Characterization of Rice Blast Resistance Gene *Pi61(t)* in Rice Germplasm

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Abstract

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Identification of resistance (*R*) genes to races of *Magnaporthe oryzae* in rice (*Oryza sativa*) germplasm is essential for the development of rice cultivars with long-lasting blast resistance. In the present study, one major quantitative trait locus, *qPi93-3*, was fine mapped using a recombinant inbred line (RIL), F₈ RIL171, derived from the cross between ‘Nipponbare’ and ‘93-11’. RIL171 contained a heterozygous *qPi93-3* allele which was found to be resistant against nine U.S. common races—ID1, IA1, IB49, IE1, IA45, IB1, IC17, IB45, and IH1—of *M. oryzae*. An F₂ mapping population consisting of 2,381 individuals derived from RIL171 was evaluated with a field isolate (race) ARB82 (IA1) of *M. oryzae* under greenhouse conditions. Disease reaction of a

resistant/susceptible ratio of 3:1 was identified with F₂:F₃ families. In total, 12 simple sequence repeat markers spanning *qPi93-3* were used for fine mapping. Consequently, *qPi93-3* was delimited to 4.2 Mb between RM3246 and RM7102. Three insertion-deletion (InDel) markers located between RM3246 and RM7102, that had previously used to map *Pi61(t)*, showed that *qPi93-3* was *Pi61(t)*. The existence of *Pi61(t)* in 136 rice germplasm lines from the United States Department of Agriculture rice core collection was evaluated using *Pi61(t)*-specific InDel markers. *Pi61(t)* was identified as a source of resistance in 5 of the 136 lines. The characterized germplasm will be useful for rice breeders to use for improving blast resistance.

Rice blast, caused by the filamentous fungal pathogen *Magnaporthe oryzae*, is one of the most devastating diseases that reduces rice yield around the world. Reliance on host resistance (*R*) is the most economical and environmentally friendly strategy to control rice blast. After the first blast *R* gene, *Pia*, was identified in 1967 (14), about 100 blast *R* genes have been mapped on rice chromosomes (1,22). Blast *R* genes were found in all chromosomes except chromosome 3, and some of them are clustered (i.e., chromosomes 6, 11, and 12) (1,18,22). However, major *R* genes are often rapidly overcome by new virulent *M. oryzae* isolates. Efforts have been made worldwide to identify a broad spectrum of resistance to blast (3,18).

In many crops, the majority of traits are regulated by multiple genes rather than a single gene. These multiple genes, each with a relatively minor effect, are referred to as quantitative trait loci (QTLs; 4). Identification and mapping QTLs is an important strategy to discover blast *R* genes. Up to now, about 350 QTLs for blast resistance have been identified, and a few have been validated (1). In addition, 23 blast resistance genes have been mapped on rice chromosomes within identified QTL regions (22).

DNA markers tightly linked to known *R* genes are vital for breeding via a marker-assisted selection (MAS) approach. *Pik-m* and *Pik* were differentiated by allele-specific DNA markers (2), while *Pi-z* and *Pi-b* were identified in rice germplasm from United States Department of Agriculture (USDA) core collections using simple sequence repeat (SSR) markers or *Pib*-dominant markers (5,20,21,26). The presence of *Pi-b* and *Pi-ta* in 42 *indica*-type varieties from the International Rice Research Institute in the Phil-

ippines were investigated using polymerase chain reaction (PCR)-based dominant markers (6,12,26). Moreover, single-nucleotide polymorphism markers identified six *R* genes (*Piz*, *Piz-t*, *Pik*, *Pik-m*, *Pik-p*, and *Pit*) in aromatic rice accessions (13).

Pi-ta is an important blast *R* gene that initially was introduced to the United States from landrace *indica* varieties ‘Tetep’ and ‘Tadukan’. It confers resistance to the major blast races (isolates) in the United States (11). The *Pi-ta* gene was identified in the USDA core collection by a designed *Pi-ta* insertion-deletion (InDel) marker (12,25). The presence of *Pi-ta* was verified in 89 rice accessions using various DNA markers and also by disease reactions of *M. oryzae* to races IB49 and IE1K (25). These results illustrated that DNA markers linked to *R* genes were useful for detecting the function of *R* genes and, thus, could be widely used in rice improvement programs utilizing MAS.

Cultivated rice, *Oryza sativa* L., has two major subspecies, *japonica* and *indica*. Genomes of both ‘Nipponbare’ (*japonica*) and ‘93-11’ (*indica*) have been sequenced (9,31) and, thus, there is fundamental genomic information available to investigate blast *R* genes. Nipponbare contains two *R* genes, *Pish* and *Pia*, on chromosome 1 (8) and 11 (28), respectively. Moderate resistance of Nipponbare to *M. oryzae* race PO6-6 has been reported (23). Rice 93-11 is resistant to the major Chinese *M. oryzae* isolates and contains three blast *R* genes: *Pi41*, *Pi60(t)*, and *Pi61(t)* (17,30).

Four major QTLs for resistance to six isolates of *M. oryzae* were reported in a recombinant inbred line (RIL) population, containing 259 F₇ RILs, which was developed from a cross of Nipponbare and 93-11 (29). Three of these genes—*qPi93-1*, *qPi93-2*, and *qPi93-3*—originated from the resistant donor 93-11, and *qPi93-3* was linked to SSR marker RM7102 on chromosome 12. In all, 16, 53, and 28% of the phenotypic variation in resistance to isolates CHE 86, ARB52 (IA1), and ARB94, respectively, was explained (29). In addition, the *R* gene *Pi61(t)* was mapped to a 200-kb region on chromosome 12 using an F₂ population derived from a cross between susceptible *japonica* ‘Lijiangxintuanheigu’ (LTH) and resistant 93-11 (17). The *Pi61(t)* gene co-segregated with InDel marker M9 and was flanked by InDel markers M2 and S29 (17).

The objectives of this study were to (i) fine map the blast resistant QTL, *qPi93-3*, on chromosome 12, using F_{2:3} progeny families of RIL171; (ii) determine the resistance spectrum of RIL171 to 14 commonly occurring races of *M. oryzae* in the United States; and (iii) analyze resistance genes in rice germplasm collections.

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*The e-Xtra logo stands for “electronic extra” and indicates that two supplementary tables are available online.

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Materials and Methods

Selection of plant materials, growth. In all, 259 RILs derived from Nipponbare × 93-11 were developed at the USDA Agricultural Research Service Dale Bumpers National Research Center, Stuttgart, AR (G. Wang and Y. Jia, unpublished data). One RIL, F₈ RIL171, which contained a heterozygous *qPi93-3* allele, was selected for fine mapping. In total, 2,381 F₂ individuals, derived from RIL171, were sown in plastic trays with 96 wells per tray. Ten seeds each of Nipponbare and 93-11 were also planted as parental checks. The trays were completely filled with water and placed in the greenhouse. When the seedlings reached three- to four-leaf stage, they were used for pathogenicity assays and samples were collected for DNA extraction. In addition, the presence of *Pi-ta* in a total of 89 accessions from the 183 USDA rice core collection lines that contained the *Pi-ta* InDel marker associated with the presence of *Pi-ta* based on previous study (25) was verified. Furthermore, 136 accessions from the core collection were used for an R gene survey.

Pathogenicity assay. The field isolate (race) ARB82 (IA1) of *M. oryzae* (previously named ARB52 [IA1]), virulent to Nipponbare but avirulent to 93-11, was selected for pathogenicity assays. Plants were inoculated with the pathogen using a modified procedure according to Valent et al. (24). Briefly, each tray was initially placed in a clear polyethylene autoclave bag, then sprayed with 30 ml of spore suspension (4 to 5 × 10⁵ spores/ml, 0.25% gelatin) using an airbrush connected to an air compressor. Inoculated seedlings were maintained at approximately 98% relative humidity at room temperature (21°C). After 24 h, inoculated seedlings were moved to the greenhouse and blast disease was scored 7 days post inoculation based on a rating scale described by RoyChowdhury et al. (20). This scale is based on the lesion size and the percentage of the total leaf area that has lesions and is defined as 0 = no lesion formation, 1 = lesions restricted at the infection sites or covering 5% of total leaf area, 2 = restricted spindle lesions with diameter <2 mm or covering 5 to 10% of the total leaf area, 3 = lesions merge to form eye-shaped brown areas with diameter >2 mm, 4 = lesions cover more than 50% of the total leaf area, and 5 = lesions cover more than 70% of the total leaf area. Disease ratings of 0 to 2 are rated resistant and ratings of 3 to 5 are susceptible.

To determine the resistance spectrum of RIL171, 14 races (isolates) of *M. oryzae* that are common in the United States—IG1(ZN39), IB33(FL9), ID1(ZN42), IA1(ARB82), IB49(ZN61), IB54, IE1K(TM2), IC1(ARB86), IE1(ZN13), IA45(75L14), IB1, IC17(ZN60), IB45, and IH1—were used to test disease reactions of Nipponbare, 93-11, and RIL171. Ten seeds of each (parent Nipponbare, 93-11, and RIL171) were planted in individual pots with

holes at the bottom. All the pots were placed in trays filled with water. These experiments were repeated at three different times.

DNA extraction. Two methods were used to extract DNA. The rapid DNA extraction method described by Xin et al. (27) was used for DNA preparation for analysis of the F₂ population. For additional marker analysis, large quantities of DNA from selected rice recombinants were extracted following the cetyltrimethylammonium bromide method described by Hulbert and Bennetzen (19).

Marker selection and analysis. Two sets of SSR makers were used to construct a linkage map. One set, developed by McCouch et al. (19), was used to screen the entire population and included five SSR makers: RM6998, RM7003, RM3246, RM7102, and RM519 (Table 1). The marker interval for *qPi93-3* occurred between SSR markers RM3246 and RM7102 (29). After recombinants were identified, a second set of SSR markers—OSM89, RM27940, RM27941, XW137/138, XW249/250, XW121/122, and RM1261—were used to define the resistant region. Three SSR markers—XW137/138, XW249/250, and XW121/122—were then designed based on the sequence of Nipponbare. (http://www.gramene.org/Oryza_sativa/) (Table 1). The genetic map was constructed based on MapDisto software (MapDisto. v. 1.7.7).

The PCR mixture contained 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.) with 20 ng of template DNA in a total of 25 µl. For each SSR marker, the forward primer was labeled with fluorescent dyes 6FAM, NED, and Hex from Applied Biosystems or Integrated DNA Technologies. Reverse primers were unlabeled. PCR amplification were performed using MJ Research Tetrad thermocyclers under the following conditions: (i) initial denaturation at 94°C for 5 min; (ii) 35 cycles of 94°C for 1 min, 55 to 61°C for 1 min, and 72°C for 2 min; and (iii) 5 min of final extension at 72°C. Two or three PCR products were combined based on the color and size of amplified PCR products following the manufacturer's instructions. PCR products were diluted between 50 and 500 times, and 2 µl of the diluted PCR products were added to 9 µl of formamide containing the LIZ-labeled size standard (Applied Biosystems). The PCR products were run on an ABI 3730 DNA Analyzer according to the manufacturer's protocol (Applied Biosystems). Amplified fragment sizing and SSR marker genotype were analyzed using Gene Mapper software (version 3.7; Life Technologies Corp.).

Three InDel makers—M2, M9, and S29 (17; Table 1)—also were used for analyses of the selected recombinants and the 136 rice germplasm collections. The PCR conditions were the same as previously described, and gene-specific PCR products were separated on 3.0% agarose gels. Gels were stained with 10% SYBR

Table 1. Sequence information of simple sequence repeat (SSR) and insertion-deletion (InDel) markers used for fine mapping the *Pi61(t)* gene^a

Marker	Forward primer (5′–3′)	Reverse primer (5′–3′)	Physical position (bp)		Size (bp) ^b	T (°C) ^c
			Start	End		
RM6998	AGATGATAAGCTTGGCGACC	ATGCAGATGAGTCCCTCCAC	4,745,091	4,745,301	210	55
RM7003	GGCAGACATACAGCTTATAGGC	TGCAAATGAACCCCTCTAGC	6,775,083	6,775,183	100	55
OSM89	TTGGTCAAAGTTAGCCATGGGAGGG	TTTGAACCGGGTGGCCACATG	8,826,555	8,826,854	299	67
RM3246	GCCACTCATATAAGCAAATG	TGGTTAATGGTCAGAACCTG	9,092,467	9,092,762	295	55
M2	GGCAGAGCGAGTGCTTGTCGAG	TGCCCAGACAGTTGGATTGGACA	9,925,293	9,924,675	662	58
M9	CGATTTTCCTTCTCACCTGGGGG	TGGCCTATGGCTGCTGGAAGT	9,985,472	9,984,645	880	58
S29	CATCTCCCCCTAGTCGCTCT	AGCAACAGGAACCAACACC	10,124,186	10,124,419	234	60
XW137F/XW138R	ATCCTTTCATCTGTGTGGCCC	TACGCACCTTCCTATCGTCTT	10,124,634	10,124,946	312	55
XW249F/XW250R	AAGTTGTGTGTCGCTTGCTG	TCTGCTTGAAGTTGGCA	10,739,366	10,739,473	107	55
XW121F/XW122R	CGACAGGAAAACTTGTTAGGAA	AGTTGTGTGTCGCTTGCTGT	10,739,382	10,739,491	109	55
RM27940	CGAAGGCTATCTTATCTCCGAACC	AAGAAGATCACGCCGAGGTAGG	10,760,041	10,760,144	103	55
RM27941	GCTGTTTCGATGCGTATTTCTGC	GCGTGAATCTAACCGAGAAACG	10,768,383	10,768,568	185	55
RM7102	TTGAGAGCGTTTTTAGGATG	TCGGTTTACTTGGTTACTCG	13,211,325	13,211,536	211	55
RM1261	GTCCATGCCCAAGACACAAC	GTTACATCATGGGTGACCCC	17,531,111	17,531,328	217	55
RM519	AGAGATGCCCTAAATTCCG	AGGTACGCTACCTGTGGAC	19,903,791	19,903,912	121	55

^a SSR markers included RM6998, RM7003, OSM89, RM3246, XW137F/XW138R, XW249F/XW250R, XW121F/XW122R, RM27940, RM27941, RM7102, RM1261, and RM519. InDel markers included M2, M9, and S29 (17).

^b Expected size.

^c Annealing temperature.

Safe DNA gel Stain (Invitrogen) for visualizing the sizes of PCR products.

Results

Broad-spectrum resistance found in RIL171. Parents Nipponbare and 93-11, and RIL171, exhibited differential disease reactions to 14 common blast races of *M. oryzae* in the United States (Table 2). Nipponbare was susceptible to all 14 *M. oryzae* races. However, 93-11 was resistant to most *M. oryzae* races (78.6%), except for IB33 (FL9), IB54, and IE1K (TM2). RIL171 was resistant to nine races of *M. oryzae* (64.3%) and susceptible to IG1 (ZN39), IB33 (FL9), IB54, IE1K (TM2), and IC1 (ARB86).

Identification of a major blast *R* gene in RIL171. Among all F₂ RIL171 progeny, 1,723 individuals were resistant and 658 were susceptible to IA1 (ARB82). The segregation of resistant/susceptible fitted a 3:1 ratio ($\chi^2 = 8.820$, $P < 0.01$), indicating that RIL171 carries a single dominant *R* gene. Because the locus *qPi93-3* was previously mapped between RM3246 and RM7102 (29), five SSR markers at the *qPi93-3* locus were used for recombinant tests (Fig. 1A). In all, 63 recombinants were selected and an additional 7 polymorphic SSR markers were used for fine mapping (Fig. 1A). The *R* gene was located within 4.1 Mb between RM3246 and RM7102.

Three InDel markers—M2, M9, and S29 (17)—were used to determine whether the *R* gene to ARB82 (IA1) of *M. oryzae* in RIL171 was *Pi61(t)*. The data results indicated that M2 and S29 were co-dominant markers for Nipponbare and 93-11 and flanked *Pi61(t)*, while M9 was a dominant marker for Nipponbare and co-segregated with *Pi61(t)* (Fig. 2). The allele sizes of M2, M9, and S29 for Nipponbare were 620, 850, and 250 nucleotides (nt), respectively, and the allele sizes of M2 and S29 for 93-11 were 150 and 220 nt, respectively (Fig. 2). All 35 resistant recombinants were predicted to have 93-11 alleles for all three InDel markers (Supplementary Table S1), suggesting that the *R* gene in RIL171 is *Pi61(t)*, which was about 200 kb between 9.93 and 10.12 Mb on chromosome 12 (Fig. 1B).

Analysis of *Pi61(t)* in rice germplasm. The *Pi61(t)*-specific markers M2, M9, and S29 were used to survey 136 rice germplasm collections. The 93-11 allele was not found in 125 rice germplasm collections, suggesting that these collections lack *Pi61(t)* (Supplementary Table S2). However, the M2 93-11 allele was found in 11 rice germplasm collections (Table 3). Five of these germplasms (P881-12-4-B, Ai Chueh Li, 519, SL 22-613, and Gazi) were resistant to IA1, suggesting that they contain *Pi61(t)*. The remaining accessions (Esperanza, Laat, D 6-2-2, Gaza, Shima, and WB 103) were susceptible to IA1, suggesting that they do not carry *Pi61(t)*.

Discussion

In the present study, the previously identified major QTL, *qPi93-3* (30), in 93-11 was determined to be a major *R* gene in RIL171, a progeny derived from the cross of Nipponbare with 93-11. The *qPi93-3* allele was delimited to a 4.1-Mb region between RM3246 (9.1 Mb) and RM7102 (13.2 Mb), whereas the *Pi61(t)* was located at 9.92 to 10.12 Mb on chromosome 12 (17). We analyzed 2,381 F₂ individuals and were unable to identify recombinants between RM3246 and RM7102 due to reduced recombination (10). Using DNA markers and pathogenicity assays, we determined that *qPi93-3* is *Pi61(t)*, a major gene resistant to prevalent Chinese isolates of *M. oryzae* (17). After testing M2, M9, and S29 in the selected 63 key recombinants from 2,381 F₂ individuals, the results indicated resistance co-segregated with 93-11 alleles while the susceptibility co-segregated with Nipponbare alleles, suggesting that *qPi93-3* is *Pi61(t)*. Another *R* gene, *Pi58(t)*, was located within 3.8 Mb between 7.46 and 11.26 Mb, flanked by SSR markers RM6905 and RM27954 and co-segregated with SSR markers RM3103 and RM27933 (16). The genomic region of *Pi61(t)* was delimited within *Pi58(t)*. It remains to be determined whether *Pi61(t)* is an allele of *Pi58(t)*. Nevertheless, there were five nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins between 9.92 and 10.12 Mb; four of which were amplified in 93-11 (17). Further analysis of these NBS-LRR genes will help to identify superior DNA markers and, eventually, allow cloning of *Pi61(t)*.

Table 2. Disease reactions of RIL171, Nipponbare, and 93-11 to 14 common races (isolates) of *Magnaporthe oryzae* in the United States^a

Cultivar or line	Blast races													
	IG1	IB33	ID1	IA1	IB49	IB54	IE1K	IC1	IE1	IA45	IB1	IC17	IB45	IH1
RIL171	S 4	S 3	R 2	R 2	R 2	S 3	S 3	S 4	R 1	R 2	R 2	R 1	R 2	R 1
Nipponbare	S 4	S 3	S 3	S 5	S 4	S 3	S 3	S 3	S 3	S 3	S 3	S 3	S 3	S 3
93-11	R 0	S 4	R 0	R 0	R 0	S 3	S 3	R 0	R 2	R 0	R 0	R 0	R 0	R 0

^a Disease reactions: 0 to 2 = resistant (R) and 3 to 5 = susceptible (S). Common races (isolates) were IG1(ZN39), IB33(FL9), ID1(ZN42), IA1(ARB82), IB49(ZN61), IB54 (unnamed), IE1K(TM2), IC1(ARB86), IE1(ZN13), IA45(75L14), IB1(unnamed), IC17(ZN60), IB45(unnamed), and IH1(unnamed).

Fig. 1. Genetic and physical map of *Pi61(t)* with simple sequence repeat (SSR) and insertion-deletion (InDel) markers. **A**, Genetic map of *Pi61(t)*, unit for numbers below the line is centimorgan (cM); *Pi61(t)* was fine mapped within 200 kb flanked by InDel markers M2 and S29 and co-segregated with M9. SSR markers RM6998, RM7003, RM3246, RM7102, and RM519 were used to screen for recombinants; SSR markers OSM89, RM27940, RM27941, XW137/138, XW249/250, XW121/122, and RM1261, were used to define the resistant region. **B**, Numbers below markers were physical distances with the unit of megabases (Mb).

Historically, genetic resistance to different pathogens or different races of the same pathogen has been mapped within a small genetic interval. Rice varieties carrying *Pi58(t)* were resistant to 17 of 20 standard blast races or isolates (15,16). Similarly, 93-11 rice carrying *Pi61(t)* was resistant to 9 of 14 U.S. common races of *M. oryzae*: ID1(ZN42), IA1(ARB82), IB49(ZN61), IE1(ZN13), IA45(75L14), IB1, IC17(ZN60), IB45, and IH1. Another blast *R* gene, *Pi-ta*, was predicted to prevent infections by nine U.S. blast races (isolates): IA45(75L14), IB1, IB45, IB49(ZN61), IC17(ZN60), IG1(ZN39), ID1(ZN42), IE1(ZN13), and IH1 (11). Consequently this gene has been widely used for breeding tropical

japonica blast-resistant cultivars in the southern United States (10). The physical location of *Pi-ta* is between 10.60 and 10.61 Mb on chromosome 12. These findings suggest that there is a cluster of blast *R* genes—*Pi-ta*, *Pi36(t)*, *Pi58(t)*, and *Pi61(t)*—providing resistance to different races or isolates of *M. oryzae* in the central region of chromosome 12.

In summary, based on the pathogenicity test using the same germplasm collections examined for *Pi-ta*, 65 germplasm collections were resistant and 71 rice germplasm collections were susceptible to isolate IA1 of *M. oryzae* (Fig. 3). Only 5 rice germplasm collections (P881-12-4-B, Ai Chueh Li, 519, SL 22-

Fig. 2. Gel showing polymerase chain reaction (PCR) products with three insertion-deletion (InDel) markers in selected recombinants and parents. Sizes of PCR products amplified with each of InDel markers M2, M9, and S29. Lane L was obtained from the 1-kb-plus DNA ladder; lanes 8 to 94 are recombinants; and Nipponbare (NBP) and 93-11 are parents. PCR products were separated on a 3% agarose gel and stained with 10% SYBR Safe DNA gel stain.

Table 3. Analysis of blast resistance (*R*) genes in rice germplasm collections after inoculation with *Magnaporthe oryzae* isolate IA1

Accession	Cultivar	InDel markers ^a			DR ^c	Predicted <i>R</i> genes ^b		Gene ^d	Country	Region
		M2	M9	S29		<i>Pi-ta</i>	<i>Pi61(t)</i>			
PI372046	P881-12-4-B	150	850	250	R 0	+	+	2	Colombia	South America
PI244727	Esperanza	150	850	250	S 5	+	–	1	Cuba	Central America
PI392702	Gazi	150	850	250	R 1	+	+	2	Fiji	South Pacific
PI403369	D 6-2-2	150	850	250	S 5	+	–	1	India	Subcontinent
PI430982	Shima	150	850	250	S 5	+	–	1	Iraq	Mideast
PI403401	Gaza	150	850	250	S 5	+	–	1	Mozambique	Africa
PI431111	WB 103	150	850	250	S 5	+	–	1	Pakistan	Subcontinent
PI433797	SL 22-613	150	850	250	R 0	+	+	2	Sierra Leone	Africa
PI276860	Laat	150	850	250	S 5	+	–	1	Suriname	South America
PI389036	Ai Chueh Li	150	850	250	R 0	+	+	2	Taiwan	China
PI400276	519	150	850	250	R 0	+	+	2	Uruguay	South America

^a M2 and S29 were co-dominant insertion-deletion (InDel) markers and flanked the *Pi61(t)* gene; M9 was a dominant InDel marker and co-segregated with *Pi61(t)*. Allele sizes of M2, M9, and S29 were 620, 850, and 250 nucleotides (nt), respectively, for Nipponbare; and allele sizes of M2 and S29 were 150 and 220 nt, respectively, for 93-11.

^b Symbols + and – indicate the presence and absence, respectively of the *Pi-ta* gene, based on Wang et al. (25), or *Pi61(t)*.

^c Disease reaction (DR): 0 to 2 = resistant (R) and 3 to 5 = susceptible (S). The isolate for *M. oryzae* was 09S38-227R1-C (IA1).

^d Number of *R* gene.

Fig. 3. Analysis of the existence of *Pi61(t)* and *Pi-ta* in 136 rice germplasm collections. The diagram shows the germplasm collection number and the results of disease reactions after inoculation with the race (isolate) IA1(09S38-227R1-C) of *Magnaporthe oryzae*. R = resistant; S = susceptible; + and - indicate the presence and absence, respectively, of *Pi61(t)* or *Pi-ta*.

613, and Gazi) from 136 tested germplasm collections contained both *Pi61(t)* and *Pi-ta*. This suggests that *Pi61(t)* and *Pi-ta*, in these germplasms, may share the same ancestors and can be used as donors for both R genes. DNA markers M2, M9, and S29 co segregated with *Pi-61(t)* could be used for improving blast resistance via MAS.

Acknowledgments

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