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A Set of Standard Differential Blast Isolates (*Magnaporthe grisea* (Hebert) Barr.) from the Philippines for Rice (*Oryza sativa* L.) Resistance

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Abstract

A total of 119 blast (*Magnaporthe grisea* (Hebert) Barr.) isolates collected from the Philippines, were characterized for their pathogenicities using 19 rice (*Oryza sativa* L.) differential varieties (DVs) targeting for 18 resistance genes. These isolates were classified into 31 groups based on the reaction patterns to 9 DVs for targeting 9 resistance genes, *Pia*, *Pib*, *Pii*, *Pit*, *Pita*, *Pish*, *Piz-t*, *Pi3*, and *Piz-5*, at first, and then further divided into 70 pathotypes considering the reaction pattern of the other 9 DVs for the other resistance genes, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pik-s*, *Pita-2*, *Piz*, *Pi1*, and *Pi20*(t). Twenty isolates that have differentiating ability, stable reactions and good sporulating ability were selected. The reactions of these isolates were confirmed using the monogenic lines as a set of DVs for targeting 24 resistance genes, *Pia*, *Pib*, *Pii*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*,*Pik-s*, *Pish*, *Pit*, *Pita*, *Pita-2*, *Piz*, *Piz-t*, *Piz-5*, *Pi1*, *Pi3*, *Pi5*(t), *Pi7*(t), *Pi9*(t), *Pi11*(t), *Pi12*(t), *Pi19*(t), and *Pi20*(t). Several resistance alleles of *Pik* locus, *Pik*, *Pik-h*, *Pik-m*, and *Pik-p*, and *Pi1* had the same reaction patterns and could not be differentiated by these selected blast isolates. No avirulent isolate for *Pit* and *Pi19* was found. The three genes, *Pish*, *Piz-5*, and *Piz*, showed a wide spectrum of moderate resistances to these isolates. These findings suggested the existence of a wide variation of blast pathogens in the Philippines. This information on pathogenicity of blast isolates from the Philippines will be also useful to understand the differentiation and relationship between blast races and resistance genes. The monogenic lines as the DVs and the selected 20 blast isolates can be used as the first differential system, which can characterize the resistances of rice varieties and pathogenicities of blast isolates in the tropics.

Discipline: Genetic resources
Additional key words: differential system, diversity, pathogenicity, resistance gene

Introduction

Blast is a serious disease caused by a fungal pathogen, *Magnaporthe grisea* (Hebert) Barr.[23] in rice (*Oryza sativa* L.) growing regions worldwide[2,15,22]. The use of resistant varieties is known as one of the most efficient ways for crop protection from the disease. Control of the disease is not always durable because the resistance of rice varieties often breaks down a few years after being released[7]. The relationship between virulence genes of pathogens and resistance genes of rice varieties is explained by the gene-for-gene theory[5,24]. Based on the gene-for-gene theory, differential systems consisting of rice resistant varieties and blast isolates have been developed in some countries (Table 1). These differential systems can be used for the advanced studies in the identifications of pathogenicities of blast pathogens, estimation of resistance gene(s) in rice varieties, and clarifications of these variations.

The first set of differentials for rice blast includes 7 representative varieties, Zenith, NP 125, Dular, Kanto 51, Caloro, Raminad Str. 3, and Usen, as the International Differentials Varieties (IDVs)[1]. These IDVs were used in

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Table 1. Differential varieties harboring blast resistance gene

Target gene	Differential varieties					
	Atkins et al. (1967)[a)]	Yamada et al. (1976)	Kiyosawa (1981, 1984)	Mackill et al. (1992)	Tsunematsu et al. (2000)[b)]	Ling et al. (2001)
Pia	Zenith	Aichi Asahi*	Aichi Asahi*	–	IRBLa-A IRBLa-C	–
Pib	–	–	BL1*	–	IRBLb-B	F145-2
Pii	–	Ishikari shiroke	Fujisaka 5*	–	IRBLi-F5	–
Pik	NP125, Dular, Kanto 51*	Kanto 51*	Kusabue	–	IRBLk-K	F80-1
Pik-m	–	Tsuyuake*	Tsuyuake*	–	IRBLkm-Ts	F98-7
Pik-p	–	–	K60*	–	IRBLkp-K60	F129-1
Pik-h	–	–	K3*	–	IRBLkh-K3	–
Pik-s	Caloro*	Shin 2*	Shin 2*	–	IRBLks-F5 IRBLks-S	–
Pish	–	–	–	–	IRBLsh-S IRBLsh-B	–
Pit	–	–	K59*	–	IRBLt-K59	–
Pita (= *Pi4*(t))	Usen	Yashiromochi*	K1	C105TTP2L9 C101PKT	IRBLta-K1 IRBLta-CT2 IRBLta-CP1	–
Pita-2	–	Pi No. 4*	Pi No. 4*	–	IRBLta2-Re IRBLta2-Pi	F182-1 F124-1
Piz	–	Fukunishiki *	Fukunishiki*	–	IRBLz-Fu	–
Piz-5 (= *Pi2*)	–	–	–	C101A51*	IRBLz5-CA	–
Piz-t	–	Toride 1*	Toride 1*	–	IRBLzt-T	–
Pi1	–	–	–	C101LAC*	IRBL1-CL	–
Pi3	–	–	–	C104PKT*	IRBL3-CP4	–
Pi5 (t)	–	–	–	–	IRBL5-M	–
Pi7 (t)	–	–	–	–	IRBL7-M	–
Pi9 (t)	–	–	–	–	IRBL9-W	–
Pi11 (t)	–	–	–	–	IRBL11-Zh	–
Pi12 (t)	–	–	–	–	IRBL12-M	–
Pi19 (t)	–	–	–	–	IRBL19-A	–
Pi20 (t)	–	–	–	–	IRBL20-IR24	–
Unknown	Raminad strain 3	–	–	–	–	–

a): International differential varieties.
b): Monogenic line harboring single resistance gene in each genetic background.
*: Variety used for the reaction patterns to blast isolates from the Philippines.

various countries like United States, Japan, Taiwan, Korea, and Philippines, to assess the pathotypic variability of the blast fungus, but the genetic compositions of resistance genes were unknown. It is estimated that these include only five resistance genes, *Pia* and *Piz* in Zenith, *Pik* in three varieties, NP125, Dular and Kanto 51, *Pik-s* in Caloro, and *Pita* in Usen (unpublished data). Two sets of DVs targeted for 13 resistance genes, *Pia*, *Pib*, *Pii*, *Pik-s*, *Pik*, *Pik-p*, *Pik-h*, *Pik-m*, *Pit*, *Pita*, *Pita-2*, *Piz*, and *Piz-t*, were developed in Japan[11,13], and have been used widely in many countries. A set of near isogenic lines (NILs) with Indica-type genetic background CO 39 was developed in International Rice Research Institute (IRRI), but it covered only four resistance genes, *Pita*, *Piz-5*, *Pi1*, and *Pi3*[18]. In China, a set of NILs with five resistance genes, *Pib*, *Pik*, *Pik-m*, *Pik-p*, and *Pita-2*, using a susceptible and Japonica-type variety, Lijiangxintuanheigu (LTH) was developed[16].

The numbers and kinds of resistance genes targeted in IDVs, Japanese DVs, CO 39 NILs, and LTH NILs,

were still limited. Moreover, the comparisons of results using the sets of DVs were very difficult and several DVs still have some problems. Japanese DVs by Yamada et al.[27] and Kiyosawa[11,13] can systematically differentiate the Japanese blast isolates, but several DVs were not suitable for differentiating the Philippine fungus strain[11]. Inukai et al.[10] reported that three Japanese DVs, Shin 2, Fukunishiki and Kusabue, showed the unique reactions of moderate resistance to almost all blast isolates from the Philippines, and an additional resistance gene was assumed to harbor these genetic backgrounds. Noda et al.[21] had identified 12 kinds of blast races among 129 isolates collected all over the Mekong River Delta area in Vietnam using the Japanese DVs for 12 resistance genes by Yamada et al.[27] and Kiyosawa[11,13], and also reported that several DVs among them were not suitable for differentiations of blast isolates in Vietnam.

However, there were limitations for numbers or kinds of the targeted resistance genes and the abilities of differentiation in several DVs, and the pathogenicity analyses for blast isolates have been done in several countries. The population of blast isolates in North Vietnam appears to be highly diverse wherein 23 pathotypes were found in 25 selected from 114 isolates[20]. Mekwatanakarn et al.[19] had investigated the pathogenicities of 527 isolates from Thailand using LTH NILs, and a total of 175 pathotypes were identified. In Bhutan, 110 isolates were differentiated into 53 pathotypes based on the two sets of NILs, CO 39 and LTH NILs[25]. Furthermore in China, 792 isolates were classified into 344 pathotypes using LTH NILs for six resistance genes *Pita-2*, *Pib*, *Pik*, *Pik-m*, *Pita*, and *Pik-p*, and CO 39 NILs for four resistance genes, *Pita*, *Piz-5*, *Pi3*, and *Pi1*[3]. These results indicated that these DVs in each study could explain the wide variations of pathotypes in the blast populations, and demonstrate the differential abilities for the pathogens.

Under the IRRI-Japan Collaborative Research Project, a set of DVs, monogenic lines for 24 resistance genes — *Pia*, *Pib*, *Pii*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pik-s*, *Pish*, *Pit*, *Pita*, *Pita-2*, *Piz*, *Piz-5*, *Piz-t*, *Pi1*, *Pi3*, *Pi5*(t), *Pi7*(t), *Pi9*(t), *Pi11*(t), *Pi12*(t), *Pi19*(t), and *Pi20(*t) — was developed from the crossing between a susceptible LTH and previous DVs as the donor varieties, to eliminate the effects of additional resistance genes in these genetic backgrounds[14,26].

In the Philippines, the diversity analyses based on the pathogenicity of blast isolates have not been done well, and suitable standard blast isolates for a differential system were also not available for systematic identification of blast resistance genes. We tried to clarify the variation of blast pathogens and to select the representative differential isolates using the previous DVs of Atkins et al.[1], Yamada et al.[26], Kiyosawa[11,13], Mackill et al.[18], and some other varieties, Shin 2 for *Pish*, Reiho for *Pita-2*[8], and IR 24 for *Pi20*(t)[9] in the Philippines. Moreover, the detailed pathogenicities of the selected representative isolates were confirmed and characterized using the monogenic lines[26]. From these results, the pathogenicity of blast isolates from the Philippines will be discussed.

Materials and methods

1. Blast isolates

A total of 119 blast isolates collected from several regions of the Philippines and maintained at Entomology and Plant Pathology Division of International Rice Research Institute (IRRI), were used (Table 2). In the southern regions of Luzon Island, 43 and 52 isolates were collected from the nursery fields for blast evaluation at IRRI, Los Baños and Caliraya, respectively. The percentage of total isolates from these two sites was almost 80.0% of all isolates. The other 7 isolates were derived from three provinces, Batangas, Bicol and Masapang, in the southern region of Luzon Island. Seven and two isolates were collected from the central and northern regions of Luzon Island, respectively. Three isolates from Palawan and Mindanao Islands were also used.

2. Differential varieties

Nine differential varieties (DVs), Aichi Asahi for *Pia*, BL1 for *Pib*, Tsuyuake for *Pik-m*, K60 for *Pik-p*, Shin 2 for *Pish*, Fujisaka 5 for *Pii*, K59 for *Pik-s* and *Pit*, Fukunishiki for *Piz*, and Toride 1 for *Piz-t*[12], three CO 39 near isogenic lines (NILs), C104PKT for *Pi3*, C101LAC for *Pi1* and C105A51 for *Piz-5*[17], Caloro for *Pik-s*[1], two DVs, Yashiromochi for *Pita* and Kanto 51 for *Pik*[27], K3 for *Pik-h*, Pi No. 4 and Reiho for *Pita-2*[8,11], and IR 24 for *Pi20*(t)[9] were used to determine the pathogenicities of the blast isolates (Table 1). It means that a total of 18 resistance genes were targeted in the evaluation of pathogenicities.

3. Inoculation and evaluation

The set of 19 DVs were sown in a plastic tray (33 × 11 × 11 cm) half-filled with sieved garden soil. Three grams of ammonium sulfate were applied as basal fertilizer. Twelve varieties were sown with 10 seeds, and a Chinese Japonica-type variety, LTH, and an Indica-type variety, CO 39, were also planted in the two end rows as the susceptible checks. One gram of ammonium sulfate was added on each tray 1–2 day(s) before inoculation.

Inoculation of blast isolate was done following basically the method of Bonman et al.[2]. The spore concentration was standardized to 1×10^5 spores per ml. DVs were

Table 2. Numbers of blast pathotypes in different sites of the Philippines

	Luzon Is.											
			Southern									
					Laguna Prov.							
Isolate's group	Northern	Central	Batangas Prov.	Bicol Prov.	Caliraya	Los Baños	Masapang	Palawan Is.	Mindanao Is.	Un known	Total	(%)
I	2	1	1		5	1					10	(8.4)
II					2	1					3	(2.5)
III			1			4					5	(4.2)
IV		1	1	1	6	5					14	(11.7)
V					1	3					4	(3.3)
VI		1			2	2					5	(4.2)
VII		1			1	2					4	(3.3)
VIII					2	2					4	(3.3)
IX		1			1	1				1	4	(3.3)
X		1				1			1		3	(2.5)
XI					2	2					4	(3.3)
XII			1		3	3	1				8	(6.7)
XIII						2					2	(1.6)
XIV						3					3	(2.5)
XV					2						2	(1.6)
XVI					3	1					4	(3.3)
XVII		1									1	(0.8)
XVIII										1	1	(0.8)
XIX					1	5		1		1	8	(6.7)
XX					2						2	(1.6)
XXI					2						2	(1.6)
XXII					3	1				1	5	(4.2)
XXIII										1	1	(0.8)
XXIV			1		2	3					6	(5.0)
XXV					1						1	(0.8)
XXVI					1				1		2	(1.6)
XXVII					1						1	(0.8)
XXVIII					6						6	(5.0)
XXIX					2						2	(1.6)
XXX						1					1	(0.8)
XXXI					1						1	(0.8)
Total	2	7	5	1	52	43	1	1	2	5	119	

inoculated at 14–15 days after sowing or approximately the 4–5th leaf stages by spraying 40–50 ml spore suspension on each tray using a fine atomizer. The degree of disease of each seedling was evaluated 6–7 days after inoculation. Each seedling was examined and rated according to the IRRI *Standard Evaluation System* (SES, 1996) for rice blast. The reactions of DVs were categorized and summarized into three reaction classes wherein 0–2 were resistant (R), 3 was moderately resistant (M), and scores 4–5 were susceptible (S).

4. Classification of isolates based on reaction patterns against differential varieties

Based on the reaction patterns to 19 DVs, the isolates

were classified following two steps. At first, 9 DVs, Aichi Asahi for *Pia*, Yashiromochi for *Pita*, Shin 2 for *Pish*, K59 for *Pit* and *Pik-s*, C104PKT for *Pi3*, Fujisaka 5 for *Pii*, Toride 1 for *Piz-t*, BL1 for *Pib*, and C1015A51 for *Piz-5*, were used in the primary classification. In the next step, these isolates' groups were classified further into subgroups based on the reactions of DVs, Pi No. 4 and Reiho for *Pita-2*, Fukunishiki for *Piz*, Kanto 51 for *Pik*, C101LAC for *Pi1*, K3 for *Pik-h*, Tsuyuake for *Pik-m*, K60 for *Pik-p*, Caloro for *Pik-s*, and IR 24 for *Pi20*(t).

Reactions of 'S' and 'R' were considered as compatible and incompatible, respectively, and 'M' reaction was evaluated as compatible to avoid missing an evaluation of some other genes in the genetic backgrounds of DVs. The classification is governed by the gene-for-gene theory[5], and it was proved to be exhibited in the pathosystem of rice blast by Silue et al.[24].

5. Selection and characterization for representative blast isolates from the Philippines

Representative isolates were selected from several pathotypes that distinguished DVs targeting 18 resistance genes — *Pia*, *Pib*, *Pii*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pik-s*, *Pish*, *Pit*, *Pita*, *Pita-2*, *Piz*, *Piz-5*, *Piz-t*, *Pi1*, *Pi3*, and *Pi20*(t). These pathogenicities of selected blast isolates were confirmed using the monogenic lines that introduced a single resistance gene into the LTH genetic background and targeted 24 kinds of resistance genes — *Pia*, *Pib*, *Pii*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pik-s*, *Pish*, *Pit*, *Pita*, *Pita-2*, *Piz*, *Piz-5*, *Piz-t*, *Pi1*, *Pi3*, *Pi5*(t), *Pi7*(t), *Pi9*(t), *Pi11*(t), *Pi12*(t), *Pi19*(t), and *Pi20*(t)[14,26]. These monogenic lines were developed during the selection of the standard differential blast isolates, and could be used for the confirmation.

Results and discussion

1. Compatibility of blast isolates against differential varieties

The frequencies of incompatible reactions (R) of 119 isolates varied from 8.4 to 91.5% depending on the DVs (Table 3).

Aichi Asahi for *Pia* showed resistance to only 10 isolates with the frequency of 8.4%, and it was the lowest one among those of DVs. The six DVs, K59 for *Pit* (*Pik-s*), Yashiromochi for *Pita*, Shin 2 for *Pish*, C104PKT for *Pi3*, Fujisaka 5 for *Pii*, Toride 1 for *Piz-t*, and BL1 for *Pib*, showed incompatibility to the blast isolates with frequencies ranging from 14.7 to 70.1%, as compared with the other DVs'. Among them, three DVs, Shin 2, Toride 1 and BL1, showed the moderate resistance (M) to a lot of blast isolates. The other DVs, C101A51 for *Piz-5*, Pi No. 4 and Reiho for *Pita-2*, Fukunishiki for *Piz*, Kanto 51 for *Pik*, C101LAC for *Pi1*, K3 for *Pik-h*, Tsuyuake for *Pik-m*, and K60 for *Pik-p*, were highly resistant ranging from 79.0 to 91.5%. Among them, the four DVs for *Pik* multiple alleles, *Pik*, *Pik-h*, *Pik-m*, and *Pik-p*, and *Pi1*, have showed similar frequencies of incompatible isolates and reactions. Two DVs, Caloro for *Pik-s* and IR 24 for *Pi20*(t), also have low frequencies of incompatibility to blast isolates, but the numbers used for the inoculations were only 32 and 20, respectively.

We estimated that Caloro harbored only *Pik-s* (unpublished data), and it showed similar reactions as those of K59 to several incompatible isolates. From these results, almost all reactions of resistance in K59 to blast isolates were estimated to be due to *Pik-s* and not to *Pit.*

In several DVs, additional resistance genes were found to be harbored in these genetic backgrounds. Kiyosawa[11] and Inukai et al.[10] reported that a resistance gene, *Pish*, was found in four DVs, Shin 2, Toride 1, BL1, and Fukunishiki, genetic backgrounds. Kiyosawa[11] also indicated that *Pish* showed a wide and incomplete spectrum of resistance to the blast isolates from the Philippines, but was susceptible to Japanese isolates. The same reaction patterns were shown in the four DVs, Shin 2, Toride 1, BL1, and Fukunishiki in this study. The Indica-type variety, CO 39, which was used for the recurrent parent of NILs for blast resistance genes, *Pi1*, *Pi3* and *Piz-5* by Mackill et al.[17], harbors *Pia* in the genetic background[9]. The other three DVs, Aichi Asahi, K59, and IR 24, were found to harbor the additional genes, *Pi19*(t) by Hayashi et al.[6], *Pik-s* (unpublished data) and three genes, *Pia*, *Pib* and *Pik-s* by Imbe et al.[9] and Ebron et al.[4], respectively. However, these additional genes might make it difficult to evaluate the true reaction of a target gene. In particular, the blast isolates from the Philippines showed higher compatibility for 9 DVs, Aichi Asahi, Yashiromochi, Shin 2, K59, C104PKT, Fujisaka 5, Toride 1, BL1, and C101A51. For these reasons, the reaction data of these 9 DVs can be used for the primary classification of blast isolates.

2. Classification of blast isolates based on the reaction patterns against differential varieties

The blast isolates from the Philippines showed specific reaction patterns to DVs targeting 18 resistance genes. As the first step of classification, these isolates were classified into 31 primary groups, I–XXXI, based on compatibility of isolates to 9 DVs, Aichi Asahi, Yashiromochi, Shin 2, K59, C104PKT, Fujisaka 5, Toride 1, BL1, and C101A51. These primary groups were further divided into several sub-groups considering the other reaction patterns of 10 DVs, Kanto 51, K3, Tsuyuake, K60, C101LAC, Reiho, Pi No. 4, Fukunishiki, Caloro,

Table 3. Reaction patterns of representative blast isolates in each pathotype to differential varieties

					Differential variety and resistance gene harboring in each genetic background																	
				Isolates	Aichi Asahi	Yashiromochi	Shin 2	K59	C104 PKT	Fujisaka 5	Toride 1	BL1	C101 A51	Pi No. 4	Reiho	Fukunishiki	Kanto 51	C101 LAC	K3	Tsuyuake	K60	Caloro	IR 24
				Designation	*Pia*	*Pita*	*Pik-s*	*Pit*	*Pi3*	*Pii*	*Piz-t*	*Pib*	*Piz-5*	*Pita-2*	*Pita-2*	*Piz*	*Pik*	*Pi1*	*Pik-h*	*Pik-m*	*Pik-p*	*Pik-s*	*Pi20*(t)
	Group[a]	Subgroup[b]	Number		*Pi19*[c]	–	*Pish*[d]	*Pik-s*[e]	*Pia*[f]	–	*Pish*[d]	*Pish*[d]	*Pia*[f]	–	–	*Pish*[d]	–	*Pia*[f]	–	–	–	–	*Pia*[g] *Pib*[g] *Pik-s*[g] *Piz-t*[g]
Pathotype	I	a	2	Ca82	S	M	S	S	S	S	M	M	M	R	R	R	R	R	R	R	R	–	–
		b	2	Ca89*	S	S	S	S	S	S	M	M	R	M	M	M	R	R	R	R	R	S	S
		c	2	JMB840806	S	S	S	S	S	S	M	M	R	R	R	M	R	R	R	R	R	–	–
		d	4	Ca41*	S	M	M	S	S	S	M	M	R	R	R	R	R	R	R	R	R	S	S
	II	–	3	IK81-3*	S	M	S	S	S	S	M	R	M	R	R	R	R	R	R	R	R	M	S
	III	a	2	M39-1-3-8-1*	S	M	S	S	M	M	R	M	M	R	R	R	S	M	S	S	S	S	S
				M64-1-3-9-1*	S	M	S	S	S	S	R	M	R	R	R	R	S	M	S	S	M	S	S
		b	1	M39-7-3-4-1	S	M	M	S	S	S	R	M	R	S	S	R	S	M	S	S	S	–	–
		c	2	V850256*	S	M	M	S	S	S	R	M	M	R	R	R	R	R	R	R	R	S	S
	IV	a	1	B90324	S	M	M	S	S	S	R	R	M	R	R	R	S	R	R	R	R	–	–
		b	7	C9240-4	S	M	M	S	S	S	R	R	S	R	R	R	R	R	R	R	R	–	–
		c	1	B90038	S	S	M	S	S	S	R	R	R	S	S	M	R	R	R	R	R	–	–
		d	3	JMB84050	S	S	M	M	S	S	R	R	R	R	R	M	R	R	R	R	R	–	–
		e	1	V86010*	S	S	M	R	S	S	R	R	M	R	R	R	R	R	R	R	R	R	S
		f	1	JMB840334	S	S	M	S	M	M	R	R	R	R	R	R	R	R	R	R	R	–	–
	V	a	2	BN111*	S	S	M	S	M	R	M	M	R	R	R	M	R	R	R	R	R	S	R
		b	1	PO6-6*	S	S	M	M	M	R	M	M	R	S	S	R	R	R	R	R	R	S	S
		c	1	B90190	S	S	S	S	M	R	M	M	R	R	R	R	R	R	R	R	R	–	–
	VI	a	4	V86016	S	S	M	S	S	R	M	R	R	R	R	R	R	R	R	R	R	–	–
		b	1	M39-1-2-21-2*	S	S	M	M	M	R	M	R	R	R	R	R	S	S	S	S	M	S	R

Pathotype																							
VII	a	1	92318-4	S	S	M	S	M	R	R	M	R	R	R	S	M	S	S	M	–	–
	b	1	M64-7-3-5-2	S	S	M	S	M	R	R	M	R	M	M	R	M	R	S	S	–	–
	c	2	C921-13	S	S	M	S	M	R	R	M	R	R	R	R	R	R	R	–	–	–
VIII	a	1	92319-6	S	S	M	M	M	R	R	R	R	R	R	M	M	S	S	M	–	–
	b	1	M64-7-3-12-1	S	S	M	S	M	R	R	R	R	M	M	R	R	R	R	R	–	–
	c	2	JMB840349	S	S	M	S	M	R	R	R	R	R	R	R	R	R	R	R	–	–
IX	a	1	BN98	S	S	M	S	R	R	M	M	R	M	M	R	R	R	R	R	–	–
	b	3	V85020	S	S	M	S	R	R	M	M	R	R	R	R	R	R	R	R	–	–
X	a	2	B90187	S	S	M	S	R	R	M	R	R	R	R	R	R	R	R	R	–	–
	b	1	JMB8401102	S	M	S	R	R	R	M	R	R	R	R	R	R	R	R	R	–	–
XI	a	1	92429-13	S	S	M	M	R	R	R	M	R	R	R	S	M	M	M	S	–	–
	b	1	92319-4	S	S	M	R	R	R	R	M	R	M	M	R	S	R	M	R	–	–
	c	1	B90024	S	S	M	S	R	R	R	M	R	R	R	R	R	M	S	S	–	–
	d	1	M64-7-2-8-2	S	S	M	S	R	R	R	M	R	R	R	R	R	R	R	R	–	–
XII	a	1	JMB8401*	S	S	M	S	R	R	R	R	R	R	R	R	S	S	S	S	M	S	S	S
	b	1	Ca92	S	M	M	S	R	R	R	R	R	R	R	M	R	R	R	R	–	–
	c	2	M36-7-3-5-1	S	S	M	S	R	R	R	R	R	S	S	R	R	R	R	R	–	–
	d	3	M39-7-3-5-1	S	S	M	S	R	R	R	R	R	R	R	R	R	R	R	R	–	–
	e	1	JMB840286	S	S	M	R	R	R	R	R	R	R	R	R	R	R	R	R	–	–
XIII	–	2	M36-7-3-6-2	S	S	R	S	S	S	M	R	R	R	R	R	R	R	R	R	–	–
XIV	a	2	B90350	S	M	R	M	S	S	R	R	R	R	R	R	R	R	R	R	–	–
	b	1	B90165	S	M	R	R	S	–	R	R	R	R	R	R	R	R	R	R	–	–
XV	–	2	C9240-8	S	M	R	R	S	R	M	R	S	R	R	R	R	R	R	R	–	–
XVI	a	3	C9240-10	S	M	R	R	S	R	R	R	S	R	R	R	R	R	R	R	–	–
	b	1	92512-3	S	S	R	S	M	R	R	R	R	R	R	R	R	R	R	R	–	–
XVII	–	1	JMB840487	S	S	R	R	R	M	R	R	R	R	R	R	R	R	R	R	–	–
XVIII	–	1	43*	S	S	R	S	R	R	M	R	R	M	M	R	R	R	R	S	S	S
XIX	a	1	CBN921411	S	S	R	S	R	–	R	R	R	R	R	M	S	S	R	M	–	–
	b	1	M36-1-3-10-1*	S	S	R	S	R	R	R	R	R	R	R	R	S	S	R	M	–	R
	c	1	V86046	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	S	–	–
	d	1	BN112	S	S	R	R	R	R	R	R	R	M	M	R	R	R	–	R	–	–
	e	2	M64-7-2-1-1	M	M	R	M	R	R	R	R	R	M	M	R	R	R	R	R	–	–
	f	2	B90185	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	–	–

Pathotype	XX	a	1	B90019	S	R	M	S	S	S	M	M	R	S	S	R	R	R	R	R	R	–	–
		b	1	B90027	S	R	S	S	S	S	M	M	R	R	R	R	R	R	R	R	R	–	–
	XXI	–	2	V850229	S	R	M	S	S	S	M	R	R	R	R	R	R	R	R	R	R	–	–
	XXII	a	3	BN209*	S	R	M	M	S	S	R	R	M	R	R	R	R	R	R	R	R	S	S
				M101-1-2-9-1*	S	R	M	S	S	S	R	R	S	R	R	R	R	R	R	R	R	–	S
		b	1	V850231	S	R	S	S	S	S	R	R	R	R	R	M	R	R	R	R	R	–	–
		c	1	B90076	S	R	–	S	S	–	R	R	R	R	R	R	R	R	R	R	R	–	–
	XXIII	–	1	IK81-25*	S	R	M	S	R	R	R	R	R	R	R	R	S	S	S	S	S	S	S
	XXIV	a	1	M101-1-3-3-2	S	R	R	S	S	S	R	R	S	R	R	R	R	R	R	R	R	–	–
		b	5	JMB840610*	S	R	R	S	S	S	R	R	R	R	R	R	R	R	R	R	R	S	S
	XXV	–	1	B90033	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	R	R	–	–
	XXVI	a	1	JMB840385	S	R	R	R	M	R	M	M	M	R	R	R	R	R	R	R	R	–	–
		b	1	Ca34	S	R	R	R	S	R	M	M	M	R	R	M	R	R	R	R	R	–	–
	XXVII	–	1	V850196*	S	R	R	R	R	R	M	M	M	R	R	R	R	R	R	R	R	R	S
	XXVIII	a	1	B90010	R	R	M	S	R	S	R	R	R	M	M	R	R	R	R	R	R	–	–
		b	5	B90002* C923-49*	R	R	M	S	R	S	R	R	R	R	R	R	R	R	R	R	R	S	R
	XXIX	–	2	B90022	R	R	R	S	R	S	R	R	R	R	R	R	R	R	R	R	R	–	–
	XXX	–	1	92520-7	R	R	R	–	S	S	R	R	R	R	R	R	R	R	R	R	R	–	–
	XXXI	–	1	B90036	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	–	–
Total	31	70	119	Virulent	109	89	83	99	79	58	39	35	25	19	19	15	15	14	12	13	10	22	16
				(%)	91.6	74.7	70.2	85.3	66.3	50.3	33.0	29.9	21.0	16.0	16.0	12.6	12.5	11.7	10.1	10.9	8.5	68.8	80.0
				Avirulent	10	30	35	17	40	57	79	82	94	100	100	104	104	105	107	106	107	10	4
				(%)	8.4	25.2	29.6	14.7	33.6	49.7	66.9	70.0	78.9	84.0	84.0	87.4	87.4	88.2	89.9	89.1	91.4	31.2	20.0

a): Blast isolates were classified based on the reactions against seven differential varieties, Aichi Asahi, Yashiromochi, Shin 2, C104PKT, Fujisaka 5, Toride 1, and BL1, in the primary classification.

b): These isolates' groups in primary classification were divided further into subgroups based on the reactions against Kanto 51, K3, Tsuyuake, K60, C101LAC, C101A51, Fukunishiki, Pi No.4, Reiho, K59, Caloro, and IR 24.

c^{6}, d^{8}, $e^{10,12}$, f^{26}, and g^{4}: Superscripts indicate additional resistance genes which were found in the genetic backgrounds of differential varieties except for the target ones.

An underline and asterisk indicate representatives of pathotypes and final selections as the differential blast isolates, respectively.

R: resistance, M: moderate resistance, S: susceptible.

–: Indicates non-data for reaction to each differential variety.

and IR 24, and these blast isolates were classified into a total of 70 pathotypes, finally. In each group, the numbers of pathotypes and blast isolates varied from 1–6 and 1–14, respectively. Many kinds of pathotypes were found, but the number of isolates was few in each pathotype (Table 3). These numbers of pathotypes suggest the existence of wide genetic variability of blast races in the Philippines. This result agreed with the cases of Vietnam[20,21], Thailand[19] and China[3].

The five DVs, Kanto 51 for *Pik*, K3 for *Pik-h*, Tsuyuake for *Pik-m*, K60 for *Pik-p*, and C101LAC for *Pi1*, showed similar reaction patterns. In other words, very few isolates could differentiate among these *Pik* allele genes and *Pi1* in the Philippines. An isolate, 92319-4, was incompatible to *Pi1* and *Pik-p* and compatible to three genes *Pik*, *Pik-h*, and *Pik-m*. The two isolates, M64-7-3-5-2 and CBN921411, were incompatible to *Pi1* and *Pik-m*, respectively. The two isolates, V86046 and B90036, showed compatibility to *Pik-p* only. Using these five isolates, 92319-4, M64-7-3-5-2, CBN921411, V86046, and B90036, the three kinds of genes, *Pi1*, *Pik-m* and *Pik-p*, and the remaining two genes, *Pik* and *Pik-h*, could be differentiated, but the classification between *Pik* and *Pik-h* was impossible. These isolates could partially differentiate some of the *Pik* alleles. Three DVs, Shin 2, K59 and Caloro, for *Pik-s*, showed a narrow spectrum of resistance, and the reactions were different from those of other *Pik* alleles.

3. Variation of pathotypes in blast isolates

Many kinds of pathotypes were found in the blast isolates collected from Caliraya and Los Baños, Laguna Province of Southern Luzon Island (Table 2).

Caliraya and Los Baños included 23 (52 isolates) and 19 (43 isolates) pathotypes, respectively. In both sites, any tendency of predominant pathotypes was not recognized. These two sites were planted with highly diverse sets of varieties, new germplasm and some developed breeding lines, and the evaluations for blast resistance were carried out for a long period. The wide variations of pathotypes of blast isolates might have been created due to the pressures caused by the diversities of rice varieties.

The number of isolates used in each region was not uniform and limited. It will be necessary to evaluate many more isolates from each area: irrigated, rainfed lowland, and upland ecosystems, to confirm more details about the characters of pathogenicities and distributions of races in the Philippines.

4. Selection of representative isolates

Based on the evaluations using the DVs targeting 18 blast resistance genes, *Pia*, *Pib*, *Pii*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pik-s*, *Pish*, *Pit*, *Pita*, *Pita-2*, *Piz*, *Piz-t*, *Piz-5*, *Pi1*, *Pi3*, and *Pi20*(t), a total of 20 isolates from 70 pathotypes were selected, as the representative standard differential blast isolates in the Philippines, tentatively. These 20 blast isolates had always good sporulating ability and could differentiate the DVs. We have tried to use them for selection in rice breeding from the 1990s, and the pathogenicity of each of these blast isolates has been stable. Among five blast isolates which could differentiate the four *Pik* alleles, *Pik*, *Pik-h*, *Pik-m*, and *Pik-p*, and two genes, *Pi7*(t) and *Pi1*, the isolates CBN921411, V86046, and B90036 were not selected because their pathogenicities were not stable after storage for long periods.

5. Confirmation and characterization of selected blast isolates

These 20 blast isolates might not directly show the true reactions of the targeted resistance genes because several DVs harbored one or more additional resistance genes in these genetic backgrounds[10,12]. To confirm and evaluate further the differentiating ability of the 20 selected isolates, monogenic lines[14,26] for 24 kinds of resistance genes, *Pia*, *Pib*, *Pii*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pik-s*, *Pish*, *Pit*, *Pita*, *Pita-2*, *Piz*, *Piz-t*, *Piz-5*, *Pi1*, *Pi3*, *Pi5*(t), *Pi7*(t), *Pi9*(t), *Pi11*(t), *Pi12*(t), *Pi19*(t), and *Pi20*(t), were investigated for their reaction patterns.

The selected blast isolates showed high differentiating abilities to all these resistance genes, except for several cases (Table 4). A monogenic line, IRBLa-A for *Pia*, confirmed the resistance to two isolates, B90002 and C923-49, and susceptible to the other isolates. *Pia* had a narrow spectrum of resistance for the Philippine isolates. Only two isolates, V850196 and V86010, showed incompatibility to *Pik-s* as exhibited by a monogenic line, IRBLks-F5. The gene *Pik-s* also has a narrow spectrum of resistance. These monogenic lines for the other four *Pik* alleles' genes, *Pik*, *Pik-h*, *Pik-m*, and *Pik-p*, and *Pi1*, showed the same reaction patterns to 20 isolates. It means that these four genes, *Pik*, *Pik-h*, *Pik-m*, and *Pik-p*, could not be differentiated by the selected isolates. The monogenic line, IRBLzt-T for *Piz-t*, is resistant to four isolates and has a specific resistance spectrum. The other three different alleles of *Piz*, *Piz-5* in IRBL5z-CA, *Piz* in IRBLz-Fu, and the *Pi9*(t) in IRBL9-W[26] showed moderate or complete resistance with wide spectrums of resistance to almost all isolates. One isolate, M101-1-2-9-1, was compatible to IRBLz5-CA for *Piz-5* and not IRBLz-Fu for *Piz*, and could differentiate these two genes. There were no compatible isolates for *Pi9*(t). IRBLsh-S harboring *Pish* also showed moderate reactions to almost all isolates and a wide spectrum of resistance. The reaction of *Pish*

Table 4. Reaction patterns of monogenic lines to blast isolates from the Philippines

Target gene	Designation[a)]	Blast isolates and pathotypes																			
		Ca89	Ca41	IK81-3	M64-1-3-9-1	M39-1-3-8-1	V850256	V86010	BN111	PO6-6	M39-1-2-21-2	JMB8401	43	M36-1-3-10-1	BN209	M101-1-2-9-1	IK81-25	JMB840610	V850196	B90002	C923-49
		Ib	Id	II	IIIa	IIIa	IIIc	IVe	Va	Vb	VIb	XIIa	XVIII	XIXb	XXIIa	XXIIa	XXIII	XXIVb	XXVII	XXVIIIb	XXVIIIb
Pia	IRBLa-C	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R
Pita	IRBLta-K1	S	R	R	S	R-M	R	S	S	MS	S	M	S	MS	S	S	R	S	R	R	S
Pik-s	IRBLks-F5	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	R	S	S
Pit	IRBLt-K59	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Pi3	IRBL3-CP4	S	S	S	S	M	S	S	R-M	R	R	R	R	S	S	S	R	S	R	S	S
Pii	IRBLi-F5	S	S	S	S	M	S	S	R	R	R-M	R	R	S	S	S	R	S	R	S	S
Piz-t	IRBLzt-T	S	S	S	R	R	S	R	S	S	S	S	S	S	S	S	S	R-M	S	R	R
Pib	IRBLb-B	S	S	S	S	S	S	M	S	S	S	S	S	S	R-M	R	S	R	S	R	R
Piz-5	IRBLz5-CA	M	R	R-M	M	M	M	M	R	R	R-M	R	M	R	M	S	R	R	R-M	M	M
Pita-2	IRBLta2-Re	S	R	R	R-M	R	R	R	R	S	R	R	S	R	R	R	R	R	R	R	S
Piz	IRBLz-Fu	M	M	R	R-M	R-M	M	R-M	S	R-M	R-M	R-M	R	R	R-M	R	R	M	R	M	R
Pik	IRBLk-ka	R	R	R	S	S	R	R	R	R	S	S	R	S	R	R	S	R	R	R	R
Pi1	IRBL1-CL	R	R	R	S	S	R	R	R	R	S	S	R	S	R	R	S	R	R	R	R
Pik-h	IRBLkh-K3	R	R	R	S	S	R	R	R	R	S	S	R	MS	R	R	M	R	R	R	R
Pik-m	IRBLkm-Ts	R	R	R	S	S	R	R	R	R	S	S	R	M	R	R	MS	R	R	R	R
Pik-p	IRBLkp-K60	R	R	R	S	S	R	R	R	R	S	S	R	S	R	R	S	R	R	R	R
Pi20(t)	IRBL20-IR24	S	S	S	S	S	S	R	R	S	R	S	S	R	S	S	MS	S	S	R	R
Pi5(t)	IRBL5-M	S	M	R-M	M	R	MS	S	R-M	R	R	R	R	R-M	MS	R	R	M	R	M	MS
Pi7(t)	IRBL7-M	R	R	R	S	S	R	S	R	R	S	S	R	S	R	R	S	R	R	R	R
Pi9(t)	IRBL9-W	R	R	R	R-M	M	R	S	R	R	R-M	R	R	R	R	R	R	R	R-M	R	R
Pi11(t)	IRBL11-Zh	S	S	M	S	S	S	R	S	S	S	MS	S	MS	R-M	R	S	R	S	R	R
Pi12(t)	IRBL12-M	S	S	S	S	S	S	M	S	S	S	S	S	S	R	R	S	R	M	R	R
Pi19(t)	IRBL19-A	S	S	S	S	S	S	S	S	S	S	MS	S	MS	S	S	S	S	S	S	S
Pish	IRBLsh-S	M	R-M	R-M	M	M	R-M	M	R-M	R-M	R	S	M	R	M	R-M	R	M	R-M	R-M	M

a): Monogenic lines are designated as IRBL followed by the resistance gene then the abbreviation of the resistant donor variety.
R: resistant, M: moderately resistant, MS: moderately susceptible, S: susceptible, R-M: varied from moderately resistant to resistant.

was difficult to distinguish from those of *Piz*. Two MLs, IRBLt-K59 for *Pit* and IRBL19-A for *Pi19*(t), showed susceptible reactions to all isolates. There were no incompatible blast isolates for *Pit* and *Pi19*(t). It is necessary to find the available blast isolates that can differentiate *Pik* alleles, *Piz*, *Piz-5*, *Pi9*(t), *Pit*, and *Pi19*(t) in the Philippines. The compatible isolates for resistance genes, *Pik* alleles, *Pish*, *Pit*, and *Pi19*(t) were found in Japan and China, respectively. These differences of pathogenicities between these countries and the Philippines might be related with the adaptations or differentiations of blast races.

The differential system consisting of differential varieties and blast isolates is a useful guide to study the resistance and avirulence gene interaction. Identification of genotype and pathotype of a certain variety and blast isolate, respectively, is made possible. By using a standard set of DVs like the monogenic lines and NILs with a single resistance gene, we demonstrated that it would be easier to characterize and differentiate the races of blast pathogens in the Philippines. Differential systems would also help us in monitoring and characterizing pathogen populations that could facilitate the identification of appropriate breeding strategies and proper gene deployment to rice blast infected areas for effective control measures. This study will provide information that serves as a starting point to build up a durable resistance system of rice cropping in the tropics.

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