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Estimation of Genes in Blast Resistance in Elite Indica-type Rice (*Oryza sativa* L.) Varieties-bred at the International Rice Research Institute

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The presence of blast resistance genes in the elite Indica-type rice (*Oryza sativa* L.) varieties bred at the International Rice Research Institute (IRRI) was estimated based on a differential system using Philippines isolates of the rice blast fungus *Pyricularia grisea* (Cooke) Sacc., according to the gene-for-gene theory. Based on the presence of the three resistance genes, *Pi20*, *Pita* and one of the *Pik* alleles (other than *Pik-s*), the 42 varieties were classified into seven groups. A group that did not harbor these three genes contained seven varieties derived from the progenies of the hybrids with IR24 as a parental variety. The largest group harboring *Pita* consisted of 17 varieties, including IR36 and its sister lines or progenies. The group harboring by *Pi20* had seven varieties that included IR8, IR24 and their hybrid progenies. Thus, most of the IRRI varieties were classified into these three groups that included IR8, IR24, IR36 or their hybrid progenies in their pedigree. The presence of a total of seven resistance genes *Pib*, *Pita*, *Piz-t*, *Pi20*, *Pik-s*, one of the *Pik* alleles (other than *Pik-s*) and one of the two genes, *Pii* or *Pi3*, was estimated in these varieties. In some cases, the presence of genes like *Pib*, *Pik-s* and *Piz-t* could not be confirmed due to the masking effect of *Pita*, *Pik* allele, or *Pi20*. The number and kind of blast resistance genes in IRRI varieties were limited compared with previously reported blast resistance genes. Since the presence of *Pik-s* and another *Pik* allele was estimated in 17 varieties belonging to five groups, and that of *Pib* in 38 varieties belonging to four groups, it appeared that these genes were widely distributed in IRRI-bred varieties.

Key Words: ***Oryza sativa* L., *Pyricularia grisea* (Cooke) Sacc., resistance gene, differential system, IRRI-bred variety.**

Introduction

Blast, caused by *Pyricularia grisea* (Cooke) Sacc. (*P. oryzae* Cavara), teleomorph *Magnaporthe grisea* (Hebert) Barr, is one of the most destructive diseases of rice (*Oryza sativa* L.) worldwide. Particularly, the threat is serious in the temperate and sub-tropical rice production areas (Bonman 1992). In the tropics, the occurrence of blast is severe in upland and rainfed lowland environments. Susceptible varieties can be severely damaged by blast even under irrigated conditions (Bonman and Mackill 1988). The control of blast disease can be achieved by the cultivation of resistant varieties (Yu *et al.* 1991, Bonman *et al.* 1992). Hence, breeding for blast resistance is a major objective in a rice improvement program.

Resistance to rice blast has been explained by the gene-for-gene theory between the resistance gene in the host and the avirulence gene in *P. grisea* (Kiyosawa 1972, Silue *et al.* 1992). To date, around 40 blast resistance genes and their chromosomal locations have already been reported (RGC 1998). In spite of the wide distribution of many known genes in rice varieties grown in different countries, genetic studies of blast resistance are limited in the tropics. This is partly attributed to the lack of a suitable differential system for the efficient identification of those genes. Additionally, the presence of several resistance genes in Indica-type varieties may account for the complexion of genetic studies on blast resistance genes difficult (Mackill *et al.* 1985, Yu *et al.* 1987).

The information about blast resistance genes in Indica-type varieties as well as IRRI-bred varieties and lines is limited. Several resistance genes, *Pita* (Kiyosawa 1966), *Pita-2* (Kiyosawa 1967), *Pik-h* (Kiyosawa and Murty 1969), *Pik-p* (Kiyosawa 1969), *Piz-t* (Yokoo and Kiyosawa 1970), *Pib* and *Pit* (Yokoo *et al.* 1978), *Pi6*(t) (Yu 1991), *Pi11*(t)[*Piz-h*] (Zhu *et al.* 1993), *Pi8* (Pan *et al.* 1996), *Pi16*(t) (Pan *et al.* 1999) and *Pi17*(t) (RGC 1996) have been identified in Indica-type varieties. Regarding IRRI-bred varieties, Flores-Gaxiola *et al.* (1983) reported the presence of two complementary genes and one dominant gene in the genetic analysis using hybrid progenies derived from a cross between IR8 and Tetep. Yu *et al.* (1987) showed that the resistance in five varieties, IR36, IR46, IR54, IR56 and IR60, bred of the

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International Rice Research Institute (IRRI) and four traditional varieties, Carreon, Paikantao, Pankhari 203 and Tetep, were controlled by one or two dominant genes. They also identified a recessive gene that controlled the resistance in IR54 against one blast isolate, although the genotypes in each variety could not be characterized. Yamaguchi *et al.* (1996) reported that IR50 harbored two resistance genes, *Pia* and *Pib*, based on the analysis using Japanese blast isolates. Imbe *et al.* (1997) reported that four resistance genes, *Pia*, *Pib*, *Pik-s* and *Pi20*, were present in IR24 based on the genetic analysis using blast isolates from the Philippines. The information about the genotypes of IRRI-bred and Indica-type varieties is very limited.

The lack of a differential system for the identification of blast resistance genes has been recently addressed using blast isolates from the Philippines. Yanoria *et al.* (2000) examined the pathogenicity of around 150 blast isolates from IRRI's stock collections using differential varieties and lines. Several isolates that showed distinct reaction patterns to known resistance genes, were selected for use in a differential system. Moreover, Tsunematsu *et al.* (2000) studied in detail the pathogenicity of 12 isolates, which were selected by Yanoria *et al.* (2000), using 29 monogenic lines that were targeting 23 known blast resistance genes and harboring a single gene in each genetic background. The set of monogenic lines and selected blast isolates from the Philippines can be used as a differential system to analyze the pathogenicity of isolates and genotypes of rice varieties.

IRRI-bred varieties have been distributed worldwide and used by farmers and breeders as important parental varieties in breeding programs. One IRRI variety, IR8, released in 1966, triggered the Green Revolution in tropical countries of Asia (Hossain 1995). IR36 once dominated rice production in several Asian countries in the 1970s (IRRI 1982). Released in 1985, IR64 has been widely accepted as a high-quality rice variety in many countries because of its desirable combination of intermediate amylose content and intermediate gelatinization temperature (Khush 1987). A more recent variety, IR72, displays a high yield potential, shorter growth duration, and enhanced resistance to several diseases and insect pests (Kropff *et al.* 1990). In addition to these outstanding traits, IRRI-bred varieties may be carrying important blast resistance genes that could be used for deployment in blast- prone environments or for the breeding of blast-resistant rice varieties. However, this has not been well understood. For effective utilization of these genes, it is necessary to analyze the genetic constitution of these varieties for blast resistance. In the present study, it were made to identify the kinds of blast resistance genes in IRRI-bred varieties using a differential system developed by Yanoria *et al.* (2000) and Tsunematsu *et al.* (2000).

Materials and Methods

IRRI-bred varieties

A total of 42 Indica-type varieties bred at IRRI were used for estimating the presence of blast resistance genes. These included IR8, IR24, IR36, IR64 and IR72, which have been widely distributed and used in many countries because of their outstanding traits. Eight varieties, PSBRc1, PSBRc2, PSBRc4, PSBRc10, PSBRc18, PSBRc20, PSBRc28 and PSBRc30, developed by IRRI and designated by the Philippine Seed Board were also included.

Inoculation and evaluation

Pre-germinated seeds were sown in 26 × 35-cm plastic trays, with seven seedlings for each variety and two replications. A ten gram of ammonium sulfate was applied to each tray as basal fertilizer and 1 g was added one week before inoculation. In all the cases, two susceptible varieties, CO39 and Lijiangxintuanheigu (LTH), were placed in each tray to determine the success of inoculation and virulence of the Philippine blast isolates used.

Fourteen blast isolates previously selected by Yanoria *et al.* (2000) were used for inoculation. Virulence genotypes of these isolates were confirmed and the diversity in pathogenicity was revealed. At the fourth-leaf stage, seedlings in each tray were sprayed with 40–50 ml of spore suspension adjusted to 10^5 spores/ml. These seedlings were placed inside wet jute sacks for 18–24 h and transferred to an air-conditioned glasshouse room at 23 ± 3°C/30 ± 5°C night and day temperatures. Disease reactions on the seedlings were examined 6–7 days after inoculation. The reactions were evaluated using a 0–5 scale with slight modifications as described by Mackill and Bonman (1992). Scores of 0 to 2, 3 and 4 to 5 corresponded to resistant (R), moderately resistant (M) and susceptible (S) reactions, respectively.

Estimation of genotypes for resistance genes

The genotypes of the blast resistance genes in the IRRI varieties were estimated based on the differential system developed by Yanoria *et al.* (2000) and Tsunematsu *et al.* (2000). The estimation was carried out based on the reaction patterns of nine kinds of monogenic lines to 14 blast isolates from the Philippines (Table 1). Each monogenic line carried a different single resistance gene, *Pi20*, *Pita*, *Pik-s*, *Pia*, *Pib*, *Piz-t*, *Pii*, *Pi3*, and one of the *Pik* alleles (other than *Pik-s*). In the pathogenicity tests of blast isolates, *Pik-s* could be distinguished from the other four *Pik* alleles, *Pik*, *Pik-h*, *Pik-m* and *Pik-p*. However, no isolates were available to classify the four alleles (Tsunematsu *et al.* 2000, Yanoria *et al.* 2000). The four alleles were tentatively designated as *Pik*† in this study.

The IRRI varieties were classified into seven groups with distinct reaction patterns. The differences in the reaction patterns of the groups were based mainly on the presence of three genes *Pi20*, *Pita* and *Pik*†. These three genes displayed a relatively wider spectrum of resistance than the other genes. These genes demonstrated an incompatibility with several blast isolates but were easily identified by reactions to three isolates, M36-1-3-10-1, IK81-25 and PO6-6. Each isolate was known to be avirulent to only one of the

Table 1. Classification of IRRI-bred varieties and estimation of the presence of blast resistance genes for each vareity group following the reaction patterns of the monogenic lines to Philippine blast isolates of *Pyricularia grisea* Sacc.

Isolate	Monogenic line									Variety group (VG)									
										1		2			3	4	5	6	7	
										a	b	a	b	c					a	b
	IRBL20-IR24 (*Pi20*)	IRBLta-CP1 (*Pita*)	IRBLk-Ka (*Pik*†)[4]	IRBLks-F5 (*Pik-s*)	IRBLa-A (*Pia*)	IRBLb-B (*Pib*)	IRBLzt-T (*Piz-t*)	IRBLi-F5 (*Pii*)	IRBL3-CP4 (*Pi3*)	(-, -, -)[5]		(*Pi20*, -, -)			(-, *Pita*, -)	(-, -, *Pik*†)	(*Pi20*, -, *Pik*†)	(-, *Pita*, *Pik*†)	(*Pi20*, *Pita*, -)	
PO6-6[1]	S	S	R	S	S	S	S	R[3]	R	S	S	S	S	S	S	R	R	R	S	R
IK81-25[1]	S	R	S	S	S	S	S[3]	R-M	R	S	R-M	S	S	S	R	S	S	R	R	R
M36-1-3-10-1[1]	R	S	S	S	S	S	S	S	S	S	S	R	R	R	S	M	R	S	R	R
IK81-3[2]	S	R	R	S	S	S	S	R-M	M	S	R-M	S	S	S	R	M	R	R	R	R
BN111[1]	R	S	R	S	S	S	S	R	R	S	S	R	R	R	S	R	R	R	R	R
Ca89[1]	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S
V850256[2]	S	R	R	S	S	S	S	S	S	S	S	S	S	S	M	R	R	R	R	M
M39-1-3-8-1[2]	S	R	S	S	S	S	R	M	R-M	S	R	S	R	R	R	S	S	R	R	R
M64-1-3-9-1[1]	S	R-M	S	S	S	S	R	S	S	S	R	S	M	R	R	S	S	R	R	R
M39-1-2-21-2[2]	R	S	S	S	S	S	S	R-M	R	S	S	R	R	R	S	S	R	S	R	R-M
BN209[1]	S	S	R	S	S	R-M	S	S	S	R	R	R	R	R	R	R	R	R	R	R
V850196[1]	S	R	R	R	S	S	S	R	R	R	R	R	M	R	R	R	R	R	R	R
B90002[1]	R	M	R	S	R	R	R	S	S	R	R	R	R	R	R	R	R	R	R	R
C923-49[1]	R	S	R	S	R	R	R	S	S	R	R	R	R	R	R	R	R	R	R	R
Variety										IR20 IR28 IR30 IR45 IR66	IR29 IR34	IR8 IR22 IR24 IR26 PSBRc30	IR43	PSBRc2	IR5, IR32 IR36, IR38 IR40, IR42 IR44, IR50 IR52, IR54 IR58, IR60 IR62, IR65 IR68, IR72 PSBRc4	PSBRc1	IR74	IR56 IR70	IR46 IR48 IR64 PSBRc28	PSBRc10 PSBRc18 PSBRc20
Additional estimated genes										*Pib* *Pik-s*	*Pib* *Pik-s* *Piz-t* Unknown	*Pib* *Pik-s*	*Pib* Unknown	*Pib* *Pik-s* *Piz-t*	*Pib*	Unknown	–	–	*Pib*	*Pib* *Pii or Pi3*

[1] Tsunematsu *et al.* (2000)

[2] Additional blast isolates used by our group

[3] New data obtained using advanced generation of the materials

[4] Also includes monogenic lines carrying other *Pik* alleles, *Pik-p*, *Pik-h* and *Pik-m*, showing similar reaction patternsto that of IRBLk-Ka.

[5] Genotype of three kinds of blast resistance genes, *Pi20*, *Pita* and *Pik*†

three genes, i.e., M36-1-3-10-1(avirulent to *Pi20*), IK81-25 (avirulent to *Pita*) and PO6-6 (avirulent to *Pik*†). Hence, the presence or absence of the genes *Pi20*, *Pita* and *Pik*† was estimated based on a R (resistant) or S (susceptible) reaction to M36-1-3-10-1, IK81-25 and PO6-6 in a particular variety. The other isolates used in the study exhibited different combinations of avirulence to known blast resistance genes other than *Pi20*, *Pita* and *Pik*†.

Reclassification of the groups into subgroups was based on the presence of *Pib*, *Pik-s*, *Piz-t*, and *Pii* or *Pi3*. Isolates M36-1-3-10-1, IK81-25 and PO6-6 were virulent to *Pib*, *Pik-s* and *Piz-t*, hence, reactions to other isolates were analyzed to estimate the presence of these genes, which included resistance to BN209, V850196, B90002, C923-49, M39-1-3-8-1 and M64-1-3-9-1. The presence of one of the two genes, *Pii or Pi3*, was estimated based on the reaction to PO6-6 and Ca89. Both isolates showed similar pathogenicity, except for the virulence to *Pii* and *Pi3*.

Results

The 42 IRRI rice varieties were classified into seven variety groups, VG 1 to VG 7. In some cases, subgroups within a group were also identified whenever the variety reacted differently to a particular isolate. Three variety groups, VG 1, VG 2 and VG 7, were further divided into two, three, and two subgroups, respectively. The number of varieties in each group ranged from 1 to 17 (Table 1).

The VG 1 group was characterized by the absence of the three genes, *Pi20*, *Pita* and *Pik*† and seven varieties were included in this group. All the varieties were resistant to four blast isolates, BN209, V850196, B90002 and C923-49. These results suggested that two genes, *Pib* and *Pik-s*, were commonly present in the VG1 group. Among these varieties, IR29 and IR34 were additionally resistant to four isolates, IK81-25, IK81-3, M39-1-3-8-1 and M64-1-3-9-1. These reactions to M39-1-3-8-1 and M64-1-3-9-1 were attributed to the presence of *Piz-t*, while those of the other two isolates, IK81-25 and IK81-3, did not enable to identify the corresponding resistance genes. Based on these results, the VG1 group was reclassified into sub-groups, VG1a (IR20, IR28, IR30, IR45 and IR66) and VG1b (IR29 and IR34). Although the presence of *Pia* might also be assumed in the VG1 group, but the reactions to two isolates, B90002 and C923-49 were masked in the presence of *Pib* or *Piz-t*.

Seven varieties, IR8, IR22, IR24, IR26, PSBRc30, IR43 and PSBRc2, were included in the VG2 group harboring *Pi20*. These showed resistant (R) and susceptible (S) reactions to isolate, M36-1-3-10-1, which was avirulent to *Pi20*, and to two isolates, IK81-25 and PO6-6, which were virulent to *Pi20*, respectively. Additionally, all the varieties showed a R reaction to three isolates, BN209, B90002 and C923-49, that were avirulent to *Pib*. These results indicated that all the varieties in the VG 2 group were likely to harbor *Pib*.

The VG 2 group was divided into three subgroups, VG 2a, VG 2b and VG 2c, differentiated by the reactions to three isolates, M39-1-3-8-1, M64-1-3-9-1 or V850196. The varieties in two subgroups, VG 2a and VG 2c, showed a resistance to V850196 which the VG 2b variety displayed a moderately resistance to it. *Pita* and *Pik*† were not present in the among VG 2 varieties, and the R reaction to V850196 was estimated to be associated with the presence of *Pik-s*. The moderate resistance to the same isolate in VG 2b could be attributed to the presence of an unknown gene. Variety PSBRc2 in the VG 2c subgroup was resistant to both isolates, M39-1-3-8-1 and M64-1-3-9-1, and was estimated to harbor *Piz-t*. VG 2b variety showed a resistance to only M39-1-3-8-1 and a moderate resistance to M64-1-3-9-1. These kinds of resistance could not be associated with the reaction patterns in the differential system using the 14 isolates.

The VG 3 group contained the largest number of varieties characterized by the presence of *Pita* including seventeen varieties, IR5, IR32, IR36, IR38, IR40, IR42, IR44, IR50, IR52, IR54, IR58, IR60, IR62, IR65, IR68, IR72 and PSBRc4. In addition to the resistance to IK81-25, the resistance to isolate IK81-3 suggested the presence of *Pita* in the VG 3 group. All the varieties in this group showed a R reaction to two isolates, BN209 and C923-49. Since these isolates were avirulent to *Pib*, it was estimated that this gene was also present in the VG 3 group.

The VG 4 group harbored *Pik*† and only one variety, PSBRc1, was identified. PSBRc1 was moderately resistant to M36-1-3-10-1 but was susceptible to M39-1-2-21-2 and IK81-25 indicating that PSBRc1 did not carry the other genes, *Pi20* and *Pita*. It also showed a moderate resistance to isolate IK81-3. It was estimated that additional unknown gene(s) conferred the moderate resistance observed in this variety.

Only IR74 was included in the VG 5 group, which was estimated harbor two genes, *Pik*† and *Pi20*. The S reaction to IK81-25 indicated the absence of *Pita* in the variety.

The VG 6 group harbored *Pita and Pik*† and contained two varieties, IR56 and IR70. Although the resistance of IR56 and IR70 corresponded nearly to the reaction patterns of *Pita* and *Pik*†, the reaction of the varieties to isolate V850256 ranged from M to R.

The VG 7 group harboring *Pi*20 and *Pita*, was divided into two subgroups, VG 7a and VG 7b. All the seven varieties also showed a R reaction to isolate BN209, suggesting that the VG 7 varieties harbored *Pib*. The two subgroups were differentiated by the reaction to isolate, PO6-6. The VG 7a varieties were susceptible and the VG 7b varieties were resistant to the isolate. The R reaction was estimated to be associated with one of the two genes, *Pii* or *Pi3*.

Discussion

The 42 IRRI-bred varieties were classified into seven groups based on the estimated presence of three resistance genes, *Pi20*, *Pita* and *Pik*†. Moreover, the presence of other

additional genes, *Pib*, *Pik-s*, *Piz-t*, and *Pii or Pi3*, was inferred based on the differential system by using the reaction patterns of the monogenic lines to blast isolates from the Philippines.

Among 42 varieties, fifteen that harbored *Pi20* were classified into three groups, VG 2, VG 5 and VG 7. IR8 and its derived progeny IR24 were included in the VG 2 group. All the other varieties in the VG 2 group were bred from crosses with IR8, IR24 or Peta. In the other groups, IR8, IR24 and Peta and their progenies were similarly found in the pedigrees of the varieties. In additing to Peta, the variety Sigadis was also included in the pedigree of IR24. Peta and Sigadis were estimated to carry *Pi20*, based on an analysis using the differential system with the Philippine blast isolates (unpublished data). Moreover, Imbe *et al.* (1997) had identified *Pi20* in IR24. These results suggested that *Pi20* was introduced from Peta or Sigadis at the early stage of the IRRI breeding activities.

Pita was harbored by the largest group of varieties, VG 3. IR36, once the most popular semi-dwarf variety (IRRI 1982), and its sister lines were identified in the pedigree of these varieties. IR36 was selected from the progeny of IRRI breeding line, IR2071, derived from crosses of the progenies of IR24 and IR8. The five varieties, IR32, IR38, IR40, IR42 and IR44, were also selected from IR2071 and IR2070 that had the same parental line, CR94-13. As for the other varieties, IR50, IR52, IR54, IR58, IR60, IR62, IR65, IR68 and IR72, have IR36, IR2071 or IR36 hybrid progenies were included in their pedigree. In the case of several more recent varieties from other groups harboring *Pita*, such as IR56 and IR70 in the VG 6 group, and IR64, PSBRc10, PSBRc18, PSBRc20 and PSBRc28 in the VG 7 group, also IR36 or its progenies were identified in their pedigree. IR36 was probably responsible for transmitting *Pita* to these varieties. CR94-13 was considered to carry *Pita*, which is effective against Philippine blast isolates (unpublished data). Kiyosawa (1966) reported that a rice variety from the Philippines, Tadukan, harbored *Pita*. Tadukan was also carried in the breeding of IR36. Base on these findings, it was assumed that CR94-13 or Tadukan could be the source of *Pita* in the IRRI varieties.

Kiyosawa (1972) and Kiyosawa and Ando (1997) reported that five alleles, *Pik*, *Pik-h*, *Pik-m*, *Pik-p* and *Pik-s*, were present at the *Pik* locus. Although *Pik*, *Pik-h*, *Pik-m* and *Pik-p* could not be distinguished from each other using blast isolates from the Philippines, one of them was found in PSBRc1 belonging to the VG 4 group, IR74 belonging to the VG 5 group, and IR56 and IR70 belonging to the VG 6 group. Dawn, Tadukan and Tetep, were included in the pedigrees of these IRRI varieties. Kiyosawa (1981) reported that *Pik-h* was identified in Dawn, Tadukan and Tetep. On this basis, the gene of the *Pik* allele in the VG 4, 5 and 6 gropus was estimated to be *Pik-h*.

During the development of IRRI rice varieties, Peta was used in a cross with the semi-dwarf variety Dee-geo-woo-gen (DGWG), from which IR8 was selected. Peta and its derivatives such as IR8 were used for the subsequent development of many IRRI varieties. With the use of blast isolates from the Philippines, Peta and DGWG were estimated to carry *Pib* and *Pik-s*, respectively (unpublished data). These findings suggested that Peta and DGWG were the sources of the *Pib* and *Pik-s* genes of the IRRI varieties.

Yokoo and Kiyosawa (1970) reported that *Piz-t* conferred a broad resistance spectrum against the races of blast fungus and was transmitted by an Indian variety, TKM1. With the use of blast isolates from the Philippines, *Piz-t* was estimated to be present in TKM6, which was one of the lines in the TKM breeding series (unpublished data). The presence of *Piz-t* was estimated in IR29 and IR34 belonging to the VG 1b subgroup and PSBRc2 belonging to the VG 2c subgroup. TKM6 and its progeny were included in the pedigrees of these three varieties. Although IR28 in the VG 1a subgroup is a sister variety of IR29 and IR34, it failed to inherit *Piz-t*. Thus, TKM6 was estimated to be a source of *Piz-t* gene of these varieties.

Among the seven resistance genes, two genes, namely *Pib* and one of the *Pik* alleles, *Pik-s* or *Pik-h*, were harbored by almost all the varieties. The genes of the *Pik* allele were estimated to be present in 17 varieties belonging to five groups and *Pib* in 38 varieties belonging to four groups. These results indicate that the two, *Pib* and *Pik* allele genes are commonly harbored by the IRRI-bred varieties.

The gene *Pia* may also be harbored by the varieties, as evidenced by the resistance of the varieties to the avirulent blast isolates B90002 and C923-49. However, *Pia* was masked by the presence of *Pib*, *Pik*† or *Pi20* in the varieties. Blue Bonnet, a variety from the United States, was introduced during the development of IR24 and was found to carry *Pia* (Kiyosawa 1972). IR24 could have inherited *Pia* from Blue Bonnet and likely transmitted the gene to its progenies. Although *Pib* was assumed to be present in belonging to the varieties in VG 1, 2, 3 and 7 groups, it may also be present in the VG 4, 5, and 6 groups. Since *Pik-h* and *Pi20* in the varieties might have masked the effect of *Pib*, this gene could not be identified. *Pik-s* was present in the VG 1 and 2 groups, whereas the other *Pik* alleles were assumed to be present in the VG 4, 5 and 6 groups. *Pik-s* was also possibly present in the VG 3 and 7 groups, because the reaction to an isolate, V850196, was masked by that of *Pita*. In our study based on the differential system using blast isolates from the Philippines, the presence of these genes in IRRI varieties could not be confirmed.

Around 40 blast resistance genes have already been reported (RGC 1998). Comparatively, we could postulate the presence of a limited number of blast resistance genes in the IRRI varieties, probably due to the genetic relatedness among these varieties. During the development of IRRI varieties, IR8, IR24 and IR36 or their progenies were often included. Because of this breeding methodology, a large number of IRRI varieties showed similar reaction patterns to the blast isolates. This study is the first to elucidate the genetic constitution of important Indica-type rice varieties in the

tropics for blast resistance. Previous studies on a few IRRI varieties and lines had focused on the inheritance of resistance to particular blast isolates from the Philippines. The number of genes conferring the resistance to the isolates was determined based on segregation ratios. However, the genotypes of the blast resistance genes were unknown. In the present study, the presence of at least seven kinds of blast resistance genes was estimated in the varieties by analyzing the reaction patterns to well characterized blast isolates. We could also demonstrate the ability of the differential system to estimate the genotypes of multiple genes for blast resistance carried in a variety. The use of reaction pattern data, on the other hand, is also faced with limitations because resistance may result from the masking of some genes. It is difficult to infer the presence of masked genes in some varieties based on reaction pattern and genetic analysis should be carried out to detect them. However, their genotypes in a variety may be assumed by examining the avirulence genotypes of the isolates used. In the present study, since we used isolates in which avirulence genotypes had been determined, the kinds of blast resistance genes to which they exhibited incompatible reactions could be researched. We also examined the pedigree of the varieties in relation to presence of estimated genes (masked genes included) using the parental varieties involved in the development of these varieties. Although pedigree data may not provide conclusive evidence, nevertheless, they are also important for inferring the presence and the kind of genes in the varieties. Genetic analysis based on segregation and allelism analyses will be necessary to confirm the presence of estimated and masked gene(s). Wang *et al.* (1999) and Jia *et al.* (2002) published information about the sequence of two genes, *Pib* and *Pita*, and molecular markers, which can detect directly these genes, have been developed. These molecular markers will also be useful to confirm and identify the resistance genes. The reaction patterns to well characterized blast isolates from the Philippines were particularly useful for estimating the presence of blast resistance genes harbored by the varieties. More importantly, the information derived from this study should also be useful for rice blast resistance breeding programs using IRRI-bred rice varieties.

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