Haplotype Diversity at the *Pi-ta* Locus in Cultivated Rice and Its Wild Relatives

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Accepted for publication 3 September 2008.

ABSTRACT

Wang, X., Jia, Y., Shu, Q. Y., and Wu, D. 2008. Haplotype diversity at the *Pi-ta* locus in cultivated rice and its wild relatives. *Phytopathology* 98:1305-1311.

The *Pi-ta* gene in rice confers resistance to races of *Magnaporthe oryzae* that contain *AVR-Pita*. *Pi-ta* encodes a predicted cytoplasmic receptor protein with a nucleotide-binding site and a leucine-rich domain. A panel of 51 *Oryza* accessions of AA genome species *Oryza sativa*, *O. glaberrima*, *O. rufipogon*, *O. nivara*, and *O. barthii*, and CC genome species *O. officinalis* were sequenced to investigate the diversity present in the exon and intron regions of the *Pi-ta* gene. Two major clades were identified, consisting of 16 different sequences with numerous insertion and deletions. Only one *Pi-ta* resistance allele was identified despite DNA

sequences revealing 16 *Pi-ta* variants. Most differences were identified in the intron region, and obvious selection of any motif was not observed in the coding region of *Pi-ta* variants. Reverse-transcription polymerase chain reaction analysis of seedlings revealed that all *Pi-ta* variants were expressed with or without pathogen inoculation. The 15 *Pi-ta* variants can be translated into nine proteins highly similar to the *Pi-ta* protein. Resistance to *M. oryzae* expressing *AVR-Pita* correlates with alanine and susceptibility correlates with serine at position 918 of *Pi-ta* in most accessions examined. These data confirm that a single amino acid controlling resistance specificity underlies the evolution of resistance of *Pi-ta* genes in rice.

Additional keywords: blast resistance gene, *Oryza* spp.

Blast disease caused by the filamentous ascomycete fungus *Magnaporthe oryzae* (formerly *Magnaporthe grisea* (T. T. Hebert) M. E. Barr, anamorph *Pyricularia grisea* (Cooke) Sacc.) is one of the most serious threats to rice (*Oryza sativa* L.) production worldwide (48,54). Intensive studies of interactions between *O. sativa* and *M. oryzae* worldwide have made the rice blast system one of the best experimental models for understanding the molecular mechanisms of host–parasite interactions (22,43,49). The genome sequences of *M. oryzae* and knowledge on the adaptability of *M. oryzae* have been accumulating rapidly (11,44). Available genome sequences of two subspecies of rice (*O. sativa* subsp. *indica* and subsp. *japonica*) have allowed genome-wide analysis of critical resistance (*R*) genes involved in interactions with pathogens (20). Historically, blast *R* genes (*Pi* genes) in rice accessions were found to prevent infections by races of *M. oryzae* that contain the corresponding avirulence genes (15,17,44). To date, 40 *Pi* genes have been identified (8,18,53), and 7 *Pi* genes—*Pi-b*, *Pi-ta*, *Pi-d2*, *Pi9*, *Pi2*, *Piz-t*, and *Pi-36*—have been molecularly characterized (6,9,32,40,52,55). *Pi-b*, *Pi9*, *Pi2*, and *Piz-t* are

known to be members of gene families encoding receptor proteins containing centrally localized nucleotide-binding sites (NBS) and leucine-rich repeats (LRRs) at the carboxyl termini (40,52,55). *Pi-36* and *Pi-d2* are single genes each encoding an NBS-LRR-type receptor (32) and a receptor-like kinase (9), respectively.

The *Pi-ta* gene prevents infections by races of *M. oryzae* that contain the *AVR-Pita* gene. *Pi-ta* encodes a predicted cytoplasmic protein with a typical NBS domain and a leucine-rich domain (LRD)—an atypical LRR domain (6,26). The *AVR-Pita* gene is a telomeric-associated putative metalloprotease (39), and is known to be unstable in some isolates of *M. oryzae* (48,56). The *Pi-ta* and *AVR-Pita* gene pair was the first matched *R* and *AVR* genes characterized from the *Oryza*–*Magnaporthe* pathosystem (6,39). The *Pi-ta* protein was shown to physically interact with a product of *AVR-Pita* (*AVR-Pita*₁₇₆) in triggering resistance (26). A single amino acid, alanine (A) at amino acid 918 (LRD) of the *Pi-ta* protein was shown to govern interaction specificity, and substitution of alanine with serine (S) impaired interaction and resulted in susceptibility (6,26). This system provides a unique opportunity to examine the degree of structural variation at the *Pi-ta* locus that contributes to recognition specificity (6,25,26). Although the origin of the *Pi-ta* gene is unclear, early reports indicated that the *Pi-ta* gene was introgressed into elite cultivars from landrace *indica* cultivars (Tetep and Taducan) for the control of infection by races of *M. oryzae* that contain *AVR-Pita* (25,29,38). Thus far, *Pi-ta* has been effectively deployed to prevent blast for the past 40 years and is still effective in the United States and worldwide (22,28,29).

Thus far, characterized *R* genes are mainly from *Arabidopsis*, tomato, rice, and flax. Allelic variation and ancient polymorphisms in *Arabidopsis* (*RPS2*, *RPM1*, *RPS5*, and *RPP13*), flax (*L*), and *Solanum* spp. (*Cf-9*) were correlated with the specificity

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Sequence data from this article have been deposited with EMBL/GenBank data libraries under accession nos. EU770205-EU770220.

*The e-Xtra logo stands for “electronic extra” and indicates that the online version contains three tables showing the primers used for the study, nucleotide polymorphism among the *Pi-ta* variants in all *Oryza* accessions, and a summary of the key features of DNA sequences among all *Pi-ta* variants in all accessions.

doi:10.1094/PHYTO-98-12-1305

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of pathogen recognition (4,7,13,19,37,47,50). Similar to *Pi* genes, most cloned *R* genes are members of small gene families found to cluster within short genomic intervals (2,5,20,24,40,45). A few of them are in simple loci composed of single genes (3,31,32,47). Most *R* genes are predicted to encode NBS-LRR class receptor proteins, suggesting a conservative role in signal recognition and transduction. Knowledge of the diversity of sequence variation at an *R* locus is useful in understanding specificity of the protein, and protein interactions triggering effective resistance responses.

In rice, the greatest genetic diversity has been commonly observed in accessions of *Oryza* spp. (33,51). A broader and detailed analysis of DNA sequence variation at the *Pi-ta* locus in *Oryza* spp. should help to determine the functional role of alanine in the *Pi-ta* protein and to understand the origin of the *Pi-ta* gene. The objectives of this study were to determine the DNA sequences of

the coding regions of *Pi-ta* variants in a panel of 51 accessions composed of cultivated rice (*O. sativa* and *O. glaberrima*), invasive weedy species of rice (*O. sativa*), and wild relatives, including *O. rufipogon* (AA), *O. nivara* (AA), *O. barthii* (AA), and *O. officinalis* (CC); and evaluate expression patterns of *Pi-ta* variants using reverse-transcription polymerase chain reaction (RT-PCR) and functional determination using disease assays.

MATERIALS AND METHODS

Plant materials. A panel of 51 accessions consisting of six *Oryza* spp. (*O. sativa*, *O. glaberrima*, *O. rufipogon*, *O. nivara*, *O. barthii*, and *O. officinalis*) was assembled to investigate sequence variation at the *Pi-ta* locus in the coding and intron regions (Table 1). The panel is composed of: (i) *O. sativa* Tetep, a

TABLE 1. Rice accessions in the panel and their reactions to *Magnaporthe oryzae*

Accessions ^a	Plant name	Origin	<i>Oryza</i> spp.	Disease evaluation ^b		Seed source
				+AVR- <i>Pita</i>	–AVR- <i>Pita</i>	
PI277414	Red Khosha Cerna	Afghanistan	<i>sativa</i>	S	S	W. Yan
PI460635	Pratao	Brazil	<i>sativa</i>	R	R	W. Yan
PI536047	Te Qing	China	<i>sativa</i>	R	R	W. Yan
PI615197	Nanjing 11	China	<i>sativa</i>	S	S	W. Yan
PI615199	Luhongzao	China	<i>sativa</i>	R	R	W. Yan
PI584668	Oryzica Llanos 5	Colombia	<i>sativa</i>	R	R	K. Moldenhauer
PI160476	Raminad str. 3	Colombia	<i>sativa</i>	R	R	K. Moldenhauer
PI430981 ^c	Nema	Iraq	<i>sativa</i>	S	S	W. Yan
PI415656	BALDO	Italy	<i>sativa</i>	S	S	W. Yan
...	Yashiro-mochi	Japan	<i>sativa</i>	R	S	B. Velant
...	Tsuyake	Japan	<i>sativa</i>	S	S	B. Velant
PI514663	Nipponbare	Japan	<i>sativa</i>	S	S	K. Moldenhauer
PI408473	IR 2151-745-3-1	Philippines	<i>sativa</i>	R	R	W. Yan
PI373122 ^c	Hatadawee	Sri Lanka	<i>sativa</i>	S	S	W. Yan
PI373331 ^c	Patchaipurumal	Sri Lanka	<i>sativa</i>	S	S	W. Yan
PI373335 ^c	AMANE	Sri Lanka	<i>sativa</i>	S	S	W. Yan
PI373346	Kaluwee	Sri Lanka	<i>sativa</i>	S	S	W. Yan
...	Banks	United States	<i>sativa</i>	R	S	K. Moldenhauer
...	TX4	United States	<i>sativa</i> Red rice	R	S	D. Gealy
...	StgS	United States	<i>sativa</i> Red rice	R	S	D. Gealy
...	StgB	United States	<i>sativa</i> Red rice	S	S	D. Gealy
...	MS4	United States	<i>sativa</i> Red rice	S	S	D. Gealy
...	LA3	United States	<i>sativa</i> Red rice	R	S	D. Gealy
...	#1997-6	United States	<i>sativa</i> Red rice	S	S	D. Gealy
...	#1997-33	United States	<i>sativa</i> Red rice	R	R	D. Gealy
...	#1997-2	United States	<i>sativa</i> Red rice	S	S	D. Gealy
...	#1996-05	United States	<i>sativa</i> Red rice	R	S	D. Gealy
...	#1996-11	United States	<i>sativa</i> Red rice	S	S	D. Gealy
...	#1996-1	United States	<i>sativa</i> Red rice	R	R	D. Gealy
...	#1995-14	United States	<i>sativa</i> Red rice	R	S	D. Gealy
...	#1995-12	United States	<i>sativa</i> Red rice	R	S	D. Gealy
...	18A	United States	<i>sativa</i> Red rice	S	S	D. Gealy
PI494105	M202	United States	<i>sativa</i>	S	S	K. Moldenhauer
PI584902	Katy	United States	<i>sativa</i>	R	S	K. Moldenhauer
PI431324	Tetep	Vietnam	<i>sativa</i>	R	S	K. Moldenhauer
PI430387	Botika S/R	Zaire	<i>sativa</i>	R	R	W. Yan
PI430388	Goli	Zaire	<i>sativa</i>	S	S	W. Yan
...	S1007	China	<i>rufipogon</i>	–	–	Y. Wang
...	Guangdong	China	<i>rufipogon</i>	–	–	D. Wu
...	Jiangxi	China	<i>rufipogon</i>	–	–	D. Wu
PI590404	IRRI-IRGC-101524	India	<i>nivara</i>	S	S	H. Bockelman
PI590409	IRRI-IRGC-101510	India	<i>nivara</i>	S	S	H. Bockelman
PI590400	PI223065R	United States	<i>barthii</i>	S	S	H. Bockelman
PI369447	Kwadwo Amoa	Ghana	<i>glaberrima</i>	S	S	H. Bockelman
PI369455	Bankoram	Ghana	<i>glaberrima</i>	R	R	H. Bockelman
PI432555	GPNO 22236	Guinea	<i>glaberrima</i>	R	R	H. Bockelman
PI450246	TOg 6244	Liberia	<i>glaberrima</i>	S	S	H. Bockelman
PI450252	TOg 6250	Liberia	<i>glaberrima</i>	S	S	H. Bockelman
PI450298	TOg 6327	Liberia	<i>glaberrima</i>	S	S	H. Bockelman
PI590412	IRRI-IRGC-101073	Philippines	<i>officinalis</i>	R	R	H. Bockelman

^a Numbers of accessions not assigned indicated by ‘...’.

^b Isolate ZN 60 (race IC-17) containing AVR-*Pita* was used as an avirulent isolate; isolate TM2 (race IE1k) lacking AVR-*Pita* was used as an virulent isolate; R indicates resistance, S indicates susceptibility, and – indicates disease reaction was not determined due to the absence of germplasm.

^c Accession containing *Pi-ta* was susceptible to *M. oryzae* that contains AVR-*Pita*.

landrace indica cultivar from Vietnam, the donor of *Pi-ta* for the U.S. cultivars (38); (ii) *O. sativa japonica* cv. Yashiro-Mochi, containing the original reference *Pi-ta* gene from landrace cv. Tadukan from the Philippines (6), and *O. sativa japonica* cv. Tsuyake from Japan, from which the susceptible *pi-ta* was cloned (23); (iii) *O. sativa indica* cvs. Raminad str. 3 and Oryzica Llanos no. 5, which confer resistance to all common races of *M. oryzae* in the United States; (iv) *O. sativa* cvs. Katy, Te Qing, Banks, and M202 and eight accessions of *O. sativa* selected from eight countries in a United States Department of Agriculture collection; (v) six accessions of African cultivated rice (*O. glaberrima*); (vi) 14 biotypes of *O. sativa* red rice; and (vii) wild rice relatives with the AA genome: one accession of *O. barthii* (PI590400), two accessions of *O. nivara* (PI590404 and PI590409), three accessions of *O. rufipogon* (Guangdong, Jiangxi, and S10007) and one accession of *O. officinalis* with the CC genome.

Plant growth, pathogen races, and pathogenicity assays. Rice seedlings grown in a greenhouse to the three- to four-leaf stage were used for DNA and RNA extraction and for pathogen inoculation. Standard pathogenicity assays were performed using a method described by Valent et al. (49). Race IC17 (isolate ZN60) of *M. oryzae* containing *AVR-Pita* and race IE1k (isolate TM2) lacking *AVR-Pita* were used for disease testing. Conidial concentration was measured using a hemacytometer and the final concentration for inoculation was adjusted to 1.3×10^6 conidia/ml. Rice seedlings were inoculated with 20 ml of the conidial suspension using an Airbrush Sprayer. After inoculation, the seedlings were placed in sealed plastic bags to maintain 70 to 90% humidity at room temperature at 24°C. After 24 h of incubation, seedlings were moved to a greenhouse. Disease reactions were determined 7 days postinoculation using an evaluation standard described by Valent (48).

DNA isolation, DNA primers, amplification, and sequencing. DNA was isolated using a Qiagen Plant DNA Mini Kit (Valencia, CA) from 0.1 g of fresh or frozen leaves following the manufacturer's instructions. DNA was suspended in 50 µl of GIBCO ultrapure distilled DNase, RNase-free water. Primers based on DNA sequences of the *Pi-ta* gene (GenBank accession no. AF207842) (Fig. 1) were designed to amplify *Pi-ta* variants using PCR. The standard protocol was 95°C for 5 min; 29 cycles of 95°C for 30 s, 50 to 60°C for 30 s, and 72°C for 90 s; followed by 72°C for 10 min. PCR products were separated on a 1% agarose gel and purified using a Qiagen PCR Purification Kit following the manufacturer's instructions. DNA was sequenced in the genomic center at the University of Arkansas Medical Center, Little Rock.

RNA preparation and expression analysis using RT-PCR. Total RNA was extracted from 0.1 g of leaves at 24 h post-pathogen inoculation using a Qiagen Plant RNA Mini Kit. Total RNA was treated with DNase using a DNA-free Ambion Kit (Austin, TX) for RT-PCR. One-step RT-PCR was performed with reagents provided in an Invitrogen SuperScript One-Step RT-PCR System with Platinum Taq DNA Polymerase kit (Carlsbad, CA). A pair of primers (XW13 and XW14) flanking the intron was designed for RT-PCR. Using XW13 and XW14, genomic contamination can be readily ruled out in RT-PCR. RT-PCR was performed as follows: 50°C for 30 min; 35 cycles of 94°C for 30 s, 53°C for 30 s, and 72°C for 30 s; followed by 72°C for 5 min. The amplified products were separated on a 1.5% agarose

Fig. 1. Graphic presentation of the structure of the *Pi-ta* gene with indicated locations for exons, intron, and the *Pi-ta* primers (GenBank accession no. AF 207842).

gel and visualized under ultraviolet lights using a BioRad ChemDoc (Hercules, CA).

Data analysis. DNA sequences of 4,275 bp consisting of an open reading frame (ORF) and an intron were aligned using Clustal W of MEGA 3.0 software (30). The sequences of DNA and amino acids were assembled by Vector NTI (Carlsbad, CA). The phylogenetic tree was constructed by neighbor-joining statistical analyses (30). A sliding window was built by DNAsp software to reveal the nucleotide variation at the *Pi-ta* locus (42). BioEdit software (available online) was used to calculate the identity of DNA sequences and the identity and similarity of amino acid sequences.

Fig. 2. Phylogenetic analysis of the *Pi-ta* variants in 51 accessions using neighbor-joining tree showing major differences between accessions in clade I and II. The differences within each clade were one to several nucleotides. The tree was drawn based on the nucleotide sequences of the *Pi-ta* variants from 51 accessions using MEGA 3.0 (28). In all, 1,000 bootstrap replicas were applied. The number 1-15 indicates 15 *Pi-ta* variants based on nucleotide sequence and I to IX indicate different predicted proteins. The DNA sequences for all *Pi-ta* variants can be accessed at GenBank accession nos. EU770205 to EU770220 and key features of each variant are summarized in Supplementary Table 3. Note: few errors in the intron region of the reference *Pi-ta* gene in AF207842 were noted. Position 3546 should be G not T, position 3549 should be G not A, position 3554 should be G not A, position 3574 should be T not C, and position 5810 should be C not T. The corrected *Pi-ta* gene sequences were used for analysis in the present study, and the new GenBank accession number is EU770205. R stands for resistance and S susceptible.

RESULTS

Natural variation at the *Pi-ta* locus. In total, 51 sequence assemblies, each containing 4,275 bp composed of the ORF and the intron of the *Pi-ta* locus, were analyzed. *Pi-ta* variants were found in all 51 accessions from six *Oryza* spp. (Table 1). These *Pi-ta* variants were further classified into two major clades that distinguish some Asian cultivated rice cultivars (*O. sativa*) from some African cultivated rice (*O. glaberrima* cultivars and its progenitor, *O. barthii*) (Fig. 2). The large clade possessed the longer intron, including the original *Pi-ta* allele and a small clade containing accessions such as *O. barthii* PI 590400, with a shorter intron derived from a total of 225 bp of deletions within the longer intron. Within the larger clade, greater differences were observed between some indica and japonica cultivars than within indica and japonica cultivars. For example, japonica cvs. M202 and Nipponbare were more similar to each other than to indica cvs. Teqing and Tetep (Fig. 2).

Overall, DNA sequences at the *Pi-ta* locus were highly similar and the sequence identity ranged from 92 to 99% among all pairs (data not shown). These variants translated into 10 different proteins, including the Pi-ta protein (Table 2). Similarly, the identity and similarity of protein sequences were the same for all pairs and ranged from 97.6 to 99.9% (data not shown). Among the *Pi-ta* variants, 105 polymorphic sites (excluding gaps) were identified, with 27 sites resulting in amino acid substitutions and 78 sites resulting in silent substitutions (19 in the exons and 59 in the intron). The sliding window shows clearly that most variation occurred in the intron region and less variation was observed randomly across the entire coding region (Fig. 3).

Expression of *Pi-ta* variants. To determine whether the *Pi-ta* variants in 51 accessions were expressed, one or two accessions of each *Pi-ta* variant 24 h after inoculation were examined by RT-PCR using a pair of *Pi-ta*-specific primers, XW13 and XW14. This pair of primers specifically amplifies portions of two exons adjacent to the intron. Products of 160 bp were amplified from all tested accessions that contained different *Pi-ta* variants (Fig. 4). These results suggest that the intron was spliced out in the transcript as predicted. Similarly, expression of the transcript was detected in inoculated plants in each accession analyzed (data not shown).

Each predicted protein (Pi-ta I to IX) contained a centrally located NBS and an LRD at the carboxyl terminus. The substitutions of the amino acids across different regions and domains of the entire protein are summarized in Table 2. The proteins predicted from clade II contain more polymorphic amino acids than the proteins predicted from clade I (Table 2). At position 918, serine was maintained among all susceptible *pi-ta* variants but was substituted by alanine in the Pi-ta protein to become

resistant plants; isoleucine was maintained at position 6 except Pi-ta and variant I. These results suggest that alanine at position 918 of LRD is critical for the integrity of the Pi-ta protein and isoleucine at position 6 could result in an insignificant non-synonymous change of the Pi-ta protein (Table 2).

Functional correlation with *Pi-ta* variants. The function of *Pi-ta* variants in each accession was examined by a standard pathogenicity assay using races of *M. oryzae* that contain and lack AVR-Pita (Table 1; Fig. 2). Eleven accessions containing the *Pi-ta* gene exhibited the expected incompatible resistant response and four accessions containing the *Pi-ta* gene exhibited a compatible susceptible interaction. This finding suggests that these accessions may contain defects in *Pi-ta*-mediated resistance. In a compatible interaction, where *avr-pita* will avoid the recognition by *Pi-ta*, susceptibility was expected and observed in most accessions. However, 12 accessions in groups 1, 4, 5, 6, and 9 that contain different *Pi-ta* variants were resistant to a race of *M. oryzae* that contains AVR-Pita. These findings suggest that these accessions may contain different *R* genes. To verify whether disease reactions were specific to AVR-Pita, 48 rice accessions (seed of three *O. rufipogon* accessions, Guangdong, Jiangxi, and S1007, were not available) were inoculated using a virulent race IE1k that

Fig. 3. Sliding window of the *Pi-ta* variants. Sliding window was built by DNAsp software to reveal the nucleotide variation among all *Pi-ta* variants. **A**, Window length: 100 steps; size: 251 to 944 (exon 1) and 2,433 to 4,275 (exon 2), gaps were not counted. **B**, Schematic presentation of the intron and exons of the *Pi-ta* gene.

TABLE 2. Protein variation among 15 *Pi-ta* variants^a

Protein ^b	Variant ^c	6	61	79	118	148	158	162	174	176	218	230	234	315	386	395	403	416	466	479	571	644	711	724	816	887	911	918	React ^d
Pi-ta	N/A	I	Y	A	G	R	H	H	E	D	V	K	I	R	V	H	M	I	H	K	A	L	T	R	L	H	P	A	R/S
I	1,2	I	Y	A	G	R	H	H	E	D	V	K	I	R	V	H	M	I	H	K	A	L	T	R	L	H	P	S	S
II	3,4,6,7,8	S	Y	A	G	R	H	H	E	D	V	K	I	R	V	H	M	I	H	K	A	L	T	R	L	H	P	S	R/S
III	5	S	Y	A	S	R	H	H	E	D	V	K	I	R	V	H	M	I	H	K	A	L	T	R	L	H	P	S	R/S
IV	10	S	Y	A	G	S	Q	H	E	V	A	K	I	R	V	H	M	I	H	K	A	L	T	R	L	H	P	S	S
V	9,11	S	Y	A	G	S	Q	H	E	V	V	K	I	R	V	H	M	I	H	K	A	L	T	R	L	H	P	S	R/S
VI	12	S	H	V	G	R	H	D	Q	D	V	K	V	K	V	Y	I	I	H	K	S	I	N	C	F	N	P	S	S
VII	13	S	H	V	G	R	H	D	E	D	V	K	V	K	V	Y	I	I	H	K	S	I	N	C	F	N	P	S	S
VIII	14	S	Y	V	G	R	H	D	E	D	V	R	V	K	F	Y	I	T	R	R	S	I	N	C	F	N	L	S	S
IX	15	S	Y	V	G	R	H	D	E	D	V	R	V	K	F	Y	I	I	R	R	S	I	N	C	F	N	L	S	S

^a Different amino acids and their positions, the letter code used for each amino acid. Number indicates a position of amino acid in the Pi-ta protein (AF207842) and bold letters indicate differences in amino acids in comparison with the Pi-ta protein.

^b Groups of *Pi-ta* variants based on amino acid sequences.

^c Groups of *Pi-ta* variants based on nucleotide (EU770205-EU770220); N/A = not applied.

^d Disease reactions. R indicates resistance, S indicates susceptible, and R/S indicates that accessions in this group that were resistant and susceptible. *M. oryzae* race IC-17 (ZN60) that contains AVR-Pita was used for inoculation (56).

lacks *AVR-Pita* (Table 1). If the disease reaction was specific to *AVR-Pita*, these accessions containing the resistance *Pi-ta* allele should be susceptible to IE1k. Most accessions were susceptible (Table 1; Fig. 2); however, the same accessions in groups 1, 4, 5, 6, and 9 were still resistant to IE1k (TM2) (Fig. 2). Together, these data support the hypothesis that additional *R* genes in these accessions may recognize the corresponding unknown avirulence gene or genes in race IE1k.

DISCUSSION

In the present study, 16 different DNA sequence assemblies of the *Pi-ta* variants in 51 selected accessions of six *Oryza* spp. were identified and their predicted roles in resistance were examined using RT-PCR and pathogenicity assays. All *Pi-ta* variants were found to fall into two major clades, with significant differences between the intron regions. These *Pi-ta* variants were translated into nine putative proteins, including three previously reported proteins (23). Pathogenicity assays revealed that an alanine amino acid at position 918 of the LRD of the *Pi-ta* protein correlated with resistance, and substitution of alanine with serine correlated with susceptibility in most accessions. Four accessions (PI 430981, PI 373122, PI 373331, and PI 373335) expressing *Pi-ta* were found to be susceptible to a race of *M. oryzae* that contains *AVR-Pita*. Although other regulation mechanisms may exist in these accessions, one possibility is that these accessions may contain defects in *Pi-ta*-mediated resistance. Recently, an additional component, *Ptr(t)*, was identified in *Pi-ta*-mediated signal recognition and transduction in an induced mutant of rice cv. Katy (25). It would be useful to determine whether defects in these accessions are allelic to *Ptr(t)*. On the other hand, accessions that were resistant to both avirulent and virulent races of *M. oryzae* may contain novel *R* genes.

Molecular characterization of plant *R* genes has shed light on the degree of structural variation that affects their abilities to detect their corresponding pathogen (3,5,12,15,16,19,20,36,41,46,53). Most *R* genes are members of small gene families and different family members may reside within small genomic intervals and detect different races of the same pathogen or different pathogens (5,10,16,24,34,35,45,50). *Pi-ta* resides at a locus that comprises a single gene, raising the possibility that different *Pi-ta* variants in nature may have evolved to detect different races of *M. oryzae* or different pathogens. Specificity of interaction of *Pi-ta* with *AVR-Pita* proteins in triggering resistance requires matched structures of *Pi-ta* and *AVR-Pita* (6,26). Alanine at position 918 was conserved among all *Pi-ta* variants. Substitution of isoleucine to serine at position 6 should move the *Pi-ta* variant from group II to group I, and further replacement of serine with alanine at position 918 should create the *Pi-ta* protein that recognizes *AVR-Pita* (Table 2). In the *Pi-ta* protein, position 918 falls in the LRD, a region similar to LRRs. The LRR of an *R* protein is thought to be involved in ligand binding (5,37). Diversified LRR domains are predicted to ensure the integrity of the structure of *R* proteins

and to recognize a diversity of molecules from the pathogens that trigger resistance (13,15,16,20,56). In the present study, no obvious selections on any particular exon regions were noticed; instead, we observed highly conserved *Pi-ta* variants with rare amino acid variation across different domains and regions of the *Pi-ta* protein (Table 2). A predicted β sheet can be altered significantly with the substitution of alanine at position 918 with serine of the *Pi-ta* protein (X. Wang and Y. Jia, unpublished data). Although an LRR repeat is not maintained in the *Pi-ta* protein, alanine at position 918 in the LRD is predicted to be involved in maintaining the integrity of the structure of the *Pi-ta* protein, and other regions of *Pi-ta* may determine recognition specificity to *AVR-Pita*. Single amino acids of two other blast resistance genes, *Pi-36* and *Pi-d2*, were also shown to determine recognition specificity to the pathogen-signaling molecule (9,32). We suggest that *R* genes in rice may have evolved this simple and efficient mechanism to cope with rapidly adapting pathogens.

Historically, it is known that *Pi-ta* was introgressed into cultivated rice from indica landrace cvs. Tetep from Vietnam and Taducan from the Philippines. Although some questions still remain on the origin of *Pi-ta* in Yashiro-Mochi, it was clear that Tetep was the donor for *Pi-ta* in all U.S. cultivars analyzed (38). The *Pi-ta* gene was identified in *O. sativa* cultivars and several red rice biotypes in the United States, and more diverse *Pi-ta* variants were found in *O. glaberrima* cultivars, suggesting that the origin of the *Pi-ta* gene is specific to *O. sativa*, not *O. glaberrima*. The presence of *Pi-ta* variants highly similar to the *Pi-ta* protein in all accessions of six *Oryza* spp. studied suggests that the homologous sequences of *Pi-ta* were conserved before the divergence of these *Oryza* spp. In particular, *Pi-ta* was identified from some accessions of the AA genome of *O. sativa*. The presence of a *Pi-ta* variant in accession PI 590412 of *O. officinalis* is interesting because *O. officinalis* has the CC genome. In contrast, the presence of highly homologous sequences of *Pi-ta* variants is less likely in accessions PI 590397 and PI 590398 of *O. alta*, PI 590413 of *O. glumaepatula*, and PI 269727 of *O. latifolia*, and the situation in PI 237987 of *O. barthii* and S 1020 of *O. rufipogon* is not known because they did not yield PCR products using most combinations of the *Pi-ta* primers (data not shown). Together, these data support a hypothesis that *Pi-ta* has evolved from *O. sativa* subsp. *indica* landrace cultivars. Interestingly, screening *Oryza* spp. accessions using a marker for *Pi-ta* identified the *Pi-ta* gene in two accessions, PI 590420 and PI 590424 of *O. rufipogon* (14,27), and sequencing confirmed the presence of the *Pi-ta* gene. In addition, the *Pi-ta* gene was also identified in one accession of *O. barthii* (PI 590399) (X. Wang and Y. Jia, unpublished data). However, *Pi-ta* variants with different sequences were found in other accessions of *O. rufipogon* and *O. barthii*. Analysis of *Pi-ta* in additional accessions of *O. rufipogon* and *O. barthii* might help to understand the origin of *Pi-ta*. It can be seen in Table 2 that *Pi-ta* seems to be closely related with *Pi-ta* variants in groups I and II. Whether or not one of them is the immediate ancestor of *Pi-ta* needs to be further

Fig. 4. Expression analysis of 15 *Pi-ta* variants using reverse-transcription polymerase chain reaction (RT-PCR). Total RNA used for RT-PCR were prepared from the following: lane 1, *Oryza sativa* YT 16; lane 2, *O. sativa* AMANE; lane 3, *O. sativa* Luhongzao; lane 4, *O. sativa* Nanjing 11; lane 5, *O. rufipogon* 105941; lane 6, *O. sativa* YT 14; lane 7, *O. sativa* Nipponbare; lane 8, *O. sativa* prato; lane 9, *O. glaberrima* PI 369447; lane 10, *O. sativa* IR 215-745-3-1; lane 11, *O. sativa* Botika SR; lane 12, *O. sativa* M202; lane 13, *O. sativa* RNS3; lane 14, *O. barthii* PI 590400; lane 15, *O. glaberrima* PI 450346; lane 16, *O. nivara* PI 590404; and lane 17, *O. sativa* Kaluwee. The size was estimated using a DNA marker (DNA ladder, Glen Burnie, MD). The products of RT-PCR were separated using a 1.5% agarose gel and the picture was captured using a BioRad ChemDoc Analyzer.

investigated. Currently, *Pi-9* is the only known blast *R* gene introduced by breeding into elite cultivars from *O. minuta*, a tetraploid wild species of the *Oryza* genus (1,21). Further analysis of the genetic background surrounding *Pi-ta* in Tetep and Tadukan should help to determine the origin of the *Pi-ta* gene.

The findings that different *Pi-ta* variants were in precursors of modern cultivated rice can also help to understand the biogeography and evolution of cultivated rice. Sequence identities among the *Pi-ta* variants are consistent with the hypothesis that *O. rufipogon* and *O. nivara* were the progenitors of *O. sativa* and also are consistent with a new theory on the independent domestications of cultivated rice, *O. sativa*, from Asian wild rice, *O. rufipogon* (33). More importantly, sequence diversity of the *Pi-ta* variants also reflects the prediction that wide divergence does exist between Asian AA genome *Oryza* and progenitors of African rice, *O. barthii* (51).

In conclusion, nucleotide polymorphism identified in this study should allow us to design variant specific primers for genetic studies. Four accessions that may contain defects in other components in *Pi-ta* pathway identified in this study may be useful to dissect *Pi-ta*-mediated resistance. Resistant cultivars that do not contain *Pi-ta* can be used to tag novel *R* genes. In the future, a better understanding of molecular interaction of *AVR-Pita* with *Pi-ta* variants should help to understand the co-evolutionary relationship of *Pi-ta* and *AVR-Pita*. The resulting knowledge will be useful for the development of effective strategies to control the rice blast disease.

ACKNOWLEDGMENTS

This material is based on work partially supported by NP301 "Genomic Characterization of Rice Germplasm" of the USDA-Agricultural Research Service, Dale Bumpers National Rice Research Center and National Science Foundation grant no.0638820, and Arkansas Rice Research and Promotion Board supported project "Development of Molecular Strategies to Control Rice Blast Disease." We thank M. Lin for help with disease evaluation; K. Gubrij for designing primers and PCR amplification; K. Pratt and R. Clemons for excellent technical support; W. Yan, H. Rockleman, D. Gealy, and H. Black for providing red rice biotypes; F. N. Lee for helpful discussions on disease evaluations; S. McCouch, K. Olsen, A. Caicedo, S. Brooks, and S. Lee for their critical reviews; M. H. Jia for the verification of rice accessions using SSR marker analysis and proofreading of the manuscript; and Asia Rice Travel Grant Foundation, U.S.A. for supporting X. Wang's partial traveling expenses.

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