Determination of Resistance Spectra of the *Pi-ta* and *Pi-k* Genes to U.S. Races of *Magnaporthe oryzae* Causing Rice Blast in a Recombinant Inbred Line Population

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ABSTRACT

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Molecular tagged resistance (*R*) genes are useful for developing improved cultivar resistance using marker-assisted breeding. In the present study, *R* genes to common races of *Magnaporthe oryzae*, the causal agent of blast disease of rice (*Oryza sativa*), were mapped using an F₁₀ recombinant inbred line (RIL) population derived from a cross of tropical japonica cv. Katy with breeding line RU9101001. Katy was resistant to 10 common U.S. races: IA-45, IB-1, IB-45, IB-49, IB-54, IC-17, ID-1, IE-1, IG-1, and IH-1 of *M. oryzae*. RU9101001 was resistant to races IA-45, IB-45, IB-54, IG-1, and IH-1. Katy and RU9101001 were susceptible to race IE-1k. Twenty-three polymorphic simple sequence repeat (SSR) markers were used to map *R* genes. Segregation ratios of 1:1 (resistant/susceptible) to races IB-1, IB-49, IC-17, ID-1, and IE-1 indicated the presence of a single dominant *R* gene in Katy. Ratios of 3:1 (resistant/susceptible) to races IA-45, IB-45, IG-1, and IH-1 indicated that a single *R* gene was present in Katy and a different *R* gene was present in RU9101001. Resistance to the abovementioned races was correlated with the presences of the *Pi-ta* gene and 11 Katy SSR alleles, suggesting that *Pi-ta* confers resistance to IA-45, IB-1, IB-45, IB-49, IC-17, IG-1, ID-1, IE-1, and IH-1. Katy, RU9101001, and all RILs were resistant to race IB-54, which was consistent with the presence of *Pi-k^s* in Katy and *Pi-k^h* in RU9101001. Resistance to IA-45, IB-45, IG-1, and IH-1 correlated with the presence of *Pi-k^h*, suggesting that *Pi-k^h* confers resistance to IA-45, IB-45, IG-1, and IH-1. These data suggest that *Pi-ta* and *Pi-k^h* are effective *R* genes with overlapped resistance to the 10 common races of *M. oryzae*.

Blast disease, caused by the ascomycete fungus *Magnaporthe oryzae* B.C. Couch (formerly *M. grisea* (T.T. Hebert) M.E. Barr.) (10,37,44,47), remains the most threatening disease of rice (*Oryza sativa* L.) worldwide (19). *M. oryzae* infects the aboveground tissue of rice plants during all developmental stages (24). The fungus is airborne and can also be transmitted through seed (14). Thus far, blast has been found in rice production areas worldwide (19) and is primarily controlled by the use of resistance (*R*) genes in comprehensive crop-protection systems worldwide (33). *R* genes effectively prevent infection by races of *M. oryzae* in a gene-for-gene specificity

(12,39). Over 40 such *R* genes have been identified from diverse germplasm worldwide (33,42,48). Most blast *R* genes were found in clusters on chromosomes 6, 9, 11, and 12 (13,33,42). Several blast *R* genes, including *Pi-b*, *Pi-ta*, *Pi-9*, *Pi-36*, *Pi-37*, and *Pi-k^m*, were isolated (1,2,5,36,43). All isolated blast *R* genes except *Pi-ta* were known to provide resistance to wide ranges of races of *M. oryzae* (1,2,5,28,35,36,43). However, resistance provided by an *R* gene is often short lived because resistance can be overcome by an increase in frequency of the virulent alleles in the *M. oryzae* population (9,18,21,25,49).

Studies on the spectrum of *R*-gene-mediated resistance in the greenhouse and outdoor blast nursery should help to identify effective blast *R* genes for breeders to use. In the United States, 10 common races (IA-45, IB-1, IB-45, IB-49, IB-54, IC-17, ID-1, IG-1, IE-1, and IH-1) of *M. oryzae* were identified in the 1960s (2) and are still present in contemporary rice fields (6–9,29,30,45). To date, all of these races have been recovered from southern rice fields and IB-49 and IC-17 are still the predominant races (9). A number of genetic approaches were used to determine the effectiveness of *R* genes, including the utilization of multiple sets of near-isogenic

lines to separate and identify *R* genes (2,27). F₂ mapping populations with the detached leaf inoculation method (16), doubled haploid (DH), and recombinant inbred line (RIL) populations (3,42,48). Without a single gene line, DH and RIL populations are ideal to study resistance spectra of *R* genes because the disease reaction of each race to each DH or RIL line can be repeated, and many races of the same pathogen or different pathogens can be accurately tested in segregated progeny. Using these methods, a number of *R* genes, including *Pi-ta*/*Pi-ta²*, *Pi-b*, and two alleles of *Pi-k*, *Pi-k^s*, and *Pi-k^h*, were identified and are now successfully utilized to prevent blast in U.S. rice cultivars (11,32,34,43).

The *Pi-ta* gene was first named by Kiyosawa (20). Rice cultivars containing *Pi-ta²* provide a wider resistance spectrum than rice cultivars containing *Pi-ta* (38). *Pi-ta* and *Pi-ta²* were introgressed into tropical cv. Katy from Vietnamese cv. Tetep after U.S. blast epidemics in the 1990s (24,34). *Pi-ta* was known to encode a predicted nucleotide binding site (NBS) leucine rich domain (LRD) protein (3). Simple sequence repeats (SSR) and allelic-specific markers for *Pi-ta* were developed for marker-assisted selection (MAS) (11,17). A number of blast *R* genes, including *Pi20 (t)*, *Pi39(t)*, *Pi-ta²*, and a *Pi-ta*-required gene, *Ptr (t)*, were mapped at the *Pi-ta* region of chromosome 12 (4,15,26,28). *Pi-ta* in Katy was shown to confer resistance to races IB-49 and IC-17 of *M. oryzae* using an F₂ population and the detached-leaf inoculation method (16). However, whether the gene is effective against other races has not been determined.

Pi-k^h was first documented by Kiyosawa and Murty (22) and was commonly found in some U.S. long grains, whereas the *Pi-k^s* allele was predominantly found in medium-grain cultivars (11). The *Pi-k^h* gene is allelic to *Pi-k^s* and was recently mapped with several SSR markers on chromosome 11 that have been widely utilized in U.S. breeding programs (11,29,31). Resistance to races IB-45, IB-54, IG-1, and IH-1 of *M. oryzae* was associated with the presence of *Pi-k^h*, whereas resistance to IB-54 was associated with *Pi-k^s* (29,31). However, it was unknown whether the alleles confer resistance to other races.

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All rice materials for the present study are deposited at GSOR for distribution as GSOR 100361 (RU9101001-RIL GSOR100600 (Katy) (<http://www.ars.usda.gov/main/docs.htm?docid=8318>))

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RU9101001 is an extremely early and cold-tolerant breeding line and Katy is a midseason, long-grain U.S. cultivar with a good package of disease resistance (31). Both Katy and RU9101001 have been important breeding parents for MAS for rice-breeding programs in the southern United States. Knowing the resistance spectra of both *Pi-ta* and *Pi-k* to the common races of *M. oryzae* should benefit the development of improved resistance using marker-assisted *Pi-ta* and *Pi-k* incorporation. Because single gene lines were not available for genes *Pi-ta* and *Pi-k*, an advanced RIL population should be suitable to determine their resistance spectrum. The objective of the present study was to determine the resistance spectra of both *Pi-ta* and *Pi-k* genes using an F₁₀ RIL population from the RU9101001 × Katy cross.

MATERIALS AND METHODS

Plant material, pathogen isolate, growth, and spore suspension. Tropical japonica cv. Katy containing the *Pi-ta* gene (16,34), early and cold-tolerant breeding line RU9101001 lacking the *Pi-ta* gene (Y. Jia and Moldenhauer, unpublished data), and present races (isolates) of *M. oryzae* IA-45 (75L14), IB-1 (isolate unnamed), IB-45 (isolate unnamed), IB-49 (ZN61), IB-54 (isolate unnamed), IC-17 (ZN60), IE-1k (TM2), ID-1 (ZN42), IE-1 (ZN13), IG-1 (ZN39), and IH-1 (isolate unnamed) from the southern United States were used for the present study (6,29). The fungal isolates were grown in oatmeal agar plates for producing spores using conditions previously described (41). Five spore-suspension concentrations were determined using a hemacytometer for each inoculation, with the final spore concentration for inoculation being from 4×10^5 to 1.6×10^6 spores/ml.

Greenhouse inoculation and evaluation. Eleven races of *M. oryzae* were used to inoculate separately on plants of Katy and RU9101001 to determine their resistance spectra to the races. Subsequently, a total of 235 F₁₀ RILs of the cross RU9101001 × Katy (Y. Jia and Moldenhauer, unpublished data) was inoculated separately with each of 11 races for determining resistance spectra of *R* genes. All RILs were evaluated using each race or mixed races of IB-1, IB-49, IC-17, and IG-1 in a greenhouse in Stuttgart from 2005 to 2006. In all, 10 to 15 seedlings of each line

at the three- to four-leaf stage were inoculated following a protocol described by Valent et al. (41), and disease reaction was evaluated 7 days after inoculation following a rating scale described in Valent (40). Disease reactions of each RIL to each race of *M. oryzae* were repeated three times.

Disease evaluation in outdoor blast nurseries. To verify greenhouse assays, seedlings of all RILs were evaluated using mixed races (IB-49, IC-17, and IE-1k [TM2]) in outdoor blast nursery plots following the procedure of Marchetti (31) in 2006 in Beaumont, TX. Briefly, all 235 RILs were seeded in single-row plots (60 cm in length). Every two entries were separated by susceptible spreader cv. M-201. The parents, RU9101001 and Katy, were seeded every 20th row as internal controls. Spreader rows were planted along the windward side to enhance the spread of spores to nearby lines. Plots were not inoculated with any specific races but were allowed to be wind inoculated with a natural mix of races (IB-49, IC-17, and IE-1k [TM2]) from the spreader during morning and evening hours to maximize leaf wetness, which maximizes natural infection.

Disease reactions were evaluated using a method described by Marchetti (31) on seedlings at three- to four-leaf stages starting 20 days after seeding until completion of the study.

To compare results from the outdoor blast nurseries from Beaumont, TX, a mixture of races IB-1, IB-49, IC-17, and IG-1 was used to inoculate plots following a procedure described below in the summer of 2006, in Stuttgart, AR. Briefly, corn was seeded around the plots and blast-susceptible cvs. Francis and M202 were planted throughout plots to create conditions conducive for blast development. Ten seeds of each RIL were planted in hills in a completed randomized block design with three replications. Seedlings at the three- to four-leaf stage in plots were inoculated with a mixture of races from spreaders by natural wind dispersal. Field disease reactions were recorded 15 days after inoculation according to a scale of 0 to 5, where 0 to 2 is resistant and 3 to 5 is susceptible, as described by Valent (40).

SSR marker amplification and analysis. In all, 190 SSR markers were tested on both parents, and 26 polymorphic SSRs

Table 2. Segregation analysis of resistance to races of *Magnaporthe oryzae* of 235 recombinant inbred lines (RILs) of the rice cultivar cross of RU9101001 with Katy

Race of <i>M. oryzae</i>	No. of RILs ^a		Expected ratio	χ^2	P
	R (<i>Pi-ta</i>) ^b	S			
IA-45	173 (84)	62	3:1	0.203	0.676
IB-1	133 (128)	102	1:1	3.322	0.073
IB-45	175 (84)	60	3:1	0.023	0.888
IB-49	132 (128)	103	1:1	3.336	0.072
IB-54 ^c	235 (130)	0			
IC-17	132 (128)	103	1:1	3.336	0.072
ID-1	133 (128)	102	1:1	3.322	0.073
IE-1	130 (128)	105	1:1	2.451	0.112
IE-1k	0	235			
IG-1	177 (84)	58	3:1	0.023	0.888
IH-1	177 (84)	58	3:1	0.023	0.888
Multiple ^d	130 (130)	105	1:1	2.876	0.093
Field blast ^e	129 (130)	106	1:1	2.059	0.170
Field blast ^f	129 (130)	106	1:1	2.059	0.170

^a R = resistant and S = susceptible.

^b Numbers in parentheses indicate RILs containing *Pi-ta*. The presence of the *Pi-ta* gene was determined using a marker for *Pi-ta* as described in (17).

^c *R* gene to IB-54 cannot be determined in this study because both parents are resistant and no segregation was observed in all RIL.

^d Mixed races (IB-1, IB-49, IC-17, and IG-1) were used for inoculation in the greenhouse.

^e Mixed races (IB-49, IC-17, and IE-1k/TM2) were used for inoculation in a field blast nursery in Beaumont, TX. The discrepancies of disease reaction with *Pi-ta* could due to the local environmental effects on the disease rating.

^f Mixed races (IB-1, IB-49, IC-17, and IG-1) were used for inoculation in Stuttgart, AR. Field leaf blast was evaluated 15 days after inoculation using a rating scale of 0 to 2 = resistant and 3 to 5 = susceptible (42).

Table 1. Disease reactions of Katy and RU9101001 to U.S. races of *Magnaporthe oryzae*^a

Name	Race (isolate)											
	IA-45 (75L14)	IB-1 (UI)	IB-49 (ZN61)	IC-17 (ZN60)	ID-1 (ZN42)	IE-1 (ZN13)	IG-1 (ZN39)	IB-45 (UI)	IB-54 (UI)	IH-1 (UI)	IE-1k (TM2)	Mixed races ^b
Katy	R	R	R	R	R	R	R	R	R	R	S	R
RU9101001	R	S	S	S	S	S	R	R	R	R	S	S

^a All blast races were described in Correll et al (8) and unnamed isolates (UI) were collected by Fleet Lee. Disease reaction was determined using 0 to 2 = resistant (R) and 3 to 5 = susceptible (S), as described in Valent (42).

^b Races of IB-1, IB-49, IC-17, and IG-1 of *M. oryzae* were mixed.

that were polymorphic between the two parents were used to genotype all 235 RILs for the establishment of the linkage between markers and traits. The primer sequences and map position of the SSR markers were obtained from the publicly available Gramene database (version 21; <http://www.gramene.org>). Polymerase chain reaction (PCR) was performed in 25 µl of 20 ng of genomic DNA; 10 mM Tris-HCl, pH 8.3; 50 mM KCl; 2.5 mM MgCl₂; 300 nM each primer; and 1 unit of Taq DNA polymerase (Promega Corp., Madison, WI). For each marker, the forward primer was labeled with either 6FAM, NED, or Hex (Applied Biosystems, Foster City, CA or Integrated DNA Technologies, Coralville, IA). The reverse primers were unlabeled. DNA was amplified under the following conditions: (i) initial denaturation at 94°C for 5 min; (ii) 35 cycles of 94°C for 1 min, 55 to 67°C (marker dependent) for 1 min, and 72°C for 2 min; and (ii) 5

min of final extension at 72°C. PCR products were pooled based on color and size range of PCR fragments (three markers per run along with ROX-labeled size standard), and heating samples at 94°C for 5 min denatured the DNA. The samples were separated on an ABI Prism 3730 DNA analyzer according to the manufacturer's instructions (Applied Biosystems). The sizes of SSR fragments were determined and alleles were binned using GeneMapper software (version 3.7; Applied Biosystems).

Data analysis. The goodness-of-fit of the observed number of RU9101001 and Katy alleles to the expected ratio was evaluated with a χ^2 test. Physical locations of SSR markers at the *Pi-ta* region were obtained from public databases (e.g., <http://www.gramene.org>). The segregation patterns of marker genotypes were compared with disease reactions for estimating their genetic distances from *R* genes using

JoinMap 4.0 (Kyazma BV, Wageningen, Netherlands). The map units (centimorgans) were derived using the Kosambi function (23).

RESULTS

Disease reactions of Katy and RU9101001. In the greenhouse tests, Katy was found to be resistant to races IA-45, IB-1, IB-45, IB-54, IB-49, IC-17, ID-1, IE-1, IG-1, and IH-1 and susceptible to IE-1k. RU9101001 was found to be resistant to IA-45, IG-1, IB-45, IB-54, and IH-1 but susceptible to IB-1, IB-49, IC-17, ID-1, IE-1, and IE-1k (Table 1). These results confirmed the resistance sources of respective parents.

***Pi-ta* confers resistance to races IB-1, IB-49, IC-17, ID-1, and IE-1.** A 1:1 segregation ratio of resistance to susceptibility was observed in 235 RIL for IB-1, IB-49, IC-17, ID-1, and IE-1 (Table 2). The ratio of 1:1 in F₁₀ RILs suggests that a single

Fig. 1. Map locations of the *Pi-ta* gene on chromosome 12 and *Pi-k* on chromosome 11 in rice. **A**, Map location of the *Pi-ta* gene in rice. Physical locations of *Pi20(t)*, *Pi39(t)*, and *Ptr(t)* were also shown (15,26,28). Blast races conditioned by *Pi-ta* were also indicated on the map. Map location of the *Pi-k* gene in rice, genetic distance was estimated using JoinMap 4.0 (Kyazma BV, Wageningen, Netherlands). **B**, Physical location of *Pi-k* was based on Ashikawa et al (1). Physical locations of simple sequence repeat markers and chromosome numbers were obtained from the Gramene database at www.gramene.org.

dominant *R* gene confers resistance to IB-1, IB-49, IC-17, ID-1, and IE-1 and the resistance factor was also mapped at the *Pi-ta* locus (Fig. 1). Because *Pi-ta* was previously known to confer resistance to IB-49 and IC-17 (14), a codominant marker for *Pi-ta* was used to genotype all 235 RILs. The presence of the Katy *Pi-ta* allele was found to be consistent with resistance with each of the abovementioned races. Moreover, 11 SSR markers were found to be the Katy allele in resistant RILs, indicating that the entire region of chromosome also cosegregated with resistance (Fig. 1). Similarly, the absence of the Katy allele and the presence of RU9101001 were found to cosegregate with the susceptibility to races IB-1, IB-49, IC-17, ID-1, and IE-1 of *M. oryzae* (Table 2). Together, our observations suggest that the *Pi-ta* gene in Katy confers resistance to IB-1, IB-49, IC-17, ID-1, and IE-1.

***Pi-ta* confers resistance to races IA-45, IB-45, IG-1, and IH-1.** A ratio of 3:1 segregation of resistance to susceptibility was observed in the F₁₀ RIL population (Table 2). These results suggest that two *R* genes independently control resistance to these four races. Because Katy and RU9101001 were resistant to races IA-45, IB-45, IG-1, and IH-1, one *R* gene was predicted in Katy and the resistance was found to coexist with the presence of the *Pi-ta* gene. Thus, resistance in Katy was due to the *Pi-ta* gene.

***Pi-k* confers resistance to races IA-45, IB-54, IG-1, and IH-1.** All RILs that contain *Pi-ta* were excluded to analyze another *R* gene in Katy. Resistance to IA-45, IG-1, and IH-1 was from RU9101001. The presence of the RU9101001 SSR allele of RM224 correlated with resistance to races IA-45, IB-45, IG-1, and IH-1, and the *Pi-k* allele from RU9101001 was mapped between RM224 and RM144 within one megabase region on chromosome 11 (Table 3 and 4; Fig. 1).

The RU9101001 SSR allele for RM224 being 139 bp in length was previously used to indicate the presence of the *Pi-k^h* blast *R* gene (11). Thus, *Pi-k^h* was found in RU9101001 and the existence of the *Pi-k^h* gene was found to correlate with the resistance in non-*Pi-ta*-containing RILs; thus, resistance to these four races was likely due to *Pi-k^h* (Table 2). Both Katy and RU9101001 were resistant to race IB-54

and all their RIL progeny were resistant to this race, inferring that they carry allelic *R* genes conferring resistance to IB-54.

Consistency of *R*-gene-mediated resistance. The same disease reactions of 235 F₁₀ RILs were observed in the greenhouse and outdoor blast nurseries in Arkansas and Texas. A ratio of 1:1 of resistance with *Pi-ta* to susceptibility with *pi-ta* (the recessive *Pi-ta* allele) was observed (Table 2). These results suggest that *Pi-ta* was responsible for resistance to races IB-1, IB-49, IC-17, and IG-1. If IE-1k (TM2) was used in Texas in the mixed races, then all seedlings should be susceptible; however, a ratio of 1:1 (resistance/susceptibility) was observed in the 235 F₁₀ RILs population. The presence of *Pi-ta* correlated with resistance to the mixed races. Taken together, these data suggest that resistance observed in the greenhouse is consistent with that in the outdoor blast nurseries.

DISCUSSION

In the present study, the *Pi-ta* gene was determined to confer resistance to races IA-45, IB-49, IB-1, IB-45, IC-17, ID-1, IE-1, IG-1, and IH-1 of *M. oryzae* using a RIL population from a cross with Katy that contains *Pi-ta* with classical pathogenicity assays. *Pi-ta* conferring resistance to the major races IB-49 and IC-17 of *M. oryzae* (16) was confirmed in the present study. We also extended the resistance spectrum of the *Pi-ta* gene to seven additional common races of *M. oryzae* in the United States. The gene conditioning resistance to IB-54 was undetermined due to the presence of *Pi-k^s* in Katy conferring resistance to IB-54 and *Pi-k^h* in RU9101001 confirming resistance to IB-54 (11; this study). Isolate TM2 belonging to race IE-1k lacking avirulence (*AVR*)-*Pita* was used to verify the specificity of resistance mediated by *Pi-ta* (35). If the *Pi-ta* gene confers resistance to races that contain *AVR-Pita*, then the susceptibility of Katy to TM2 was expected. Similarly, RU0101001 also was susceptible. Together, our observations suggest that the *Pi-ta* gene in Katy is responsible for resistance to nearly all contemporary *M. oryzae* pathotypes in the United States (16; this study).

Pi-ta and *Pi-ta²* were mapped at the centromeric region of chromosome 12 and have not been separated in several mapping populations due to linkage drag

(3,15,16; Y. Jia, unpublished data). Katy was known to contain both *Pi-ta* and *Pi-ta²*, and DNA sequence of the *Pi-ta* gene in Katy is identical to that of Yashiro-mochi where the *Pi-ta* gene was cloned (3,16). Thus, resistance spectrum of *Pi-ta²* may include resistance spectrum of the *Pi-ta* gene and other *R* genes; for example, *Pi20(t)* and *Pi39(t)* (26,28,38). However, only one NBS leucine-rich repeat (LRR) gene (12 kb) was identified between the *Pi-ta* gene and the centromere of chromosome 12 within two megabase regions (www.tigr.org). One possibility is that rice cv. Nipponbare used for sequencing does not contain homologous alleles of *Pi20(t)*, *Pi39(t)*, and *Pir(t)*, or they were alleles of the same *R* gene in different rice cultivars. Another possibility is that this region may contain atypical *R* genes.

The *Pi-k* gene is located in a complex locus that comprises several *R* alleles on chromosome 11. The map location of *Pi-k* was determined with RILs with the same disease reactions to all races IA-45, IB-45, IG-1, and IH-1 of *M. oryzae*. The *Pi-k* locus was mapped between RM224 and RM144 in this study. Searching the rice genome database, www.tigr.org, we found that several clustered NBS-LRR genes are located within the region delimited by RM224 and RM144. NBS-LRR is a classical *R* gene product and, hence, one of them could be the candidate gene for *Pi-k*. Ashikawa et al. (1) reported that two adjacent NBS-LRR class genes are required to confer *Pik^m*-specific rice blast resistance and both NBS-LRR genes are located within RM224 and RM144. Therefore, recombinant inbred lines that contain *Pi-k^h* can be used to determine the allelic relationship between *Pi-k^h* and *Pik^m*.

The presence of RM224 in the present study did not cosegregate perfectly with resistant reactions, which may be due to distorted segregation favoring the Katy allele. The segregation distortion on chromosome 11 in a RIL rice population was located at or near previously identified gametophytic gene loci (*ga*) or sterility loci (*S*) (46). It is possible that the segregation distortion of markers on chromosome 11 in this study is likely due to the peculiar effects of *ga* or *S* genes.

The fact that Katy carries a 120-bp allele at RM224 is consistent with findings of Fjellstrom et al. (11) that associated the

Table 3. Segregation analysis of simple sequence repeat markers for *Pi-k* alleles with the disease reactions^a

<i>Pi-k</i> markers	Total no.	No. of alleles ^b		Expected ratio	χ^2	<i>P</i>
		RU9101001	Katy			
RM224	83	30	53	1:1	6.58	0.01
RM144	80	34	46	1:1	1.80	0.18

^a Recombinant inbred lines that contain the *Pi-ta* gene were excluded for this analysis. Disease reactions were the same for races IA-45, IB-45, IG-1, and IH-1. *P* value does not support 1:1 segregation for RM224 showing the presence of the distorted segregation favoring the Katy allele.

^b RU9101001 = resistant and Katy = susceptible.

Table 4. Mapping the *Pi-k* gene in rice using simple sequence repeat (SSR) markers

SSR marker	No of recombinant plants ^a	Genetic distance
RM144	2/79	1.2
RM224	4/79	2.9

^a Recombinant plants were determined if an individual was susceptible with SSR marker (allele size = 257 bp), and resistance without SSR markers was linked to the *Pi-k* gene (120 bp) in a plant.

presence of the 120-bp allele with the *Pi-k^s* gene at the *Pi-k* locus. However, in the present study, the *Pi-k* gene was mapped 2.9 centimorgans (cM) between RM224 and *Pi-k* at one side and 1.3 cM between *Pi-k* and RM144 at another side. The *Pi-k^s* and *Pi-k^h* genes from Katy and RU9101001, respectively, are allelic, and both confer resistance to race IB-54 (29); therefore, their progeny should not segregate for IB-54 resistance, which we observed (Table 2). The results showed that *Pi-k^h* was effective against races IA-45, IB-45, IG-1, IH-1, and IB-54 while *Pi-k^s* was effective only against IB-54.

In the absence of a monogenic line carrying *Pi-ta* and *Pi-k* genes, RILs that are homozygous segregation lines that contain either *Pi-ta* or *Pi-k* should be useful for determining plant-pathogen interactions at the population level and gene-specific level. In preventing the complete loss of resistance conferred by either *Pi-ta* or *Pi-k*, RILs that contain both *Pi-ta* and *Pi-k* and thus provide overlapped resistance to 10 races of *M. oryzae* are therefore recommended as breeding parents for stacking additional agronomically important traits.

MAS increases the precision and speed of selection for the existence of an *R* gene compared with classical pathogenicity assays with each of the races of the pathogen. From this study, it was demonstrated that the presence of the *Pi-ta* gene in rice is adequate to prevent infections by races IA-45, IB-1, IB-45, IB-49, IC-17, ID-1, IG-1, IE-1 and IH-1 of *M. oryzae*, and the presence of the *Pi-k* gene is adequate to prevent infections by the races IA-45, IB-45, IG-1, and IH-1. However, available polymorphisms for any pair of breeding parents, and ease of detection of the existence of markers with conventional PCR technology, are two major constraints for MAS. Based on the present study, all 11 SSR markers and the dominant markers (Fig. 1A) can be used as candidates for MAS for the *Pi-ta* gene while RM144 and RM224 can be used for MAS for the *Pi-k* gene in U.S. rice breeding materials (11,17). In the future, better markers will be needed for increasing the precision of prediction of the alleles at the *Pi-k* locus.

In conclusion, data from this study show that *R* genes *Pi-ta* and *Pi-k* confer resistance to 10 races of *M. oryzae* and provide important knowledge for their use in crop protection through MAS techniques in U.S. rice breeding programs.

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