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## DIFFERENTIATION OF PATHOGENIC RACES OF RICE BLAST FUNGUS BY USING NEAR-ISOGENIC LINES WITH *INDICA* GENETIC BACKGROUND<sup>1)</sup>

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### Introduction

As for other specific pathogens, the race of a *Pyricularia grisea* isolate is determined by assaying its infection spectrum on a set of cultivars carrying different resistance genes, which are termed differential cultivars or "differentials." The ideal differential set would consist of cultivars differing in single resistance genes<sup>3)</sup>. A differential set named "Kiyosawa's differentials," consisting of 12 Japanese cultivars or breeding lines carrying single resistance genes was developed in Japan<sup>8)</sup>. No proper differential set, however, has been developed for use in the tropics.

Although some differential sets were developed and used in tropical countries<sup>12)</sup>, the genetic constitution for blast resistance of many cultivars in the sets were not well understood. These cultivars are suspected to carry more than one resistance gene. While Kiyosawa's differentials and the International differentials<sup>1)</sup> are useful in temperate regions, they are not suitable for use in the tropics because some cultivars in the differentials may have more than one resistance gene against Philippine isolates, and the *japonica* cultivars/lines do not grow well in the tropics.

For those reasons, a set of near-isogenic lines (NILs) with single resistance genes was developed in an *indica* genetic background as a differential set for the

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tropics<sup>11</sup>). The set of 22 NILs developed was reduced to five NILs carrying distinct resistance loci, and the allelism relationships between these loci and resistance genes in other cultivars was studied in our previous study<sup>5</sup>). In this study, the differentiating ability of the set of NILs was evaluated by comparison with those of Kiyosawa's and the International differentials.

## Materials and Methods

### Differentials

The set of 5 NILs (Table 2), Kiyosawa's differentials (Table 4) and the International differentials (Table 5) were used. The recurrent parent of the NILs was the highly susceptible *indica* cultivar CO39. The donor parents were resistant *indica* cultivars Tetep (TTP) and 5173 (A51) and *japonica* cultivars Pai-kan-tao (PKT) and LAC23 (LAC). According to our previous study, *Pi-1*(t) in C101LAC and *Pi-3*(t) in C104PKT were different from any resistance genes in Kiyosawa's differentials. *Pi-2*(t) in C101A51 was allelic to *Pi-z*. The gene in C101PKT was identical to *Pi-ta*, and 105TTP-4L23 was found to carry *Pi-ta* and an additional resistance gene<sup>5</sup>).

### Test isolates

Forty-six Philippine isolates of *Pyricularia grisea* from the collection of the Plant Pathology Division at IRRI were used. (Table 1). These isolates were mainly collected from irrigated and upland rice areas in central Luzon and Mindanao in the Philippines from 1983 to 1986.

### Pathogenicity tests

The pathogenicity of the isolates was assayed using spraying method<sup>2</sup>). Ten plants per cultivars/lines were inoculated. The disease reactions were scored about 7 days after inoculation. Pathogenicity of isolates was rated by the criterion which was originally made for injection method<sup>15</sup>) and modified for spraying method<sup>9</sup>). Cultivars/lines rated R<sup>h</sup>, R and MR were considered resistant, and those rated M, MS and S were considered susceptible.

The pathogenicity of the isolates was first assayed by the set of 5 NILs. When 46 isolates were inoculated to the set of NILs, the isolates were classified into 7 races (Table 2). Two isolates each were chosen from the seven races (Table 3) and those isolates were inoculated to Kiyosawa's differentials and the International differentials to evaluate the ability of the NILs as a differential set. Only one isolate was available in races C and G in these pathogenicity tests. The numbering system of races defined by Kiyosawa's or the International differentials was described in previous papers<sup>1,8</sup>).

**Table 1.** Reaction of near-isogenic lines (NILs) to Philippine isolates

Isolate	Host variety	Year	Reaction of NILs to each isolates					Race
			C101LAC ( <i>Pi-1(t)</i> )	C101A51 ( <i>Pi-2(t)</i> )	C104PKT ( <i>Pi-3(t)</i> )	C101PKT ( <i>Pi-ta</i> )	C105TTP-4L23 ( <i>Pi-ta + Pi-?</i> )	
V86026	Azucena	1986	R	R	S	S	S	D
JMB840493	Barao	1984	R	R	R	R	R	A
JMB840495	Barao	1984	R	R	R	R	R	A
JMB840364	C22	1984	R	R	R	R	R	A
JMB840385	C22	1984	R	R	S	R	R	B
JMB840286	CNM539	1984	R	R	R	S	S	E
V850223	Dao Pao	1985	R	R	S	R	R	B
V850225	Dao Pao	1985	R	R	S	R	R	B
V850228	Dao Pao	1985	R	R	S	S	S	G
V850229	Dao Pao	1985	R	R	S	R	R	B
V850230	Dao Pao	1985	R	R	S	S	S	G
V850231	Dao Pao	1985	R	R	S	R	R	B
PO83-Z1-25	Denorado	1983	R	R	R	R	R	A
PO83-Z1-30	Denorado	1983	R	R	S	S	S	G
V850187	Dharial	1985	R	R	S	R	R	B
V86025	IAC25	1986	R	R	S	S	R	D
V850115	IR36	1985	R	R	R	S	S	E
V850136	IR36	1985	R	R	R	S	S	E
V86014	IR36	1986	R	R	R	S	S	E
JMB840487	IR42	1984	R	R	R	S	S	E
JMB840334	IR442	1984	R	R	R	S	S	E
V850210	IR52	1985	R	R	R	S	S	E
V85065	IR52	1985	R	R	R	S	S	E

**Table 1.** (continued)

Isolate	Host variety	Year	Reaction of NILs to each isolates					Race
			C101LAC ( <i>Pi-1(t)</i> )	C101A51 ( <i>Pi-2(t)</i> )	C104PKT ( <i>Pi-3(t)</i> )	C101PKT ( <i>Pi-ta</i> )	C105TTP-4L23 ( <i>Pi-ta + Pi-?</i> )	
JMB8401102	IR56	1984	R	R	R	S	S	E
PO83-N1-9	IR58	1983	R	R	R	S	S	E
V8607	IR58	1986	R	R	R	S	S	E
V85097	IR60	1985	R	R	R	S	S	E
V8605	IR60	1986	S	R	R	S	S	F
V85020	IR64	1985	R	R	R	S	S	E
V86041	IR64	1986	R	R	R	S	S	E
E89019	IRAT13	1989	R	R	R	S	S	E
JMB840139	KMP47	1984	R	R	S	S	S	G
IK81-3	Milyang 49	1981	R	R	S	R	R	B
V86018	RNR1446	1986	R	R	R	R	R	A
JMB840148	She-Li-Co	1984	R	R	R	S	S	E
PO83-Z5-3	Tayak	1983	R	R	S	S	S	G
PO6-6	Tetep	1983	R	R	R	S	S	E
V850190	Tiale	1985	R	R	S	S	S	G
JMB840349	Tumindog	1984	R	R	R	S	S	E
V850196	UPLRi5	1985	R	R	R	R	R	A
JMB840505	breeding line	1984	R	R	S	S	R	D
JMB8401	breeding line	1984	S	R	R	S	S	F
V86010	unknown	1986	R	R	S	S	R	D
V86046	unknown	1986	S	R	R	S	S	F
43	unknown	unknown	R	R	R	R	R	A
IK81-25	unknown	unknown	S	R	S	R	R	C

**Table 2.** Classification of pathogenic races with a set of near-isogenic lines

Race		A	B	C	D	E	F	G
Near-isogenic line	Genotype	<i>Av-1</i>	<i>Av-1</i>	<i>Av-1<sup>+</sup></i>	<i>Av-1</i>	<i>Av-1</i>	<i>Av-1<sup>+</sup></i>	<i>Av-1</i>
		<i>Av-2</i>	<i>Av-2</i>	<i>Av-2</i>	<i>Av-2</i>	<i>Av-2</i>	<i>Av-2</i>	<i>Av-2</i>
		<i>Av-3</i>	<i>Av-3<sup>+</sup></i>	<i>Av-3<sup>+</sup></i>	<i>Av-3<sup>+</sup></i>	<i>Av-3</i>	<i>Av-3</i>	<i>Av-3<sup>+</sup></i>
		<i>Av-ta</i>	<i>Av-ta</i>	<i>Av-ta</i>	<i>Av-ta<sup>+</sup></i>	<i>Av-ta<sup>+</sup></i>	<i>Av-ta<sup>+</sup></i>	<i>Av-ta<sup>+</sup></i>
C101LAC	<i>Pi-1(t)</i>	R	R	S	R	R	S	R
C101A51	<i>Pi-2(t)</i>	R	R	R	R	R	R	R
C104PKT	<i>Pi-3(t)</i>	R	S	S	S	R	R	S
C101PKT	<i>Pi-ta</i>	R	R	R	S	S	S	S
C105TTP-4L23	<i>Pi-ta + Pi-?</i>	R	S	R	R	S	S	S
No. of isolates		7	7	1	3	18	3	7

**Table 3.** Reaction of near-isogenic lines to representative isolates

Near-isogenic line	R gene	Reaction to isolate												
		A		B		C		D		E		F		G
		PO83-Z1-25	43	V850231	IK81-3	IK81-25	V86010	JMB840505	V85097	PO6-6	JMB8401	V86046	V850190	
C101LAC	<i>Pi-1(t)</i>	R <sup>h</sup>	R	R <sup>h</sup>	R <sup>h</sup>	MS	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	MS	M	R <sup>h</sup>	
C101A51	<i>Pi-2(t)</i>	R	MR	R	R	R <sup>h</sup>	R	R <sup>h</sup>	R	R	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	
C104PKT	<i>Pi-3(t)</i>	R	R	M	M	M	MS	MS	R	R	R <sup>h</sup>	R <sup>h</sup>	M	
C101PKT	<i>Pi-ta</i>	R	R	R	R <sup>h</sup>	R <sup>h</sup>	MS	M	MS	MS	MS	M	M	
C105TTP-4L23	<i>Pi-ta + Pi-?</i>	R	R	R	R <sup>h</sup>	R <sup>h</sup>	R	R <sup>h</sup>	MS	MS	MS	M	M	
CO39		M	M	M	M	M	MS	M	M	MS	MS	MS	M	

## Results

### Reaction of three differential sets to a group of Philippine isolates

#### Set of five NILs

Based on the gene-for-gene theory, each incompatible reaction to a single resistance gene should correspond to a single avirulence gene in the pathogen<sup>3,13</sup>. Except for C105TTP-4L23, which carried two resistance genes, each of the NILs carried a single effective resistance gene. Thus, the virulence genotype of each blast isolate was estimated based on their interactions with the NILs (Table 2). At least seven races were identified. Most of the isolates (39.1%) belonged to race E. Races A (15.1%), B (15.1%) and G (15.1%) were the next most predominant in the collection tested. Race C was consisted of a single isolate. The proportion of isolates compatible to *Pi-ta* was 67.4%, while 8.7% and 23.9% of the isolates were compatible to *Pi-1(t)* and *Pi-3(t)*, respectively. None of the isolates tested were compatible to *Pi-2(t)*.

CO39, the recurrent parent of the NILs, generally showed M to MS reaction to the Philippine isolates (Table 3), while Aichi asahi in Kiyosawa's differentials mostly showed MS to S reaction (Table 4). CO39 appeared to show a low level

**Table 4.** Reaction of Kiyosawa's differentials to representative isolates

Kiyosawa's differentials	R gene	Code No.	Reaction to isolate												
			A		B		C		D		E		F		G
			PO83-21-25	43	V850231	IK81-3	IK81-25	V86010	JMB 840505	V85097	PO6-6	JMB8401	V86046	V850190	
Shin 2	<i>Pi-k<sup>s</sup></i>	1	R <sup>h</sup>	R	R	M-MR	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R	R	M-MR	R	M-MR	
Aichi asahi	<i>Pi-a</i>	2	MS	S	M	S	S	MS	MS	S	MS	MS	M	MS	
Fujisaka 5	<i>Pi-i</i>	4	R <sup>h</sup>	R	M	M	M	R <sup>h</sup>	MS	R <sup>h</sup>	R	R <sup>h</sup>	R <sup>h</sup>	MS	
Kusabue	<i>Pi-k</i>	10	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	M	R	R <sup>h</sup>	
Tsuyuake	<i>Pi-k<sup>m</sup></i>	20	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	S	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	MS	M	R <sup>h</sup>	
Fukunishiki	<i>Pi-z</i>	40	R	R	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R	R <sup>h</sup>	R <sup>h</sup>	R	
K1	<i>Pi-ta</i>	100	R	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	M	M	M	MS	M	M	S	
Pi No. 4	<i>Pi-ta<sup>2</sup></i>	200	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	
Toride 1	<i>Pi-z'</i>	400	R	MR	R	M-MR	R	R <sup>h</sup>	R <sup>h</sup>	R	R	M-MR	R	R <sup>h</sup>	
K 60	<i>Pi-k<sup>p</sup></i>	0.1	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	M-MR	R <sup>h</sup>	R <sup>h</sup>	
BL 1	<i>Pi-b</i>	0.2	R	R	R	M-MR	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R	R	R	R	
K 59	<i>Pi-t</i>	0.4	R <sup>h</sup>	MS	M	M	S	R <sup>h</sup>	M	MS	MS	MS	MS	S	
	Race		002.0	002.4	006.4	006.4	026.4	102.0	106.4	102.4	102.4	122.4	122.4	106.4	

of partial resistance. The reaction of the NILs to several compatible isolates was different from that of CO39 though CO39 was the recurrent parent of the NILs (Table 3). For example, CO39 was more partially resistant than C101PKT or C105TTP-4L23 to V85097, an isolate which was compatible to the three lines. Conversely, CO39 was more susceptible to V86046 than the NILs that were compatible to the isolates.

#### *Kiyosawa's differentials*

All isolates tested were compatible to Aichi asahi carrying *Pi-a* but incompatible to Fukunishiki carrying *Pi-z* and Pi No. 4 carrying *Pi-ta<sup>2</sup>* (Table 4). Shin 2, Toride 1, K60 and BL1 showed intermediate reactions (M-MR) to some isolates, making race determination with this set of differentials difficult. Assuming that the intermediate reaction was considered resistance, 12 isolates were differentiated into 8 races.

#### *International differentials*

The reactions of the International differentials were clearer than those of Kiyosawa's differentials. The International differentials differentiated 12 isolates into 10 races (Table 5). NP125 and Dular were resistant to all isolates tested. The reaction pattern of Usen was similar to that of C101PKT carrying *Pi-ta*. Caloro showed a similar reaction pattern to K59 carrying *Pi-t*.

**Table 5.** Reaction of International differentials to representative isolates

International differentials	Reaction to isolate												
	A		B		C		D		E		F		G
	PO83-Z1-25	43	V850231	IK81-3	IK81-25	V86010	JMB 840505	V85097	PO6-6	JMB8401	V86046	V850190	
Raminad Str.3	R <sup>h</sup>	S	R <sup>h</sup>	S	R	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	
Zenith	R	R	MR	R	R	R <sup>h</sup>	R <sup>h</sup>	R	S	R <sup>h</sup>	R <sup>h</sup>	R	
NP 125	R <sup>h</sup>	R	R <sup>h</sup>	R	R	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R	R	R	R <sup>h</sup>	
Usen	R	R	R	R	R	MS	M	MS	S	M	M	M	
Dular	R <sup>h</sup>	R	R <sup>h</sup>	R	R	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R	R	R <sup>h</sup>	R <sup>h</sup>	
Kanto 51	R <sup>h</sup>	R	R <sup>h</sup>	R	S	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R	M	M	R <sup>h</sup>	
Sha-tiao-tsao-S	R	R	MS	S	R	S	MS	R <sup>h</sup>	R	R <sup>h</sup>	R <sup>h</sup>	MS	
Caloro	R <sup>h</sup>	S	M	S	S	R <sup>h</sup>	M	MS	S	M	M	M	
Race	II-1	(IA-127)	IG-1	(IA-125)	(IF-3)	ID-14	ID-13	ID-15	(IB-47)	ID-11	ID-11	ID-13	

( ): referred to Yu's PhD thesis<sup>16)</sup>.

**Table 6.** Comparison of three differentials for differentiating ability

Differentials <sup>a</sup>	Differentiation of 12 isolates by three differentials											No. of races	
	PO83-Z1-25	43	V850231	IK81-3	IK81-25	V86010	JMB 840505	V850190	V85097	PO6-6	JMB8401		V86046
NILs	A		B		C	D		G	E		F		7
KDs	002.0	002.4	006.4		026.4	102.0	106.4		102.4		122.4		8
IDs	II-1	IA-127	IG-1	IA-125	IF-3	ID-14	ID-13		ID-15	IB-47	ID-11		10

a. NILs= near-isogenic lines.

KDs= Kiyosawa's differentials.

IDs= International differentials.

### Differentiating ability of the set of near-isogenic lines

The International differentials appeared to be the most useful as a differential set in this experiment. However, the set of NILs was able to discriminate between JMB840505 and V850190, which were both classified as race ID-13 by the International differentials (Table 6). These two differential sets appeared to be complementary each other.

Kiyosawa's differentials were not suitable for the Philippine isolates because some of the differentials showed intermediate reactions to some isolates (Table 4). In addition, at least Shin 2, Fukunishiki and Kusabue appeared to carry more than one resistance gene against Philippine isolates, for the following reasons. Caloro, Zenith and Kanto 51 in the International differentials have already been found to carry *Pi-k<sup>s</sup>*, *Pi-z* and *Pi-k*, respectively<sup>6,7,15)</sup>. But the reaction patterns of those cultivars were not the same to those of Shin 2, Fukunishiki and Kusabue, known to carry the same resistance genes (Table 5). In all cases these Kiyosawa's differentials showed resistance to isolates compatible to Caloro, Zenith or Kanto 51. This indicated that Shin 2, Fukunishiki and Kusabue carried additional resistance gene(s) to Philippine isolates.

### Discussion

A set of near-isogenic lines with single resistance genes is the ideal differentials to identify pathogen races<sup>3)</sup>, because it can be used to infer the avirulence genotype of the pathogen isolates. A set of five NILs was able to differentiate 46 Philippine isolates into 7 races and to allow estimation the genotype of each isolate for avirulence. However, the differentiating ability of the NILs for pathogen isolates was not sufficient. Although the International differentials were also not a proper differential set for the tropics<sup>2)</sup>, this differential set was more capable than the NILs for description of pathogen isolates. When the International differentials were combined with the set of NILs, the integrated differential set was able to classify 12 isolates tested into 11 races. This integrated differential set appeared to be useful as a tentative differential set in the Philippines.

Since Raminad Str.3, Zenith and Caloro in the International differentials discriminated between two isolates in each of the four races A, B, D and E defined by the NILs, those cultivars might to be useful as donor of resistance genes to develop additional NILs. The reaction pattern of Raminad Str. 3 was different from those of any NILs and Kiyosawa's differentials, so this cultivar appeared to carry unknown resistance genes. Zenith has been found to carry *Pi-z* and *Pi-a*<sup>7)</sup>. Since *Pi-a* was compatible to all isolates tested, the reaction pattern of Zenith appeared to be due to only *Pi-z*. Caloro has been found to carry at least *Pi-k*<sup>s</sup>, but it was not confirmed if the resistance of Caloro to Philippine isolates was due to *Pi-k*<sup>s</sup>. Shin 2, carrying *Pi-k*<sup>s</sup>, showed a different reaction pattern to that of Caloro because of the existence of unknown resistance genes in Shin 2. As the reaction pattern of Caloro was similar to that of K 59 carrying *Pi-t*, it is necessary to test allelism between resistance genes in Caloro and K59. The reaction pattern of Usen was similar to that of C101PKT carrying *Pi-ta*. *Pi-ta* in C101PKT was derived from a Chinese cultivar Pai-kan-tao<sup>5,11)</sup>. Usen was also a Chinese cultivar, so this cultivar might have *Pi-ta*.

Kiyosawa's differentials were not suitable differential set for the tropics though this differential set has been useful for the temperate regions. The reasons were that some of Kiyosawa's differentials showed intermediate reaction to Philippine isolates and that some carried more than one resistance gene effective against the Philippine isolates. Kiyosawa et al.<sup>10)</sup> has already pointed out these problems and estimated that the intermediate reactions to Philippine isolates were due to partial resistance genes. On the other hand, Imbe et al.<sup>4)</sup> showed that Shin 2, Kusabue, Fukunishiki, Toride 1 and BL1 carried *Pi-sh* that were identified using local races on the island of Kyushu in Japan. The reaction of *Pi-sh* to isolates fluctuated with varying temperature after infection. Assuming that *Pi-sh* was effective against Philippine isolates, it may be that the intermediate reaction of the cultivars to Philippine isolates might be due to *Pi-sh*.



Kiyosawa's differentials will also be useful as donor of resistance genes for developing additional NILs. However, it is first necessary to understand the gene constitution of each cultivar for resistance to Philippine isolates.

CO39, the recurrent parent of the NILs, was considered to be a highly susceptible cultivar. However, CO39 appeared to show a low level of partial resistance. It has already been reported that CO39 showed partial resistance to Japanese isolates, too<sup>14)</sup>. Moreover, CO39 has been shown to carry at least one gene conditioning complete resistance to Japanese isolates<sup>14)</sup> and to Philippine isolates (M. Bronson, personal communication). The NILs with the genetic background of CO39 are therefore not useful in regions where races incompatible to CO39 were distributed. The reaction of some of the NILs to several compatible isolates was different from that of CO39 to the isolates. This indicated that the genetic background of the NILs was still not uniform and some of the NILs might hold chromosomal segments from donor parents, including loci for partial resistance. According to available RFLP data, most of the NILs still carried more than 10% of the chromosomal segments derived from the donor parents<sup>16)</sup>. It is necessary to eliminate the remained chromosomal segments from the NILs by more backcrossing.

### Summary

A set of near-isogenic lines (NILs) of rice was previously developed to identify pathogen races of blast fungus in the tropics. In this study the differentiating ability of the set of NILs was evaluated by comparison with those of Kiyosawa's and the International differentials. The set of 5 NILs was able to differentiate 46 Philippine isolates into 7 races. However, the differentiating ability of the NILs was not sufficient comparing with that of the International differentials. The integrated differentials of the NILs and the International differentials appeared to be tentatively useful to describe blast pathogen isolates in the Philippines. Kiyosawa's differentials developed in Japan was not useful in the Philippines because some of Kiyosawa's differentials showed intermediate reaction to Philippine isolates and that some carried more than one resistance gene to Philippine isolates.

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