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Pathogenicity Analysis of Blast (*Pyricularia oryzae* Cavara) Isolates from West Africa

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Abstract

To clarify the diversity of blast (*Pyricularia oryzae* Cavara) races in West Africa, we investigated the pathogenicity of 96 blast isolates collected from different ecosystems in six countries, Bénin, Burkina Faso, Côte-d'Ivoire, Ghana, Mali, and Nigeria, and characterized using rice (*Oryza sativa* L.) differential varieties for 23 resistance genes and a susceptible control variety Lijangxintuanheigu (LTH). Virulent blast isolates occurred with high frequencies against LTH or differential varieties carrying *Pia*, *Pik-s*, *Pi19*(t), *Pi12*(t), *Pit*, *Pii*, *Pi3* and *Pi5*(t). Conversely, they occurred at low frequencies against differential varieties carrying *Pish*, *Pi9*(t), *Piz*, *Piz-5*, *Piz-t* and *Pita-2* and at intermediate frequencies against those carrying *Pib*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, *Pita* and *Pi20*(t). The isolates were re-characterized as reaction types of five groups, U, i, k, z and ta; accordingly, LTH and the differential varieties were categorized based on the chromosome locations of the resistance genes harbored in each genetic background. Twelve, seven, thirteen, eight and seventeen reaction types were found in groups U, i, k, z and ta, respectively. Thirteen of these, namely U43, U63, i7, k100, k106, k177, z00, z03, z04, ta003, ta031, ta403 and ta431, showed high frequencies of blast isolates and were considered dominant reaction types. We used the infection types of the differential varieties against these blast isolates to conduct a cluster analysis, and the isolates were classified into two clusters, I and II. Substantial differences in frequencies between both clusters were found in the reactions of differential varieties carrying these genes in the *Pii*, *Pik* and *Pita* chromosome regions. Both clusters I and II were distributed in an upland ecosystem with high and similar frequencies, whereas group II was mainly distributed in the irrigated lowland. These results suggested that many types of blast races were distributed in the upland ecosystem, while the limited ones were in lowland in West Africa. Finally, these blast isolates were categorized into 79 races in accordance with a new designation system based on the five differential variety groups used for reaction typing.

Discipline: Plant disease
Additional Key words: differential variety, diversity, rice (*Oryza sativa* L.)

Introduction

Rice blast disease caused by *Pyricularia oryzae* Cavara is an important fungal disease of rice (*Oryza sativa* L.), which causes yield losses in most rice-producing areas of the world. The disease has caused yield losses as high as 70 to 80%, when predisposing factors (high mean temperatures, relative humidity exceeding 90%, or the presence of dew, drought stress, or excessive nitrogen fertilization) favor epidemic development (Piotti et al. 2005). Delassus (1973) reported rice yield losses of nearly 80% in West Africa. Blast causes serious damage to all ecosystems of upland, irrigated lowland, and rainfed lowland in most countries in West Africa, particularly Burkina Faso, where farmers have

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intensified production using improved cultivars and fertilizers (Séré 1999). Rice blast is therefore a major constraint to increasing rice production in many countries in West Africa.

Recently, the development of resistant cultivars has been considered the most effective strategy for protecting rice crops against this disease. In West Africa, it is also the most economical and effective way of controlling rice blast, particularly in fields of resource-poor farmers (Séré 2007). Unfortunately, the causal fungus can only overcome this resistance a few years after the wide cultivation of improved cultivars. Blast races change according to the genotypes of resistance genes in the rice cultivar, and when the breakdown of such resistance appears. The relationships between virulence genes in the blast fungus and resistance genes in the rice cultivar have been explained by the gene-for-gene theory (Flor 1971, Silué 1992).

Pathogenicity studies of blast isolates have been performed in Asian countries using several differential variety (DV) sets. Noda et al. (1999) identified 12 kinds of blast race among 129 isolates collected from all over the Mekong River Delta area in Vietnam; they used 12 Japanese DVs developed for *Pia*, *Pik-s*, *Pii*, *Pik*, *Pik-m*, *Piz*, *Pita*, *Pita-2*, *Piz-t*, *Pik-p*, *Pib* and *Pit* by Yamada et al. (1976) and Kiyosawa (1981, 1984). Mekwatanakarn et al. (2000) investigated the pathogenicities of 527 blast isolates from Thailand using two kinds of set of near isogenic lines (NILs) for the resistance genes *Pi1*, *Pi1-LAC*(t), *Pi1-TTP*(t), *Piz-5*, *Pi3*, *Pi4a* and *Pi4b*(t) (*Pita*), *Pi4a-PKT*(t), *Pi4a-TTP*(t), *Pia*, *Pik-m*, *Pita*, *Pita-2*, *Pib*, *Pik-p* and *Pik* as DVs; the isolates were classified into 175 races. In Bhutan, 110 isolates were differentiated into 53 races based on the reactions of NILs for *Pi4b* (*Pita*), *Pi2* (*Piz-5*), *Pi3*, *Pi4a* (*Pita*), *Pi1*, *Pib*, *Pita-2*, *Pik-m* or *Pik-p* (Thinlay et al. 2000). In China, 792 isolates were classified into 344 races using NILs for *Pita-2*, *Pib*, *Pik*, *Pik-m*, *Pita*, *Pik-p Piz-5*, *Pi3* or *Pi1*3 (Chen et al. 2001). The pathogenicities of 119 blast isolates collected from the Philippines have been characterized using 18 Japanese DVs developed 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) by Yamada et al. (1976) and Kiyosawa (1981, 1984); the isolates were categorized into 70 races (Telebanco-Yanoria et al. 2008). Because different numbers and kinds of DVs were used in these previous studies, the results are difficult to compare. However, the results indicate that variations in the DVs used in each study could explain the wide-ranging scope of reaction in the blast populations of Southeast Asian counties and China, and demonstrate the usefulness of DVs in pathogen analysis.

Conversely, in West Africa, pathological studies of blast fungus have been performed using Asian DVs with known resistance genes or NILs of the cultivar CO 39 developed by Mackill and Bonman (1992); these studies have focused mainly on nursery trap analysis when screening of sites for durable resistance (Séré et al. 2004, Nutsugah et al. 2008, Odjo et al. 2011). Nutsugah et al. (2008) identified 25 reaction types among 71 blast isolates from Ghana using the international DVs CO 39, M201, and Yashiro-mochi (Valent et al. 1991, Ling & Ou 1969), the resistance genes of which were unknown. Therefore, pathogenicity studies of blast isolates in West Africa have been limited, and the diversity and differentiation of blast races remain to be clarified. To develop a durable system to protect against blast fungus, we need to understand the differentiation and distribution of blast races in the first step.

Tsunematsu et al. (2000) and Kobayashi et al. (2007) developed monogenic lines as a set of international DVs for targeting 23 kinds of resistance gene. These monogenic lines were produced by introducing single resistance genes into the genetic background of a Chinese Japonica-type rice cultivar, Lijangxintuanheigu (LTH). As advanced DVs, Telebanco-Yanoria et al. (2010) bred LTH NILs targeting 11 resistance genes. These monogenic lines and LTH NILs are minimally influenced by genetic background and are the most effective materials for use as international DVs. Here, we used these monogenic lines and LTH NILs to elucidate the pathogenicity of blast isolates collected in West Africa and thus understand the diversity and differentiation of blast races in this area.

Materials and Methods

Differential varieties

To clarify the pathogenicity of blast isolates, we used the susceptible Chinese rice cultivar LTH, along with DVs in the form of 23 monogenic lines (Tsunematsu et al. 2000, Kobayashi et al. 2007) and two NILs (Telebanco-Yanoria et al. 2010) with the genetic background of LTH and targeting 23 resistance genes. The monogenic lines were IRBLsh-B for *Pish*, IRBLb-B for *Pib*, IRBLt-K59 for *Pit*, 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*(t), IRBLz-Fu for *Piz*, IRBLz5-CA for *Piz-5*, IRBLzt-T for *Piz-t*, IRBLta2-Pi for *Pita-2*, IRBLta2-Re for *Pita-2*, IRBL12-M for *Pi12*(t), IRBLta-K1 for *Pita*, IRBLta-CP1 for *Pita*, IRBL19-A for *Pi19*(t), and IRBL20-IR24 for *Pi20*(t) and the two LTH NILs were IRBLkh-K3[LT] for *Pik-h* and IRBLk-K[LT] for *Pik* (Fig. 1).

Blast isolate collection

Blast samples on rice plants were collected from three different ecosystems — upland, rainfed lowland, and irrigated lowland — in five countries (Bénin, Burkina-Faso, Côte-d'Ivoire, Ghana, Mali, and Nigeria) in West Africa

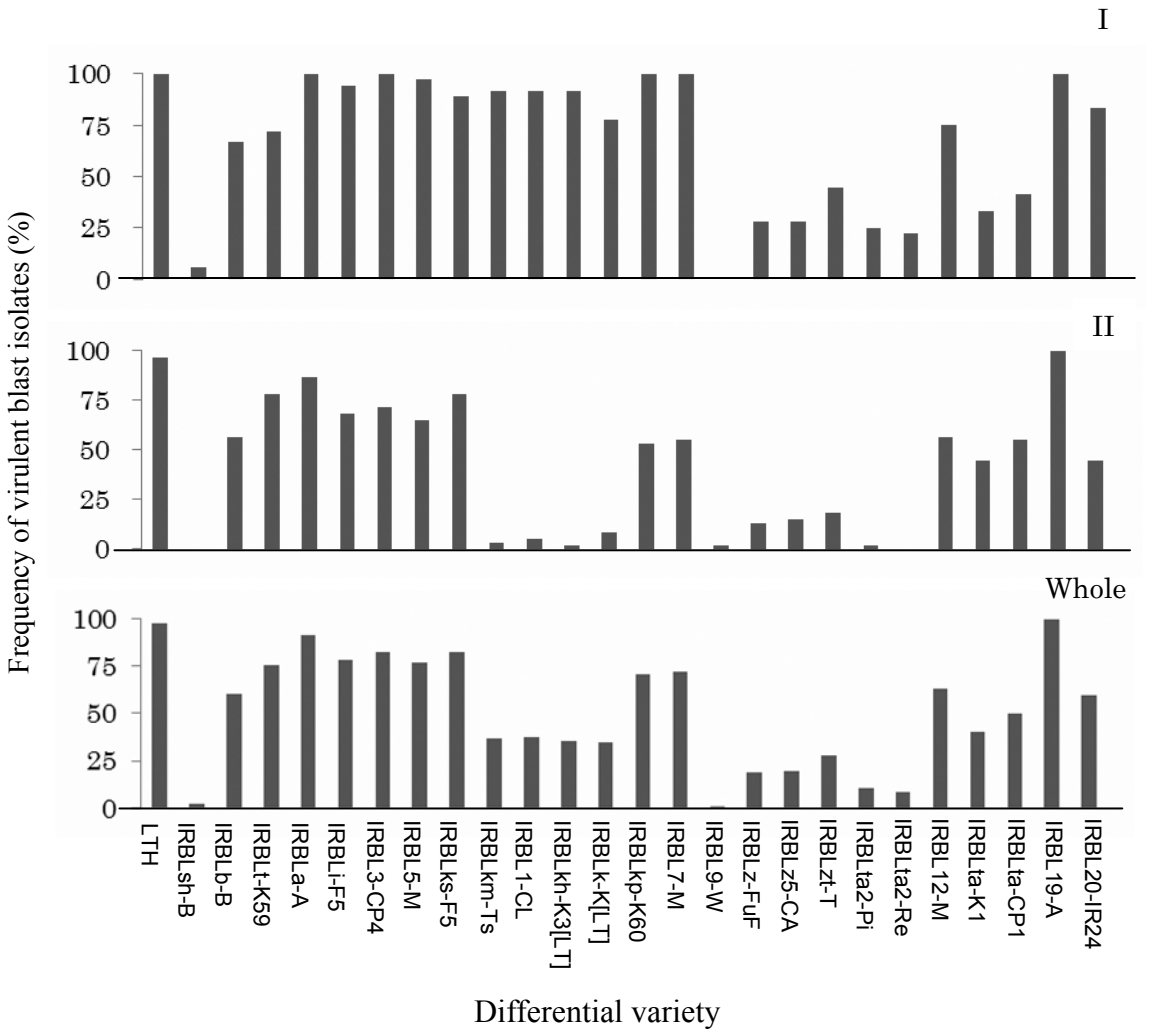

Fig. 1. Frequencies of occurrence of virulent blast isolates from West Africa

Cluster analysis using Ward's hierarchical clustering method was performed using data on the reaction patterns of 25 differential varieties and a susceptible variety, Lijangxintuanheigu (LTH), to blast isolates. Whole (n = 96), Cluster I (n = 36), Cluster II (n = 60)

Table 1. Blast isolates for West Africa

Ecosystem	No. of blast isolates (%)						
	Countries						
	Benin	Burkina- Faso	Côte-d'Ivoire	Mali	Nigeria	Ghana	Total
Upland	36	0 (0.0)	7 (7.3)	15 (15.6)	0 (0.0)	0 (0.0)	58 (60.4)
Rainfed lowland	1 (1.0)	1 (1.0)	0 (0.0)	0 (0.0)	3 (3.1)	0 (0.0)	5 (5.2)
Irrigated lowland	0 (0.0)	8 (8.3)	0 (0.0)	23 (24.0)	0 (0.0)	2 (2.1)	33 (34.4)
Total	37 (38.5)	9 (9.4)	7 (7.3)	38 (4.0)	3 (3.1)	2 (2.1)	96 (100.0)

(Table 1). The samples from Bénin and Côte-d'Ivoire were collected only from uplands. In Burkina Faso and Ghana, blast isolates were collected from irrigated lowlands, in Mali, from both uplands and irrigated lowlands and in Nigeria, from rainfed lowlands. A total of 96 blast isolates — 58 (60.4%) from upland, 5 (5.2%) from rainfed lowland, and 33 (34.4%) from irrigated lowland — were used to investigate pathogenicity (Table 2). Monoconidial isolation was

Table 2. Relationships between rice cultivation ecosystems and clusters of blast isolates from West Africa

Rice cultivation ecosystem	No. of blast isolates (%)		
	Cluster		
	I	II	Total
Upland	31 (32.3)	27 (28.1)	58 (60.4)
Rainfed lowland	3 (3.1)	2 (2.1)	5 (5.2)
Irrigated lowland	2 (2.1)	31 (32.3)	33 (34.4)
Total	36 (37.5)	60 (62.5)	96 (100.0)

Table 3. Number of blast isolates in each reaction type categorized based on the reactions of differential varieties and LTH in group U

Resistance gene harbored by DV			No. of virulent isolates (%)												Diversity index
			Reaction types											Total	
			U00	U01	U03	U21	U23	U33	U40	U41	U43	U61	U63	U73	
Reaction	*Pish*	LTH	- -	- +	- +	- +	- +	+ +	- -	- +	- +	- +	- +	+ +		
	Pib	*Pia*	- -	- -	- +	+ -	+ +	+ +	- -	- -	- +	+ -	+ +	+ +		
	Pit		-	-	-	-	-	-	+	+	+	+	+	+		
Cluster	I		0 (0.0)	0 (0.0)	5 (5.2)	0 (0.0)	4 (4.2)	1 (1.0)	0 (0.0)	0 (0.0)	7 (7.3)	0 (0.0)	18 (18.8)	1 (1.0)	36 (37.5)	0.68
	II		1 (1.0)	2 (2.1)	3 (3.1)	1 (1.0)	6 (6.3)	0 (0.0)	1 (1.0)	1 (1.0)	18 (18.8)	2 (2.1)	25 (26.0)	0 (0.0)	60 (62.5)	0.72
	Total		1 (1.0)	2 (2.1)	8 (8.3)	1 (1.0)	10 (10.4)	1 (1.0)	1 (1.0)	1 (1.0)	25 (26.0)	2 (2.1)	43 (44.8)	1 (1.0)	96 (100.0)	0.71

A total of four DVs — IRBLsh-B for *Pish*, IRBLb-B for *Pib*, IRBLt-K59 for *Pit* and IRBLa-A for *Pia* — and a susceptible control, LTH, were included in group U by Hayashi and Fukuta (2009).
Reaction types were classified according to the susceptibility reactions of these DVs and LTH.
+: pp. susceptible; -: pp. resistant.
Diversity index was calculated by the method of Simpson (1949).

performed in accordance with the method of Hayashi et al (2009).

Inoculation and disease assessment

Three seeds of LTH and each of the DVs were sown in a plastic tray with garden soil. Two-three seeding plants of each were prepared for pathogenicity testing and average scoring codes (see below) of two duplications were used to indicate the degrees of infection.

Blast isolates were inoculated using the method of Hayashi et al. (2009). The spore concentration was standardized to 1×10^5 conidia per milliliter. DVs were inoculated approximately 19 days after seed sowing (at the 4- to 5-leaf stage) by spraying 20 ml of spore suspension on each tray with a fine atomizer. Inoculated plants were incubated for 1 day at 25 ºC and 100% relative humidity and then transferred to a greenhouse for 7 days. The degree of disease of each seedling was evaluated 7 days after inoculation. The reactions of DVs or rice varieties in the genetic evaluation for resistance were categorized into eight scoring codes and summarized into 0-2, resistant (R) and 3-5, susceptible (S). However, IRBLta2-Pi for *Pita-2* was evaluated as 0-3 resistant (R) and 4-5 susceptible (S), whereas IRBL5-M for *Pi5*(t) was evaluated as 0-1 resistant (R) and 2-5 susceptible (S), according to the evaluation method of Hayashi at al. (2009).

Characterization of blast isolates and race designation

Virulent blast isolates were designated using the method of Hayashi and Fukuta (2009) as an international differential system using LTH, the monogenic lines, or LTH NILs. The blast isolates were designated by the reaction patterns of 25 monogenic lines targeting the 23 resistance genes and LTH. The lines were categorized into five groups: pp. group U, with five lines, LTH, IRBLa-A, IRBLsh-B, IRBLb-B and IRBLt-K59 (Table 3); group i, with three lines with the *Pii* locus on chromosome 9 (Table 4); group k, with seven lines with the *Pik* region on chromosome 11 (Table 5); group z, with four lines with the *Piz* region on chromosome 6 (Table 6); and group ta, with seven lines with the *Pita* region on chromosome 12 (Table 7). We replaced two monogenic lines, IRBLkh-K3 and IRBLk-K, of Hayashi and Fukuta (2009) with two LTH NILs, IRBLkh-K3[LT] and IRBLk-K[LT], respectively. Each group comprised some varietal

Table 4. Number of blast isolates in each reaction type categorized based on the reactions of differential varieties in group i

Resistance gene harbored by DV		No. of virulent isolates (%)								
		Reaction types							Total	Diversity index
		i0	i1	i2	i3	i5	i6	i7		
Reaction	*Pii*	-	+	-	+	+	-	+		
	Pi3	-	-	+	+	-	+	+		
	Pi5(t)	-	-	-	-	+	+	+		
Cluster	I	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)	2 (2.1)	33 (34.4)	36 (37.5)	0.16
	II	14 (14.6)	1 (1.0)	3 (3.1)	3 (3.1)	2 (2.1)	2 (2.1)	35 (36.5)	60 (62.5)	0.6
	Total	14 (14.6)	1 (1.0)	3 (3.1)	4 (4.2)	2 (2.1)	4 (4.2)	68 (70.8)	96 (100.0)	0.47

A total of three DVs — IRBLi-F5 for *Pii*, IRBL3-CP4 for *Pi3* and IRBL5-M for *Pi5*(t) — were included in group i by Hayashi and Fukuta (2009).
Reaction types were classified according to the susceptibility reactions of these DVs.
+: pp. susceptible; -: pp. resistant.
Diversity index was calculated by the method of Simpson (1949).

Table 5. Number of blast isolates in each reaction type, categorized based on the reactions of differential varieties in group k

Resistance gene harbored by DV				No. of virulent isolates (%)														
				Reaction types													Total	Diversity index
				k000	k006	k076	k077	k100	k104	k106	k107	k116	k126	k157	k176	k177		
Reaction	*Pik-s*	*Pik-m*	*Pik*	- - -	- - -	- + -	- + +	+ - -	+ - -	+ - -	+ - +	+ + -	+ - -	+ + +	+ + -	+ + +		
		Pi1	*Pik-p*	- -	- +	+ +	+ +	- -	- -	- +	- +	- +	+ +	- +	+ +	+ +		
		Pik-h	*Pi7*(t)	- -	- +	+ +	+ +	- -	- +	- +	- +	- +	- +	+ +	+ +	+ +		
Cluster	I			0 (0.0)	0 (0.0)	1 (1.0)	3 (3.1)	0 (0.0)	0 (0.0)	3 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (4.2)	25 (26.0)	36 (37.5)	0.49
	II			8 (8.3)	5 (5.2)	0 (0.0)	0 (0.0)	19 (19.8)	1 (1.0)	18 (18.8)	4 (4.2)	1 (1.0)	3 (3.1)	1 (1.0)	0 (0.0)	0 (0.0)	60 (62.5)	0.78
	Total			8 (8.3)	5 (5.2)	1 (1.0)	3 (3.1)	19 (19.8)	1 (1.0)	21 (21.9)	4 (4.2)	1 (1.0)	3 (3.1)	1 (1.0)	4 (4.2)	25 (26.0)	96 (100)	0.83

A total of seven DVs — IRBLks-F5 for *Pik-s*, IRBLkm-Ts for *Pik-m*, IRBL1-CL for *Pi1*, IRBLkh-K3[LT] for *Pik-h*, IRBLk-K[LT] for *Pik*, IRBLkp-K60 for *Pik-p* and IRBL7-M for *Pi7*(t) — were included in group k categorized by Hayashi and Fukuta (2009).
Reaction types were classified according to the susceptibility reactions of these DVs.
+: pp. susceptible; -: pp. resistant.
Diversity index was calculated by the method of Simpson (1949).

Table 6. Number of blast isolates in each reaction type, categorized based on the reactions of differential varieties in group z

Resistance gene harbored by DV			No. of virulent isolates (%)									
			Reaction types								Total	Diversity index
			z00	z01	z02	z03	z04	z05	z07	z10		
	Pi9	*Piz*	- -	- +	- -	- +	- -	- +	- +	+ -		
Reaction		*Piz-5*	-	-	+	+	-	-	+	-		
		Piz-t	-	-	-	-	+	+	+	-		
Cluster	I		11 (11.5)	0 (0.0)	1 (1.0)	8 (8.3)	14 (14.6)	1 (1.0)	1 (1.0)	0 (0.0)	36 (37.5)	0.70
	II		38 (39.6)	1 (1.0)	2 (2.1)	7 (7.3)	11 (11.5)	0 (0.0)	0 (0.0)	1 (1.0)	60 (62.5)	0.55
	Total		49 (51.0)	1 (1.0)	3 (3.1)	15 (15.6)	25 (26.0)	1 (1.0)	1 (1.0)	1 (1.0)	96 (100.0)	0.65

A total of four DVs — IRBL9-W for *Pi9*, IRBLz-Fu for *Piz*, IRBLz5-CA for *Piz-5* and IRBLzt-T for *Piz-t* — were included in group z categorized by Hayashi and Fukuta (2009).
Reaction types were classified according to the susceptibility reactions of these DVs.
+: pp. susceptible; -: pp. resistant.
Diversity index was calculated by the method of Simpson (1949).

Table 7. Number of blast isolates in each reaction type, categorized based on the reactions of differential varieties (DVs) in group ta

Resistance gene harbored by DV			No. of virulent isolates (%)									
			Reaction types									
			ta001	ta003	ta021	ta023	ta031	ta033	ta233	ta333	ta401	ta403
Pita-2	*Pita*	*Pi19*(t)	- - +	- - +	- - +	- - +	- + +	- + +	- + +	+ + +	- - +	- - +
Pita-2	*Pita*	*Pi20*(t)	- - -	- - +	- + -	- + +	- + -	- + +	+ + +	+ + +	- - -	- - +
Pi12(t)			-	-	-	-	-	-	-	-	+	+
Cluster	I		1 (1.0)	4 (4.2)	0 (0.0)	1 (1.0)	1 (1.0)	1 (1.0)	1 (1.0)	1 (1.0)	3 (3.1)	13 (13.5)
	II		3 (3.1)	8 (8.3)	1 (1.0)	1 (1.0)	13 (13.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	15 (15.6)
	Total		4 (4.2)	12 (12.5)	1 (1.0)	2 (2.1)	14 (14.6)	1 (1.0)	1 (1.0)	1 (1.0)	3 (3.1)	28 (29.2)

(Table 7 continued)

Resistance gene harbored by DV			No. of virulent isolates (%)							Total	Diversity index
			Reaction Types								
			ta413	ta421	ta423	ta431	ta433	ta523	ta733		
Pita-2	*Pita*	*Pi19*(t)	- + +	- - +	- - +	- + +	- + +	+ - +	+ + +		
Pita-2	*Pita*	*Pi20*(t)	- - +	- + -	- + +	- + -	- + +	- + +	+ + +		
Pi12(t)			+	+	+	+	+	+	+		
Cluster	I		0 (0.0)	1 (1.0)	1 (1.0)	0 (0.0)	1 (1.0)	0 (0.0)	7 (7.3)	36 (37.5)	0.81
	II		1 (1.0)	3 (3.1)	1 (1.0)	13 (13.5)	0 (0.0)	1 (1.0)	0 (0.0)	60 (62.5)	0.82
	Total		1 (1.0)	4 (4.2)	2 (2.1)	13 (13.5)	1 (1.0)	1 (1.0)	7 (7.3)	96 (100.0)	0.85

A total of seven DVs — IRBLta2-Re and IRBLta2-Pi for *Pita-2*(2), IRBL12-M for *Pi12*(t), IRBLta-K1 and IRBLta-CP1 for *Pita*(2), IRBL19-A for *Pi19*(t) and IRBL20-IR24 for *Pi20*(t) — were included in group ta categorized by Hayashi and Fukuta (2009).
Reaction types were classified according to the susceptibility reactions of these DVs.
+: pp. susceptible; -: pp. resistant. Diversity index was calculated by the method of Simpson (1949).

units, each of which had one to three DVs or LTH allocated (i.e. one to three genes). We applied codes 1, 2, and 4 to the susceptibility reactions of the respective differential varieties to the blast isolates. Blast races were designated by the combined sum of the codes representing the reactions of the differential varieties in each unit, using Gilmor's method (Gilmor 1973). Isolates classified this way were designated as "reaction types" within each differential variety unit and as "races" using the set of all five reaction types.

Classification of blast isolates

The reaction patterns to the blast isolates by LTH and the 25 DVs harboring the 23 resistance genes were used for the cluster analyses. The analysis was performed using Ward's hierarchical clustering method (Ward 1963) and the computer program PROC CLUSTER of Statistical Analysis System software (SAS vs. 9.1).

The diversity of blast isolates in each cluster group was calculated using Simpson's index method (λ): pp.

$$\lambda = \sum_{i=1}^{s} P_i^2$$

$P_i = \frac{x_i}{\sum_{i=1}^{s} x_i}$ (proportion of blast isolates belonging to reaction type i),

x_i : Number of blast isolates per reaction type,

s: pp. Number of reaction types (in the cluster group),

Diversity = 1– λ (the likelihood of two randomly chosen individuals being different species (Simpson 1949).

Results

Out of the 116 blast isolates collected in West Africa, 96 (upland: pp. 58; rainfed lowland: pp. 5; and irrigated lowland: pp. 33) could be used and their pathogenicity determined using LTH and the DVs in accordance with the method of Hayashi et al. (2009) (Table 2).

The percentage occurrences of virulent blast isolates varied from 0 to 100% among DVs and LTH. Virulent isolates were found at high frequencies on LTH and on DVs carrying *Pia*, *Pik-s*, *Pi19*(t), *Pi12*(t), *Pit*, *Pii*, *Pi3*, and *Pi5*(t). Conversely, they were found at low frequencies on DVs bearing *Pish*, *Pi9*(t), *Piz*, *Piz-5*, *Piz-t* and *Pita-2*(2 lines) and at intermediate frequencies on DVs carrying *Pib*, *Pik-m*, *Pi1*, *Pi-k-h*, *Pik*, *Pik-p*, *Pita* (2 lines) and *Pi20*(t) (Fig. 1).

The cluster analysis revealed that the 96 blast isolates

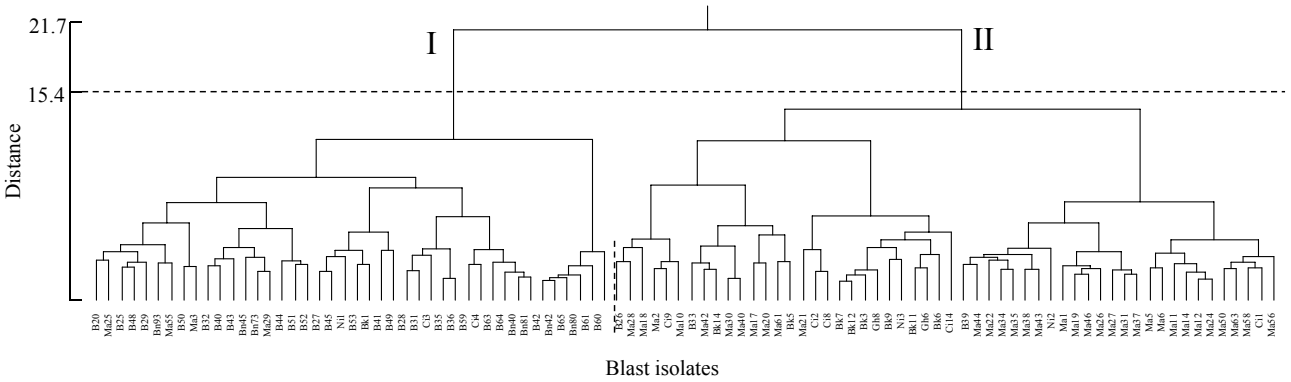

Fig. 2. Classification of blast isolates from the West Africa based on the reaction patterns to monogenic lines as the differential varieties

A total of 96 blast isolates were classified into two cluster groups I and II.
Cluster analysis was carried out using a Statistical Analysis System (SAS vs. 9.1) program

could be classified into two major clusters, I and II, based on the reaction patterns of the 25 DVs and LTH (Fig. 2). The frequencies of virulent blast isolates against DVs were higher in cluster I than II (Fig. 1). Substantial differences in frequencies emerged between clusters I and II in the reactions of DVs carrying *Pii* groups genes; *Pii*, *Pi3*, and *Pi5*(t), *Pik* allele genes; *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p* and *Pi7*(t), *Piz* allele genes; *Pi9*(t), *Piz*, *Piz-5*, and *Piz-t*, and *Pita* allele genes; *Pita-2* and *Pi20*(t).

The total numbers of blast isolates in clusters I and II were 36 (37.5%) and 60 (62.5%), respectively. In the uplands, the numbers of blast isolates in clusters I and II were 31 (32.3%) and 27 (28.1%), respectively. Conversely, two (2.1%) and 31 (32.3%) blast isolates from irrigated lowlands were classified into clusters I and II, respectively. These frequencies between upland and lowland ecosystems differed significantly. From the rainfed lowland, three blast isolates (3.1%) emerged in cluster I and two (2.1%) in cluster II (Table 2). These results indicated that both isolates in clusters I and II were distributed in upland ecosystem, but those of cluster II were mainly found in irrigated lowland ecosystems.

Reaction types of blast isolates

Several major reaction types emerged in each group of DVs, as proposed by the method of Hayashi and Fukuta (2009).

Based on the reactions of LTH and the DVs for *Pish*, *Pib*, *Pit*, and *Pia*, 96 blast isolates were classified into 12 reaction types, U00, U01, U03, U21, U23, U33, U40, U41 U43, U61, U63 and U73 (Table 3). Among these, four reaction types, U03, U23, U43 and U63, emerged in both clusters I and II. Notably, U43 and U63 had 25 (26.0%) and 43 blast isolates (44.8%), respectively, and were the dominant reaction types in group U, while the other 10 reaction types had fewer than 11 blast isolates. There were no significant differences between clusters I and II in terms of the numbers of blast isolates in each reaction type, which means that blast isolates with genes virulent to DVs for *Pia*, and one or both of *Pib* and *Pit*, were distributed commonly in the countries of West Africa. Cluster I had six reaction types and cluster II had 10. The overall diversity index value was 0.71, and those for clusters I and II were 0.68 and 0.72, respectively, differing only slightly.

A total of seven reaction types against DV group "i", namely i0, i1, i2, i3, i5, i6 and i7, were found. One reaction type, i7, was found in both cluster I (33 isolates, 34.4%) and cluster II (35 isolates, 36.5%), and dominated (Table 4). The second most abundant was reaction type i0, categorized by 14 blast isolates (14.6%) and found only in cluster II. The other five reaction types had fewer than five blast isolates. The high number of isolates in i0 was one of the characteristics of cluster II, which means that virulent blast isolates to three genes; *Pii*, *Pi3* and *Pi5*(t) were distributed widely and avilurent ones to them were also differentiated in West Africa. The total number of reaction types in both clusters and the numbers in clusters I and II were seven, three, and seven, respectively. The overall diversity index was 0.47, and those of clusters I and II were 0.16 and 0.60, respectively. The diversity of cluster I was lower than that of cluster II.

A total of 13 reaction types, k000, k006, k076, k077, k100, k104, k106, k107, k116, k126, k157, k176 and k177, emerged against DVs; *Pik-s*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p* and *Pi7*(t), in group k (Table 5). Reaction type k106 (virulent to *Pik-s*, *Pik-p* and *Pi7*(t)) included 21 blast isolates (21.9%). Only three blast isolates were classified into cluster I; the other 18 isolates were categorized into cluster II. The reaction types k100 (virulent to *Pik-s*), and k000 (avirulent to all DVs in group k) included 19 (19.8%) and eight (8.3%) blast isolates, respectively, and were categorized only into cluster II. Conversely, the 25 blast isolates (26.0%) categorized in

k177 (virulent to all DVs in group k) emerged in cluster I. The other nine reaction types had fewer than six isolates each. These results indicated that the virulence genes in four reaction types differed between clusters I and II. In other words, the reactions of the DVs for *Pik-m*, *Pi1*, *Pik-h* and *Pik* differentiated the two clusters. The number of reaction types and diversity index values in the whole DV group and in clusters I and II were 13 and 0.83, five and 0.49, and nine and 0.78, respectively. The diversity in cluster I was lower than that in cluster II.

A total of eight reaction types, z00, z01, z02, z03, z04, z05, z07 and z10, emerged against DVs for *Pi9*(t), *Piz*, *Piz-5* and *Piz-t* in group z (Table 6). A total of 49 blast isolates (51.0%) were categorized into z00 (avirulent to all DVs); in cluster I there were 11 isolates (11.5%) and in cluster II there were 38 (39.6%), and this reaction type dominated in this DV group. The two reaction types; z03 and z04, included 15 and 25 blast isolates, respectively, and emerged in both clusters. The other five reaction types had four blast isolates each. The numbers of reaction types in the whole group and in clusters I and II were eight, six, and six, respectively, and the respective diversity index values were 0.65, 0.70, and 0.55, respectively. The diversities in clusters I and II did not differ substantially.

Seven DVs, in group ta

A total of 17 reaction types, ta001, ta003, ta021, ta023, ta031, ta033, ta233, ta333, ta401, ta403, ta413, ta421, ta423, ta431, ta433, ta523 and ta733, emerged against DVs for *Pita-2* (2 lines), *Pi12*(t), *Pita* (2 lines), *Pi19*(t) and *Pi20*(t), in group ta (Table 6). A dominant reaction type, ta403 (virulent to DVs for *Pi12*(t), *Pi19*(t) and *Pi20*(t), 28 isolates, 29.2%) was found. Thirteen (13.5%) and 15 isolates (15.6%) were included in clusters I and II, respectively. Twelve blast isolates (12.5%) were categorized into ta003 (virulent to DVs for *Pi19*(t) and *Pi20*(t)), which was also found in both clusters I and II. A total of 14 blast isolates (14.6%) were categorized into ta031 (virulent DVs for *Pita* and *Pi19*(t)), and almost all (13 isolates) emerged in cluster II. Reaction type ta431 (virulent to *Pi12*(t), *Pita* and *Pi19*(t)), 13 blast isolates, 13.5%) was found only in cluster II. Conversely, reaction type ta733 (virulent all DVs in group ta, 7 blast isolates, 7.3%) was found only in cluster I. These results indicated that the virulence genes in three reaction types, ta031, ta431 and ta733, differed between the two clusters. In other words, the reactions of DVs containing *Pita-2* and *Pi12*(t) differed between the clusters. The other 12 reaction types had fewer than five blast isolates each. The numbers of reaction types in the whole DV group and in clusters I and II were 17, 13 and 11, respectively, and the respective diversity index values were 0.85, 0.81 and 0.82, respectively, which showed similar diversities in the two clusters. Based on pathotyping in five DV groups using the method proposed by Hayashi and Fukuta (2009), we characterized 96 blast isolates and found 12, 7, 13, 8 and 17 reaction types in the DV groups U, i, k, z, and ta, respectively. Among these, 13, namely U43, U63, i0, i7, k100, k106, k177, z00, z03, z04, ta003, ta031, ta403 and ta431, showed high frequencies of blast isolates and were the dominant reaction types, while eight of these reaction types, namely U43, U63, i7, z00, z03, z04, ta003 and ta403, were common to both clusters. Thus blast isolates with virulence genes against DVs for *Pib*, *Pit*, *Pia*, *Pii*, *Pi3*, *Pi5*(t), *Pik-s*, *Pik-p*, *Pi7*(t), *Piz*, *Piz-5*, *Piz-t*, *Pi19*(t), *Pi20*(t) or *Pi12*(t) were widely distributed in West Africa. Reaction type k177 was found only in cluster I, whereas reaction types i0, k100, k106, ta031 and ta431 emerged mainly in cluster II. Thus these reactions of avirulence to *Pii*, *Pi3* and *Pi5*(t), or virulence to *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pita* and *Pi19*(t) differentiated between clusters I and II.

Finally, the 96 blast isolates were classified into 79 races (Table 7); of these, nine races, U63-i7-k177-z04-ta733, U43-i7-k106-z00-ta031, U63-i0-k100-z00-ta403, U63-i7-k106-z00-ta431, U03-i7-k000-z03-ta003, U63-i7-k177-z04-ta403, U63-i7-k106-z04-ta431, U63-i7-k177-z03-ta403 and U43-i7-k100-z03-ta403, had some blast isolates. These races comprised the major reaction types in each DV group. The other 70 races had only one isolate each. Cluster II always had greater numbers of reaction types and higher diversity index values than cluster I, with the exception of DV group z.

Discussion

A wide variation in virulence frequencies of blast isolates was observed in our pathogenicity analysis performed using monogenic lines and LTH NILs as DVs, and LTH (Fig. 1). The 96 isolates were characterized into 79 blast races (Table 8). These results indicated a high diversity of blast fungal pathogen populations in West Africa. To our knowledge, this is the first information to become available on blast isolates from West Africa and reveals the blast pathogen distribution.

Nineteen reaction types, U03, U23, U43, U63, i3, i6, i7, k106, z00, z02, z03, z04, ta001, ta003, ta023, ta031, ta403, ta421 and ta423, emerged in both clusters I and II. Notably, U43, U63, i7, z00, z04 and ta403 included many blast isolates. Thus blast isolates virulent to DVs with the resistance gene *Pib*, *Pit*, *Pia*, *Pii*, *Pi3*, *Pi5*(t), *Pik-s*, *Pik-p*, *Pi7*(t), *Pi12*(t), *Pi19*(t) or *Pi20*(t), and isolates avirulent to all allele genes of group z, or virulent to *Piz-t*, were distributed widely with high frequencies together in West Africa. Conversely, six reaction types, i0, k100, k106, k177, ta031 and ta431, differed in frequency between clusters I and II, while the frequency of k177 in cluster I exceeded that in cluster II. The other reaction types were lower in frequency in cluster I than II and thus differentiated these clusters. In other words, blast isolates virulent to DVs with the resistance gene *Pik-m*,

Table 8. Blast races in West Africa

Designation			Total no. of blast isolates (No. of races,%)	Cluster
U63i7k177z04ta733			5 (1, 1.3%)	I
U43i7k106z00ta031	U63i0k100z00ta403		8 (2, 2.5%)	II
U63i7k106z00ta431			3 (1, 1.3%)	II
U03i7k000z03ta003, U63i7k106z04ta431,	U63i7k177z04ta403, U63i7k177z03ta403	U43i7k100z03ta403	10 (5, 6.3%)	I
U03i7k177z00ta003, U43i7k177z00ta403, U63i6k177z04ta403, U03i7k177z00ta401, U03i7k177z00ta031, U23i7k177z00ta401, U03i7k176z00ta001, U63i7k177z03ta401, U03i7k177z03ta421, U43i7k177z00ta423, U23i6k077z04ta403, U43i7k177z07ta023, U43i7k177z03ta403, U43i7k106z00ta333, U63i7k177z05ta733, U63i7k106z00ta733, U00i0k000z00ta001, U03i7k000z00ta006, U43i7k106z02ta403, U40i7k157z00ta001, U63i7k107z04ta403, U61i6k126z00ta003, U43i7k116z00ta403, U43i3k106z00ta023,	U63i7k177z03ta003 U63i7k176z02ta003 U43i7k176z03ta003 U63i7k076z04ta433 U63i7k177z00ta403 U43i7k177z03ta033 U23i7k077z04ta403 U63i7k176z00ta233 U23i3k077z04ta403 U33i7k177z04ta403 U73i7k106z04ta403 U23i7k006z04ta021 U63i5k106z00ta431 U43i7k100z00ta003 U63i7k126z00ta431 U43i7k106z03ta031 U23i7k106z04ta431 U63i7k100z00ta431 U63i3k000z00ta421 U23i7k100z00ta001 U63i3k000z00ta403 U43i0k100z00ta031 U43i0k100z00ta431	U43i1k126z00ta003, U23i7k006z04ta031, U41i5k100z00ta031, U61i2k100z00ta003, U43i7k107z03ta031, U43i7k106z00ta431, U21i0k006z04ta031, U23i7k106z04ta421, U63i0k106z00ta431, U63i7k107z00ta431, U43i7k107z01ta031, U63i2k006z00ta403 U63i2k100z00ta403 U63i0k100z00ta523 U63i0k000z00ta403 U63i0k100z02ta403 U63i0k100z10ta403 U23i7k006z04ta421, U63i7k100z04ta423, U01i6k000z03ta003, U43i7k104z00ta031, U63i7k106z04ta413, U01i0k100z00ta031	70 (70, 88.6%)	I II
	Total: 96(79)			

Pi1, *Pik-h*, or *Pik* were abundant in cluster I, while those virulent to DVs with *Pita*, *Pi12*(t) or *Pi19*(t), and avirulent to DVs with *Pii*, *Pi3* or *Pi5*(t), were abundant in cluster II.

Cluster II always had more reaction types and higher diversity index values than cluster I, except in DV group z. The blast isolates in cluster II were characterized by genes for avirulence to DVs for *Pii*, *Pi3*, *Pi5*(t), *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Piz-5*, *Piz-t* or *Pita-2* and genes for virulence to DVs for *Pik-s*, *Pik-p*, *Pi7*(t), *Pi12*(t), *Pita* or *Pi19*(t). They maintained high diversity and were distributed with similar frequencies in both upland and irrigated lowland. The distribution of cluster I blast isolates, for which the reactions to DVs carrying *Pii*, *Pi3*, *Pi5*(t), *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pita*, *Pi12*(t) or *Pi19*(t) differed from those of cluster II, was limited to the irrigated lowland. These results suggest that blast isolates of cluster II are distributed widely as a mother population in West Africa, whereas those of cluster I have become differentiated in the uplands in accordance with the adaptation of rice cultivars to these different ecosystems. The different distributions of clusters I and II between the ecosystems may correspond to the different distributions of the genotypes of resistance genes in rice cultivars, as indicated by the gene-for-gene theory (Flor 1971, Silué 1992). It will be necessary to confirm the relationships between the diversity and differentiation of blast races and the genetic variation in rice cultivars in each ecosystem.

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