Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Genomic organization and sequence dynamics of the *AvrPiz-t* locus in *Magnaporthe oryzae**

Ping LI[1,2], Bin BAI[1], Hong-yan ZHANG[1], Heng ZHOU[1,2], Bo ZHOU[†‡1]

([1]*State Key Laboratory Breeding Base for Zhejiang Sustainable Pest and Disease Control, Institute of Virology and Biotechnology, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China*)

([2]*Institute of Biotechnology, Zhejiang University, Hangzhou 310058, China*)

[†]E-mail: bzhzd@zju.edu.cn

Received Nov. 4, 2011; Revision accepted Jan. 18, 2012; Crosschecked May 7, 2012

Abstract: Plants utilize multiple layers of defense mechanisms to fight against the invasion of diverse pathogens. The *R* gene mediates resistance, in most cases, dependent on the co-existence of its cognate pathogen-derived avirulence (*Avr*) gene. The rice blast *R* gene *Piz-t* corresponds in gene-for-gene fashion to the *Magnaporthe oryzae Avr* gene *AvrPiz-t*. In this study, we determined and compared the genomic sequences surrounding the *AvrPiz-t* gene in both avirulent and virulent isolates, designating as AvrPiz-t-ZB15 and avrPiz-t-70-15 regions, respectively. The sequence of the AvrPiz-t-ZB15 region is 120966 bp whereas avrPiz-t-70-15 is 146292 bp in length. The extreme sequence similarity and good synteny in gene order and content along with the absence of two predicted genes in the avrPiz-t-70-15 region were observed in the predicted protein-coding regions in the *AvrPiz-t* locus. Nevertheless, frequent presence/absence and highly dynamic organization of transposable elements (TEs) were identified, representing the major variation of the *AvrPiz-t* locus between different isolates. Moreover, TEs constitute 27.3% and 43.2% of the genomic contents of the AvrPiz-t-ZB15 and avrPiz-t-70-15 regions, respectively, indicating that TEs contribute largely to the organization and evolution of *AvrPiz-t* locus. The findings of this study suggest that *M. oryzae* could benefit in an evolutionary sense from the presence of active TEs in genes conferring avirulence and provide an ability to rapidly change and thus to overcome host *R* genes.

Key words: *Magnaporthe oryzae*, Synteny, Transposon complex, Dynamics, Recombination
doi:10.1631/jzus.B1100338 **Document code:** A **CLC number:** Q343.1

1 Introduction

Rice blast disease caused by the fungus pathogen *Magnaporthe oryzae* is one of the most serious threats to rice production worldwide. Utilization of rice cultivars in which resistance is mounted by co-expression of a resistance (*R*) gene in rice and its cognate avirulence (*Avr*) gene in *M. oryzae* has been practiced as the most economical and efficient way to control this disease. The knowledge gained from recently cloned *R* and *Avr* genes is of critical importance for understanding how rice recognizes and blocks the invasion of *M. oryzae*. To date, a total of 13 dominant *R* genes and 2 major quantitative trait loci (QTLs) have been cloned in rice, and most of them encode proteins containing nucleotide-binding site (NBS) and leucine-rich repeat (LRR) domains (Liu *et al*., 2010). Meanwhile, a total of 7 *Avr* genes have been cloned in the rice pathogenic race of *M. oryzae* in the last decade. *AvrPi-ta* (Orbach *et al*., 2000), *Avr1-CO39* (Leong, 2008), *ACE1* (Böhnert *et al*., 2004), and *AvrPiz-t* (Li *et al*., 2009) were isolated by map-based cloning whereas *AvrPii*, *AvrPia*, and *AvrPik/km/kp* (Yoshida *et al*., 2009) were isolated by

‡ Corresponding author

* Project supported by the National Natural Science Foundation of China (Nos. 30700450 and 30971878) and the Fundamental Research Funds for the Central Universities (No. 2009QNA6024), China

genome resequencing and association genetics. Miki *et al*. (2009) described an independent isolation of *AvrPia* by identification of a deletion in a DNA fragment due to a spontaneous gain-of-virulence mutant. The isolation of these 7 *Avr* genes reiterated the common theme that most *Avr* genes encode small novel secreted proteins, generally having no significant homology to known proteins.

Inherent variability of *M. oryzae* poses a challenge to the utilization of *R* genes in the rice breeding program, which is attributed largely to the genetic instability of *Avr* genes. It is intriguing that *M. oryzae Avr* genes are often tightly associated with diverse transposable elements (TEs) (Farman *et al*., 2002; Fudal *et al*., 2005; Khang *et al*., 2008). Moreover, the insertion of TEs in either coding or promoter regions of avirulence genes was reported in some gain-of-virulence *M. oryzae* isolates, indicating that TEs were involved in the instability of *Avr* genes (Kang *et al*., 2001; Zhou *et al*., 2007; Li *et al*., 2009). Most recently, Yoshida *et al*. (2009) examined DNA polymorphisms of 1032 predicted secreted protein genes in the reference isolate 70-15 among 46 worldwide isolates and found extremely low sequence diversity in secreted protein genes. The presence/absence polymorphisms of secreted protein genes also appear to be more significant in *M. oryzae.* Interestingly, this type of polymorphism tends to be more frequent in candidate effector genes linked to TEs than in those that are not linked to TEs. Therefore, diverse TEs contribute to the instability of *M. oryzae Avr* genes and help to explain how they can be gained or lost during evolution of this pathogen.

We utilized a map-based cloning strategy to clone *AvrPiz-t* gene from an avirulent field isolate 81278ZB15 (Li *et al*., 2009). The sequence comparison of *AvrPiz-t* locus in various isolates revealed that virulent isolates have either an insertion of a Pot3 element in the promoter region or a single nucleotide substitution in the coding sequence (Li *et al*., 2009). However, no sequence variations of *AvrPiz-t* in the promoter or coding region were identified in any avirulent isolate (Li *et al*., 2009). In this study, we compared the sequences of *AvrPiz-t* locus in both avirulent and virulent isolates and identified a large transposon complex at each locus. Moreover, this transposon complex is quite divergent with respect to its composition and organization between the avirulent and virulent isolates. The sequence recombination events at the *AvrPiz-t* locus referred by single nucleotide polymorphism (SNP) patterns in diverse isolates suggest that parasexual recombination probably plays a role in the evolution of the *AvrPiz-t* locus.

2 Materials and methods

2.1 Rice cultivars, *M. oryzae* isolates, culture, pathogenicity tests, and disease assessments

Two *Piz-t*-containing rice cultivars (*Piz-t*-transformed Nipponbare and *Piz-t* donor line Toride 1) and two *Piz-t*-lacking cultivars (CO39 and Nipponbare) were used for pathogenicity assays. The *M. oryzae* isolates stored at −20 °C on filter paper were grown in a growth chamber at 28 °C under 12 h of constant light with high intensity on oatmeal agar plates for inducing conidial production. Standard pathogenicity tests and disease assessments were performed as described by Zhou *et al*. (2006).

2.2 DNA preparation and SNP pattern analysis

Genomic DNA of *M. oryzae* was isolated using a modified cetyl trimethyl ammonium bromide (CTAB) method as described by Saghai-Maroof *et al*. (1984). Polymerase chain reaction (PCR) analyses of the SNP patterns of different *M. oryzae* isolates were carried out using 10 ng of genomic DNA as template in 50 μl amplification reactions containing respective pair of primers targeting five genomic locations in the *AvrPiz-t* locus. Thirty-five cycles of PCR were performed and amplified products were directly used for sequence determination. The sequences were compared against the references of both avirulent and virulent isolates and SNP patterns were then determined.

2.3 Bacterial artificial chromosome (BAC) sequencing, sequence annotation and classification of TEs

The sequence of the BAC clone was obtained using a shotgun strategy as described by Zhou *et al*. (2006). The sequence reads were assembled with the Phred and Phrap software packages (Ewing and Green, 1998; Ewing *et al*., 1998) and the derived assembly was edited with the Consed program (Gordon *et al*., 1998). The final consensus sequence of the BAC clone BAC07bg07 was obtained after all the gaps and low-quality regions were filled. The

BAC sequence was annotated by using gene prediction program and homology search. The Fgenesh program (http://www.softberry.com) was used for gene prediction. The predicted genes that either encode proteins smaller than 50 amino acids (aa) or correspond to repetitive sequences were not annotated as gene models. The repetitive sequences were identified by searching the BAC sequence against the NCBI database and classified into respective groups. The target sequence duplicate (TSD) of each TE was determined manually.

3 Results

3.1 Establishment of the *AvrPiz-t* locus in both avirulent and virulent isolates

A BAC clone BAC07bg07 from an avirulent isolate 81278ZB15 harboring the *AvrPiz-t* gene along with neighboring sequences was completely sequenced, which resulted in a 120966-bp consensus sequence, for convenience, designated as AvrPiz-t-ZB15 in this study (GenBank accession No. JN639897). The equivalent allelic sequence in the virulent isolate 70-15 designated as avrPiz-t-70-15 was retrieved from the genomic sequence of chromosome 7 [corresponding to the genomic interval: 858371–1004652 bp of the sequence (GenBank: CM000230)]. However, we found that the published sequence of the avrPiz-t-70-15 region contains an inversion of two sequence fragments compared to the one of AvrPiz-t-ZB15 (Fig. 1). Interestingly, these two sequence fragments include two TEs, an LINE retrotransposon MGL and a composite retrotransposon MINE (Fig. 1). Moreover, we found that the homologous regions of these two TEs are almost identical to each other (only 14 nucleotide differences out of the 3412-bp compared region). Nevertheless, two pairs of TSDs, i.e., tggcggcgatggg and gacacttttgagcta, the footprints of transposition of these two TEs, were distributed at unexpected positions in the retrieved sequence (Fig. 1). Therefore, we speculated that the genomic inversion in the avrPiz-t-70-15 region in the retrieved sequence could represent a sequence mis-assembly due to the highly related sequences of these two TEs. By comparing genomic sequence of AvrPiz-t-ZB15 region and the positions of the TSDs, we re-established the avrPiz-t-70-15 region by relocating the genomic interval flanked by these two TEs at the allelic position in the corresponding AvrPiz-t-ZB15 region (Fig. 1). The upstream MGL element was also relocated accordingly (Fig. 1). We used a PCR approach to validate this sequence re-assembly and synthesized six pairs of primers spanning these two TEs (Fig. 1 and Table 1). An expected PCR amplification pattern deduced from the re-established sequence of the avrPiz-t-70-15 region (Fig. 2 and Table 1) was obtained, which indicated that the re-established sequence could most likely represent the correct sequence in the virulent isolate 70-15. The sequences of PCR products from 70-15 were further determined and mapped on the corresponding regions, further validating the correctness of the sequence re-assembly (data not shown). The re-established sequence of the avrPiz-t-70-15 region is 146292 bp in length and contains a single gap represented by 200 'N's as indicated in the released sequence (GenBank: CM000230).

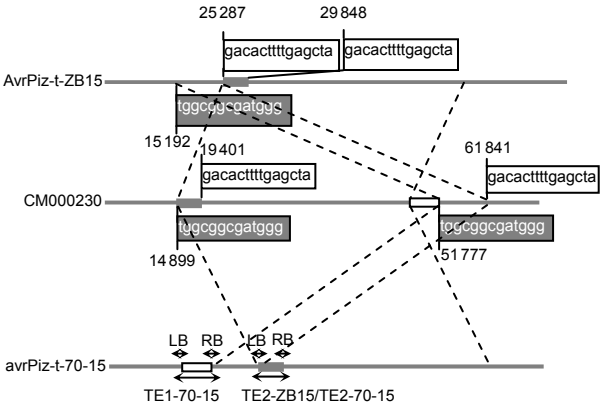

Fig. 1 Re-establishment of the avrPiz-t-70-15 region by referring both the genomic sequence of the AvrPiz-t-ZB15 region and the target sequence duplicate (TSD) surrounding the respective TE

AvrPiz-t-ZB15, CM000230, and avrPiz-t-70-15 represent the genomic sequences in an avirulent isolate 81278ZB15, a virulent isolate 70-15 for which the sequence was released in GenBank (accession No. CM000230), and the re-established 70-15, respectively. The genomic sequence of each locus is indicated by solid lines. The allelic fragments in both CM000230 and AvrPiz-t-ZB15 are indicated by dashed lines. An MGL and an MINE element are indicated by unfilled and filled boxes, respectively. The same TSD is indicated by identical boxes with start positions along the genomic regions. The target regions used for PCR confirmation of the presence of two TEs are indicated by bi-directional arrows. LB: left border; RB: right border

Table 1 Target regions surrounding an LINE retrotransposon MGL (TE1-70-15) and a composite retrotransposon MINE (TE2-ZB15/TE2-70-15) selected for the validation of sequence re-assembly using a PCR method

Target region	Primer (5′–3′)	Expected size (bp)	
		70-15	81278ZB15
TE1-70-15-LB	F: aaaggcgttgaaggcggtcg	500	NA
	R: cttaggcacggggtcgcgtc		
TE1-70-15-RB	F: gctgcaaagacctcttcgag	550	NA
	R: aacggggcgttttccgtcgg		
TE1-70-15	F: aaaggcgttgaaggcggtcg	NA	444
	R: aacggggcgttttccgtcgg		
TE2-ZB15/TE2-70-15-LB	F: ggtggtcagtgtcaatctcg	810	822
	R: cagacaagcttcgggcaacc		
TE2-ZB15/TE2-70-15-RB	F: tgtgcgcctctatgcgggtg	451	451
	R: caggcccattttcaagggcc		
TE2-ZB15/TE2-70-15	F: ggtggtcagtgtcaatctcg	NA	NA
	R: caggcccattttcaagggcc		

LB: left border; RB: right border; F: forward; R: reverse; NA: not applicable

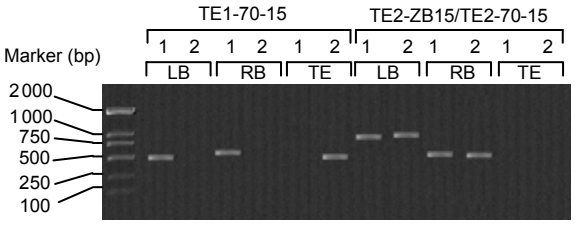

Fig. 2 PCR amplification pattern at two genomic sites containing possible inversions due to mis-assembly in the avrPiz-t-70-15 region

The PCR products corresponding to the left border (LB), right border (RB), and the TE itself of both TE1-70-15 and TE2-ZB15/TE2-70-15 were resolved on 0.8% agarose gel. Lanes 1 and 2 show the PCR templates from the genomic DNA of 70-15 and 81278ZB15, respectively. The primers and expected sizes of the PCR products are listed in Table 1

3.2 Genomic organization of the *AvrPiz-t* locus

The sequences of the AvrPiz-t-ZB15 and avrPiz-t-70-15 regions were annotated to identify protein encoding genes and repetitive sequences. A total of 26 and 24 gene models encoding proteins larger than 50 aa in length were predicted in the avirulent and virulent isolates, respectively (Fig. 3 and Table 2). Twenty-four gene models in each genomic region (GR) are alleles and almost identical to each other. On the contrary, two gene models (7bg7.17 and 7bg7.18) are only present in the AvrPiz-t-ZB15 region. However, a 3′ portion of 7bg7.17 allele (approximately 1/3 of the entire gene) was identified at the allelic region, suggesting that it was probably partially deleted in 70-15 due to unknown reason. The gene model 7bg7.18 was identical to another gene model MC_09064 that was putatively mapped on chromosome 3 (http://www.mgosdb.org) and located in the WGS cont5.268 (GenBank: AACU 02000513), indicating that the same allele is present in the genome of 70-15 albeit at another location. As listed in Table 2, only four gene models (7bg7.4, 7bg7.7, 7bg7.16, 7bg7.25) have confirmed expressed sequence tags (ESTs) released in the public database (http://www.mgosdb.org), indicating a relatively low number of gene models in this locus that were experimentally confirmed to be expressed. A total of 15 gene models share significant sequence homology in different organisms and 11 of them have putative biochemical functions (Table 2).

Virtually all the repetitive sequences identified in both loci belong to diverse families of TEs. As listed in Table 3, a total of 7 and 8 TEs were identified in the AvrPiz-t-ZB15 and avrPiz-t-70-15 regions, respectively. Based on the sequence homology and conserved TSD, four pairs, i.e., TE2-ZB15/TE2-70-15, TE3-ZB15/TE6-70-15, TE5-ZB15/TE7-70-15, and TE6-ZB15/TE8-70-15, are allelic to each other (Fig. 3 and Table 3), indicating their localizations at the *AvrPiz-t* locus prior to the geographical isolation of these two isolates. The other TEs, on the contrary, are present in one isolate or another, suggesting that they were most likely transposed to the observed locations after the geographical isolation of these two isolates. The most TEs are distributed as singletons except for TE5-ZB15 and TE7-70-15 that are nested transposon complexes. Seven families of TEs including

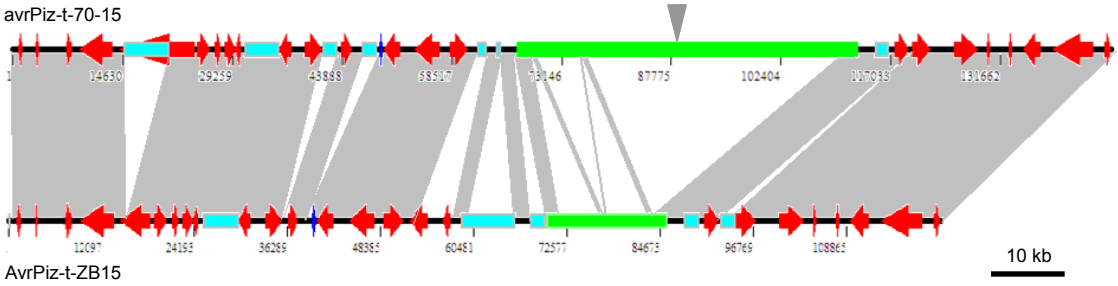

Fig. 3 Genomic structure of the *AvrPiz-t* locus in the virulent isolate 70-15 (upper panel) and the avirulent isolate 81278ZB15 (lower panel)

The predicted gene models are indicated in red arrows except the *AvrPiz-t* gene that is highlighted in blue. The singleton TEs are indicated in blue boxes whereas the nested complexes are in green boxes. The extremely identical regions are indicated in shadow. The single gap in the avrPiz-t-70-15 region is indicated by an arrow

Table 2 Predicted gene models at the *AvrPiz-t* locus in 81278ZB15 and 70-15

Gene model in the AvrPiz-t-ZB15 region	Allele in the avrPiz-t-70-15 region	Sequence variation	Annotation
7bg7.1	v7bg7.1	None	Hypothetical protein
7bg7.2	v7bg7.2	None	Hypothetical protein
7bg7.3	v7bg7.3	None	Hypothetical protein
7bg7.4[1,2]	v7bg7.4	None	Ankyrin repeat-containing protein; ESTs confirmed
7bg7.5[1,2]	v7bg7.5	A 30-bp indel; v7bg7.5 contains an MGL element in its coding sequence	Choline transporter protein
7bg7.6	v7bg7.6	A 18-bp indel; 2 SNPs	Hypothetical protein
7bg7.7	v7bg7.7	None	Hypothetical protein; ESTs confirmed
7bg7.8[1,2]	v7bg7.8	1 SNP	Acyl-CoA dehydrogenase
7bg7.9[1]	v7bg7.9	None	Conserved protein
7bg7.10[1,2]	v7bg7.10	None	Phenylacetate-coenzyme A ligase
7bg7.11[1,2]	v7bg7.11	1 SNP	P450 monooxygenase
7bg7.12	v7bg7.12	None	Hypothetical protein
7bg7.13	v7bg7.13	None; v7bg7.13 contains a Pot3 element in its promoter region (462 bp)	AvrPiz-t
7bg7.14[1]	v7bg7.14	1 SNP	Conserved protein
7bg7.15	v7bg7.15	1 SNP	Hypothetical protein
7bg7.16[1]	v7bg7.16	None	Urea active transporter; ESTs confirmed
7bg7.17	NP	NA	Hypothetical protein
7bg7.18[1,2]	NP	NA	Protein kinase
7bg7.19[1]	v7bg7.19	1 SNP	Conserved protein
7bg7.20[1,2]	v7bg7.20	None; 7bg7.20 contains a Pot2-B element in its promoter region (200 bp)	Quinate permease
7bg7.21[1,2]	v7bg7.21	None	Extracellular protease
7bg7.22	v7bg7.22	None	Hypothetical protein
7bg7.23	v7bg7.23	None	Hypothetical protein
7bg7.24[1,2]	v7bg7.24	None	Endoglucanase
7bg7.25[1,2]	v7bg7.25	None	Conserved protein; ESTs confirmed
7bg7.26[1,2]	v7bg7.26	None	Mitochondrial GTPase; 3′ partial

[1] Significant homologues found in other organisms; [2] Predicted gene products having putative biological functions. NP: not present; NA: not applicable; SNP: single nucleotide polymorphism; ESTs: expressed sequence tags

Pot2-A/B, Pot3, Pyret, MINE, MGL, RETRO5, and MAGGY (the last two elements are embedded within the nested transposon complex described in the following section) were classified based on their sequences similar to known TEs (Tables 3 and 4). As described by Li *et al*. (2009), the insertion of a Pot3 element at the promoter region of *AvrPiz-t* in the virulent isolates including 70-15 resulted in the gain of virulence. Another two gene models (v7bg7.5 and 7bg7.20) were found to have MGL and Pot2-B within the coding sequence and the promoter region, respectively. It is evident that v7bg7.5 is truncated compared with its homolog 7bg7.5 due to a premature stop codon introduced by the MGL element. The total sizes of TEs in the AvrPiz-t-ZB15 and avrPiz-t-70-15 regions are 33016 and 63148 bp in length, constituting approximately 27.3% and 43.2% of the sequenced regions, respectively.

3.3 Divergence of the *AvrPiz-t* locus

The availability of the sequences of the AvrPiz-t-ZB15 and avrPiz-t-70-15 regions allows us to investigate the divergence of this locus between avirulent and virulent isolates. As described above, TEs account for large genomic content surrounding the *AvrPiz-t* locus and virtually all of them are intact in structure. We, therefore, removed all of them from each locus for analyzing the divergence of the non-repetitive sequences in this study. The derived sequences are 87919 and 83099 bp in length for the AvrPiz-t-ZB15 and avrPiz-t-70-15 regions, respectively. Comparison of these two sequences revealed a total of 18 SNPs and 25 InDel (insertion and deletion) sites. An average SNP rate is only 1 SNP per 4.7 kb approximately, indicating that the SNP rate in the non-repetitive sequences surrounding the *AvrPiz-t*

Table 3 Transposable elements at the *AvrPiz-t* locus in 81278ZB15 and 70-15

TEs		Classification	TSD
AvrPiz-t-ZB15	avrPiz-t-70-15		
TE1-ZB15		Pot2-B, partial at 3′	TA
	TE1-70-15	MGL	TGGCGGCGATGGG
TE2-ZB15	TE2-70-15	MINE	GACACTTTTGAGCTA
	TE3-70-15	Pot3	TA
	TE4-70-15	Pot3	TA
	TE5-70-15	MGL, partial at 5′	
TE3-ZB15	TE6-70-15	TE3-ZB15 is an intact Pyret element whereas TE6-70-15 is a solo-LTR	TATAA
TE4-ZB15		Pot2-B	TA
TE5-ZB15	TE7-70-15	Nested transposon complex	TCCAC
TE6-ZB15	TE8-70-15	Pot2-A	TA
TE7-ZB15		Pot2-B	TA

Allelic elements are listed in the same rows

Table 4 Interior transposable elements in both TE5-ZB15 and TE7-70-15

TEs		Classification	TSD
TE5-ZB15	TE7-70-15		
TE5-ZB15-1	TE7-70-15-1	Solo-LTR of Pyret	GATAG
TE5-ZB15-2		MAGGY	ATTTC
	TE7-70-15-2	MAGGY	GTTGG
TE5-ZB15-3		MAGGY	ATAAT
	TE7-70-15-3	Solo-LTR of RETRO5	AATAT
	TE7-70-15-4	MAGGY	TTTAA
	TE7-70-15-5	RETRO5, inserted in TE7-70-15-4	TAACC
	TE7-70-15-6	A complicated MAGGY resulting from unequal recombination of two MAGGYs, inserted in TE7-70-15-5	CTTTT
	TE7-70-15-7	Solo-LTR of RETRO5, inserted in TE7-70-15-6	
	TE7-70-15-8	Inago	GTTAT
	TE7-70-15-9	MAGGY	AGTGT
TE5-ZB15-4	TE7-70-15-10	Solo-LTR of RETRO5	

Allelic TEs are listed in the same rows

locus is low. On the other hand, 25 InDels account for 4913 bp in length and the largest is 4828 bp. Most of the InDels, however, are represented as different repeats of single nucleotides (20 out of 25 sites). Therefore, the region of DNA surrounding the *AvrPiz-t* gene is highly conserved in the non-repetitive sequences between these two isolates except the 4828-bp InDel as described above. Interestingly, the corresponding region of this large InDel in the avrPiz-t-70-15 region is TE5-70-15, which is a 5′ partial MGL element. Thus, the allele of 7bg7.18 located in 4828-bp InDel in 81278ZB15 is relocated in different locus in 70-15. It is therefore reasonable to speculate that jumping of an MGL element in the avrPiz-t-70-15 region might be responsible for this genomic variation.

Contrasting to the high sequence conservation in the non-repetitive region, TEs represent major genomic variations with respect to their distribution and composition between the AvrPiz-t-ZB15 and avrPiz-t-70-15 regions. As described above, insertions of TEs in the avrPiz-t-70-15 region caused either truncation of genes, e.g., TE1-70-15 in v7bg7.5, or genomic rearrangement, e.g., TE5-70-15 in the 4828-bp InDel (Fig. 3). TE3-ZB15 is an intact Pyret element whereas its allele TE6-70-15 remains as a solo-LTR (Table 3), indicating that an unequal recombination between two LTRs of this Pyret element could have occurred in 70-15. In addition, three Pot2-B elements (TE1-, TE4-, and TE7-ZB15) were present only at the AvrPiz-t-ZB15 locus whereas two Pot3 elements (TE3- and TE4-70-15) were only in the avrPiz-t-70-15 region. Moreover, we calculated the SNP rates of two pairs of TE alleles, TE2-ZB15/TE2-70-15 and TE6-ZB15/TE8-70-15, and found approximately 1 SNP per 120 and 931 bp on average, respectively. The TEs appear to be more sequence divergent as compared to non-repetitive sequences.

It is worthwhile to highlight the impact of the centrally localized nested transposon complexes (TE5-ZB15/TE7-70-15) on the divergence of the *AvrPiz-t* locus. TE5-ZB15 is 15616 bp whereas TE7-70-15 is 45406 bp in length, representing almost 30000 bp size difference. TE5-ZB15 and TE7-70-15 consist of 4 and 10 diverse families of interior elements, respectively (Fig. 4 and Table 4). The exterior TE of both complexes is highly degenerated since it only contains few fragments of known Pyret elements. However, we found that both exterior TEs share overall 99% identity in nucleotides to each other except for 682-bp InDel in TE7-70-15 (Fig. 4). The AT contents of the exterior TEs of TE5-ZB15 and TE7-70-15 were approximately 70.2% and 70.4%, respectively, indicating that both of them consist of relatively high AT contents. Based on sequence similarity and identical TSDs, TE5-ZB15-1 and TE7-70-15-1 were speculated to be allelic to each other (Table 4). TE5-ZB15-4 and TE7-70-15-10 could be allelic to each other since they are almost identical and are located in the same genomic position. However, TSDs were not identified for both elements. The rest of them are not allelic to each other since they are located in different positions. For example, TE5-ZB15-2 and TE7-70-15-2 are almost identical to each other albeit in different locations, which indicates that they were probably integrated at different times within each complex. Compared to the TE5-ZB15 complex, the TE7-70-15 underwent an extremely dynamic TE burst, indicating two different evolutionary fates between these two complexes.

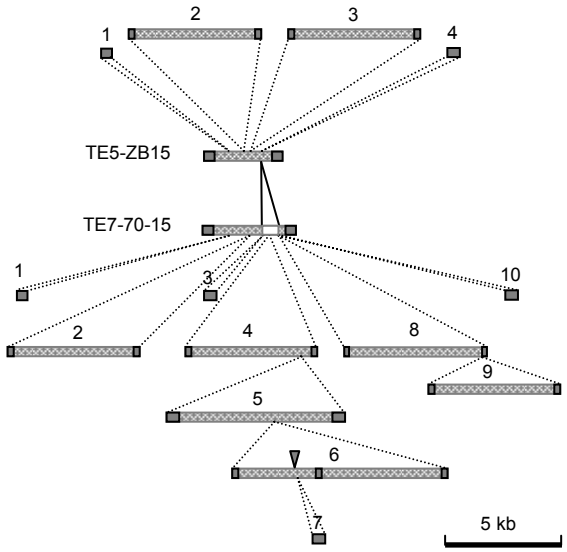

Fig. 4 Genomic organization of diverse TEs in both TE5-ZB15 and TE7-70-15 nested complexes

TEs corresponding to entire retrotransposon- and LTR-like elements are indicated in gridded and filled boxes, respectively. The interior TEs are designated by number as listed in Table 4. The InDel in the exterior TE of TE7-70-15 is indicated by an unfilled box. A gap in the sequence of the avrPiz-t-70-15 region is indicated by an arrow

3.4 SNP patterns in the *AvrPiz-t* locus in different *M. oryzae* isolates

We assessed sequence variation of the *AvrPiz-t* gene in our previous study and revealed that either presence/absence of a Pot3 element in the promoter region or a single nucleotide mutation in the coding sequence of *AvrPiz-t* represented two types of sequence variation of *AvrPiz-t* in different *M. oryzae* isolates (Li *et al*., 2009). In this study, we included another 25 avirulent isolates as listed in Table 5 to investigate sequence polymorphisms of the *AvrPiz-t* gene using the same primer pairs as used previously (Li *et al*., 2009). We found that the coding sequence of the *AvrPiz-t* gene in all tested isolates is identical to those three isolates as studied previously (Li *et al*., 2009) (Table 5). We did not find any TE present in the promoter region of *AvrPiz-t* in these avirulent isolates (data not shown). We further utilized SNP sites as molecular markers to identify possible recombination events at the *AvrPiz-t* locus in all the 28 isolates as listed in Table 5. A total of six SNPs in five GRs proximal to the *AvrPiz-t* gene were selected as a reference to the sequences of the *AvrPiz-t* locus in 81278ZB15 and 70-15 (Table 6). The SNP pattern in each GR, was determined by sequencing the PCR

Table 5 SNP patterns at five genomic regions (GRs) in different *M. oryzae* strains

Isolate[1]	Origin	Phenotype[2]	Provider[4]	Genotype[5]					
				GR1	GR2	*AvrPiz-t*	GR3	GR4	GR5
81278ZB15	China	R	Our study	P-I	P-I	NA	P-I	P-I	P-I
CHE86061	China	R	Wang G.L.	P-I	P-I	−	P-I	P-I	P-I
2539	Laboratory strain	R	Wang G.L.	P-I	P-I	−	P-I	P-I	P-I
Br18	Brazil	R	Terauchi R.	P-I	P-I	−	P-I	P-I	P-I
Shin85.86	Japan	R	Terauchi R.	P-I	P-I	−	P-I	P-I	P-I
Mar-05	Japan	R	Terauchi R.	P-I	P-I	−	P-I	P-I	P-I
22-4-1-1	Japan	R	Terauchi R.	P-I	P-I	−	P-I	P-I	P-I
TH68-126	Japan	R	Terauchi R.	P-I		−	P-I		
Sasa2	Japan	R	Terauchi R.	P-I	P-I	−	P-I	P-I	P-I
1836-3	Japan	R	Terauchi R.	P-I	P-I	−	P-I	P-I	P-I
2403-1	Japan	R	Terauchi R.	P-I	P-I	−	P-I	P-I	P-I
Ina168	Japan	R	Terauchi R.	P-I	P-I	−	P-I	P-I	P-I
Ina72	Japan	R	Terauchi R.	P-I	P-I	−	P-I	P-I	P-I
TH68-140	Japan	R	Terauchi R.	P-I	P-I	−	P-I	P-I	P-I
TH87-20-BII	Japan	R	Terauchi R.	P-I	P-I	−	P-I	P-I	P-I
TH69-8	Japan	R	Terauchi R.	P-I	P-I	−	P-I	P-I	P-I
KJ105	Korea	R[3]	Jeon J.S.	P-I	P-I	−	P-I	P-I	P-I
KJ197	Korea	R[3]	Jeon J.S.	P-I	P-I	−	P-I	P-I	P-I
ROR1	Korea	R[3]	Jeon J.S.	P-I	P-I	−	P-I	P-I	P-I
HoKu1	Japan	R	Terauchi R.	P-I	P-I	−	P-I	P-I	P-II
Ina86-137	Japan	R	Terauchi R.	P-I	P-I	−	P-I	P-I	P-II
TH78-15	Japan	R	Terauchi R.	P-I	P-I	−	P-I	P-I	P-II
CHNOS-60-2-3	Japan	R	Wang G.L.	P-I		−	P-I	P-II	P-II
P-2b	Japan	R	Terauchi R.	P-II	P-I	−	P-I	P-II	P-II
C9240	Philippines	R[3]	Wang G.L.	P-II		−	P-I		P-II
75-1-127-5	Columbia	R[3]	Wang G.L.	P-II	P-I	−	P-II	P-II	P-II
GUY11	French Guiana	S	Wang G.L.	P-II	P-II	NA	P-II	P-II	P-II
70-15	Laboratory strain	S	Wang G.L.	P-II	P-II	NA	P-II	P-II	P-II
Recombinants from the *AvrPiz-t* gene (rate)				3 (11.5%)	0 (0%)		1 (3.8%)	3 (11.5%)	7 (26.9%)

[1] Three isolates including 81278ZB15, GUY11 and 70-15 were assessed by Li *et al*. (2009). [2] Phenotype indicates the disease resistance to *AvrPiz-t*-harboring strains. [3] The phenotypes of these marked strains were determined in this study. [4] Provider: Dr. Guo-liang WANG (the Ohio State University, USA), Dr. Ryohei TERAUCHI (the Iwate Biotechnology Research Center, Japan), and Dr. Jong-Seong JEON (the Kyung Hee University, Korea). [5] Genotypes identical to the ones of 81278ZB15 and 70-15 are indicated by P-I and P-II, respectively. '–' indicates no sequence variation of the coding region of the *AvrPiz-t* in 81278ZB15 and other three isolates described by Li *et al*. (2009). NA: not applicable

Table 6 Five genomic regions (RGs) containing SNPs between the sequences of AvrPiz-t-ZB15 and avrPiz-t-70-15 regions

Genomic region	Genomic position (bp)[1]	SNP (genomic location)[2]	Primer (5′–3′)
GR1	20114–21094	C/T (20283 bp), A/G (20900 bp)	F: GGATGGAAACATGTTATCGG R: ATGCCTACTATGAAGGATGC
GR2	37224–37633	G/A (37414 bp)	F: CTGACATTGCCACATATAGG R: GTGGTAGCAGCAAGGTCAAC
GR3	40341–40707	G/C (40526 bp)	F: CTCCTTGTCAAGGACTCGTG R: GACGAGGAGTGGTTCTACAG
GR4	41076–41420	G/C (41264 bp)	F: CGATGATGGTCATTGCGGCC R: AGTTCATCATCGACTACAAC
GR5	118561–118800	T/G (118682 bp)	F: GGAGCCAAAAAGCGACATTG R: GTTTCGCCTCGACCGTCGCC

[1] The genomic position was determined based on the sequence of the *AvrPiz-t* locus. [2] The former and latter nucleotides represent the ones in the AvrPiz-t-ZB15 and avrPiz-t-70-15 regions, respectively

products and designated as either P-I or P-II, representing the pattern identical to the ones in 81278ZB15 and 70-15, respectively. We found that most avirulent isolates exhibited the P-I pattern in all these five GRs, which was identical to 81278ZB15 (Table 5). On the other hand, the virulent isolate GUY11 showed an identical pattern to 70-15 (Table 5). However, seven avirulent isolates had P-II SNP patterns in at least one GR (Table 5). Moreover, P-II patterns in three GRs (GR1, GR4, and GR5) were observed in several isolates from different localities, suggesting that these SNPs could be conserved prior to their geographical separation. In this case, the different SNP patterns observed in these isolates could be inherited from different isolates rather than from spontaneous mutations. It was also found that a greater frequency of P-II pattern was observed in the GR more distal to the *AvrPiz-t* gene in the avirulent isolates. For example, seven avirulent isolates exhibited a P-II pattern in GR5 which is approximately 80 kb from the *AvrPiz-t* gene (Table 5). By contrast, only one avirulent isolate (75-1-127-5) exhibited a P-II pattern in GR3 which is about 1 kb away from the *AvrPiz-t* gene (Table 5). None of the avirulent isolates had a P-II SNP even at the GR2 (Table 5).

4 Discussion

4.1 Genomic structure and dynamics of the *AvrPiz-t* locus

The studies on the genomic organization and evolution of *Avr* genes are always valuable for investigating the mechanisms underlying their high instability in the co-evolution of *M. oryzae* and rice. The genomic context of *Avr* genes is hypothesized to be critical for their instability. It has been found that *Avr* genes of *M. oryzae* reside within genetically unstable regions. For example, both *Avr-Pita1* and *Avr-Pita2* are situated in the subtelomeric regions and are highly variable among different isolates (Orbach *et al*., 2000; Khang *et al*., 2008; Dai *et al*., 2010). Insertions, deletions, point mutations, and insertions of diverse TEs were found to mediate the variation of *Avr-Pita* family members (Kang *et al*., 2001; Zhou *et al*., 2007; Khang *et al*., 2008; Dai *et al*., 2010). Another *Avr* gene, *Avr-Pii* genetically delimited into the telomeric region was found to have an MAGGY element nearby (Yasuda *et al*., 2006; Yoshida *et al*., 2009). In addition to *Avr-Pita1*, *Avr-Pita2*, and *Avr-Pii*, *M. oryzae* has several *Avr* genes that also mapped near the telomeric region, e.g., *Avr-Pia*, *Avr-Pit*, *Avr1-Ku86*, *Avr1-MedNoi*, and *PWL1* (Kang *et al*., 1995; Dioh *et al*., 2000; Chen *et al*., 2007). It was found that the *Avr1-CO39* locus was deleted partially or entirely in the J and G type isolates compared to the W type isolate harboring an entire *Avr* gene (Farman *et al*., 2002). The repetitive sequences including REP1, RETRO5, and MGR691/MGR508 were identified at the 5′ terminus of the *avr1-CO39* locus in virulent isolates (Farman *et al*., 2002). Another *Avr* gene, *ACE1*, which encodes a polyketide synthase fused to a nonribosomal peptide synthetase (PKS-NRPS), is located in a gene cluster encoding different enzymes potentially involved in secondary metabolism (Böhnert *et al*., 2004; Collemare *et al*., 2008).

In this study, we determined the complete sequences of a portion of *M. oryzae* genome that includes the *AvrPiz-t* gene in both avirulent and virulent

isolates, providing an in-depth view of its genomic structure and dynamics. It was located in a region that harbors the second cluster as designated by Thon *et al*. (2006) that comprises relatively high TE content on chromosome 7. It was found that the predicted gene alleles at the *AvrPiz-t* locus are identical or have only 1 or 2 SNPs, indicating that the genic sequences are almost identical to each other in the two isolates. Yoshida *et al*. (2009) conducted a large-scale study on the sequence variation of putative secreted protein genes in 46 isolates collected worldwide and found that the majority of analyzed genes (78% of 1032 loci) are monomorphic. Therefore, we believe that nucleotide polymorphisms of most *M. oryzae* genes could be very low in different isolates. On the contrary, we also found that repetitive sequences are highly dynamic with respect to their composition and sequence divergence surrounding the *AvrPiz-t* locus. As illustrated in Fig. 3, all nine non-syntenic sites are exclusively attributed to the presence of TEs at one of the two loci. Both class I elements or retrotransposons that mediate transposition by the reverse transcription of an RNA intermediate, and class II elements or DNA transposons that mediate transposition by a DNA form (Daboussi and Capy, 2003), were involved in these processes. It has been proposed that rice isolates of *M. oryzae* have a small effective population size and likely have arisen from a founder population recently (Yoshida *et al*., 2009). Given the fact that the *AvrPiz-t* locus is almost identical in two isolates after removing those TEs that are only present at one locus, we speculate that the *AvrPiz-t* locus in the founder isolate could have fewer TEs. The finding that most TEs have a perfect pair of TSDs further implies that they have occurred by integration rather than excision. Other genetic events, including an unequal recombination between two LTR sequences of TE6-70-15, nested transpositions of different TEs in TE7-70-15/TE6-ZB15, and a possible genomic rearrangement mediated by the occurrence of TE6-70-15, have also been hypothesized to be involved in the evolution of the *AvrPiz-t* locus.

4.2 Role of TEs in regulating the host specificity of *M. oryzae*

In the warfare between rice and rice blast, the instability of avirulence genes has been proposed as one of the major strategies employed by *M. oryzae* to defeat rice blast resistance genes (Khang *et al*., 2008). Isolation of various avirulence genes has made it possible to investigate their dynamics in natural populations, which could reveal the molecular mechanisms underlying the instability of avirulence genes. It was found that some TEs are closely associated with different *M. oryzae Avr* genes, e.g., three families of TE-like sequences including MGR619, MGR608, and REP1 were identified within the *PWL*, *Avr1-CO39*, and *Avr-Pita* loci (Kang *et al*., 1995; Farman *et al*., 2002; Khang *et al*., 2008). The insertion of TEs at either the promoters or coding regions of several *Avr* genes was found to be responsible for the conversion from avirulence to virulence in *M. oryzae*. For example, the insertion of a Pot3 element was identified at the promoter and coding region of the *Avr-Pita*1 gene in two different virulent isolates (Kang *et al*., 2001; Zhou *et al*., 2007). A 1.9-kb MINE element was inserted in the last exon of *ACE1* in the virulent isolate 2/0/3 (Fudal *et al*., 2005). In our previous study (Li *et al*., 2009), we identified a Pot3 element present at the promoter region of *AvrPiz-t*, which was hypothesized to be responsible for the loss of avirulence in GUY11. Yoshida *et al*. (2009) has revealed that putative secreted protein genes in *M. oryzae* have significantly more presence/absence polymorphisms than nucleotide polymorphisms. Insertion of a TE in or near genes has been hypothesized as one of the mutagens that usually create a null phenotype by blocking transcription or causing premature translation of target genes in fungi (Daboussi and Capy, 2003). In this case, inactivation of an *Avr* gene by insertion of a TE could mimic the same effect as its absence in the genome because the cognate plant resistance gene is unable to recognize its presence any longer. Interestingly, we found that 40% of those TEs, which are distributed as singletons in the genome of *M. oryzae* and are polymorphic with respect to their presence/absence in different isolates, are targeted in or adjacent to predicted genes. Moreover, some of the predicted genes encode putative secreted proteins (unpublished data). Therefore, it is reasonable to postulate that TE families could have played an important role in protecting *M. oryzae* from recognition by its host by their transposition in or adjacent to avirulence genes.

4.3 Origin of the nested transposon complex at the *AvrPiz-t* locus

Nested TEs, the outcomes of successive integration events by additional TEs within the boundaries of existing ones, are widely observed across grass genomes (SanMiguel *et al*., 1996; Wei *et al*., 2002). It is a high likelihood that clustered TE families are arranged into the forms of nested elements. Even if insertion is random, TE families that are distributed into clusters are more likely to form into nests because insertion between elements becomes less and less likely as the cluster expands. Given the fact that TEs are relatively abundant and not randomly distributed in *M. oryzae* (Thon *et al*., 2004; 2006; Ma *et al*., 2009), it is reasonable to speculate that nested TEs could be quite common in this fungus. Indeed, MAGGY, MGL, and Mg-SINEs were found in nested forms within previously existing Pot2 elements in different loci (Kachroo *et al*., 1995; Thon *et al*., 2004). In this study, we identified and reconstructed the original TEs of a nested TE complex located near the *AvrPiz-t* locus in two isolates. These two nested elements have rapidly evolved separately from each other with respect to the constitution and chronology of insertion of interior TE families. We believe that most of the interior TE families were inserted within the common pre-existing families after the geographical isolation of these two isolates. However, the nested TE complex in the isolate 70-15 might not represent the one in either of the parental isolates since 70-15 was developed from the cross between GUY11 and another rice isolate 66-10 (Lau *et al*., 1993). It was also found that the exterior TEs are almost identical to each other except for 682-bp InDel, indicating it could exist in the founder isolate. Interestingly, AT contents of both exterior TEs are much higher than the average for the genome of *M. oryzae* (70% versus 48.4 % (Dean *et al*., 2005)). This finding reiterates the hypothesis that TEs prefer to integrate within the GR having high AT content in different organisms including *M. oryzae* (Thon *et al*., 2004; Yant *et al*., 2005). The presence of different nested TE complexes represents the major disruption of the colinearity at the *AvrPiz-t* locus between 81278ZB15 and 70-15, which was also observed in other organisms. For example, a 53-kb nested transposon block in the *bz* region from the maize line carrying the *Bz-McC* allele, is missing in the corresponding region in another maize line carrying the *Bz-B73* allele (Fu and Dooner, 2002). Likewise, a 53-kb nested TE complex in the *Pi2/9* region in Nipponbare is absent in the allelic region in C101A51 in rice (unpublished data). Thon *et al*. (2006) reported a positive correlation between the content of TEs and recombination frequency in *M. oryzae*. However, our study indicated that the nested TE complex could have a little effect on recombination frequency because the recombinant rate is not altered significantly between GR4 and GR5. Nevertheless, a relatively good correlation between the recombination rate and genomic distance was observed at the *AvrPiz-t* locus in natural population.

Acknowledgements

We gratefully acknowledge Dr. Ryohei TERAUCHI at the Iwate Biotechnology Research Center, Japan, for providing the genomic DNA samples listed in Table 5. We also thank Dr. Jong-Seong JEON at the Kyung Hee University, Korea, and Dr. Guo-liang WANG at the Ohio State University, USA, for providing the isolates listed in Table 5.

References

Böhnert, H.U., Fudal, I., Dioh, W., Tharreau, D., Notteghem, J.L., Lebrun, M.H., 2004. A putative polyketide synthase/peptide synthetase from *Magnaporthe grisea* signals pathogen attack to resistant rice. *Plant Cell*, **16**(9):2499-2513. [doi:10.1105/tpc.104.022715]

Chen, Q.H., Wang, Y.C., Li, A.N., Zhang, Z.G., Zheng, X.B., 2007. Molecular mapping of two cultivar-specific avirulence genes in the rice blast fungus *Magnaporthe grisea*. *Mol. Genet. Genomics*, **277**(2):139-148. [doi:10.1007/s00438-006-0179-8]

Collemare, J., Pianfetti, M., Houlle, A.E., Morin, D., Camborde, L., Gagey, M.J., Barbisan, C., Fudal, I., Lebrun, M.H., Böhnert, H.U., 2008. *Magnaporthe grisea* avirulence gene *ACE1* belongs to an infection-specific gene cluster involved in secondary metabolism. *New Phytol.*, **179**(1):196-208. [doi:10.1111/j.1469-8137.2008.02459.x]

Daboussi, M.J., Capy, P., 2003. Transposable elements in filamentous fungi. *Annu. Rev. Microbiol.*, **57**(1):275-299. [doi:10.1146/annurev.micro.57.030502.091029]

Dai, Y., Jia, Y., Correll, J., Wang, X., Wang, Y., 2010. Diversification and evolution of the avirulence gene *AVR-Pita1* in field isolates of *Magnaporthe oryzae*. *Fungal Genet. Biol.*, **47**(12):973-980. [doi:10.1016/j.fgb.2010.08.003]

Dean, R.A., Talbot, N.J., Ebbole, D.J., Farman, M.L., Mitchell, T.K., Orbach, M.J., Thon, M., Kulkarni, R., Xu, J.R., Pan, H., *et al*., 2005. The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature*, **434**(7036):980-986. [doi:10.1038/nature03449]

Dioh, W., Tharreau, D., Notteghem, J.L., Orbach, M., Lebrun, M.H., 2000. Mapping of avirulence genes in the rice blast fungus, *Magnaporthe grisea*, with RFLP and RAPD markers. *Mol. Plant Microbe Interact.*, **13**(2):217-227. [doi:10.1094/MPMI.2000.13.2.217]

Ewing, B., Green, P., 1998. Base-calling of automated sequencer traces using phred. II. Error probabilities. *Genome Res.*, **8**(3):186-194. [doi:10.1101/gr.8.3.186]

Ewing, B., Hillier, L., Wendl, M.C., Green, P., 1998. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. *Genome Res.*, **8**(3):175-185. [doi:10.1101/gr.8.3.175]

Farman, M.L., Eto, Y., Nakao, T., Tosa, Y., Nakayashiki, H., Mayama, S., Leong, S.A., 2002. Analysis of the structure of the *AVR1-CO39* avirulence locus in virulent rice-infecting isolates of *Magnaporthe grisea*. *Mol. Plant Microbe Interact.*, **15**(1):6-16. [doi:10.1094/MPMI.2002.15.1.6]

Fu, H., Dooner, H.K., 2002. Intraspecific violation of genetic colinearity and its implications in maize. *PNAS*, **99**(14):9573-9578. [doi:10.1073/pnas.132259199]

Fudal, I., Böhnert, H.U., Tharreau, D., Lebrun, M.H., 2005. Transposition of MINE, a composite retrotransposon, in the avirulence gene *ACE1* of the rice blast fungus *Magnaporthe grisea*. *Fungal Genet. Biol.*, **42**(9):761-772. [doi:10.1016/j.fgb.2005.05.001]

Gordon, D., Abajian, C., Green, P., 1998. Consed: a graphical tool for sequence finishing. *Genome Res.*, **8**(3):195-202. [doi:10.1101/gr.8.3.195]

Kachroo, P., Leong, S.A., Chattoo, B.B., 1995. Mg-SINE: a short interspersed nuclear element from the rice blast fungus, *Magnaporthe grisea*. *PNAS*, **92**(24):11125-11129. [doi:10.1073/pnas.92.24.11125]

Kang, S., Sweigard, J.A., Valent, B., 1995. The *PWL* host specificity gene family in the blast fungus *Magnaporthe grisea*. *Mol. Plant Microbe Interact.*, **8**(6):939-948. [doi:10.1094/MPMI-8-0939]

Kang, S., Lebrun, M.H., Farrall, L., Valent, B., 2001. Gain of virulence caused by insertion of a Pot3 transposon in a *Magnaporthe grisea* avirulence gene. *Mol. Plant Microbe Interact.*, **14**(5):671-674. [doi:10.1094/MPMI.2001.14.5.671]

Khang, C.H., Park, S.Y., Lee, Y.H., Valent, B., Kang, S., 2008. Genome organization and evolution of the *AVR-Pita* avirulence gene family in the *Magnaporthe grisea* species complex. *Mol. Plant Microbe Interact.*, **21**(5):658-670. [doi:10.1094/MPMI-21-5-0658]

Lau, G.W., Chao, C.T., Ellingboe, A.H., 1993. Interaction of genes controlling avirulence/virulence of *Magnaporthe grisea* on rice cultivar Katy. *Phytopathology*, **83**(4):375-382. [doi:10.1094/Phyto-83-375]

Leong, S.A., 2008. The Ins and Outs of Host Recognition of *Magnaporthe oryzae*. *In*: Gufstason, J.P., Taylor, J., Stacey, G. (Eds.), The Genomics of Disease. Springer Science+Business Media, New York, p.119-216.

Li, W., Wang, B., Wu, J., Lu, G., Hu, Y., Zhang, X., Zhang, Z., Zhao, Q., Feng, Q., Zhang, H., *et al*., 2009. The *Magnaporthe oryzae* avirulence gene *AvrPiz-t* encodes a predicted secreted protein that triggers the immunity in rice mediated by the blast resistance gene *Piz-t*. *Mol. Plant Microbe Interact.*, **22**(4):411-420. [doi:10.1094/MPMI-22-4-0411]

Liu, J., Wang, X., Mitchell, T., Hu, Y., Liu, X., Dai, L., Wang, G.L., 2010. Recent progress and understanding of the molecular mechanisms of the rice-*Magnaporthe oryzae* interaction. *Mol. Plant Pathol.*, **11**(3):419-427. [doi:10.1111/j.1364-3703.2009.00607.x]

Ma, L.J., Ibrahim, A.S., Skory, C., Grabherr, M.G., Burger, G., Butler, M., Elias, M., Idnurm, A., Lang, B.F., Sone, T., *et al*., 2009. Genomic analysis of the basal lineage fungus *Rhizopus oryzae* reveals a whole-genome duplication. *PLoS Genet.*, **5**(7):e1000549. [doi:10.1371/journal.pgen.1000549]

Miki, S., Matsui, K., Kito, H., Otsuka, K., Ashizawa, T., Yasuda, N., Fukiya, S., Sato, J., Hirayae, K., Fujita, Y., *et al*., 2009. Molecular cloning and characterization of the *AVR-Pia* locus from a Japanese field isolate of *Magnaporthe oryzae*. *Mol. Plant Pathol.*, **10**(3):361-374. [doi:10.1111/j.1364-3703.2009.00534.x]

Orbach, M.J., Farrall, L., Sweigard, J.A., Chumley, F.G., Valent, B., 2000. A telomeric avirulence gene determines efficacy for the rice blast resistance gene *Pi-ta*. *Plant Cell*, **12**(11):2019-2032. [doi:10.1105/tpc.12.11.2019]

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A., Allard, R.W., 1984. Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. *PNAS*, **81**(24):8014-8018. [doi:10.1073/pnas.81.24.8014]

SanMiguel, P., Tikhonov, A., Jin, Y.K., Motchoulskaia, N., Zakharov, D., Melake-Berhan, A., Springer, P.S., Edwards, K.J., Lee, M., Avramova, Z., *et al*., 1996. Nested retrotransposons in the intergenic regions of the maize genome. *Science*, **274**(5288):765-768. [doi:10.1126/science.274.5288.765]

Thon, M.R., Martin, S.L., Goff, S., Wing, R.A., Dean, R.A., 2004. BAC end sequences and a physical map reveal transposable element content and clustering patterns in the genome of *Magnaporthe grisea*. *Fungal Genet. Biol.*, **41**(7):657-666. [doi:10.1016/j.fgb.2004.02.003]

Thon, M.R., Pan, H., Diener, S., Papalas, J., Taro, A., Mitchell, T.K., Dean, R.A., 2006. The role of transposable element clusters in genome evolution and loss of synteny in the rice blast fungus *Magnaporthe oryzae*. *Genome Biol.*, **7**(2):R16. [doi:10.1186/gb-2006-7-2-r16]

Wei, F., Wing, R.A., Wise, R.P., 2002. Genome dynamics and evolution of the *Mla* (powdery mildew) resistance locus in barley. *Plant Cell*, **14**(8):1903-1917. [doi:10.1105/tpc.002238]

Yant, S.R., Wu, X., Huang, Y., Garrison, B., Burgess, S.M., Kay, M.A., 2005. High-resolution genome-wide mapping of transposon integration in mammals. *Mol. Cell. Biol.*, **25**(6):2085-2094. [doi:10.1128/MCB.25.6.2085-2094.2005]

Yasuda, N., Noguchi, M.T., Fujita, Y., 2006. Partial mapping of avirulence genes *AVR-Pii* and *AVR-Pia* in the rice blast fungus *Magnaporthe oryzae*. *Can. J. Plant Pathol.*, **28**(3):494-498. [doi:10.1080/07060660609507325]

Yoshida, K., Saitoh, H., Fujisawa, S., Kanzaki, H., Matsumura, H., Tosa, Y., Chuma, I., Takano, Y., Win, J., Kamoun, S., *et al*., 2009. Association genetics reveals three novel avirulence genes from the rice blast fungal pathogen *Magnaporthe oryzae*. *Plant Cell*, **21**(5):1573-1591. [doi:10.1105/tpc.109.066324]

Zhou, B., Qu, S., Liu, G., Dolan, M., Sakai, H., Lu, G., Bellizzi, M., Wang, G.L., 2006. The eight amino-acid differences within three leucine-rich repeats between *Pi2* and *Piz-t* resistance proteins determine the resistance specificity to *Magnaporthe grisea*. *Mol. Plant Microbe Interact.*, **19**(11):1216-1228. [doi:10.1094/MPMI-19-1216]

Zhou, E., Jia, Y., Singh, P., Correll, J.C., Lee, F.N., 2007. Instability of the *Magnaporthe oryzae* avirulence gene *AVR-Pita* alters virulence. *Fungal Genet. Biol.*, **44**(10): 1024-1034. [doi:10.1016/j.fgb.2007.02.003]