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Pathogenicity of Rice Blast (*Pyricularia oryzae* Cavara) Isolates from Cambodia

Yoshimichi FUKUTA[1,6]*, Ikumi KOGA[1]*, Tochi UNG[2], Khay SATHYA[2], Akiko KAWASAKI-TANAKA[3,1], Yohei KOIDE[1], Nobuya KOBAYASHI[4], Mitsuhiro OBARA[1], Hun YADANA[2] and Nagao HAYASHI[5]

[1] Japan International Research Center for Agricultural Sciences (Tsukuba, Ibaraki 305-8686, Japan)
[2] Cambodian Agricultural Research and Development Institute (Sangkat Prateah Lang, Khan Dangkor, Phnom Penh, Kingdom of Cambodia)
[3] Tottori University (Tottori, Tottori 680-8553, Japan)
[4] National Institute of Crop Science (Tsukuba, Ibaraki 305-8602, Japan)
[5] National Institute of Agrobiological Sciences (Tsukuba, Ibaraki 305-8602, Japan)

Abstract

The evaluation of a total of 122 blast (*Pyricularia oryzae* Cavara) isolates collected from the Tonle Sap and Mekong river regions of Cambodia revealed wide variation. Using a new designation system, the blast isolates were categorized into 92 races based on the reaction patterns of rice (*Oryza sativa* L.) differential varieties (DVs) harboring 23 resistance genes and of 1 susceptible cultivar, Lijiangxintuanheigu (LTH). Cluster analysis was used to classify the blast isolates into 3 groups — I, IIa, and IIb — using data from these reaction patterns of the DVs and LTH. We used the classifications established under the new designation system, alongside cluster analysis and the geographical distribution of blast isolates, to investigate the diversity and differentiation of blast races in the Tonle Sap and Mekong river regions. The distributions of the blast races differed between the 2 regions, although blast isolates of group IIa were distributed commonly in both regions and groups I and IIb occurred at higher frequencies in the Tonle Sap region rather than the Mekong region. The blast isolates in groups I and IIb were also less diverse than those in group IIa. Accordingly, Group II blast isolates overall were distributed in both regions with high diversity, but some modified blast isolates were additionally distributed in the Tonle Sap region. We also investigated the pathogenicities of blast isolates from wild rice (*Oryza rufipogon* Griff) weeds neighboring the cultivated rice, and discuss the relationship between these isolates and those from cultivated rice.

Discipline: Plant disease
Additional key words: cluster analysis, differential variety, diversity, pathotype, resistance gene

Introduction

Rice blast, caused by the pathogen *Pyricularia oryzae* Cavara is one of the most significant diseases affecting rice worldwide[22]. The use of resistant cultivars is the most practical and economical method to control this blast disease in rice (*Oryza sativa* L.). However, using such varieties has limited effect owing to the breakdown of resistance genes, with more and more blast races overcoming resistance in rice[7]. The dynamic interaction between host resistance and fungus virulence in rice blast pathosystems can be explained by the gene-for-gene theory: every resistance gene in the host corresponds to an avirulence gene in the pathogen[2,15]. Based on this theory, differential varieties (DVs), which can be used to distinguish pathotypes (races) by their reaction patterns to each pathogen strain, have been developed to identify the blast pathogen population structure and predict

This paper reports the results obtained in the JIRCAS research projects "Blast Network for Stable Rice Production" from 2006 to 2010 and "Rice Innovation for Environmentally Sustainable Production Systems" from 2011 to 2015
Present address:
[6] Tropical Agricultural Research Front, Japan International Research Center for Agricultural Sciences (Ishigaki, Okinawa 907-0002, Japan)
*Corresponding author: e-mail zen@affrc.go.jp
*These persons contributed equally to this work.
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the emergence of new blast races.

Using several sets of DVs, pathogenicity studies of blast isolates have been performed in China and Southeast Asia. Using 12 Japanese differential varieties (DVs) (for *Pia*, *Pik-s*, *Pii*, *Pik*, *Pik-m*, *Piz*, *Pita*, *Pita-2*, *Piz-t*, *Pik-p*, *Pib* and *Pit*) developed by Yamada et al.[21], Kiyosawa[8,9], and Kiyosawa et al.[11], Noda et al.[14] identified 12 kinds of blast race among 129 isolates collected from all over the Mekong river Delta area of Vietnam. Mekwatanakarn et al.[13] then investigated the pathogenicities of 527 blast isolates from Thailand using, as DVs, CO 39 near-isogenic lines (NILs) for *Pi1*, *Pi1-LAC*(t), *Pi1-TTP*(t), *Piz-5*, *Pi3*, *Pi4a*, *Pi4b*(t) (*Pita*), *Pi4a-PK*T(t), *Pi4a-TTP*(t) and *Pia* and Lijiangxintuanheigu (LTH) NILs for *Pik-m*, *Pita*, *Pita-2*, *Pib*, *Pik-p* and *Pik*. They classified the isolates into 175 races. In Bhutan, 110 isolates were differentiated into 53 races based on the reactions of CO 39 NILs for *Pi4b* (*Pita*), *Pi2* (*Piz-5*), *Pi3*, *Pi4a* (*Pita*), and *Pi1* and LTH NILs for *Pita*, *Pib*, *Pita-2*, *Pik-m* and *Pik-p*[19]. In China, 792 isolates were classified into 344 races using LTH NILs for *Pita-2*, *Pib*, *Pik*, *Pik-m*, *Pita*, and *Pik-p* and CO 39 NILs for *Pita*, *Piz-5*, *Pi3* and *Pi1* [1]. The pathogenicities of 119 blast isolates collected from the Philippines were characterized by Yamada et al.[21], Kiyosawa[8,9], and Kiyosawa et al.[10] using 18 Japanese DVs (for *Pia*, *Pib*, *Pii*, *Pit*, *Pita*, *Pish*, *Piz-t*, *Pi3*, *Piz-5*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pik-s*, *Pita-2*, *Piz*, *Pi1* and *Pi20*(t)); the isolates were categorized into 70 races[17]. In these previous studies, the use of different numbers and kinds of DVs hindered comparison of the results. However, the results indicate that DVs in each study can be used to explain wide variations in pathotypes in the blast populations of Southeast Asia and China and demonstrate the differential capacities of the pathogens. However, there has been no research into blast races in Cambodia, nor has any information on blast disease or genotypes of rice varieties there been collected.

To establish a durable system to protect against blast disease, since 2006 the Japan International Research Center for Agricultural Sciences (JIRCAS) has been conducting a collaborative study, "Blast Research Network for Stable Rice Production," targeting Southeast and East Asia. This international collaborative research aims to develop a differential system that can identify the pathogenicities of blast fungi and genotypes of resistance genes in rice varieties based on the gene-for-gene theory. The differential system comprises matrix data for the reactions between DVs and standard differential blast isolates. Twenty-five monogenenic lines harboring 23 resistance genes, namely *Pish*, *Pib*, *Pit*, *Pia*, *Pii*, *Pi3*, *Pi5*(t), *Pik-s*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, *Pi7*(t), *Pi9*, *Piz*, *Piz-5*, *Piz-t*, *Pita-2*, *Pita*, *Pi12*(t), *Pi19*(t) or *Pi20*(t), were developed as a new set of international DVs by several backcrosses using the Chinese susceptible rice variety LTH[20], and a set of LTH NILs carrying 11 resistance genes (*Pib*, *Piz-5*, *Pi9*, *Pi3*, *Pia*, *Pik-s*, *Pik*, *Pik-h*, *Pi7*(t), *Pita* or *Pita-2*) developed by Telebanco-Yanoria et al.[18]. These DVs had the common genetic background of LTH, meaning the influence of genetic background on the appearance of blast symptoms was minimized. The targets of the DV sets and other useful materials released are major blast resistance genes, which have been used internationally as part of JIRCAS's research. These monogeneic lines and LTH NILs are being used to develop differential systems in each country through 1) pathogenicity analysis of blast isolates; 2) elucidation of blast race distribution; and 3) selection of standard differential blast isolates.

As a first step in developing a differential system for Cambodia, to clarify the distribution and differentiation of blast races we used the above-mentioned DVs to elucidate the pathogenicities of blast isolates collected from that country. Wild rice (*Oryza rufipogon* Griff) has always been conserved as a neighboring weed of cultivated rice in Cambodia, and we observed its infection with blast disease on our research trips. However, little information is available on the pathogenicity of blast races in wild rice and their interrelationships in *O. rufipogon* and *O. sativa*. We therefore investigated isolates collected from *O. rufipogon* as part of our preliminary research.

Materials and Methods

1. Differential varieties

As a set of DVs, we used 23 monogenic lines[6,20] carrying 21 resistance genes (IRBLsh-B for *Pish*, IRBLt-K59 for *Pit*, IRBLb-B for *Pib*, IRBLa-A for *Pia*, IRBLi-F5 for *Pii*, IRBL3-CP4 for *Pi3*, IRBL5-M for *Pi5*(t), IRBLks-F5 for *Pik-s*, IRBLkm-Ts for *Pik-m*, IRBL1-CL for *Pi1*, IRBLkp-K60 for *Pik-p*, IRBL7-M for *Pi7*(t), IRBL9-W for *Pi9*, IRBLz-Fu for *Piz*, IRBLz5-CA for *Piz-5*, IRBLzt-T for *Piz-t*, IRBLta2-Re and IRBLta2-Pi for *Pita-2*, IRBL12-M for *Pi12*(t), IRBLta-K1 and IRBLta-CP1 for *Pita*, IRBl19-A for *Pi19*(t) or IRBL20-IR24 for *Pi20*(t)) and 2 NILs (IRBLkh-K3[LT] for *Pik-h* or IRBLk-K[LT] for *Pik*), using the Chinese Japonica-type susceptible variety LTH[18] as a control, to evaluate the pathogenicities of blast isolates from Cambodia.

2. Blast samples

A total of 122 blast isolates were collected from 2 different areas of Cambodia, namely Tonle Sap (Western region; 97 blast isolates) and the Mekong river (Eastern region; 25 blast isolates), in December 2008 and March and October 2010. Of these, 112 were collected from cultivated rice (*O. sativa* L.) and 10 from wild rice (*O. rufipogon* Griff) (Table 1). Blast isolates from wild rice were collected from plants growing as weeds near cultivated rice.

3. Inoculation and evaluation

The set of 25 DVs and LTH was sown in plastic trays (9.3×15.3×2.5cm) with garden soil, with each tray containing 3 or 4 seeds of each variety. Two sets of DVs were prepared for pathogenicity testing, and the average scores of the 2 sets were used to indicate the degrees of infection.

Blast isolates were basically inoculated in accordance with the method of Hayashi et al.[5]. The spore concentration was standardized to 1×10^5 spores/ml. DVs were inoculated approximately 19 days after sowing (at about the 4th or 5th leaf stage) by spraying 15 to 20 ml of spore suspension per tray with a fine atomizer, whereupon the degree of disease of each seedling was evaluated 7 days after inoculation. The reactions of DVs were categorized into six scoring types (0 to 5) and summarized whereby 0 to 2 was resistant (R) and 3 to 5 was susceptible (S). However, 2 DVs (IRBLsh-B for *Pish* and IRBLta2-Pi for *Pita-2*) were evaluated as 0 to 3 resistant (R) and 4 or 5 susceptible (S) and another DV, IRBL5-M for *Pi5*(t), was evaluated as 0 or 1 resistant (R) and 2 to 5 susceptible (S).

Virulent blast isolates were designated in accordance with a new international designation system by Hayashi and Fukuta[4] using LTH monogenic lines. The blast isolates were designated by the reaction patterns of LTH and the 25 monogenic lines targeting the 23 resistance genes. The lines were categorized into 5 groups: (U) LTH, IRBLa-A, IRBLsh-B, IRBLb-B, and IRBLt-K59 (Table 2); (i) 3 lines with the *Pii* locus on chromosome 9 (Table 3); (k) 7 lines with the *Pik* region on chromosome 11 (Table 4); (z) 4 lines with the *Piz* region on chromosome 6 (Table 5); and (ta) 7 lines with the *Pita* region on chromosome 12 (Table 6). We replaced the 2 monogenic lines used by Hayashi and Fukuta[4] (namely IRBLkh-K3 and IRBLk-K) with 2 LTH NILs (IRBLkh-K3[LT] and IRBLka-K[LT], respectively). Each group consisted of 1 to 3 variety units, to each of which we allocated 3 DVs (genes). We applied codes 1, 2, and 4 to the susceptible reactions of each of the 3 respective varieties to the blast isolates. Using Gilmour's method[3], blast races were designated by the combined sum of the codes representing the reactions of the 3 varieties in each unit. Isolates classified this way were designated as "pathotypes" within each variety unit and as "races" using the set of all 5 pathotypes.

4. Classification of blast isolates by cluster analysis

The reaction patterns of the 25 DVs harboring the 23 kinds of resistance gene or LTH to the blast fungus were analyzed using Ward's hierarchal analysis and JMP7.0.2 software (SAS Institute Inc., Cary, NC, USA) for Windows.

The diversity of blast isolates in each cluster group was also calculated using Simpson's index method[16].

Results

1. Reaction of DVs

We investigated the pathogenicity of a total of 122 blast isolates (112 from cultivated rice (*O. sativa* L.) and 10 from wild rice (*O. rufupugon*) using 25 DVs and LTH (Table 1).

There were high frequencies (>60%) of occurrence of blast isolates virulent to 6 DVs (for *Pib*, *Pit*, *Pia*, *Piz-t*, *Pi19*(t) and *Pi20*(t)) and LTH. There were moderate frequencies (20 to 60%) of isolates virulent to 8 DVs (for *Pii*, *Pi3*, *Pi5*(t), *Pik-s*, *Piz-5*, *Pi12*(t) and *Pita* (2)). There were low frequencies (<20%) of blast isolates virulent to the remaining 11 DVs (for *Pish*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, *Pi7*(t), *Pi9*(t), *Piz* and *Pita-2* (2)) (Fig. 1). There were no blast isolates virulent to DVs for *Pik-h.* A total of 4 blast isolates (3.3%) showed avirulence to LTH, suggesting that LTH harbored 1 or more resistance genes in its genetic background. However, these genes were estimated to be of minor importance, because no blast isolates compatible with LTH have been found in China[11] and no major resistance genes have been identified in LTH[20].

2. Classification of blast isolates based on DV reaction patterns

A total of 122 blast isolates were classified into 2 major groups (I and II) and then divided into subgroups (IIa and IIb) based on the reaction patterns of the DVs and LTH (Table 1, Fig. 1). A total of 38 blast isolates (31.1%) were classified into group I, in which the frequencies of isolates virulent to 6 DVs (for *Pii*, *Pi3*, *Pi5*(t), *Pik-s*, *Pi12*(t) and *Pita*(2)) were higher, and that of isolates virulent to 1 DV (for *Pi20*(t)) was lower, than in group II.

The other 84 blast isolates (68.9%) were classified into group II, with 48 (39.3%) and 36 (29.5%) isolates categorized into subgroups IIa and IIb, respectively. The blast isolates in subgroup IIb showed higher frequencies of virulence than those of subgroup IIa against 9 DVs (for *Pib*, *Pit*, *Pia*, *Pi9*, *Piz*, *Piz-5*, *Piz-t*, *Pi19*(t) and *Pi20*(t)).

Blast isolates from cultivated rice fell into all cluster groups, but those from wild rice (*O. rufipogon* Griff) fell into only 2 groups, namely I (6 isolates) and IIa (4 isolates). Five of the blast isolates from group I were found in the Tonle Sap region, and 1 isolate from group I and 4 isolates from group IIa were found in the Mekong river region.

The 97 blast isolates from Tonle Sap were divided into 3 cluster groups, I (n = 36, 29.%), IIa (n = 29, 23.8%) and IIb (n = 32, 26.2%) with similar frequencies. These frequencies differed among the 3 groups in the Mekong river region. Of the 25 blast isolates, 19 (15.6%) were categorized into group IIa; in the Mekong region there were very few isolates in the other 2 groups (Table 1, Fig. 2). Blast isolates of group IIa were distributed widely in the Tonle

Table 1. Numbers of blast isolates in each group from Cambodia

Region	Cultivated (Cv) or wild rice (W)	No. of blast isolates (%)				Total
		I	II			
			a	b	Sum	
Tonle Sap	Cv	31 (25.4)	29 (23.8)	32 (26.2)	61 (50.0)	92 (75.4)
	W	5 (4.1)	0 (0.0)	0 (0.0)	0 (0.0)	5 (4.1)
	SUM	36 (29.5)	29 (23.8)	32 (26.2)	61 (50.0)	97 (79.5)
Mekong river	Cv	1 (0.8)	15 (12.3)	4 (3.3)	19 (15.6)	20 (16.4)
	W	1 (0.8)	4 (3.3)	0 (0.0)	4 (3.3)	5 (4.1)
	SUM	2 (1.6)	19 (15.6)	4 (3.3)	23 (18.9)	25 (20.5)
Subtotal	Cv	32 (26.2)	44 (36.1)	36 (29.5)	80 (65.6)	112 (91.8)
	W	6 (4.9)	4 (3.3)	0 (0.0)	4 (3.3)	10 (8.2)
Total		38 (31.1)	48 (39.3)	36 (29.5)	84 (68.9)	122 (100.0)

Sampling was performed in two regions, Tonle Sap and the Mekong river, in December 2008 and March and October 2010.
The pathogenicities of a total of 122 blast isolates, including 10 from wild rice (*Oryza rufipogon*), were evaluated based on the reactions of a susceptible control (LTH) and 25 differential varieties targeting 23 resistance genes. Cluster analysis was performed using data on reaction patterns to the blast isolates.

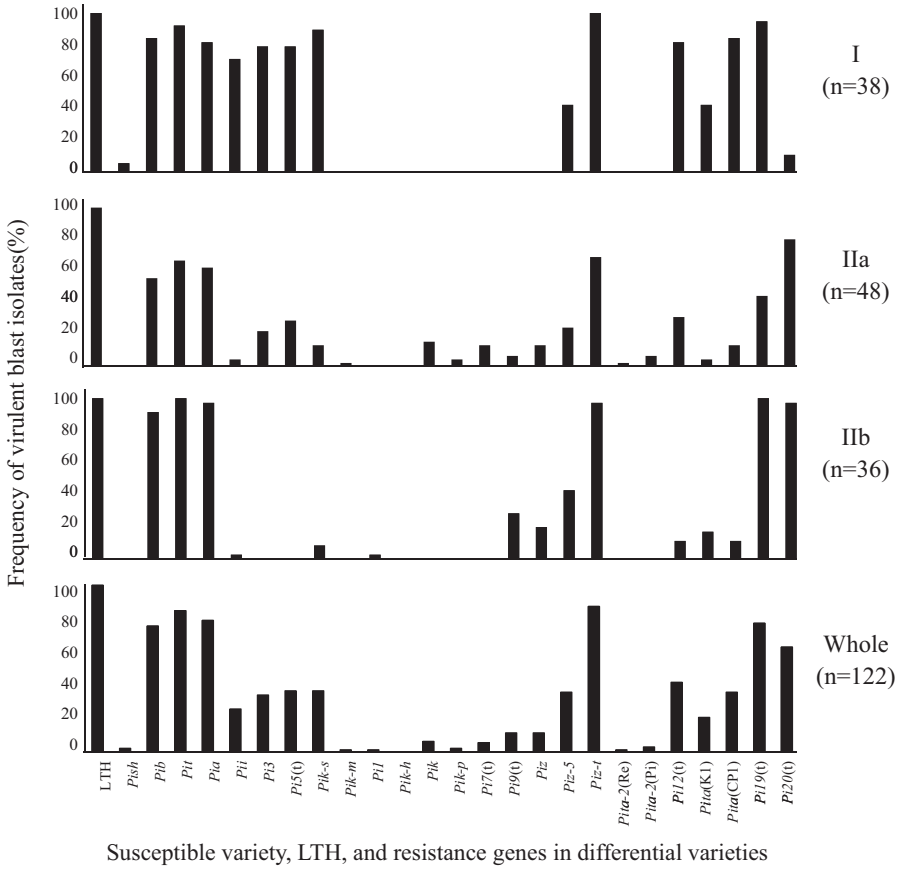

Fig. 1. Frequencies of occurrence of Cambodian blast isolates virulent against differential varieties susceptible control, LTH

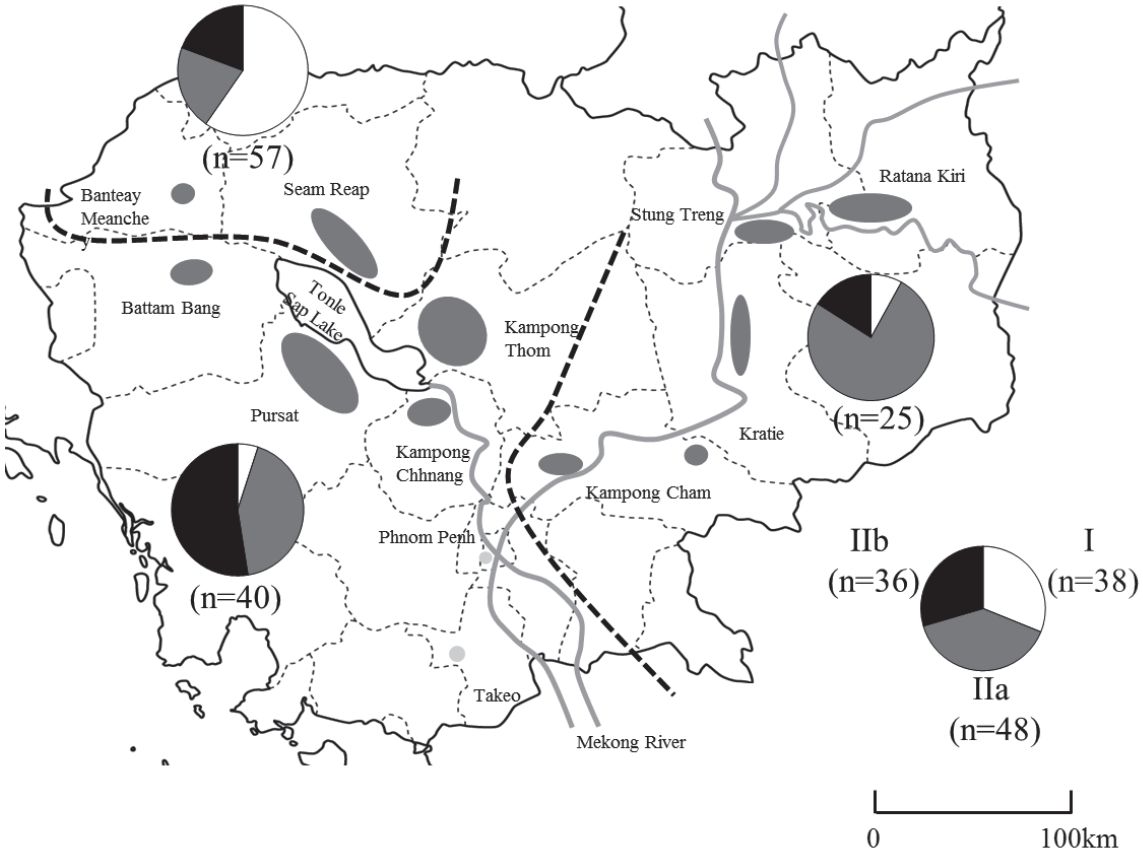

Fig. 2. Distribution of blast isolates classified into three groups; I , IIa and IIb, in Cambodia
: Area for sampling

Sap and Mekong river regions, whereas those of groups I and IIb were limited mainly to the Tonle Sap region and those of group I were particularly common in Siem Reap Province, to the north of Tonle Sap.

The blast isolates from wild rice were classified into groups I (6; 4.9%) and IIa (4; 3.3%); none fell into group IIb. The 6 and 4 blast isolates of groups I and II were sampled in the provinces of Siem Reap and Stung Treng, and Ban Lung and Stung Treng, respectively, all of which were in northern Cambodia (data not shown).

3. Pathotypes of blast isolates, based on DV reactions in the 5 variety groups

(1) LTH and 4 DVs (for *Pish*, *Pib*, *Pit* and *Pia*) in group U

Among the 122 blast isolates, we found a total of 13 pathotypes (U00, U01, U03, U21, U23, U40, U41, U42, U43, U53, U61, U63 and U73) against LTH and 4 DVs (for *Pish*, *Pib*, *Pit* and *Pia*) in variety group U (Table 2). (Resistant (R) and susceptible (S) reactions are categorized as incompatible (–) and compatible (+), respectively, in the tables.) A total of 65 blast isolates (53.3%) were categorized into pathotype U63, which was common in the 3 cluster groups, I, IIa and IIb. U43 and U61 included 13 and 12 blast isolates, respectively, and were found in all cluster groups. Each of the other pathotypes included fewer than 8 isolates (Table 2).

There were no significant differences in the numbers of blast isolates in the 13 pathotypes among the three cluster groups, but the kinds and numbers of pathotypes varied among the groups. There were 7, 11 and 3 pathotypes in groups I, IIa and IIb, respectively. The overall diversity and those of the 3 cluster groups by Simpson's index (Simpson, 1949) were 0.69, 0.62, 0.88 and 0.20, respectively.

(2) Three DVs (for *Pii*, *Pi3* and *Pi5*(t)) in group i

A total of 7 pathotypes (i0, i1, i2, i3, i4, i6 and i7) were found against variety group i. Pathotype i0 was dominant and included 69 blast isolates (56.6%). The second most common was pathotype i7, which included 23 blast isolates (18.9%). The other 5 pathotypes each had fewer than 12 blast isolates (<10%) (Table 3).

Among the 69 blast isolates of pathotype i0, 66 (54.1%) were classified into cluster group II and only 3 fell into group I. In contrast, all 23 blast isolates (18.9%) of pathotype i7 were found in group I, and none were contained in group II. Pathotypes i0 and i7 were thus differentiated by their respective occurrences in groups II and I.

Table 2. Numbers of blast isolates in pathotypes against the differential variety group U, corresponding to LTH and the resistance genes *Pish*, *Pib*, *Pit* and *Pia*, and the degrees of diversity in cluster groups

Cluster group		Number of blast isolates (%)														
		Pathotypes														
Resistance gene		U00	U01	U03	U21	U23	U40	U41	U42	U43	U53	U61	U63	U73	Total	Index of diversity
Reaction of DV *Pish*	LTH	- -	- +	- +	- +	- +	- -	- +	--	- +	+ +	- +	- +	+ +		
Pib	*Pia*	- -	- -	- +	+ -	+ +	- -	- -	- +	- +	- +	+ -	+ +	+ +		
Pit		- -	-	-	-	-	+	+	+	+	+	+	+	+		
I		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	2 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)	5 (4.1)	1 (0.8)	6 (4.9)	22 (18.0)	1 (0.8)	38 (31.1)	0.62
II	a	2 (1.6)	4 (3.3)	5 (4.1)	3 (2.5)	5 (4.1)	1 (0.8)	6 (4.9)	1 (0.8)	5 (4.1)	0 (0.0)	5 (4.1)	11 (9.0)	0 (0.0)	48 (39.3)	0.88
	b	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (2.5)	0 (0.0)	1 (0.8)	32 (26.2)	0 (0.0)	36 (29.5)	0.20
Sum		2 (1.6)	4 (3.3)	5 (4.1)	3 (2.5)	5 (4.1)	1 (0.8)	6 (4.9)	1 0.8	8 (6.6)	0 (0.0)	6 (4.9)	43 (35.2)	0 (0.0)	84 (68.9)	0.71
Total		2 (1.6)	4 (3.3)	5 (4.1)	4 (3.3)	7 (5.7)	1 (0.8)	6 (4.9)	1 (0.8)	13 (10.7)	1 (0.8)	12 (9.8)	65 (53.3)	1 (0.8)	122 (100.0)	0.69

Each pathotype was categorized in accordance with the method of Hayashi et al. (2009).
– : incompatibility reaction, + : compatibility reaction.
Indexes of diversity were calculated using the method of Simpson (1949).

Table 3. Numbers of blast isolates in pathotypes against differential variety group i, corresponding to resistance genes *Pii*, *Pi3* and *Pi5*(t), and the degrees of diversity in cluster groups

Cluster group	Number of blast isolates (%)								
	Pathotypes								
Resistance gene	i0	i1	i2	i3	i4	i6	i7	Total	Index of diversity
Reaction of DV *Pii*	-	+	-	+	-	-	+		
Pi3	-	-	+	+	-	+	+		
Pi5(t)	-	-	-	-	+	+	+		
I	3 (2.5)	1 (0.8)	1 (0.8)	3 (2.5)	4 (3.3)	3 (2.5)	23 (18.9)	38 (31.1)	0.60
IIa	31 (25.4)	2 (1.6)	2 (1.6)	0 (0.0)	5 (4.1)	8 (6.6)	0 (0.0)	48 (39.3)	0.54
IIb	35 (28.7)	1 (0.8)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	36 (29.5)	0.05
Sum	66 (54.1)	3 (2.5)	2 (1.6)	0 (0.0)	5 (4.1)	8 (6.6)	0 (0.0)	84 (68.9)	0.37
Total	69 (56.6)	4 (3.3)	3 (2.5)	3 (2.5)	9 (7.4)	11 (9.0)	23 (18.9)	122 (100.0)	0.63

Each pathotype was categorized in accordance with the method of Hayashi et al. (2009).
– : incompatibility reaction, + : compatibility reaction.
Indexes of diversity were calculated using the method of Simpson (1949).

There were 7, 5 and 3 pathotypes in groups I, IIa and IIb, respectively. The overall diversity and those of the 3 cluster groups I, IIa, and IIb were 0.63, 0.60, 0.54 and 0.05, respectively. The diversities therefore did not differ greatly between groups I and IIa, but that of group IIb was much lower.

(3) Seven DVs (for *Pik-s*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p* and *Pi7*(t)) in group k

A total of 8 pathotypes (k000, k001, k002, k004, k005, k020, k100 and k110) were found against variety group k

Table 4. Numbers of blast isolates in pathotypes against differential variety group k, corresponding to resistance genes *Pik-s*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p* and *Pi7*(t), and the degrees of diversity in cluster groups

Cluster group	Number of blast isolates (%)									
	Pathotypes								Total	Index of diversity
Reaction of DV / Resistance gene	k000	k001	k002	k004	k005	k020	k100	k110		
Pik-s *Pik-m* *Pik*	- - -	- - +	- - -	- - -	- - +	- - -	+ - -	+ + -		
Pi1 *Pik-p*	- -	- -	- +	- -	- -	+ -	- -	- -		
Pik-h *Pi7*(t)	- -	- -	- -	- +	- +	- -	- -	- -		
I	4 (3.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	34 (27.9)	0 (0.0)	38 (31.1)	0.19
IIa	32 (26.2)	2 (1.6)	2 (1.6)	1 (0.8)	5 (4.1)	0 (0.0)	5 (4.1)	1 (0.8)	48 (39.3)	0.53
IIb	32 (26.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	3 (2.5)	0 (0.0)	36 (29.5)	0.20
Sum	64 (52.5)	2 (1.6)	2 (1.6)	1 (0.8)	5 (4.1)	1 (0.8)	8 (6.6)	1 (0.8)	84 (68.9)	0.41
Total	68 (55.7)	2 (1.6)	2 (1.6)	1 (0.8)	5 (4.1)	1 (0.8)	42 (34.4)	1 (0.8)	122 (100.0)	0.57

Each pathotype was categorized in accordance with the method of Hayashi et al. (2009).
– : incompatibility reaction, + : compatibility reaction.
Indexes of diversity were calculated using the method of Simpson (1949).

(Table 4). k000 included 68 blast isolates (55.7%). Only 4 blast isolates were classified into cluster group I; the other 64 were categorized into group II. In contrast, k100 had 42 blast isolates (34.4%); 34 and 8 were categorized into groups I and II, respectively. The other 6 pathotypes each included fewer than 6 blast isolates (<5%) (Table 4). These results indicated that there were 2 dominant pathotypes, k000 and k100, and 6 minor pathotypes. The 2 dominant pathotypes could be differentiated by the differences in their distributions between cluster groups I and II — in other words, by the reaction of DVs carrying *Pik-s*.

Groups I, IIa, and IIb included 2, 7 and 3 pathotypes, respectively. The overall diversity and those of the 3 cluster groups I, IIa, and IIb were 0.57, 0.19, 0.53 and 0.20, respectively; group IIa therefore had the highest diversity.

(4) Four DVs (for, *Pi9*(t), *Piz*, *Piz-5* and *Piz-t*) in group z

A total of 11 pathotypes (z00, z01, z02, z04, z05, z06, z07, z13, z14, z16 and z17) were found against variety group z. A total of 56 blast isolates (45.9%) were categorized into z04; in groups I, IIa, and IIb there were 22 (18.0%), 19 (15.6%) and 15 (12.3%) blast isolates, respectively. z04 was dominant in all groups. A total of 14 (11.5%) and 26 (21.3%) blast isolates were categorized into z00 and z06, respectively. Group I had no z00 blast isolates, while group IIa had a greater number of isolates (13; 10.7%) than group IIb (1; 0.8%). In z06, there were 16 (13.1%) isolates in group I and only 10 (8.2%) in group II. Each of the other 8 pathotypes contained fewer than 7 blast isolates (<5%) (Table 5). The numbers of blast isolates in the 2 pathotypes z00 and z06 therefore differed between groups I and II.

The overall number of pathotypes and the numbers in groups I, IIa, and IIb were 11, 2, 10, and 8, respectively, and the respective diversity indexes were 0.72, 0.49, 0.75 and 0.76. Thus group I had the lowest diversity among the 3 groups, and there was little difference between groups IIa and IIb.

(5) Seven DVs (for *Pita-2*, *Pi12*(t), *Pita*, *Pi19*(t) and *Pi20*(t)) in group ta

A total of 26 pathotypes (ta000, ta001, ta002, ta003, ta013, ta013, ta020, ta021, ta022, ta023, ta031, ta033, ta113, ta201, ta400, ta401, ta402, ta403, ta412, ta420, ta421, ta422, ta423, ta431, ta602 and ta603) were found against variety group ta (Table 6). The dominant pathotype was ta003 (33 isolates; 27.0%): 7 (5.7%) and 25 (20.5%) isolates were included in groups IIa and IIb, respectively, but group I had only 1 isolate. Three pathotypes (ta000, ta002 and ta403) were found only in cluster group II, and 2 pathotypes (ta421 and ta431) occurred only in group I. Thus the virulence genes in 6 pathotypes differed between groups I and II, and the reactions of *Pita* and *Pita-2* were particularly divided according to these groups. Additionally, for pathotypes ta000 and ta002 there were several blast isolates in cluster group IIa, but none in group IIb. The number of isolates of ta003 was higher in group IIb than in group IIa. Thus the reactions of *Pi19*(t) and *Pi20*(t) differentiated groups IIa and IIb.

The numbers of pathotypes and diversity index values overall and for groups I, IIa, and IIb were 25 and 0.88, 12 and 0.77, 14 and 0.88, and 6 and 0.49, respectively. Thus

Table 5. Numbers of blast isolates in pathotypes against differential variety group z, corresponding to resistance genes *Pi9*(t), *Piz*, *Piz-5* and *Piz-t*, and the degrees of diversity in cluster groups

Cluster group		Number of blast isolates (%)											Index of diversity
		Pathotypes										Total	
Reaction of DV	Resistance gene	z00	z01	z02	z04	z05	z06	z07	z13	z14	z16	z17	
	Pi9(t) *Piz*	- -	- +	- -	- -	- +	- -	- +	+ +	+ -	+ -	+ +	
	- *Piz-5*	-	-	+	-	-	+	+	+	-	+	+	
	- *Piz-t*	-	-	-	+	+	+	+	-	+	+	+	
I		0 (0.0)	0 (0.0)	0 (0.0)	22 (18.0)	0 (0.0)	16 (13.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	38 (31.1)	0.49
IIa		13 (10.7)	2 (1.6)	2 (1.6)	19 (15.6)	2 (1.6)	6 (4.9)	1 (0.8)	1 (0.8)	1 (0.8)	1 (0.8)	0 (0.0)	48 (39.3)	0.75
IIb		1 (0.8)	0 (0.0)	0 (0.0)	15 (12.3)	1 (0.8)	4 (3.3)	5 (4.1)	0 (0.0)	4 (3.3)	5 (4.1)	1 (0.8)	36 (29.5)	0.76
Sum		14 (11.5)	2 (1.6)	2 (1.6)	34 (27.9)	3 (2.5)	10 (8.2)	6 (4.9)	1 (0.8)	5 (4.1)	6 (4.9)	1 (0.8)	84 (68.9)	0.78
Total		14 (11.5)	2 (1.6)	2 (1.6)	56 (45.9)	3 (2.5)	26 (21.3)	6 (4.9)	1 (0.8)	5 (4.1)	6 (4.9)	1 (0.8)	122 (100.0)	0.72

Each pathotype was categorized in accordance with the method of Hayashi et al. (2009).
– : incompatibility reaction, + : compatibility reaction.
Indexes of diversity were calculated using the method of Simpson (1949).

Table 6. Numbers of blast isolates in pathotypes against differential variety group ta, corresponding to resistance genes *Pita-2*, *Pi12*(t), *Pita*, *Pi19*(t) and *Pi20*(t), and the degrees of diversity in cluster groups

Cluster group		Number of blast isolates (%)													
		Pathotypes													
Reaction of DV	Resistance gene	ta000	ta001	ta002	ta003	ta013	ta020	ta021	ta022	ta023	ta031	ta033	ta113	ta201	ta400
	Pita-2 Pita Pi19(t)	- - -	- - +	- - -	- - +	- + +	- - -	- - +	- - -	- - +	- + +	- + +	+ + +	- - +	- - -
	Pita-2 Pita Pi20(t)	- - -	- - -	- - +	- - +	- - +	- + -	- + -	- + +	- + +	- + -	- + +	- - +	+ - -	- - -
	Pi12(t)	-	-	-	-	-	-	-	-	-	-	-	-	-	+
I		0 (0.0)	1 (0.8)	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)	1 (0.8)	0 (0.0)	1 (0.8)	2 (1.6)	1 (0.8)	0 (0.0)	0 (0.0)	1 (0.8)
II a		8 (6.6)	3 (2.5)	10 (8.2)	7 (5.7)	0 (0.0)	1 (0.8)	0 (0.0)	4 (3.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	1 (0.8)	0 (0.0)
II b		0 (0.0)	0 (0.0)	0 (0.0)	25 (20.5)	3 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	1 (0.8)	2 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)
II Sum		8 (6.6)	3 (2.5)	10 (8.2)	32 (26.2)	3 (2.5)	1 (0.8)	0 (0.0)	4 (3.3)	1 (0.8)	1 (0.8)	2 (1.6)	1 (0.8)	1 (0.8)	0 (0.0)
Total		8 (6.6)	4 (3.3)	10 (8.2)	33 (27.0)	3 (2.5)	1 (0.8)	1 (0.8)	4 (3.3)	2 (1.6)	3 (2.5)	3 (2.5)	1 (0.8)	1 (0.8)	1 (0.8)

the diversity in group IIb was the lowest, and those in the other groups did not differ greatly.

Based on the method proposed by Hayashi and Fukuta[4], we characterized 122 blast isolates based on the pathotypes in 5 variety groups. We found 13, 7, 8, 11 and 25 pathotypes in variety groups U, i, k, z and ta, respectively. Fourteen pathotypes (U63, i0, i7, k000, k100, z00, z04, z06, ta000, ta002, ta003, ta403, ta421 and ta431) had high frequencies and were considered dominant. Of these, 12 (i0, i7, k000, k100, z00, z06, ta000, ta002, ta003, ta403, ta421 and ta431) differed markedly in frequency between cluster groups I and II, while 4 (z00, ta000, ta002 and ta003) differed between subgroups IIa and IIb.

Examination of the numbers of pathotypes and the diversity indexes revealed that cluster group IIa always had the highest values, except when comparing the diversity index value against that of group I in variety group i. The diversity index values of group IIb were always the lowest among the 3 groups, except in the case of group z in comparison with group IIa.

(Table 6 continued)

Cluster group		Number of blast isolates (%)												Index of diversity
		Pathotypes											Total	
Reaction of DV	Resistance genes	ta401	ta402	ta403	ta412	ta420	ta421	ta422	ta423	ta431	ta602	ta603		
	Pita-2 Pita Pi19(t)	- - +	- - -	- - +	- + -	- - -	- - +	- - -	- - +	- + +	- - -	- - +		
	Pita-2 Pita Pi20(t)	- - -	- - +	- - +	- - +	- + -	- + -	- + +	- + +	- + -	+ - +	+ - +		
	Pi12(t)	+	+	+	+	+	+	+	+	+	+	+		
I		3 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	12 (9.8)	0 (0.0)	1 (0.8)	13 (10.7)	0 (0.0)	0 (0.0)	38 (31.1)	0.77
II	a	0 (0.0)	4 (3.3)	5 (4.1)	1 (0.8)	0 (0.0)	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)	1 (0.8)	1 (0.8)	48 (39.3)	0.88
	b	0 (0.0)	0 (0.0)	4 (3.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	36 (29.5)	0.49
	Sum	0 (0.0)	4 (3.3)	9 (9.7)	1 (0.8)	0 (0.0)	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)	1 (0.8)	1 (0.8)	84 (68.9)	0.81
Total		3 (2.5)	4 (3.3)	13 (9.7)	1 (0.8)	1 (0.8)	12 (9.8)	1 (0.8)	1 (0.8)	13 (10.7)	1 (0.8)	1 (0.8)	122 (100.0)	0.88

Each pathotype was categorized in accordance with the method of Hayashi et al. (2009).
– : incompatibility reaction, + : compatibility reaction.
Indexes of diversity were calculated using the method of Simpson (1949).

Race designation

In accordance with the methods of Hayashi and Fukuta[4], the 122 blast isolates were finally categorized into 92 races based on our pathotyping analyses in the 5 variety groups (Table 7).

One blast race, U63-i7-k100-z06-ta431 (group I) was common to 8 isolates, and U63-i0-k000-z04-ta003 (group II) was common to 7 isolates. U63-i0-k000-z07-ta003 (group II) and U63-i0-k000-z14-ta003 (group II) were common to 4 isolates in each. Each of the other blast races was common to only 1 to 3 blast isolates. The 10 blast isolates from wild rice were characterized into 10 different races.

Discussion

We evaluated a total of 122 blast isolates from Cambodia based on the reaction patterns of 1 susceptible variety, LTH, and of DVs harboring 23 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*, *Pi12*(t), *Pita*, *Pi19*(t) or *Pi20*(t)). We found high frequencies of occurrence of virulent blast isolates against 6 DVs (for *Pib*, *Pit*, *Pia*, *Piz-t*, *Pi19*(t) and *Pi20*(t)) and of LTH, and low frequencies against 11 DVs (for *Pish*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, *Pi7*(t), *Pi9*(t), *Piz* and *Pita-2*(2)). These findings indicated that blast genes virulent to the resistance genes *Pib*, *Pit*, *Pia*, *Piz-t*, *Pi19*(t) and *Pi20*(t) and avirulent to the resistance genes *Pish*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, *Pi7*(t), *Pi9*(t), *Piz* and *Pita-2* in rice were distributed widely among blast fungi in Cambodia.

In accordance with the methods of Hayashi and Fukuta[4], we classified the blast isolates into 92 races based on the pathotypes of the 5 variety groups. Noda et al.[14] identified 12 kinds of blast race among 129 isolates collected from all over the Mekong river delta of Vietnam. They used Japanese DVs carrying 12 resistance genes, *Pia*, *Pik-s*, *Pii*, *Pik*, *Pik-m*, *Piz*, *Pita*, *Pita-2*, *Piz-t*, *Pik-p*, *Pib* or *Pit*, selected by Yamada et al.[21], Kiyosawa[8,9], and Kiyosawa et al.[10]. Mekwatanakarn et al.[13] investigated the pathogenicities of 527 blast isolates from Thailand using CO 39 NILs carrying 9 genes (*Pi1*, *Pi1-LAC*(t), *Pi1-TTP*(t), *Piz-5*, *Pi3*, *Pi4a* and *Pi4b*(t) (*Pita*), *Pi4a-PKT*(t), *Pi4a-TTP*(t) or *Pia*) and LTH NILs carrying 6 genes (*Pik-m*, *Pita*, *Pita-2*, *Pib*, *Pik-p*, or *Pik*); the blast isolates were classified into 175 races. In Bhutan, 110 isolates were differentiated into 53 races based on the reactions of CO 39 NILs carrying *Pi4b* (*Pita*), *Pi2* (*Piz-5*), *Pi3*, *Pi4a* (*Pita*), or *Pi1* and LTH NILs carrying *Pita*, *Pib*, *Pita-2*, *Pik-m* or *Pik-p* (Thinlay et al. 2000). In China, 792 isolates were classified into 344 races using LTH NILs carrying *Pita-2*, *Pib*, *Pik*, *Pik-m*, *Pita* or *Pik-p* and CO 39 NILs carrying *Pita*, *Piz-5*, *Pi3* or *Pi13*[1]. The pathogenicities of 119 blast isolates collected from the Philippines were characterized using 19 DVs carrying 18 resistance genes (*Pia*, *Pib*, *Pii*, *Pit*, *Pita*, *Pish*, *Piz-t*, *Pi3*, *Piz-5*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pik-s*, *Pita-2*, *Piz*, *Pi1* or *Pi20*(t)); the isolates were categorized into 70 races[17]. Many kinds of blast races have thus been found in Bhutan[19], China[1], Thailand[13], and the Philippines[17,22], but few blast isolates were classified into each race, and no dominant blast races were found. It is difficult to compare our Cambodian data with those from these previous studies, because the numbers

Table 7. Race designation of each blast isolate in Cambodia

No.	Race	No. of isolate	Cluster group	No.	Race	No. of isolate	Cluster group	No.	Race	No. of isolate	Cluster group	No.	Race	No. of isolate	Cluster group
1	U00-i0-k000-z00-ta000	1	IIa	24	U41-i0-k000-z04-ta002	1	IIa	47	U61-i0-k110-z04-ta403[b]	1	IIa	70	U63-i0-k001-z05-ta403	1	IIa
2	U00-i0-k000-z00-ta001	1	IIa	25	U41-i0-k000-z04-ta003	1	IIa	48	U61-i1-k000-z00-ta002	1	IIa	71	U63-i0-k002-z04-ta403	1	IIa
3	U01-i0-k000-z00-ta001	1	IIa	26	U41-i4-k000-z04-ta022[b]	1[a]	IIa	49	U61-i2-k100-z04-ta421	1	I	72	U63-i0-k020-z07-ta003	1	IIb
4	U01-i0-k000-z00-ta003	1	IIa	27	U41-i6-k000-z00-ta000[b]	1[a]	IIa	50	U61-i4-k000-z04-ta401	1	I	73	U63-i0-k100-z00-ta003	1	IIb
5	U01-i0-k002-z04-ta412	1	IIa	28	U42-i0-k000-z13-ta000	1	IIa	51	U61-i4-k000-z04-ta423	1	I	74	U63-i0-k100-z04-ta003	2	IIb
6	U01-i0-k100-z00-ta020[b]	1	IIa	29	U43-i0-k000-z04-ta403	1	IIb	52	U61-i4-k100-z04-ta401	1	I	75	U63-i0-k100-z04-ta402[b]	1	IIa
7	U03-i0-k000-z04-ta003	2	IIa	30	U43-i0-k000-z05-ta403[b]	1	IIb	53	U61-i6-k100-z04-ta421	1+1[a]	I	76	U63-i0-k100-z06-ta422[b]	1	IIa
8	U03-i2-k100-z04-ta000[b]	1	IIa	31	U43-i0-k000-z06-ta000	1	IIa	54	U63-i0-k000-z04-ta002	1	IIa	77	U63-i1-k000-z04-ta003	1	IIb
9	U03-i4-k001-z06-ta002	1	IIa	32	U43-i0-k000-z06-ta002	1	IIa	55	U63-i0-k000-z04-ta003[b,c]	7	IIb	78	U63-i2-k000-z04-ta003[b]	1	IIa
10	U03-i6-k005-z02-ta002	1	IIa	33	U43-i0-k000-z16-ta003	1	IIb	56	U63-i0-k000-z04-ta023	1	IIb	79	U63-i3-k100-z06-ta421	1	I
11	U21-i0-k000-z04-ta403	1	IIa	34	U43-i0-k000-z16-ta603[b]	1	IIa	57	U63-i0-k000-z04-ta403[b]	2	IIb	80	U63-i3-k100-z06-ta431	1	I
12	U21-i0-k000-z05-ta003[b]	1	IIa	35	U43-i0-k100-z04-ta421	1	I	58	U63-i0-k000-z06-ta000[b]	1	IIa	81	U63-i4-k000-z04-ta002[b]	1	IIa
13	U21-i0-k100-z04-ta400	1[a]	I	36	U43-i1-k100-z04-ta021[b]	1	I	59	U63-i0-k000-z06-ta003	1	IIb	82	U63-i4-k100-z04-ta420	1	I
14	U21-i4-k000-z00-ta201[b]	1	IIa	37	U43-i4-k000-z04-ta022	1	IIa	60	U63-i0-k000-z06-ta013	2	IIb	83	U63-i6-k100-z04-ta421	1[a]	I
15	U23-i0-k000-z01-ta001	1	IIa	38	U43-i6-k000-z04-ta113	1	IIa	61	U63-i0-k000-z06-ta033	1	IIb	84	U63-i6-k100-z04-ta423[b]	1[a]	IIa
16	U23-i0-k100-z04-ta421	1	I	39	U43-i7-k000-z06-ta033[b]	1[a]	I	62	U63-i0-k000-z07-ta003	4	IIb	85	U63-i7-k000-z06-ta031	1	I
17	U23-i3-k100-z04-ta401	1[a]	I	40	U43-i7-k100-z04-ta001	1	I	63	U63-i0-k000-z07-ta602[b]	1	IIa	86	U63-i7-k100-z04-ta003	1	I
18	U23-i6-k005-z00-ta002	2	IIa	41	U43-i7-k100-z04-ta421	1	I	64	U63-i0-k000-z14-ta003	4	IIb	87	U63-i7-k100-z04-ta023	1	I
19	U23-i6-k005-z00-ta402	1	IIa	42	U53-i7-k100-z06-ta031	1	I	65	U63-i0-k000-z14-ta403	1	IIa	88	U63-i7-k100-z04-ta421	1+1[a]	I
20	U23-i6-k005-z02-ta402	1	IIa	43	U61-i0-k000-z04-ta003[b]	1	IIa	66	U63-i0-k000-z16-ta003	2	IIb	89	U63-i7-k100-z04-ta431	3	I
21	U40-i1-k004-z01-ta000[b]	1[a]	IIa	44	U61-i0-k000-z04-ta022[b]	1	IIa	67	U63-i0-k000-z16-ta013	1	IIb	90	U63-i7-k100-z06-ta421	2	I
22	U41-i0-k000-z00-ta000	1	IIa	45	U61-i0-k000-z04-ta031	1	IIb	68	U63-i0-k000-z16-ta033	1	IIb	91	U63-i7-k100-z06-ta431	8	I
23	U41-i0-k000-z00-ta002	1	IIa	46	U61-i0-k000-z06-ta402[b]	1	IIa	69	U63-i0-k000-z17-ta003	1	IIb	92	U73-i7-k100-z06-ta431	1	I
													Total	122	

[a] Wild rice origin.

[b] Sampled in the Mekong river region.

[c] Two blast isolates out of 7 were sampled in the Mekong river region.

Each race was categorized in accordance with the method of Hayashi et al. (2009).

and kinds of resistance genes and genetic backgrounds of the DVs used previously differed from those of monogeneic lines[20] and NILs[18]. However, as in the other reports, we found numerous races.

The blast isolates were classified into 3 cluster groups, I, IIa, and IIb, based on the reaction patterns of 25 DVs and LTH; 14 pathotypes, namely U63, i0, i7, k000, k100, z00, z04, z06, ta000, ta002, ta003, ta403, ta421 and ta431, were dominant. Of these, 2 pathotypes, U63 and z04, were found in all 3 cluster groups. Thus these 2 pathotypes, U63 and z04, were basically dominant, and 4 blast genes corresponding to the resistance genes *Pib*, *Pit*, *Pia* and *Piz-t* in rice were distributed together in Cambodia. The other 12 pathotypes, i0, i7, k000, k100, z00, z06, ta000, ta002, ta003, ta403, ta421 and ta431, occurred at different frequencies in cluster groups I and II. The blast races of group I harbored genes at high frequencies for virulence to *Pii*, *Pi3*, *Pi5*(t), *Pik-s*, *Piz-5*, *Piz-t*, *Pi12*(t) and *Pita.* In contrast, the races of group II harbored genes at high frequencies for avirulence to *Pii*, *Pi3*, *Pi5*(t), *Pik-s*, *Pi9*(t), *Piz-5* and *Piz-t* and for virulence to *Pi19*(t) and *Pi20*(t). The 3 pathotypes z00, ta002 and ta003, for avirulence genes of variety groups z and ta except for DV of *Pi19*(t), divided between subgroups IIa and IIb as additional factors. In other words, the presence or absence of the 12 pathotypes i0, i7, k000, k100, z00, z06, ta000, ta002, ta003, ta403, ta421 and ta431 plays a major role in differentiating blast races in Cambodia.

Blast isolates of groups I, IIa and IIb were distributed with similar frequencies in the Tonle Sap region, whereas group IIa was found at high frequency, and groups I and IIb at low frequency, in the Mekong river region. In terms of race, a comparison of the blast isolates in group IIa between the 2 regions revealed that the 6 found only in the Mekong river region were of pathotype k100 or k110. Thus the distributions of blast races differed between the Tonle Sap and Mekong river regions; notably, the blast isolates from the Mekong river tended to be virulent against the resistance gene *Pik-s* in variety group k. Examination of the numbers of pathotypes and diversity index values of the blast isolates indicated that cluster group IIa always had the highest values, except in the case of the diversity index values in variety group z. The numbers of pathotypes and diversity index values in group IIb were always the lowest among the 3 groups except for group z, although there was little difference in diversity index values between group IIa and IIb in group z. Among the 3 blast isolate groups, group IIa, which included genes at low frequencies for virulence to *Pib*, *Pit*, *Pia*, *Piz-5*, *Piz-t* and *Pi19*(t) and maintained a high degree of diversity, was distributed commonly in all regions. Additionally, groups I and IIb, which included several dominant pathotypes, were distributed in the Tonle Sap region. In other words, group IIa represented the basic population of blast races in Cambodia, and groups I and IIb were modified from IIa in accordance with rice cultivation conditions in the Tonle Sap region. These unique distributions of blast races among 3 regions might occur and be attributable to differences in genotypes of blast resistance genes in rice varieties cultivated based on the gene-for-gene theory[2,15]. It will be necessary to clarify the genotypes of rice varieties in each region of Cambodia and the relationship between blast races and rice varieties.

A total of 10 blast isolates from wild rice (*O. rufipogon*) were collected and analyzed for pathogenicity. These blast isolates were categorized into groups I (6 isolates) and IIa (4 isolates) and were not found in group IIb. In the Tonle Sap region, the 5 blast isolates were found only in group I. In the Mekong river region, 1 isolate occurred in group I and 4 in group IIa; their distributions might correspond to the distributions of isolates on cultivated rice in this region. These results suggested that the blast races on wild rice were differentiated according to the pathotypes of those on cultivated rice. Many more samples of blast fungus are needed to clarify the distributions of the dominant blast races in detail and the relationship between those on cultivated and wild rice respectively.

Although the blast isolates used were limited, our results are a clear first step in elucidating the diversity and differentiation of blast races in Cambodia using monogeneic lines[20,6] and LTH NILs[18] as DVs, along with the designation system proposed by Hayashi and Fukuta[4]. This information will be used to select standard differential blast isolates and develop a differential system with increasing numbers of blast isolates. It will also facilitate detailed analyses of pathogenicity. By developing and applying this differential system, the pathogenicity of blast isolates and the genotypes of resistance genes in rice varieties will be enhanced in breeding and pathological studies, enabling a system of durable blast protection to be built up in Cambodia. Information on blast races in neighboring countries such as Vietnam, Laos, Thailand, and China will be also needed if we are to understand the distribution, differentiation, and influence of races and develop future protection systems. To enhance these studies, the development of common materials, methods, and tools, such as monogeneic lines[20] and LTH NILs[18], along with a scoring system and designation system[4,5], will be useful. An international collaborative study, "Blast Research Network for Stable Rice Production," is underway and will help clarify the diversity of blast races, distribute the differential system, and establish a system to protect against blast disease on a global scale. These Cambodian data will be evaluated and compared with international information as part of this collaborative research.

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