVs of *Pit*, *Piz-t*, *Pita*, and *Pi19*(t) than those in cluster II. In contrast, isolates in cluster II showed higher frequencies of virulence to the DVs for *Pi3*, *Pi5*(t), and *Pita-2* than those in cluster I. The frequencies in cluster II were higher than those in cluster I in the three provinces of Dong Thap, Long An (and Ho Chi Minh City), and Vinh Long in the northern region of the Mekong River Delta, and the frequencies in cluster I were higher than in cluster II in the other two provinces of Tra Vinh and Bac Lieu in the southern region (Fig. 2). These results indicate that different blast races were distributed between the three provinces in the border area and the two provinces in the coastal area of the Mekong River Delta. The differences of blast races may correspond to the genotypes of resistance gene(s) in rice cultivars that adapted to the ecosystems for rice cultivation in these provinces. To understand the differentiation of blast races, the genetic variation of resistance genes in rice cultivars and the relationships between rice cultivar and blast races must be clarified.

Table 3. Estimation of resistance gene(s) in donor rice cultivars based on the reaction patterns of blast isolates collected therefrom

Rice variety											
IR 50404			OM 576			VN95-20			VND95-20		
Cluster group	Accession No.	Race	Cluster group	Accession No.	Race	Cluster group	Accession No.	Race	Cluster group	Accession No.	Race
I	VL713	U43-i0-k000-z00-ta401	I	TV363	U23-i0-k100-z04-ta401	I	LA1857	U23-i0-k000-z04-ta403	I	LA1622	U21-i0-k100-z04-ta401
I	VL777	U63-i0-k000-z04-ta421	I	TV81	U61-i0-k100-z04-ta401	I	LA1892	U63-i7-k102-z04-ta401	I	LA1731	U63-i0-k004-z04-ta421
I	VL828	U63-i0-k100-z06-ta431	I	TV49	U63-i0-k100-z04-ta401	II	LA1912	U23-i0-k100-z00-ta702	I	LA1553	U63-i0-k100-z04-ta421
I	VL811	U63-i0-k100-z07-ta401	I	TV414	U63-i0-k100-z04-ta401	II	LA1952	U23-i0-k102-z00-ta702	II	LA1565	U21-i0-k000-z00-ta002
I	VL731	U63-i0-k104-z04-ta421	I	TV193	U63-i0-k100-z04-ta401	II	LA1938	U63-i0-k000-z00-ta500	II	LA1770	U23-i0-k000-z00-ta502
I	TV175	U63-i0-k104-z04-ta421	I	TV125	U63-i0-k100-z04-ta401	II	LA1887	U63-i0-k102-z00-ta702	II	LA1584	U23-i0-k100-z00-ta402
II	LA1537	U23-i0-k000-z00-ta602	I	VL896	U63-i0-k100-z04-ta703	II	LA1872	U63-i0-k102-z00-ta702	II	LA1718	U23-i0-k100-z00-ta702
II	VL561	U23-i2-k100-z00-ta500	I	TV444	U63-i0-k104-z04-ta421				II	LA1657	U23-i4-k000-z00-ta000
II	VL581	U63-i0-k010-z04-ta401	I	TV244	U63-i0-k104-z04-ta421				II	LA1705	U63-i0-k100-z00-ta702
II	VL601	U63-i0-k100-z00-ta401	II	VL912	U61-i6-k000-z00-ta700				II	LA1668	U63-i0-k100-z00-ta702
II	LA1507	U63-i0-k100-z00-ta702	II	VL540	U63-i2-k100-z00-ta500				II	LA1645	U63-i2-k100-z00-ta700
II	VL761	U63-i4-k100-z00-ta500	II	VL521	U63-i6-k100-z00-ta700						
II	DT3262	U63-i4-k102-z00-ta700									
II	VL862	U63-i6-k100-z00-ta700									
II	VL845	U63-i6-k100-z00-ta700									
II	DT3417	U63-i6-k100-z00-ta700									
Total		16			12			7			11
Common virulence gene in blast isolates to each DV's group											
U		*Pib, Pia*			*Pib*			*Pib, Pia*			*Pib, Pia*
i		-			-			-			-
k		-			-			-			-
z		-			-			-			-
ta		*Pil2*(t)			*Pil2*(t)			*Pil2*(t)			-

-: Non-estimation of resistance gene in the DV group

Done et al. (1999) found the high frequencies of virulent blast isolates from the Mekong River Delta to the DVs for *Pik-s*, *Pia*, and *Pita*. Noda et al. (1999) also reported the distribution of blast races in the Mekong River Delta using blast isolates collected from 1995 to 1996 and indicated that almost all blast isolates were virulent to three DVs: Aichi-asahi (carrying resistance gene *Pia)*, K59 (*Pit*), and AA/S2-75 (*Pik-s*). In contrast, almost all were avirulent to five DVs: Shin 2 (*Pik-s*), Kusabue (*Pik*), Fukunishiki (*Piz*), Pi No. 4 (*Pita-2),* and BL1 (*Pib*), each harboring *Pish* in its genetic background, and to Toride 1 (*Piz-t*) and K60 (*Pik-p*). Furthermore, 38% of the isolates showed a virulent reaction in Yashiro-mochi (*Pita*). The high frequencies of blast isolates virulent to the DVs for *Pia*, *Pit*, and *Pik-s*; intermediate frequencies for the DV for *Pita*; and low frequencies for the DVs for *Pik-p* and *Pish* found in this study agreed with the results of Noda et al. (1999). These results suggest that no dramatic changes have occurred in the Mekong River Delta since 1995 or 1996 in terms of blast races. Unfortunately, Done et al. (1999) and Noda et al. (1999) were unable to clearly determine the distribution of blast races based on pathological analyses, as all blast isolates showed avirulent reactions to several DVs harboring *Pish* as an additional resistance gene in their genetic backgrounds. Our study utilized a new international set of DVs, including monogenic lines (Tsunematsu et al. 2000) and LTH NILs (Telebanco-Yanoria et al. 2010) that minimize the influence of genetic background, and clarified the reactions of additional resistance genes *Pib*, *Pi3*, *Pi5*(t), *Pik-m*, *Pik-h*, *Pik*, *Pi1*, *Pi7*(t), *Pi9*(t), *Piz*, *Piz-5*, *Pi12*(t), *Pi19*(t), and *Pi20*(t). Noda et al. (1999) found five dominant blast races that were virulent to *Pia* and *Pit*; virulent to *Pia*, *Pik-s*, and *Pit*; virulent to *Pia*, *Pik-s*, *Pita*, and *Pit*; virulent to *Pia*, *Pita,* and *Pit*; and virulent to *Pia*, and indicated that races virulent to *Pia*, *Pit*, *Pik-s*, or *Pita* were distributed in the Mekong River Delta. Based on the new designation system by Hayashi and Fukuta (2009), several dominant reaction types were found: U23, U63, i0, i2, i6, k00, k100, z00, z04, ta401, ta403, ta421, ta700, and ta702 (Table 2). These results indicate that blast isolates virulent to *Pib*, *Pit*, *Pia*, *Pi3*, *Pi5*(t), *Pik-s*, *Piz-t*, *Pi12*(t), *Pita*, *Pi19*(t), *Pi20*(t), and *Pita-2*, or avirulent to all *Piz* allele genes were distributed in the Mekong River Delta (Table 3).

By using the new set of international DVs by Tsunematsu et al. (2000) and Telebanco-Yanoria et al. (2010) and the new designation system for blast races by Hayashi & Fukuta (2009), we conducted a more detailed characterization of blast isolates compared with the study by Noda et al. (1999), and demonstrated well that blast races were differentiated between the northern and southern regions of the Mekong River Delta. However, the number of blast isolates used in this study was limited, and further studies are required to investigate many more blast isolates collected from over a wider area of southern Vietnam.

The genetic diversities of each reaction type and cluster group were calculated. The values of Simpson's diversity index for the reaction types were lower in the DV groups "U", "i", "k", and "z" than in the DV group "ta". Virulent blast isolates were dominant in the "U" group, and avirulent isolates or isolates virulent to few resistance gene(s) were dominant in the "i", "k", and "z" groups, with no great variation being recognized in these reaction type groups. These results suggest that the genetic variation among blast races was limited in the Mekong River Delta.

With its headwaters in Yunnan, China, the Mekong River flows southward through Laos, Thailand, and Cambodia, and then arrives at the Mekong Delta in southern Vietnam. To more fully understand the differentiation of blast races in southern Vietnam, the relationships among blast races in these countries and the influences thereof must be clarified. Fukuta et al. (2014) clarified the variation in blast isolates in Cambodia using the same set of DVs by Tsunematsu et al. (2000) and Telebanco-Yanoria et al. (2010), and the system of blast race designation by Hayashi & Fukuta (2009). That study confirmed the following dominant blast isolate reaction types: U43, U61, U63, i0, i6, i7, k000, k100, z00, z04, z06, ta002, ta003, ta403, ta421, and ta431. Remarkable differences were found between the Mekong River Delta and Cambodia in the reactions of DVs for *Pik-s*, *Piz-t*, *Pita-2*, *Pi12*(t), *Pita*, and *Pi19*(t). However, dominant reaction types U63, i0, i6, k000, k100, z00, z04, ta403, and ta421 were common to both Cambodia and the Mekong River Delta, and the other reaction types U43, U61, i7, z06, and ta431 were also found in the Mekong River Delta, although only in a few cases. These results indicate that the blast races were similar between Cambodia and the Mekong River Delta with small differences in the reactions for *Pik-s*, *Piz-t*, *Pita-2*, *Pi12*(t), *Pita*, and *Pi19*(t). To understand the relationships between blast races in Thailand, Laos, and China along the Mekong River and in central and northern Vietnam, pathological studies of blast isolates from these regions need to be conducted.

Based on the reactions of blast isolates collected from some common rice cultivars, we estimated the resistance genes present in their genetic backgrounds (Table 3). IR 50404 was estimated to harbor at least resistance genes *Pib*, *Pia*, and *Pi12*(t), and the other three cultivars also included two or three of these genes in their

genetic backgrounds. Blast isolates from the Mekong River Delta showed high frequencies of virulence to the DVs for these three genes. These results indicate that improved rice cultivars in the Mekong River Delta had similar genotypes in terms of such resistance genes as *Pib*, *Pia,* and *Pi12*(t) in their genetic backgrounds, and that outbreaks of virulent blast isolate corresponded with the resistance genes. Sang et al. (1996) reported that outbreaks of virulent blast races increased remarkably after IRRI rice cultivars were introduced in the early 1980s. The genetic variation among rice cultivars in southern Vietnam may be limited and could have contributed to the outbreaks of virulent blast races. Genetic analysis for the genotypes of resistance genes in these genetic backgrounds is required to confirm and refine strategies for the genetic improvement of rice cultivars. The increasing genetic variations for blast resistances in rice cultivars may be one of the important issues to establish a durable protection system in this region.

This study used a set of new international DVs and a new system of blast race designation to examine the reactions to blast isolates, determine the distributions of cluster groups of isolates and variations of blast races, and thus clarified the present variation in blast fungus in the Mekong River Delta. By using the common set of DVs and the designation system, blast isolates from the Mekong Delta could be compared with those from Cambodia. We therefore demonstrated that the DV materials developed by Tsunematsu et al. (2000) and Telebanco-Yanoria et al. (2010), and the designation system developed by Hayashi & Fukuta (2009) will be powerful tools for understanding the variation of blast races and diversities of blast isolates on a global level.

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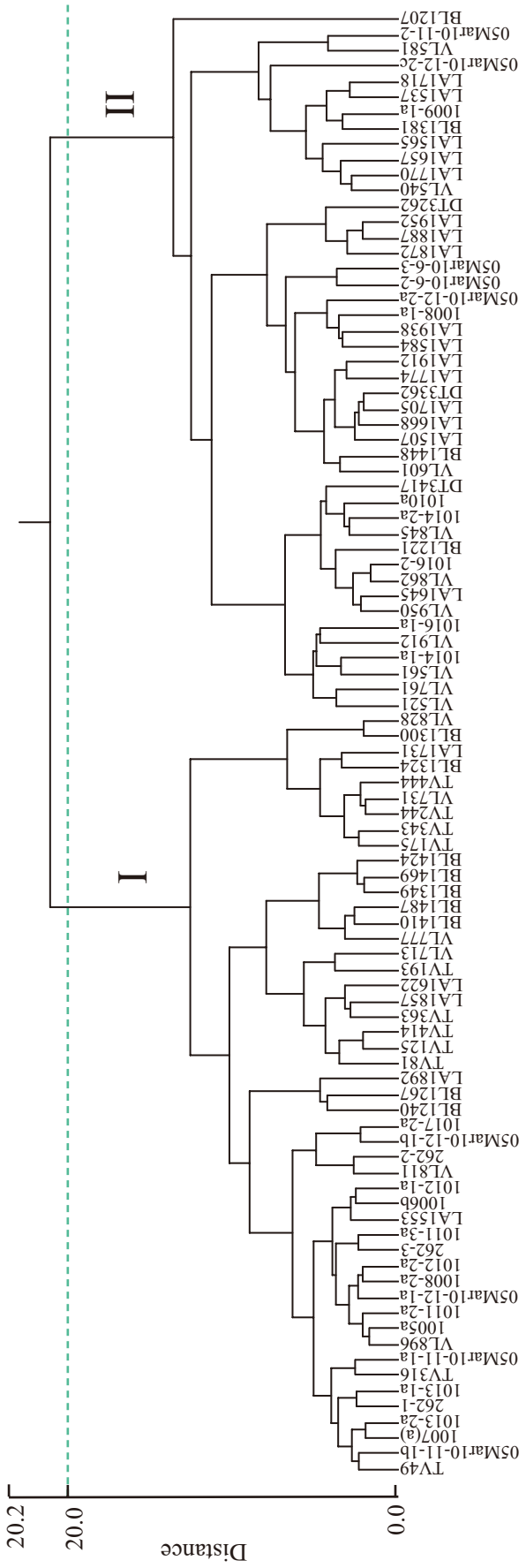

Supplemental Fig. 1. Classification of blast isolates collected from southern Vietnam
A total of 94 blast isolates were used, and cluster analysis was conducted using Ward's hierarchical method. These blast isolates were classified into two cluster groups (I and II).

Supplemental Table 1. List of blast isolates from southern Vietnam

Race No.	Race Designation	No. of isolates	Conservation No. in JIRCAS	Accession No.	Sampling Date	Province	Rice variety	Cluster group
1	U21-i0-k100-z04-ta401	1	VTN100	LA1622	2/4/2007	Long An	VND95-20	I
2	U21-i0-k000-z04-ta421	1	VTN84	BL1381	1/25/2007	Bac Lieu	Unknown	I
3	U23-i0-k100-z04-ta401	1	VTN63	TV363	1/25/2007	Tra Vinh	OM 576	I
4	U23-i0-k000-z04-ta403	1	VTN109	LA1857	2/3/2007	Long An	VN95-20	I
5	U23-i0-k100-z00-ta405	1	VTN136	13VT1008-1(a)	6/22/2013	Long An	Unknown	I
6	U23-i0-k000-z04-ta422	1	VTN140	13VT1009-1(a)	6/22/2013	Long An	Unknown	I
7	U23-i0-k100-z04-ta406	1	VTN138	13VT1008-2(a)	6/22/2013	Long An	Unknown	I
8	U23-i6-k100-z00-ta703	1	VTN141	13VT1010(a)	6/22/2013	Long An	Unknown	I
9	U43-i0-k000-z00-ta401	1	VTN71	VL713	1/22/2007	Vinh Long	IR 50404	I
10	U61-i0-k100-z04-ta401	1	VTN56	TV81	1/26/2007	Tra Vinh	OM 576	I
11	U63-i0-k000-z04-ta421	1	VTN74	VL777	1/22/2007	Vinh Long	IR 50404	I
12	U63-i0-k004-z04-ta421	1	VTN106	LA1731	2/4/2007	Long An	VND95-20	I
13	U63-i0-k004-z04-ta431	1	VTN82	BL1324	1/25/2007	Bac Lieu	OM 2718	I
14	U63-i0-k000-z04-ta702	1	VTN86	BL1424	1/27/2007	Bac Lieu	Unknown	I
15	U63-i0-k100-z04-ta401	1	VTN55	TV49	1/26/2007	Tra Vinh	OM 576	I
16	U63-i0-k100-z04-ta401	3	VTN57	TV125	1/26/2007	Tra Vinh	OM 576	I
			VTN59	TV193	1/26/2007	Tra Vinh	OM 576	I
			VTN64	TV414	1/25/2007	Tra Vinh	OM 576	I
17	U63-i0-k100-z04-ta403	4	VTN121	262-3	Unknown	Unknown	Unknown	I
			VTN125	05Mar10-11-1b	Unknown	Unknown	Unknown	I
			VTN127	05Mar10-12-1a	Unknown	Unknown	Unknown	I
			VTN134	13VT1007(a)	6/22/2013	Long An	Unknown	I
18	U63-i0-k100-z04-ta404	1	VTN131	13VT1005(a)	6/22/2013	Ho Chi Minh	Unknown	I
19	U63-i0-k100-z04-ta406	3	VTN133	13VT1006(b)	6/22/2013	Long An	Unknown	I
			VTN143	13VT1011-2(a)	6/22/2013	Long An	Unknown	I
			VTN145	13VT1011-3(a)	6/22/2013	Long An	Unknown	I
20	U63-i0-k104-z04-ta421	1	VTN58	TV175	1/26/2007	Tra Vinh	IR 50404	I
21	U63-i0-k100-z04-ta421	3	VTN88	BL1469	1/27/2007	Bac Lieu	Unknown	I
			VTN89	BL1487	1/27/2007	Bac Lieu	OM 3242	I
			VTN97	LA1553	2/2/2007	Long An	VND95-20	I
22	U63-i0-k104-z04-ta421	1	VTN60	TV244	1/27/2007	Tra Vinh	OM 576	I
23	U63-i0-k104-z04-ta421	3	VTN62	TV343	1/27/2007	Tra Vinh	OM 2514	I
			VTN65	TV444	1/25/2007	Tra Vinh	OM 576	I
			VTN72	VL731	1/22/2007	Vinh Long	IR 50404	I
24	U63-i0-k100-z04-ta431	1	VTN83	BL1349	1/25/2007	Bac Lieu	OM 3242	I
25	U63-i0-k100-z04-ta703	1	VTN94	VL896	1/24/2007	Vinh Long	OM 576	I
26	U63-i0-k100-z05-ta403	1	VTN119	262-1	Unknown	Unknown	Unknown	I
27	U63-i0-k100-z05-ta421	1	VTN85	BL1410	1/27/2007	Bac Lieu	Unknown	I
28	U63-i0-k100-z06-ta401	1	VTN61	TV316	1/27/2007	Tra Vinh	OM 2514	I
29	U63-i0-k100-z06-ta403	2	VTN120	262-2	Unknown	Unknown	Unknown	I
			VTN124	05Mar10-11-1a	Unknown	Unknown	Unknown	I
30	U63-i0-k100-z06-ta431	1	VTN91	VL828	1/22/2007	Vinh Long	IR 50404	I
31	U63-i0-k100-z07-ta401	1	VTN90	VL811	1/22/2007	Vinh Long	IR 50404	I
32	U63-i0-k100-z07-ta431	1	VTN81	BL1300	1/25/2007	Bac Lieu	OM 2717	I
33	U63-i0-k100-z14-ta403	1	VTN128	05Mar10-12-1b	Unknown	Unknown	Unknown	I
34	U63-i0-k177-z04-ta421	1	VTN77	BL1207	1/26/2007	Bac Lieu	OM 2517	I
35	U63-i5-k100-z04-ta403	1	VTN79	BL1240	1/26/2007	Bac Lieu	Unknown	I
36	U63-i7-k100-z04-ta403	1	VTN80	BL1267	1/26/2007	Bac Lieu	Unknown	I
37	U63-i7-k102-z04-ta401	1	VTN112	LA1892	2/3/2007	Long An	VN95-20	I
38	U03-i0-k000-z00-ta002	1	VTN130	05Mar10-12-2c	Unknown	Unknown	Unknown	II

Supplemental Table 1. List of blast isolates from southern Vietnam (Continued)

Race No.	Race Designation	No. of isolates	Conservation No. in JIRCAS	Accession No.	Sampling Date	Province	Rice variety	Cluster group
39	U21-i0-k000-z00-ta002	1	VTN98	LA1565	2/2/2007	Long An	VND95-20	II
40	U23-i0-k000-z00-ta602	1	VTN96	LA1537	2/2/2007	Long An	IR 50404	II
41	U23-i0-k000-z00-ta502	1	VTN107	LA1770	2/4/2007	Long An	VND95-20	II
42	U23-i0-k000-z00-ta702	1	VTN108	LA1774	Unknown	Unknown	Unknown	II
43	U23-i0-k100-z00-ta402	1	VTN99	LA1584	2/4/2007	Long An	VND95-20	II
44	U23-i0-k100-z00-ta702	4	VTN105	LA1718	2/4/2007	Long An	VND95-20	II
			VTN113	LA1912	2/3/2007	Long An	VN95-20	II
			VTN117	DT3362	2/3/2007	Dong Thap	Jasmine 85	II
			VTN115	LA1952	2/3/2007	Long An	VN95-20	II
45	U23-i2-k100-z00-ta500	1	VTN68	VL561	1/23/2007	Vinh Long	IR 50404	II
46	U23-i2-k100-z00-ta703	1	VTN153	13VT1014-1(a)	6/22/2013	Long An	Unknown	II
47	U23-i4-k000-z00-ta000	1	VTN102	LA1657	2/4/2007	Long An	VND95-20	II
48	U60-i6-k100-z00-ta503	1	VTN157	13VT1016-1(a)	6/22/2013	Long An	Unknown	II
49	U61-i6-k000-z00-ta700	1	VTN75	VL912	1/24/2007	Vinh Long	OM 576	II
50	U63-i0-k000-z00-ta002	1	VTN129	05Mar10-12-2a	Unknown	Unknown	Unknown	II
51	U63-i0-k000-z00-ta500	1	VTN114	LA1938	2/3/2007	Long An	VN95-20	II
52	U63-i0-k010-z04-ta401	1	VTN69	VL581	1/23/2007	Vinh Long	IR 50404	II
53	U63-i0-k100-z00-ta401	1	VTN70	VL601	1/23/2007	Vinh Long	IR 50404	II
54	U63-i0-k100-z02-ta402	1	VTN122	05Mar10-6-2	Unknown	Unknown	Unknown	II
55	U63-i0-k100-z12-ta402	1	VTN123	05Mar10-6-3	Unknown	Unknown	Unknown	II
56	U63-i0-k111-z00-ta402	1	VTN126	05Mar10-11-2	Unknown	Unknown	Unknown	II
57	U63-i0-k100-z04-ta404	1	VTN146	13VT1012-1(a)	6/22/2013	Long An	Unknown	II
58	U63-i0-k100-z04-ta406	3	VTN148	13VT1012-2(a)	6/22/2013	Long An	Unknown	II
			VTN149	13VT1013-1(a)	6/22/2013	Long An	Unknown	II
			VTN151	13VT1013-2(a)	6/22/2013	Long An	Unknown	II
59	U63-i0-k100-z14-ta406	1	VTN160	13VT1017(a)	6/22/2013	Long An	Unknown	II
60	U63-i0-k100-z00-ta702	6	VTN95	LA1507	2/2/2007	Long An	IR 50404	II
			VTN103	LA1668	2/4/2007	Long An	VND95-20	II
			VTN104	LA1705	2/4/2007	Long An	VND95-20	II
			VTN110	LA1872	2/3/2007	Long An	VN95-20	II
			VTN111	LA1887	2/3/2007	Long An	VN95-20	II
			VTN87	BL1448	1/27/2007	Bac Lieu	OM 2717	II
61	U63-i2-k100-z00-ta500	1	VTN67	VL540	1/23/2007	Vinh Long	OM 576	II
62	U63-i2-k100-z00-ta700	4	VTN76	VL950	1/24/2007	Vinh Long	OM 2514	II
			VTN78	BL1221	1/26/2007	Bac Lieu	OM 2717	II
			VTN101	LA1645	2/4/2007	Long An	VND95-20	II
			VTN159	13VT1016-2(a)	6/22/2013	Long An	Unknown	II
63	U63-i4-k100-z00-ta500	1	VTN73	VL761	1/22/2007	Vinh Long	IR 50404	II
64	U63-i6-k100-z00-ta605	1	VTN155	13VT1014-2(a)	6/22/2013	Long An	Unknown	II
65	U63-i6-k100-z00-ta700	3	VTN66	VL521	1/23/2007	Vinh Long	OM 576	II
			VTN92	VL845	1/22/2007	Vinh Long	IR 50404	II
			VTN93	VL862	1/22/2007	Vinh Long	IR 50404	II
66	U63-i4-k102-z00-ta700	1	VTN116	DT3262	2/4/2007	Dong Thap	IR 50404	II
67	U63-i6-k100-z00-ta700	1	VTN118	DT3417	2/3/2007	Dong Thap	IR 50404	II
	Total	94						