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Analysis of Rice Blast Resistance Genes in the National Small Grains Collection (NSGC)

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ABSTRACT

Identification of blast resistance genes in rice germplasm is one of the most important activities of rice breeding programs in worldwide. The objective of this research was to characterize two major blast resistance genes, *Pi-z* and *Pi-b,* in selected rice germplasm. A simple sequence repeat DNA marker, AP5659-1 linked to the *Pi-z* gene, and a Pib dominant marker derived from the *Pi-b* gene were first used to screen a rice core collection consisting of 1700 accessions estimated to represent 70% of the genetic diversity of rice in the US National Small Grains Collection. There were 131 rice germplasm accessions with marker profiles indicating the presence of *Pi-z,* and178 indicating the presence of *Pi-b*. This research assessed accessions using the tightly linked SSR markers - AP5659-1, AP5659-5, AP4791 and RM527 - for the *Pi-z* gene; and a *Pi-b* dominant marker plus DNA markers RM 208 and RM 166 for the *Pi-b* gene respectively. Finally, isolates of *Magnaporthe oryzae* representing differential races, including IB33, IB49 and IE1k for *Pi-z*; and IB54 and IE1k for *Pi-b*; were used to evaluate disease reactions of the inoculated rice accessions. Experimental findings indicated that the total number of lines containing *Pi-z* and *Pi-b* were reduced to 117 for *Pi-z* and 164 for *Pi-b* as a result of seed mix. Using the combination of DNA marker and pathogenicity assays, 81 germplasm accessions with *Pi-z* and 130 germplasm accessions with *Pi-b* were identified. In addition, 54 germplasm accessions with different resistance genes were also noted. These accessions carrying *Pi-z* and *Pi-b* and other unknown blast resistance genes were once elite commercial cultivars in different rice production regions and may represent valuable genetic resources for breeders to use for improving blast resistance using marker assisted and conventional breeding.

This dissertation is approved for recommendation
to the Graduate Council.

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Introduction

Rice blast, caused by the filamentous ascomycete fungal pathogen *Magnaporthe oryzae* B. Couch = (*Magnaporthe grisea* sensu Yaegashi and Udagawa), is one of the most devastating diseases affecting global rice production. Resistance to blast in most cases follows a classical gene for gene relationship, in which a resistance gene is effective against blast isolates having the corresponding avirulence gene.

Resistance in rice varieties carrying one major resistance gene has often been overcome by the emergence of the virulent races of the pathogen. Based on the gene for gene theory, an additive effect would be expected when two resistance genes were combined in one rice variety. Therefore, combining genes with overlapping resistance from different sources (stacking) has been considered an effective breeding objective to develop more durably resistant rice cultivars.

In the southern US, the major blast resistance genes *Pi-ta*, *Pi-b*, *Pi-ks*, *Pi-kh*, and *Pi-z* are effective against contemporary blast pathogen populations. *Pi-ta* was considered to be the most efficient and thus most widely used. It was effectively deployed to prevent blast for over a decade in Arkansas. In 2004, the novel blast race IE-1k was found to overcome *Pi-ta* and resulted in significant economic loss in several rice fields in Arkansas. In searching for sources of resistance, *Pi-b* and *Pi-z* were found to confer resistance to IE-1k.

The objective of this research was to characterize *Pi-z* and *Pi-b*, two resistance genes used to combat a broad spectrum of races of *Magnaporthe oryzae* in elite germplasm collections from around the world. DNA markers tightly linked to the Pi-z and Pi-b locus were chosen to eliminate the chances of recombination and identify

germplasm accessions with the genes. Pathogenicity assays were also used on these elite germplasm collections to further confirm the DNA marker findings. DNA markers and pathogenicity assays together not only provided more accurate identification of rice germplasm having both *R* genes but also facilitated the identification of novel *R* genes.

The germplasm identified as having *Pi-z* and *Pi-b* could be used as donors using marker assisted selection and conventional breeding strategies for cultivar improvement.

Structure, Function and Co-evolution of Rice Blast Resistance Genes

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Abstract

Rice blast disease caused by the fungal pathogen *Magnaporthe oryzae* is one of the most destructive rice diseases worldwide. Resistance (*R*) genes to blast encode proteins that detect pathogen signaling molecules encoded by *M. oryzae* avirulence (*AVR*) genes. *R* genes can be a single copy gene or a member of clustered gene families that have evolved through duplication and diversification. Recent advances in blast *R* gene cloning and subsequent characterization have provided useful insights into *R* gene mediated signaling transduction pathways. This review summarizes recent advances in cloning and characterization of blast *R* genes, and presents an update on evolutionary dynamics of *R* proteins, their interaction, and co-evolution with the signaling molecules encoded by the *AVR* genes, and potential implications for crop protection.

Key words

R genes, *AVR* genes, blast disease, gene interaction, *Magnaporthe oryzae*

Introduction

Rice *(Oryza sativa L.)* is the staple food for more than half of the world's population. Maintaining stable rice production is extremely important to feed the constantly growing human population. However, rice blast caused by the pathogen *Magnaporthe oryzae* B. Couch remains one of the most serious threats to secure global rice production.

M. oryzae germinates when a conidium attaches to the surface of the rice leaf, and subsequently, hyphae produce appressoria for penetration. Penetration normally occurs within 24 h of spore germination depending on strains [93]. *M. oryzae* directly penetrates the cell membranes leading to subsequent intracellular growth of mycelia that result in the destruction of a living cell [39]. Before cell death, mycelia were thought to move to the adjacent cell via unknown mechanisms including a possible utilization of plasmodesmata [45]. The growth of the fungus within the cell often results in impairment of transportation of water and minerals in the vascular system. Once inside the plant, the fungus produces thousands of spores on conidiophores emerging from stomata that can be dispersed by air currents to nearby rice plants for subsequent infection (Fig.1). The fungus is highly adaptive to its host and capable of causing infection at any growing stage. It has also been known to overcome resistance in a new rice cultivar in a few years after commercialization.

Rice blast was first reported in Asia, and is now present in more than 80 countries where rice is grown [14]. It is estimated to cause economic losses of up to USD 60 million annually in South and Southeast Asia (The International Service for the Acquisition of Agri-biotech Applications)[14]. According to the International Rice

Research Institute (IRRI), more than 266,000 tons of rice is lost due to blast disease in India annually. In Japan, the figure is similar, with an estimated 200,000 tons of rice lost annually due to blast disease [24]. In China, blast disease has been an increasing challenge for rice breeding programs and crop production for the past two decades. In the United States, blast disease occurs sporadically but causes significant crop losses in favorable years. The destructive nature of blast has drawn worldwide attention and intensive investigation. Today it is one of the best characterized model host-pathosystems for understanding molecular mechanisms of host genetic resistance. Although well characterized, *M. oryzae* is highly variable and can overcome deployed resistant cultivars in a very short time.

Due to the extensive studies conducted worldwide, rice was among the first plant species to have its complete genome sequenced [2]. A draft genome sequence of *M. oryzae* has also been determined [8, 25, 89]. Availability of genome sequences of rice and *Magnaporthe oryzae* has expedited progress in map-based cloning of resistance genes in rice and understanding the molecular bases of resistance, interaction and co-evolution of rice and *M. oryzae*. In this review, we first describe mapped and cloned blast resistance genes, then summarize the current understanding of host-pathogen interactions and co-evolution, and finally discuss the utilization of this knowledge for crop protection.

Blast *R* genes

Genetic studies of resistance to *M. oryzae* began when Goto established the differential system for races of the blast pathogen in Japan in the early 1960's [28, 29]. Interaction of *O. sativa* with *M. oryzae* in most cases follows the classical gene-for-gene

interaction where a resistance (*R*) gene named as *Pyricularia* (*Pi*) is effective in preventing *M. oryzae* races that contain the corresponding avirulence gene [33,87]. The name *Pi* was derived from the imperfect asexual fungus *Pyricularia oryzae (=Magnaporthe oryzae)*. The rules of gene nomenclature in rice blast resistance were introduced in 1993. New blast resistance genes are designated as *Pi* followed by a numeral, except for those reported before 1993. The suffix '*(t)*' (tentative) is attached until the completion of allelism tests. A total of 80 major *Pi* genes and 35 minor *R* genes have now been described [5]. Among them, *Pita*, *Pi9*, *Pi2/Piz-(t)*, *Pid2*, *Pi36*, *Pi37*, *Pike*, *Pi5*, *Pit*, *Pid3*, *pi-21*and *Pb1* have been cloned (Fig. 3). The fine mapping and cloning of the *Pikh* gene from 'Tetep' has also been reported by Sharma et al. [85, 97]. Lack of supporting evidence has raised significant doubts to this claim [96]. *Pith* in 'Tate' has now been designated *Pi54* [79].

Mapped blast *R* genes

Bellini [5,59] described fine mapping of a total of 15 blast *R* genes (*IPi*, *IPi3*, *Pb1*, *Pi15*, *Pi24*, *Pi25*, *Pi33*, *Pi39*, *Pi42*, *Pi5*, *Pigm(t)*, *Pik*, *Pi-x(t)*, *Pi-y1(t)*, and *Pi-y2(t)* (Table 3). Additional recently mapped blast *R* genes are described below:

Piz(t)*

*Piz(t)** was first identified in the US rice cultivar 'Zenith' [48]. *Piz-(t)*, another blast resistant gene, was also mapped at the same location as *Piz(t)**, close to the centromere of chromosome 6 [34, 62]. *Piz-(t)* was cloned from an *indica* rice cultivar [10, 66, 74]. The *Piz(t)**gene conferring resistance to two races of blast was mapped within a 57 kb region on the physical map of 'Nipponbare' in a location where *Pi2* was located [18]. Three simple sequence repeats (SSRs), AP5659-1 and AP5659-5 and AP5659-3,

were found to be very tightly linked to the *Piz(t)**locus, with one marker, AP5659-3, co-segregating with *Piz(t)**mediated resistance. Two simple sequence repeat (SSR) marker haplotypes, AP4791 and AP5659-1, were unique for cultivars carrying the *Piz(t)**gene, indicating these markers could be useful for selection of resistance genes at the *Pi-z(t)** locus in rice germplasm [18].

Pi42(t)* and *Pi43(t)

Pi42(t) and *Pi43(t)* were identified from the resistant *indica* cultivar 'Zhe733' [55]. These two major *R* genes provide complete resistance to IE-1k. *Pi-42(t)* was mapped between SSR markers RM310 and RM72 on chromosome 8, differing from *Pi4*2, mapped on chromosome 12 [55]. *Pi43*(*t*) was closely associated with two flanking SSR markers RM1233 and RM224 on chromosome 11 in a chromosomal region carrying the resistance gene *Pi1* [55]. Recombinant inbred lines containing *Pi42*(*t*) and *Pi43*(*t*) also tested resistant to US races IB-1, IB-45, IB-49, IB-54, IC-17, IE-1, IG-1, and IH-1. Two molecular markers, RM72 and RM1233, were also identified while identifying the genes [55] and could be used for fine mapping and facilitating incorporation of *Pi42*(*t*) and *Pi43*(*t*) into advanced breeding lines by marker-assisted selection.

Pi47(t)* and *Pi48(t)

Pi47(t) and *Pi48(t)* were identified from the resistant *indica* cultivar 'Xiangzi 3150' ('XZ3150') [38] using a recombinant inbred line (RIL). Segregation analysis suggested that resistance was conditioned by two dominant nucleic genes, tentatively designated *Pi47(t)* and *Pi48(t)* [38]. *Pi47(t)* was located between RM206 and RM224 on chromosome 11, and *Pi48(t)* was located between RM5364 and RM7102 on chromosome 12. The composite interval mapping analysis of the four resistant components of the

RILs to the field blast population revealed that *Pi47(t)* and *Pi48(t)* were also the major genetic factors responsible for field resistance observed in 'XZ3150'. The analysis showed that the two regions in chromosome 11 and 12, i.e., *Pi47* and *Pi48*, were responsible for all four blast-resistant phenotypes in the field. The peaks of the two regions closely overlapped with the regions of *Pi47* and *Pi48*, which were identified by inoculation with the isolate 193-1-1 in a growth chamber experiment performed by Huang et al[38]. The LOD score for the four resistant phenotypes at the *Pi47* region ranged from 8 to 15 while those at the *Pi48* region ranged from 7 to 18. When the RILs carrying the two major genes were removed from the CIM analysis, no QTL with LOD scores over 3.0 were identified (data not shown), suggesting that the two *R* genes were the major contributors to 'XZ3150' blast resistance in the field.

Cloned blast *R* genes

To date, a total of 14 blast *R* genes including 12 major and 2 minor *R* genes have been cloned (Table 1; Fig. 2).

Pib

Pib on chromosome 2 was the first blast *R* gene cloned [84, 94]. This gene confers high levels of resistance to most blast races in Japan. *Pib* conveys resistance to seven blast races in the US (Table 4). *Pib* has been introgressed into various *japonica* cultivars independently from two Indonesian and Malaysian cultivars. In the US, *Pib* was introgressed into rice cultivars Saber and Bolivar from the Chinese *indica* cultivar 'Teqing' [68, 69, 19]. *Pib* is a member of a small gene family encoding a cytoplasmic protein with nucleotide binding sites (NBS) and C-terminal leucine rich repeats (LRRs), but no distinct transmembrane domain. An NBS domain is a signaling motif shared by

plant *R*-gene products [32, 15, 72, 6]. A duplication of the kinase 1a, 2 and 3a motifs of the NBS region was found in the N terminal half of the Pib protein. In addition, eight cysteine residues were clustered within the LRRs, a feature not observed in any other R proteins [67, 91]. Northern blot analysis of the *Pib* gene family members (*Pib, PibH8, HPibH8-1* and *HPibH8-2*) revealed that their expression was regulated by environmental signals such as temperature, light, water and chemical treatments, including jasmonic acid, salicylic acid, ethylene and probenazole [94]. The *Pib* gene family is, to the best of our knowledge, the first plant *R* gene family to be investigated extensively at the transcription level.

Pita

Pita on chromosome 12 was the second blast *R* gene cloned [9]. *Pita* confers resistance to a wide range of blast races worldwide. 'Katy' was the first US cultivar reported to contain the *Pita* gene. The *Pita* gene in 'Katy' was derived from the landrace *indica* variety 'Tetep' [50, 72, 82]. Subsequently, a total of seven rice cultivars in the US were developed using *Pita* from 'Katy'. *Pita* encodes a putative cytoplasmic receptor with a centrally localized nucleotide-binding site and leucine-rich domain (LRD) at the C-terminus. It has a conserved internal hydrophobic domain characteristic of other NBS-class *R* gene proteins [44] between two amino acids and four potential N glycosylation sites. *Pita* differs from the dicot class of NBS *R* genes in having a unique N terminus. It does not have a leucine zipper or Toll /interleukin-1 receptor homology. The Pita carboxyl terminal domain is rich in leucine and is thus referred to as the leucine rich domain. It lacks the classical LRR motif found in other genes of this class. Susceptible rice varieties were found to contain a single amino acid difference relative to the *Pita*

resistance protein - the amino acid residue serine was in place of alanine at the 918 position.

Pi9

Pi9 on chromosome 6 is known to confer resistance to the US race IC17 [77] (Table.4). *Pi9* belongs to the NBS–LRR class of *R* genes. Although *Pi9* has two introns in its coding region, unlike other *R* genes in rice, one of the introns is much larger (5362 bp) than that of the *Pib* gene. Whether this unique feature in the *Pi9* gene has any association with its broad resistance spectrum will require further research. The Pi9 protein has a conserved nT motif (WAEQIRDLSYDIEDSLDEF) which is located 107 amino acids before the P-loop. The LRR domain in Pi9 is similar to that of the Pib protein and consists mainly of imperfect LRR repeats. A unique structural feature of the Pi9 protein is that it contains a 57-amino-acid non-LRR region at the C terminus. In contrast, the LRRs in both Pib and Pita extend to the end of the C terminus. Further research is needed to investigate whether this 57-amino-acid sequence at the C terminus of *Pi-9* has any special function in regulating resistance specificity to rice blast. Unlike Pib, the expression profile of the *Pi-9* gene showed that *Pi-9* was constitutively expressed in *Pi-9*-carrying plants and was not induced by blast infection.

Pi2/Piz-(t)

The *Pi2/Piz-(t)* gene is located on chromosome 6 and is *indica*-derived [101]. The structure of *Pi2* and *Piz-(t)* in terms of intron and exon size and position was determined by cloning the *Pi2/Piz-(t)* coding (CDS) region. The transcripts consisting of the entire CDS were cloned by reverse transcriptase-PCR with the primer pair NBS4F and NBS2R, which can amplify both *Pi2* and *Piz-(t)*. Results showed 3,332- and 3,335

-bp fragments for *Pi2* and *Piz-(t)*, respectively, that contain a 117-bp 5′ UTR and a 116-bp 3′ UTR for both *Pi2* and *Piz-(t)*. Aligning the sequences of the cloned *Pi2′* and *Piz-(t)′* transcripts with their genomic sequences revealed that *Pi2* and *Piz-(t)* contain two introns that have the same genomic position and identical sequence to each other. The first intron, 3,839 bp in length, is 116 bp downstream of the start codon, corresponding to the region before the NBS domain. The second intron, 128 bp in length, is 31 bp upstream of the stop codon, corresponding to the region after the LRR domain. A DNA fragment in the first intron was identified which was 177 bp in length and shared 93% sequence identity to the first 177 bp portion of the second exon [101]. *Pi2* and *Piz-t* encode a 1,032– and a 1,033–amino-acid protein product, respectively, belonging to a nT-NBS-LRR class of R proteins. The nT motif is located 68 to 86 amino acids away at the N-terminal region. The centralized NBS domain is located 153 to 460 amino acids away from the N terminal and has all the essential motifs [4,30]: kinase 1a or P-loop (193 to 202 amino acids), kinase 2 (281 to 287 amino acids), and kinase 3a or RNBS-B (307 to315 amino acids), and GLPL (373 to 378 amino acids). There are also 17 imperfect LRR repeats predicted based on the xxLxLxx motif representing most of the 3′ portion of the protein.

Piz-(t) showed a similar expression pattern as *Pi2*. The constitutive expression pattern of *Pi2* and *Piz-t* is quite similar to that of both *Pi-9* [77] and *Pita* [9] but different from *Pib*, which exhibits an induced expression pattern after rice blast inoculation [94].

Pid2

Pid2 on chromosome 6 was first identified in the *indica* cultivar ‘Digu’ [12]. *Pid2* represents a new class of plant resistance genes. Similar to *Pita*, *Pid2* is also a single copy

gene. *Pid2* confers resistance to numerous Chinese blast races. However, there has been no report of resistance to US blast races. *Pid2* encodes a predicted protein of 825 amino acids. The amino acid sequence of the Pid2 protein contains the domain characteristics of receptor-like kinases (RLK), an extracellular domain, a transmembrane (TM) domain, and an intracellular kinase domain. Pid2 also contains a predicted extracellular bulb-type mannose-specific lectin (B-lectin) binding domain that has not been reported in other plant R proteins. The N-terminus of Pid2, amino acids 1–32, contains a hydrophobic region with a predicted transit peptide function. The putative extracellular domain contains two regions with known motifs. First, amino acids 48–16 encode a B-lectin domain (SMART 1e-19) that was predicted to mediate protein–mannose interactions or ligand binding [94]. Additionally, amino acids 337 to 418 are predicted to encode a weak PAN domain (smart e-02) that binds proteins or carbohydrates [12]. The core of the PAN domain in the region of amino acids 337–403 was predicted to be connected with the formation of three disulphide bridges [12]. The TM-spanning region contains 23 hydrophobic amino acids (amino acids 436–458) that are associated with a membrane-spanning helix. The cytoplasmic region contains a predicted serine–threonine kinase with 11 kinase subdomains without the conserved R in subdomain VI, suggesting that Pid2 belongs to the non-RD class of kinases. The Pid2 protein is localized in the plasma membrane. Similar to Pita, a single amino acid at position 441 distinguishes resistant and susceptible alleles of *Pid2*. Both quantitative RT-PCR and northern analysis indicated that *Pid2* is constitutively expressed.

Pi36

Pi36 on chromosome 8 was first identified in the *indica* cultivar ‘Kasalth’ [63]. *Pi36* confers resistance to a wide variety of Chinese blast races (Table 4). *Pi-36* is a

single-copy gene and is more closely related to the barley powdery mildew resistance genes *Mla1* and *Mla6* than to the rice blast *R* genes *Pita*, *Pib*, *Pi-9*, and *Piz-t*. It has not been reported if the gene conveys resistance to any US blast races. The 1056-amino acid sequence of the Pi36 protein has six conserved motifs typical of NBS proteins. The GMGGLGKTT sequence (beginning at residue 206) is the kinase 1a (P loop) consensus, while IVIDDIWD (beginning at residue 286) and GSKILVTTRK (beginning at residue 310) represent the kinase 2 and kinase 3a consensus motifs [30, 90], respectively. Also, GVPLAIITIAS (beginning at residue 372) and LKNCLLYL (beginning at residue 427) represent the conserved NBS domains 2 and 3 consensus motifs [30, 90], respectively. The conserved NBS motif VHD (beginning at residue 501) is similar to the conserved MHD (methionine–histidine–aspartate) motif. The C-terminal region of the protein includes 17 imperfect LRR repeats (residues 578–1056), composed of 15% leucine. The repeats, based on an LxxLxxLxxLxL consensus, vary in length between 22 and 44 amino acids. The CC region contains three perfect hxxhxxh and one hxxhxxx motif (where h represents one of L, I, M, V, or F, and x is any residue). Together, these findings indicate that *Pi36* is a CC–NBS–LRR type *R* gene. Similar to *Pita* and *Pid2*, a single substitution event (Asp to Ser) at residue 590 was considered to be associated with the resistant phenotype. An RT–PCR analysis showed that *Pi-36* is constitutively expressed in 'Kasalath'.

Pi37

Pi37 on chromosome 1 was identified in the rice cultivar 'St. No. 1' [60]. *Pi37* confers resistance to a wide range of Chinese rice blast races but not the Japanese rice blast races (Table 4). No reports were available with regards to the response to US blas

t races. *Pi-37* was considered to be the first representative of a cereal NBS–LRR gene lacking an intron. *Pi37* encodes a cytoplasmic protein with NBS–LRR domains. In the NBS region, two substitutions (V239A and I247M) were shown to be associated with the resistance phenotype. The *Pi-37* open reading frame encoded a 1291-residue polypeptide. The N-terminal section contained three typical NBS family motifs [89], specifically GGAGKS (beginning at residue 222), LLVLDDV (beginning at residue 297), and GSRVLVTSRR (beginning at residue 327). These corresponded to the kinase 1a (P-loop), the kinase 2, and the kinase 3a consensus motifs [30, 90], respectively. The C-terminal region of the protein has 25 irregular LRRs between residues 590 and 1290. The intron positions were highly conserved over long evolutionary periods [80, 81]. Semi-quantitative expression analysis showed that in 'St. No. 1', *Pi-37* was constitutively expressed and only slightly induced by blast infection. Transient expression experiments indicated that the *Pi-37* product was restricted to the cytoplasm.

Pikm

Pikm on chromosome 11 was first identified in the Chinese *japonica* cultivar 'Hokushi Tami' [52]. Complete resistance of *Pikm* requires functions of two family members Pikm1-TS and Pikm2-TS. Pikm1-TS and Pikm2-TS reside adjacently and encode non-TIR NBS-LRR-class proteins. Although Pikm1-TS and Pikm2-TS reside adjacently at the Pikm locus as a cluster, their structures differ. First, they differed in the number and position of introns: both Pikm1-TS and Pikm2-TS contained an intron at the N-terminal side of the sequence encoding the kinase 2 motif in the NBS domain. In addition, Pikm1-TS also contained another intron upstream of the sequence encoding the NBS domain. Second, the Pikm1-TS product contained a C-terminal non-LRR region, however Pikm2-TS did not. Finally, Pikm1-TS contained well conserved repeat units

matching a consensus sequence in its LRR domain, whereas Pikm2-TS did not. All of the above-mentioned structural differences indicate that these two genes did not evolve from one another by a simple duplication event.

Expression of both Pikm1-TSand Pikm2-TS was detected in uninoculated plants. Following blast inoculation, expression of Pikm1-TS increased from 0.5 to 3 days after inoculation (DAI), and declined toward the original level by 5 DAI. Therefore, the induced expression in Pikm1-TS was not detected in the negative control inoculations, indicating that the observed induction of Pikm1-TS expression was due to the challenge of blast infection. In contrast, although expression of Pikm2-TS appeared to increase slightly from 0.5 to 3 DAI, the extent of the induction was relatively minor.

Pi5

Pi5 on the short arm of chromosome 9 was first reported in rice cultivar 'RIL260' [11, 32, 53]. *Pi5* confers resistance to numerous Korean and Philippines blast races [40] (Table 4). Similar to *Pikm*, two members, *Pi5-1* and *Pi5-2* are required for resistance. Both members were predicted to encode an N-terminal CC, a centrally located NB and LRR, and C-terminal regions. Residues 109–576 of *Pi5-1* and 109-567 of *Pi5-2* have an NB domain. The conserved internal domains characteristic of NB-containing *R*-gene products were also identified in *Pi5-1* and *Pi5-2*, including the P-loop, kinase-2, RNBS-B, GLPL, RNBS-D, and MHDV domains. The Pi5-1 and Pi5-2 proteins harbor a unique C terminus that is distinct from those of other NB–LRR proteins [12] and that does not match any known protein motif. The position of introns in the NB domain of the Pi-5 protein was studied to better understand the phylogenetic relationship between *Pi-5* and other cloned rice blast resistance genes. Notably, *Pi5-1* and *Pi5-2* harbor an intron between their RNBS-D and MHDV domains[54]. In addition, *Pi5-1* and *Pi5-2* appear to

have more introns (four and five, respectively) compared to other identified blast *R* genes. The distinctive number of introns and the genomic positions of *Pi5-1* and *Pi5-2* further validate that they belong to the same clade and are different from other NB–LRR genes [54]. Gene expression results indicated that *Pi5-1* transcripts accumulate after pathogen challenge, whereas the *Pi5-2* gene is constitutively expressed [16].

Pit

The *Pit* gene, located on chromosome 1 [36], was initially reported in the Indonesian rice variety 'Tjahaja' [37]. *Pit* confers resistance to a broad spectrum of Japanese blast races [36] (Table 4). The structure of the Pit protein is typical of NBS-LRR proteins. *Pit* contains conserved motifs indicative of an NBS domain, and the putative LRR domain (with 18 imperfect repeats) matches the cytoplasmic LRR consensus sequence. A COILS analysis of the *Pit* protein sequence detected two CC regions, located between the 27th and 54th (maximum probability: 52%), and between the 112th and 147th, amino acid positions (maximum probability: 86%) [64]. In the N-terminal region of the protein, an nT motif located between the 68th and 81st amino acid position was identified between the two CC regions [4]. These results indicate that *Pit* is a CC-NBS-LRR-type *R* gene [103].

Expression of *Pit-K59* and *Pit-Npb* was compared by RT-PCR at 0, 8, 16, 24 and 48 h after inoculation with *M. oryzae* or water (mock inoculation). In 'Nipponbare' leaves, a constitutive but low level of *Pit* expression was detected. . The decline in transcription in K59 was probably not a result of the inoculation, but a result of the experimental conditions (dark treatment with high humidity).

The increased level of *Pit* transcription in K59 compared with 'Nipponbare' was suggested to be due to the LTR retrotransposon Renovator. Renovator is known to contain a promoter in its long terminal repeat (LTR) which enhances expression, in this case of *Pit*. Renovator belongs to a family of Ty1/copia-like retrotransposons classified as rn_44 in the RetrOryza database[37]. Renovator is 5.5- kb long and is composed of two identical 114-bp LTRs, bordered by a 5-bp target site duplication[37].

Many plants have a large number of NBS-LRR-type R gene analogs (RGAs), of which only a few have been assigned functions as disease resistance genes. RGA superfamilies are thought to have been generated by tandem or segmental duplication of ancestral genes during evolution. For an RGA to function as an *R* gene, it must be expressed in an appropriate temporal and spatial manner. Therefore, duplication of the coding sequence alone is not sufficient for multiplying *R* genes. New *R* genes could be generated by duplication of a transcriptionally active *R* gene as a unit, including transcriptional regulatory sequences as well as coding sequences, followed by sequence diversification.

Another mechanism could be the transcriptional activation of otherwise transcriptionally-inactive RGAs through acquisition of promoter sequences. It was reported that *Pit* was created as a result of transcriptional activation of an inactive sleeping RGA [36]. The functional *Pit* allele was formed as a result of insertion of Renovator upstream of the *Pit* sequence. The acquisition of promoter sequences therefore seems to be a general mechanism that generates *R* genes.

Pid3

Pid3 was first reported in *indica* variety 'Digu' [84]**.** The gene is known to confer resistance to *indica* and *japonica* races collected from China (Table 4). There is no report if this gene confers resistance to US blast races. The *Pid3* gene encodes a 924-amino-acid polypeptide that contains a conserved NBS domain in positions 158–466 from the translation initiation site. The NBS domain has four sequence motifs, GMGGIGKTA (positions 202–210), KRYVLVLDDVW (positions 280–290), IGRIILTSRNYDV (positions 307–319), and GLPIAI (positions 373–378), corresponding to kinase 1a (p-loop), kinase 2, kinase 3a, and GLPL motifs, respectively. At the C terminus is the LRR region that comprises 13 imperfect LRR repeats. The MHD motif, MHDILRV (positions 502–508), and the NBS–LRR linker motif, EQNFCIVVNHS (positions 516–526), are present between the NBS domain and the LRR region. At the N terminus, there is a conserved motif, RSLALSIEDVVD (positions 78–89), but no TIR or coiled-coil motif is found. Expression studies indicated that the gene was constitutively expressed[84].

pi21

The *pi21* locus was originally identified as a major QTL that was mapped on chromosome 4 [24,59]. The resistant *pi21* allele was first identified in the *japonica* cultivar 'Owarihatamopchi'. This recessive *pi21* gene is known to confer non-race-specific resistance. The dominant *Pi21* gene encodes a proline-rich protein that has a putative heavy metal binding domain and putative protein- protein interaction motifs. Wild type *pi21* appears to slow down defense responses [24]. However, deletions in the proline-rich motif inhibit slowing of defense responses. It was reported that the deletion was in 18-and 48-bp sequences and the resistant *pi21* allele carrying the 18 and 48 bp deletion was only observed in a *japonica* cultivar. Hence it was hypothesized that the

deletion of both the 18- and 48-bp sequences resulted in a defect of the *pi21* function, which represents the consensus sequence motif PxxPxxP, the core motif for protein-protein interaction in multicellular organisms [24]. The proline rich motifs (PRMs) were thought to be associated with host defense, possibly through competitive inhibition of protein-protein interaction of the proline rich motif and its counterpart [57]. The PRMs contain several proline residues, most of which are organized in repeats of three. The heavy metal transport/detoxification protein domain has two conserved cysteines involved in metal binding. Although it conferred resistance to blast, the *pi21* allele was associated with poor flavor in cooked rice, and thus was not used in commercial cultivars. Expression results indicated that the gene is not constitutively expressed, but dependent on stress factors including humidity [24].

Pb1

The panicle resistance blast 1 gene (*Pb1*) was derived from the *indica* cultivar 'Modan' [35]. *Pb1* is located in the middle of the long arm of chromosome 11 [22, 23]. The gene confers resistance to a wide variety of Japanese blast races and a few races from Indonesia, China, the Philippines, Brazil and Thailand. There is no report of its resistance to US blast races (Table 4). The gene is partially resistant to leaf blast but is more efficient in conferring resistance to panicle blast, and resistance is quantitative.

Pb1 has two putative CC domains, CC1 and CC2, located in its N-terminus, with an nT motif-like sequence intervening them. The COILS analysis [62] did not consider these sequences as CC domains, and the periodical occurrence of leucine, or other hydrophobic amino acids, was observed in these regions. In addition, this region shared amino acid sequence similarity with CC domains of other CC-NBS-LRR proteins,

including barley MLA10 [31] and Arabidopsis RPM1 [30]. In many *R* proteins, a pentapeptide EDVID motif is located within the nT motif [4]. The EDVID motif is associated with intramolecular interaction with other parts of CC-NBS-LRR R proteins, and is needed for inducing the hypersensitive response (HR) phenotype [78]. This motif is degenerated in *Pb1* [35] so it is likely not functionally conserved in *Pb1*. In many R proteins the pentapeptide EDVID motif is conserved within the nt motif [4]. Adjacent to the CC region is the NBS domain-like region, followed by an LRR domain consisting of 14 imperfect leucine-rich repeats (residues 928–1296). The Pb1 protein differed from the previously reported R proteins, particularly in the NBS domain, which is different from the typical NBS-LRR proteins because it lacks the P loop. A long stretch of peptides with no significant sequence similarity to other sequences is located after the CC region, and a walker-like sequence is present at amino acid position 641. The NBS domain also has RNBS-B and GLPL motif-like sequences. The RNBS-D and MHD motifs near the C-terminal ends of the NBS domain, which transduce pathogen perception by LRR into R protein activation [64], are highly conserved in the Pb1 and R protein [35]. Therefore, the NBS domain of *Pb1* is homologous to those in *R* proteins in its C-terminal region, but its homology becomes weaker towards the N-terminus. Hayashi et al [35] reported that the local genome duplication of a 60-kb region placed a promoter sequence just upstream of a transcriptionally inactive 'sleeping' RGA, resulting in activation of the RGA and generation of *Pb1*. The structure of the *Pb1* locus indicates that the coding and the promoter sequences were located at the 5' and 3'-termini, respectively, of the ancestral 60-kb region before the duplication occurred. The genome duplication at this specific site was therefore critical for the generation of *Pb1*. The acquisition of promoter sequences

therefore seems to be a general mechanism that generates *R* genes, and is of particular importance in the case of *Pb1*. The characteristic temporal and spatial pattern of *Pb1* promoter activity is likely to be one of key factors contributing to the durability of its resistance and therefore its practical usefulness as a panicle resistance gene [35].

R-AVR Recognition

A simple explanation of gene for gene interaction is when products of both *R* and *AVR* interact directly [42]. The Pita/AVR-Pita interaction has been the only well characterized *R /AVR* interaction demonstrated in the rice blast system to date. Transient expression in rice cells of the *Pita* gene together with *AVR-Pita* induces a resistance response. The AVR-Pita$_{176}$ protein was demonstrated to bind specifically to the LRD of the Pita protein, both in the yeast two-hybrid system and in an *in vitro* binding assay, suggesting that the AVR-Pita$_{176}$ protein binds directly to the Pita LRD region inside the plant cell to initiate a *Pita*-mediated defense response [41, 9]. Identification of genes downstream of *R* genes has become critical for understanding the R–AVR interaction pathway. The absence of efficient NSB-LRR R protein - protein assay, gene function redundancy and lethality when mutagenesis is used all contribute to an inefficient R-AVR recognition. Using a mutagenesis approach, a *Pita*-susceptible mutant referred to as *Ptr(t)* was identified which was required for *Pita* resistance [48, 49]. *Ptr(t)* is probably specific to *Pita*-mediated signal recognition because it is not required by other R genes. Genetic analysis results showed that *Ptr(t)* segregated as a single dominant nuclear gene linked with *Pita*. Genetic analysis also revealed that *Pita* and *Ptr(t)* genes are located in a nine megabase region. Cloning this gene will improve understanding of Pita-mediated signal recognition and transduction.

AVR proteins serve as R-protein associated effectors that activate host defense responses. However, AVR proteins are involved in pathogenesis and pathogen fitness, and have provided useful information in understanding the co-evolution between fungal effectors and host *R* proteins. Thus far, 40 AVR genes of *M. oryzae* have been identified [65], nine of which have been cloned (Table 2). Effectors are protein molecules secreted by the pathogen and commonly located in unstable genomic regions. Multiple genetic mutation events, like deletion [75], point mutations [75], and transposon insertion [46], have been found to be main driving forces in the creation of new virulent races that break major *R* genes. Orbach et al [75] found that a fragment deletion from intron 3 to exon 4 in the *avr-pita*$^{-}$ mutant strain CP983; several nonsense mutations (e.g. TGG1487TAG in the mutant strain CP918 and TTA1736TGA in the mutant strain CP1615); and a missense mutation (e.g. GAA1718GGA in the mutant strain CP1635) increased the virulence of the *AvrPita* gene. In addition, transposon insertion usually leads to loss of function of *Avr* genes in the pathogen through a change in gene expression level or pattern. Fudal et al [21] found that an insertion of a 1.9-kb MINE retrotransposon in the last *ACE1* exon led to a loss of *ACE1* avirulence and activated its virulence in the virulent isolate 2/0/3. Similar events were also reported in *AvrPita* [102] and *AvrPiz* [58]. So far, *Pi-ta* and *AVR-Pita* are still the only pair of *R* and *AVR* genes that are well characterized. With more matched *R* and *AVR* genes cloned in the future, the recognition mechanisms of blast *R* genes can be further examined.

Co-evolution of *R* and *AVR* genes

M. oryzae is highly variable and can overcome deployed resistant cultivars in a very short time. The ability to overcome the resistance of one single gene is due to the

instability of *AVR* in *M. oryzae. Pita*, *Pid2*, *Pi36* are single copy genes with a single amino acid determining resistance specificity. Other blast *R* genes are members of small gene families that can evolve specificity during unequal crossing over. Most cloned blast *R* genes are predicted to encode highly similar cytoplasmic proteins with NBS-LRR domains [6]. *AVR* genes encode effector molecules which favor disease development and are under constant selection. The fundamental question for co-evolution is how *R* genes have evolved the ability to detect pathogen signals (Fig.4). Only a few studies are available due to the limited number of cloned, matched pairs of *Pi* and *AVR* genes. One of the best examples is the *Pita* and *AVR-Pita* interaction [42]. There are two predominant hypotheses for the co-evolution of *R* and *AVR* genes in plants. The ARMS race hypothesis predicts that both *R* and *AVR* genes are under diversified selection. The trench warfare hypothesis suggests that either *R* or *AVR* is under balanced selection. The Pi-ta/AVR Pi-ta interaction does not support the arms race hypothesis. *Pita* is located near the centromere, a region that is relatively stable [9]. The region is considered to be stable because it is a region that embeds fewer active genes than other regions of the chromosome. A transposon was reported at the promoter region of the *Pita* gene. Similarly, another blast *R* gene *Pit* was demonstrated to be activated by another transposon in the promoter region [37, 46]. Both cases led to a hypothesis that transposons possibly could play a positive role in regulating blast *R* genes. It may be suggested that *Pita* engages in 'trench warfare' with *AVR-Pita* where both genes have their strategies to prevent or cause disease. Additionally, *Pita* encodes 12 distinct putative products between 315 and 1033 amino acids that can function as resistance proteins [13], suggesting that posttranscriptional modification through exon skipping and alternative

splicing can play an important role in evolution of a blast *R* gene. The same linkage block (5.4-27 Mbp) was consistently found in resistant rice cultivars that carry *Pi-ta,* and possibly suggests that additional plant components needed for *Pita*-mediated resistance are clustered within a small genomic interval [41]. This is consistent with historical observations that genetic factors responsible for resistance to different pathogens are found in small genetic intervals [41]. Together, we can suggest that there are several critical components of resistance and all are important in *R* gene evolution.

In contrast, despite a lack of convincing experimental evidence, *AVR* gene products are predicted to be involved in promoting pathogen virulence and fitness. Diversification of *AVR* genes is considered to be one of the strategies that the pathogen can develop for survival [42]. Diversity can arise from partial and complete deletions, frame-shift mutations, or transposon insertions. These mutational events have been found in *AVR-Pita* and *AVR-Co39* [101, 17]. Further molecular characterization of matched pairs of blast *Pi* and *AVR* genes should help determine if these genomic rearrangements are key strategies that the pathogen has evolved in overcoming host resistance.

Strategies for preventing blast disease

R gene-mediated resistance has several attractive features for disease control. When induced in a timely manner, the responses can efficiently stop pathogen growth with minimal collateral damage to the plant. No input is required from the farmer and there are no adverse environmental effects. One approach is to sow a mixture of lines each expressing a different *R* gene(s) in the same field. A susceptible line can be included in the mixture to reduce the selection pressure for mutations in *Avr* genes [73].

A multiline protocol was tested with striking success experimentally [101], however, multiline resistance has not been widely accepted because of logistic difficulties in deployment. Many *R* genes lack durability because they can be defeated by a single loss-of-function mutation in the corresponding *Avr* gene (thereby rendering the pathogen 'invisible' to host tissue). Because individual *Avr* genes often make only incremental contributions to virulence, pathogens can alter or discard an *Avr* gene with little or no fitness penalty [53]. Traditional breeding strategies have used *R* genes 'one at a time' in crop monocultures. Such homogeneous host populations exert strong selection for mutation of the relevant *Avr* gene, and then become extremely vulnerable to the emergent pathogen. As an alternative to single-gene deployment, multiple *R* genes ('pyramids') can be bred into individual plant lines [76]. In reality, these pyramids require the pathogen to accumulate mutations in multiple *Avr* genes to escape detection, which is not likely to occur if the mutations have a strong cumulative effect on virulence. In summary, stacking major blast *R* genes in an elite rice cultivar has been the most commonly attempted strategy for rice crop protection [86].

Future perspectives

Although understanding plant diseases and their control strategies has been investigated in great detail, the global food supply is still suffering severe losses incurred by a multitude of pathogens and pests [71,97]. Plant diseases are known to reduce crop yield severely and the impact of disease is more severe in developing nations.

R genes encode putative receptors that respond to the products of *AVR* genes expressed by the pathogen during infection. In many cases, a single *R* gene can provide complete resistance to one or more strains of particular pathogen [51], when transferred

to a previously susceptible plant of the same species. *R* genes have been used in regional conventional resistance breeding programs for decades [76]. However, plants do not have the benefit of a circulating antibody system like animals so plant cells autonomously maintain constant monitoring against pathogens by expressing large arrays of *R* genes [15, 43]. Much effort in the future will be engaged towards understanding innate resistance mechanisms in order to develop innate immunity in plants.

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Table 1. Summary of chromosomal location, copy number, predicted product and expression of cloned rice blast resistance genes.

***R* gene cloned**	**Chromosome Number**	**Copy Number**	**Protein Type**	**Localization**	**Expression**	**Reference**
Pib	2	multiple	CC-NBS-LRR		Circadian, inducible by stress	*Plant J.* **19** (1999), pp. 55–64.
Pi-ta	12	1	CC-NBS-LRR	Cytoplasm	Constitutive	*Plant Cell* **12** (2000), pp. 2033–2045.
Pi9	6	multiple	CC-NBS-LRR		Constitutive	*Genetics* **172** (2006), pp. 1901–1914.
Pi2/Piz-t	6	multiple	CC-NBS-LRR		Constitutive	*Mol. Plant Microbe Interact.* **19** (2006), pp. 1216–1228.
Pi-d^2	6	1	Receptor kinase/B lectin	Membrane	Constitutive	*Plant J.* **46** (2006), pp. 794–804.
Pi36	8	1	CC-NBS-LRR		Constitutive	*Genetics* **176** (2007), pp. 2541–2549.
Pi37	1	multiple	CC-NBS-LRR	Cytoplasm	Constitutive	*Genetics* **177** (2007), pp. 1871–1880.
Pikm	11	multiple	CC-NBS-LRR		Constitutive	*Genetics* **180** (2008), pp. 2267–2276.
Pi5	9	multiple	CC-NBS-LRR		*Pi5-1* is pathogen dependent *Pi5-2* is constitutive	*Genetics* **181** (2009), pp. 1627–1638.
Pit	1	multiple	CC-NBS-LRR		Transcriptionally inactive	*Plant J.* **57** (2009), pp. 413–425
Pid3		multiple	CC-NBS-LRR		Constitutive	*Genetics* **182** (2009), pp. 1303–1311
pi21	4	multiple	Proline con-taining protein /CC-NBS-LRR	Cytoplasm	Inducible by stress	*Science.***325** (2009), pp. 998-1001
Pb1	11	multiple	CC-NBS-LRR		Transcriptionally inactive	*Plant J.* **64** (2010), pp. 498–510

Table 2. Summary of the corresponding *R* gene and predicted product of cloned *Magnaporthe oryzae AVR* gene.

***AVR* gene cloned**	***R* gene**	**Encoding protein**	**Reference**
PWL1		Glycine rich, hydrophilic protein, secreted protein	*Mol. Plant Microbe Interact.* **8** (1995), pp. 939–948; *Plant Cell*, **7** (1995), pp.1221-1233
PWL2		Glycine rich, hydrophilic protein, secreted protein	*Mol. Plant Microbe Interact.* **8** (1995), pp. 939–948; *Plant Cell*, **7** (1995), pp.1221-1233
Avr Pi-ta	*Pi-ta*	Secreted protein	*Plant Cell*. **12**(2000), pp.2019-2032
Avr1-CO39	*Pi-CO39*	Secreted protein	*Genomics of Disease.* (2008), pp199-216
ACE1	*Pi33*	Polyketide synthase/peptide synthetase	*Plant Cell.* **16**(2004), pp.2499- 2513.
AvrPiz-t	*Piz-t*	Secreted protein	*Mol. Plant Microbe Interact.* **22** (2009), pp.411-420.
AvrPia	*Pia*	Secreted protein	*Mol. Plant Pathology*.**10** (2009), pp.361–374; *The Plant Cell* **21**(2009), pp. 1573-1591.
AvrPii	*Pii*	Secreted protein	*The Plant Cell* **21**(2009), pp. 1573-1591.
AvrPik/km/kp	*Pik/km/kp*	Secreted protein	*The Plant Cell* **21**(2009), pp.1573-1591

Table 3. Mapped blast resistance genes on known chromosomal locations using DNA markers.

Gene	**Chromosome**	**DNA marker***	**Rice Germplasm**	**Reference**
IPi	12			*Genetics* **138** (1994), pp. 1251-1274.
IPi3	12			*Genetics* **138** (1994), pp. 1251-1274.
PiI5	9	RM316	GA25	*Acta Bot. Sin.* **45** (2003), pp. 871-877; *Plant Breed.***126** (2007b), pp. 287-290.
Pi24	1	RGA3620, RG241A, N7520-600	Azucena	*Theor. Appl. Genet.* **106** (2003)**,** pp. 794-803.
Pi25(t)	2	RM21	IR64	*Theor. Appl. Genet.* **106** (2003), pp. 794-803.
Pi33	8	RM72,RM331,RM404,RM483	IR64	*Theor. Appl. Genet.***107** (2003), pp. 1139-1147.
Pi34	11	RM21, RM5961	Chubu32	*Theor. Appl. Genet.* **104** (2002), pp. 547-552.
Pi39	12	RM 247,RM463	Q15	*Genetics.* 176 (2007a)**,** pp. 2541-2549.
Pi42	9	RM2529,RM1337		*Mol. Plant Microbe Int.* (2008) Q. Pan, unpublished .
Pi42(t)	8	RM310,RM72	Zhe733	*Mol. Breed.* **24** (2009), pp. 127-134.
Pi43(t)	11	RM224,RM1233	Zhe733	*Mol. Breed.* **24** (2009), pp.127-134.

Pi47(t)	11	RM206,RM224	Xiangzi 3150	*Phytopathology*.[Online] 2010.http://apsjournals.apsnet.org/doi/abs/10.1094/PHYTO-08-10-02099 (accessed January 16th 2011).
Pi48(t)	12	RM5364,RM7102	Xiangzi 3150	*Phytopathology*.[Online] 2010. http://apsjournals.apsnet.org/doi/abs/10.1094/PHYTO-08-10-02099 (accessed January 16th 2011).
Pi-z *[Piz(t)*]*	6	RM 527,AP5659-1,AP5659-3,AP5659-5,AP4791	Zenith	*Mol. Breed.* **17**, (2006), 149-157.
Pigm(t)	6		Gumei4	*Bull. Natl. Inst. Agric. Sci.* **21**(1970), pp.61-71.
Pik	11	L198, R1506, RM144, RM224	K60	Huang, unpublished; *Rice Genet. Newsl*. **22**(2005)**,** pp.76-77.
Pix(t)	2	NA	Nd	*Rice Genet. Newsl*. **22**(2005)**,** pp.76-77.
Pi-yl(t)	2	RM3248(0.8),RM208 (0.8)	Yanxian1	*Rice Genet. Newsl*. **22**(2005)**,** pp.76-77.
Pi-y2(t)	2	RM3248(1.3), RM208 (1.7)	Yanxian1	*Rice Genet. Newsl*. **22**(2005)**,** pp.76-77.
Pi22	6		Suweon 365	*Breeding and ecology.* (1997) Pages 435-436 in: 8th SABRAO Congr. Annu. Meet. Korean Breed. Soc.
Pi23	5		Suweon 365	*Breeding and ecology.* (1997) Pages 435-436 in: 8th SABRAO Congr. Annu. Meet. Korean Breed. Soc.
Pi35(t)	1	RM1216,RM1003	Hokkai 188	*Theor. Appl. Genet*. **113**(2006), pp.697-704.
Pif	11		St No.1	*Bull. Chugoku Natl. Agric. Exp. Stn.* **E6** (1970), pp.1-19.

Pikur1	4	Kuroka	*Bull. Chugoku Natl. Agric. Exp. Stn.* **A20** (1971), pp. 21-25. (In Japanese)
Pikur2	11	Kuroka	*Ann. Phytopathol. Soc. Jpn.* **54**(1988), pp. 460-465. (In Japanese, English abstract)
Pise1	11	Sensho	*Ann. Phytopathol. Soc. Jpn.* **36**(1970), pp. 304-312.

Table 4. Resistance spectrum of the cloned blast resistance gene to known *Magnaporthe oryzae* races.

R Gene	**US blast races***	**International blast races**
Pib	*IB1, IB45, IH1, IG1, IC17, IE1 and IE1K*	*003.0*
Pi-ta	*IB1, IB49, IB54, IB45, IH1, IG1, IC17and IE1*	
Pi9	*IC17*	*PH9,36B23,86061ZE39,97-4-1,95116AZ93,75-49,97-51,CHNOS,95097AZC13,87088ZE3,86062ZB15,CP16-32,R01-1,KJ201,ML25,ML8,O-249,DB-24,GUY11 and ES6*
Pi2/Piz-t	*IH1, IG1, IC17, IE1 and IE1K*	*KJ201,81278ZB15,G2,CHE86061,G2,G11,G15,CHNOS60-2-3 and ROR1*
Pi-d2		*ZB15*
Pi36		*CHL39 and CHL273*
Pi37		*CHL1159*
Pikm		*P2-b and Kyu92-22*

Pi5	*PO6-6,KJ105a,KJ107,KJ401,R01-1and K1215*
Pit	*007.0 and 777.3*
Pid3	*Zhong-10-8-14*
pi21	*007.0*
Pb1	*003.0 and MAFF101506*

Figure Legends

Fig.1. Rice blast disease. Germinated asexual conidium (A), Sporulated mycelia on disease lesion (B), typical symptom of leaf blast disease (C) and mature rice plants with panicle blast and leaf blast in a rice field (D). Photographs by Dr. Yulin Jia.

Fig.2. Phylogenetic tree of cloned blast *R* gene constructed using Vector NTI, using genomic sequence (A) and coding region (B).

Fig. 3. Schematic presentation of cloned blast resistance gene showing functional motifs.

Fig. 4. Molecular mechanisms of blast *R* gene mediated responses in compatible and incompatible interactions.

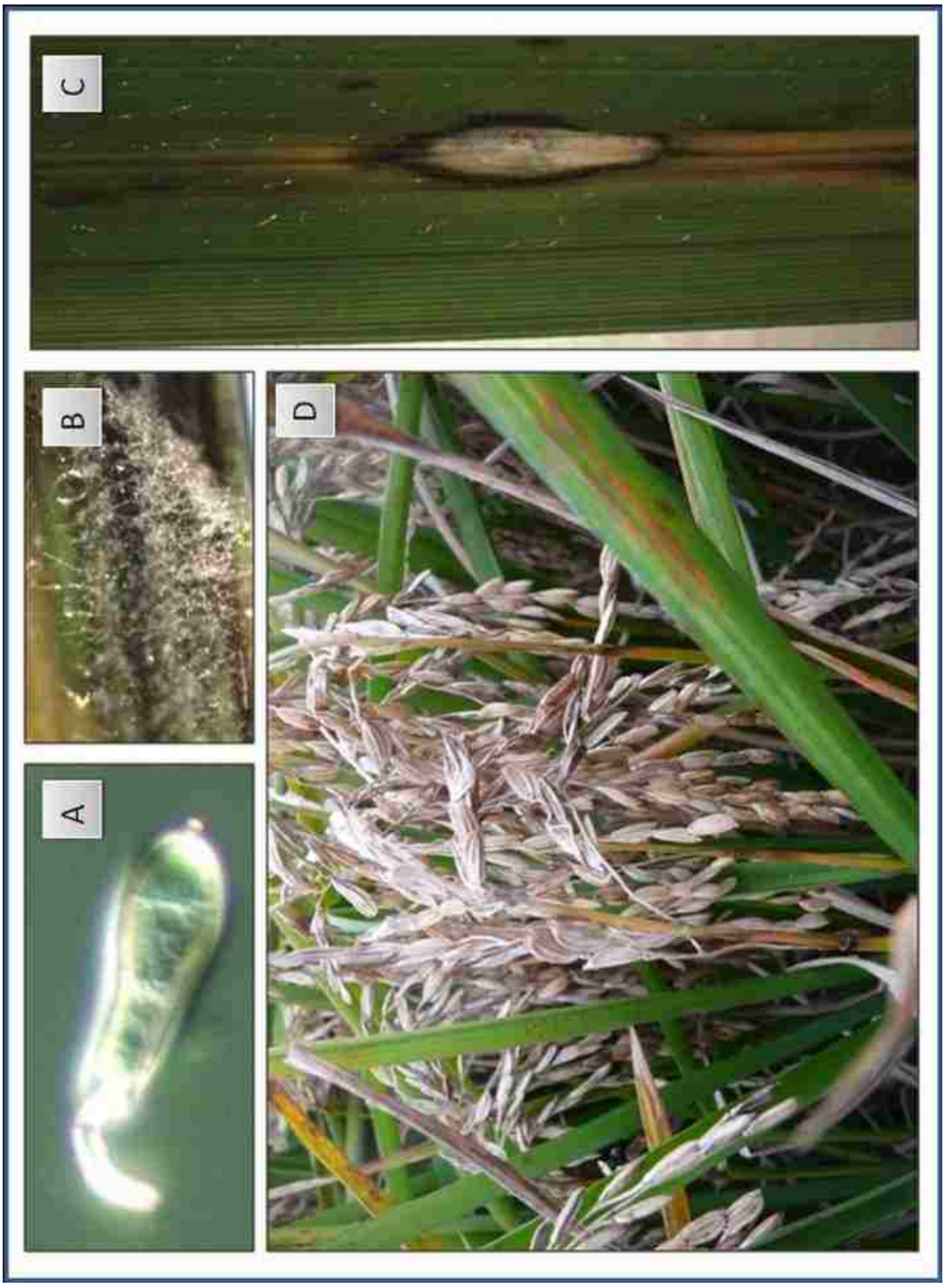

Fig. 1

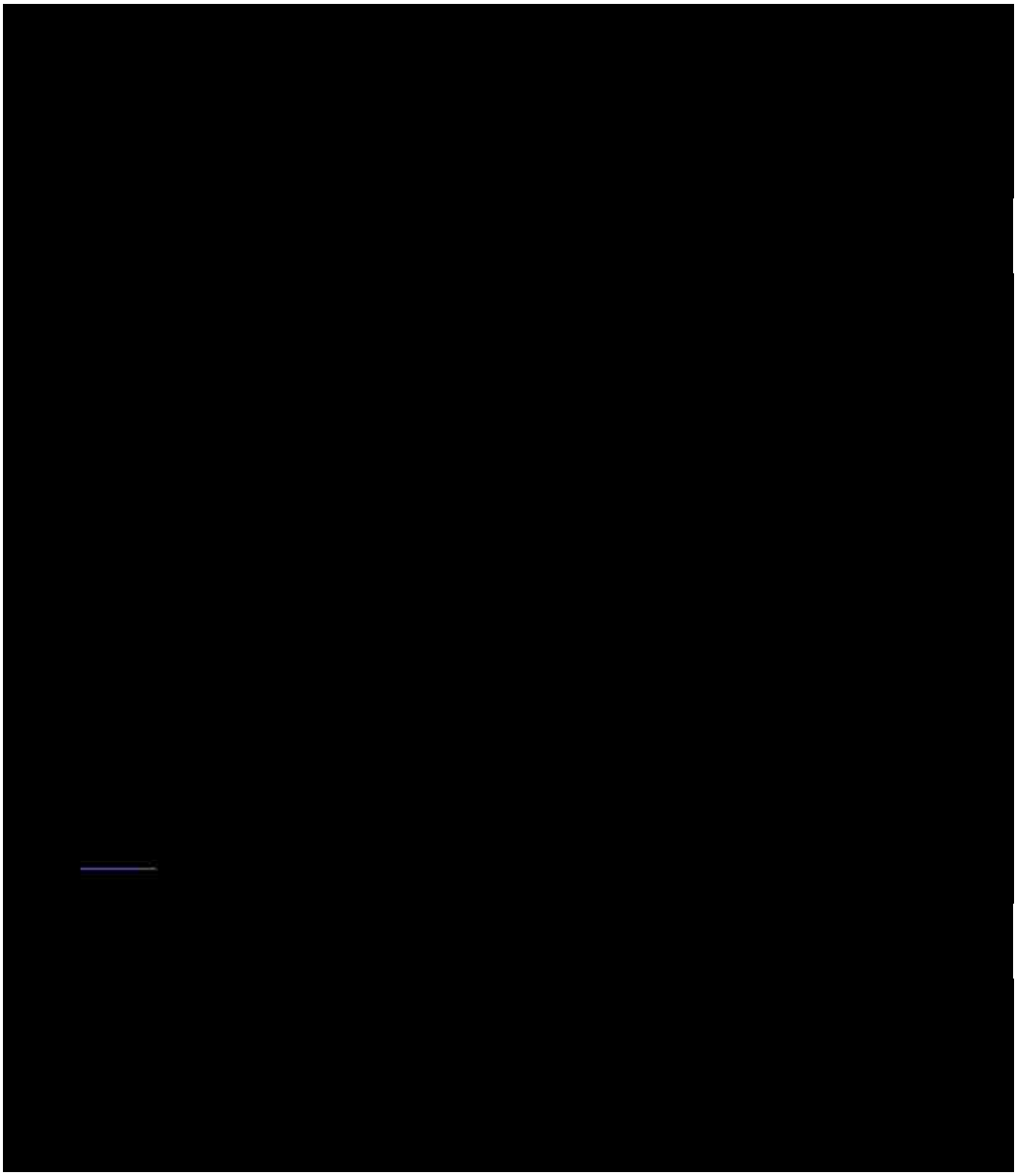

Fig. 2

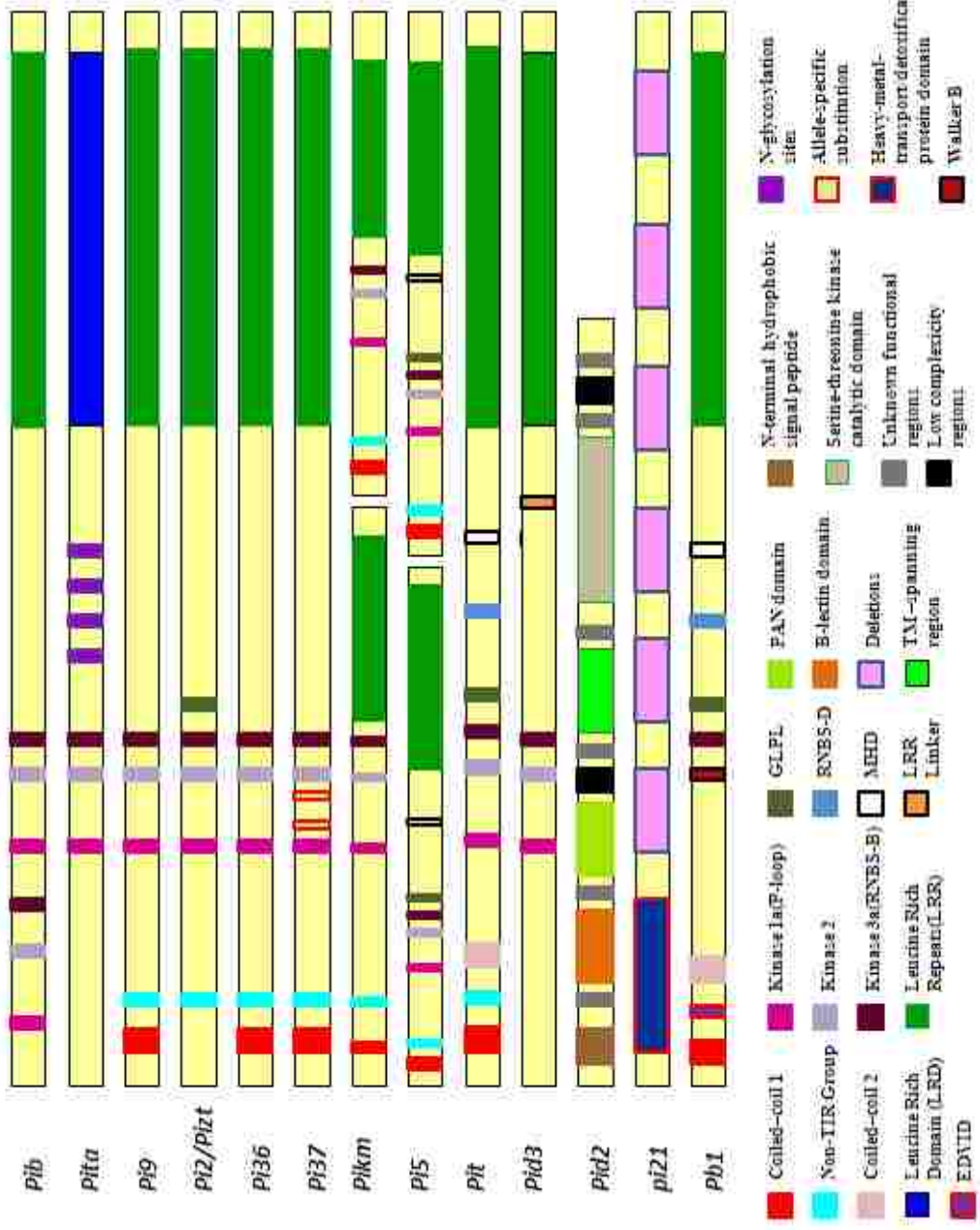
Pib
Pita
Pi9
Pi2/Piz-t
Pi36
Pi37
Pikm
Pi5
Pit
Pid3
Pid2
pi21
Pb1
Coiled-coil 1
Non-TIR Group
Coiled-coil 2
Leucine Rich Domain (LRD)
EDVID
Kinase-1a(P-loop)
Kinase 2
Kinase 3a(RNBS-B)
Leucine Rich Repeat(LRR)
GLPL
RNBS-D
MHD
LRR Linker
PAN domain
B-lectin domain
Deletions
TM-spanning region
N-terminal hydrophobic signal peptide
Serine-threonine kinase catalytic domain
Unknown functional region
Low complexity region
N-glycosylation site
Allele-specific substitution
Heavy-metal-transport detoxification protein domain
Walker B

Fig. 3

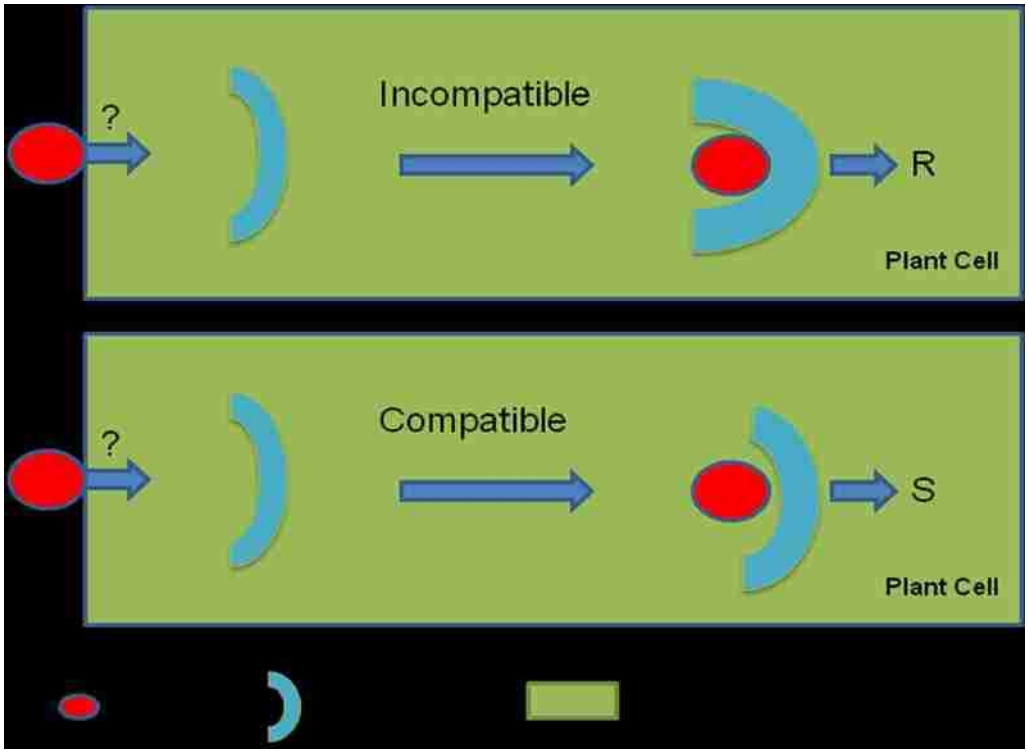

Fig. 4

Analysis of rice blast resistance gene *Pi-z* in rice germplasm using pathogenicity assays and DNA markers

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Keywords IB49• IB33• IE1k• *Magnaporthe oryzae*• *Pi-z* •SSR markers

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Abbreviations

AVR Avirulent

D Day

MAS Marker Assisted Selection

R Resistance

SSR Simple Sequence Repeat

VIR Virulent

Abstract

The *Pi-z* gene in rice confers resistance to a wide range of races of the rice blast pathogen, *Magnaporthe oryzae*. The objective of this study was to characterize *Pi-z* in 111 rice germplasm accessions using DNA markers and pathogenicity assays. The existence of *Pi-z* in rice germplasm accessions was detected by the presence of four simple sequence repeat (SSR) markers (RM527, AP4791, AP5659-1, AP5659-5) closely linked to *Pi-z* , and was verified using pathogenicity assays with an AVR strain (IE1k) and two virulent races (IB33 and IB49). Among 111 germplasm accessions evaluated, 73 were predicted to possibly contain the *Pi-z* gene using both SSR markers and pathogenicity assays. The remaining 38 germplasm accessions responded inconsistently to the blast races IB33, IEIk and IB49 with expected SSR marker alleles, suggesting the presence of unexpected SSR alleles and additional *R* gene(s). The germplasm characterized in this study can be used to expand the fundamental understanding of resistance to blast and for marker-assisted breeding to improve blast resistance.

Introduction

Rice blast, caused by the filamentous ascomycete fungus *Magnaporthe oryzae* (formerly *Magnaporthe grisea*), threatens rice production worldwide. Genetic resistance in rice to *M. oryzae* follows a typical gene-for-gene model, in which a resistance (*R*) gene prevents infection by races of *M. oryzae* containing the corresponding avirulence (*AVR*) gene (Silue et al. 1992). Resistant cultivars, fungicides, suitable planting dates, optimum fertilizer applications and adequate flood depth are useful tools to manage the disease (Bonman 1992; Lee 1994). Among these management options, the utilization of *R* genes is the most economical and environmentally benign method for control of this disease.

Thus far, more than 80 race-specific *R* genes to *M. oryzae* have been identified, and some of them have been cloned and designated for marker-assisted selection (MAS) (Yu et al. 1991; McCouch et al. 1994; Godwa et al. 2003; Ballini et al. 2008; Jia et al. 2009).

Among the known blast *R* genes, the *Pi-z* gene, first identified by Kiyosawa (1967) in the medium grain cultivar 'Zenith', has been effectively introgressed into numerous rice cultivars to prevent infection by a wide range of races of the rice blast pathogen. *Pi-z* is usually found in tropical *japonica* medium grain rice cultivars in the U.S. (Fjellstrom et al. 2006) and confers resistance to five U.S. races of blast (IH-1, IG-1, IC-17, IE-1 and IE-1k), as well as susceptibility to two races (IB49 and IB33). Historically, the AVR race IE1k and the virulent races IB49 and IB33 have been used to determine the presence of *Pi-z* in rice germplasm (Marchetti et al. 1987; Conaway-Bormans et al. 2003). The *Pi9/2/zt* complex mapped at the same chromosomal location of *Pi-z(t)* was recently characterized (Liu et al. 2002; Zhou et al. 2006), and thus "t" is removed from *Pi-z(t)* throughout this manuscript. Four simple sequence repeat (SSR) markers associated with the *Pi-z* gene have been identified and recommended for use in germplasm characterization and MAS in the U.S. (Fjellstrom et al. 2006).

Recently, MAS has become one of the most commonly used methods in breeding for improved resistance to rice blast (Jia 2003). For MAS, selections are based on DNA markers closely linked to a blast *R* gene that confers resistance to a particular race of the pathogen. MAS can be used to screen seeds or seedlings under laboratory conditions, which is much faster than traditional pathogenicity assays in which accurate selection can only be made during later stages of plant growth. In addition, MAS can avoid the overlapping effects of other matched pairs of *R* and *AVR* genes. However, disagreements

between markers and disease reactions can occur in some breeding lines due to different genetic backgrounds and/or potential recombination events between markers and trait.

Accurate identification of a particular *R* gene in diverse elite germplasm using DNA markers and differential blast races is an essential step for ensuring the accuracy of *R* gene utilization in using MAS for different rice breeding programs. Keeping this is mind the following

objectives were developed. 1) to identify the *Pi-z* gene in 111 rice germplasm accessions with SSR markers closely linked to the *Pi-z* gene; 2) to determine disease reactions of these germplasm accessions to differential U.S. blast races; and 3) to identify a better AVR race for detecting the *Pi-z* for conventional breeding. These three objectives should assist the accurate identification of *Pi-z* in diverse elite germplasm accessions from all over the world.

Material and Methods

Plant Materials

A total of 111 rice accessions were identified from a USDA core collection that consists of 1790 accessions from 113 countries representing an estimated 70% of the genetic diversity of the entire USDA collection (Yan et al. 2007) utilizing the most closely linked SSR marker AP5659-1to *Pi –z(t).* One gram of seed from each accession was provided by the Genetic Stock Collections of *Oryza* at the Dale Bumpers National Rice Research Center (DB NRRC). Four rice cultivars from the Molecular Plant Pathology Program at the DB NRRC - ‘Bengal’ (PI 561735), ‘Jefferson’ (PI 593892)[+*Pi-z*], ‘Wells’ (PI 612439), and ‘Zhe733’ (PI 629016) [-*Pi-z*] - were used as controls. Eight seeds of each accession were germinated in 96 well inserts (10 x 20 x 2

cm), (Hummert International, Missouri USA). Prior to seeding, the inserts were placed in trays (26.67 x 53.34 x 6.35[in cm], Model # INT0804, Hummert International, Missouri, USA), filled with silt loam soil (pH 5.5 – 5.8) fertilized with Osmocote Pro 15-9-12 (Scotts-Sierra Horticultural Products Company, OH), autoclaved, and stored at -20 ^{0}C for three days. The trays were completely filled with water. Rice plants were grown for 3 weeks in the greenhouse maintained at 23 -29 ^{0}C during the day in winter (November to April) and 29-32 ^{0}C in summer (May to October) and 22-25 ^{0}C during the night all year long until the 3 to 4 leaf stage, in preparation for pathogenicity assays and subsequent DNA extraction.

Pathogenicity assays

Pathogenicity assays were performed on 111 germplasm accessions and four control germplasm accessions. *M. oryzae* isolates, an avirulent (AVR) isolate ZN61 (race IB49), virulent (VIR) isolates TM2 (race IE-1k) and FL9 (race IB33) were selected for pathogenicity assays. There were four replicates for each germplasm accession. The *Pi-z* gene could be verified by the pattern of resistance or susceptibility to a pair of *AVR* and *VIR* races. Pathogen inoculation was performed using a modified procedure based on Valent et al (1991). Briefly, plants were inoculated with 40 ml of a spore suspension (5 X 10^5 spores/ml, 0.25% gelatin) using a hand atomizer connected to an air compressor (100 kPa). Inoculated plants were maintained at approximately 95% relative humidity in clear polyethylene autoclave bags (24 x 36 cm) at room temperature (Product code 018143 Fisher Scientific, USA). Approximately 24 h after inoculation, plants were moved to the greenhouse for an additional 6 d. Disease reactions were assessed 7 d after inoculation

using a visual rating scale (Fig. 1). For each accession, 7 to 8 seedlings were evaluated, and each pathogenicity assay was conducted three times.

DNA extraction

DNA was extracted using a rapid DNA extraction procedure (Xin et al. 2003) from each of four replicates for further analysis. After extraction, sample DNAs were prepared for PCR through a Biomek 2000 Lab Automation Work Station (Beckman and Coulter, Brea, CA) using manufacturer protocols.

SSR marker selection

Five SSR markers previously mapped to the *Pi-z* locus were used for marker selection (Fig. 2; Table 2, Fjellstrom et al. 2006). Four SSR markers, AP5659-1, AP5659-5, RM527 and AP4791, were selected for the present study:

1) AP5659-1 displays unique marker alleles (220 nt) in germplasm accessions carrying the *Pi-z* gene (Fjellstrom et al. 2006);

2) AP5659-5 has a 279 nt allele for all germplasm accessions with *Pi-z*, although this also was found in an accession carrying *Pi-9*, another *R* allele at the *Pi-z* locus (Liu et al. 2002).

3) RM 527 with a 217 nt allele was found in all *Pi-z* germplasm accessions but has also been found in germplasm accessions not carrying *Pi-z*;

4) AP4791 is another marker that can be used to detect association with *Pi-z* (Fjellstrom et al. 2006); and

Another marker AP5659-3 was reported to co-segregate with the *Pi-z* resistance gene, and considered the most closely linked to *Pi-z* of those identified to date. However,

this marker has a null allele in some medium and long grain cultivars and therefore not selected (Fjellstrom et al. 2006).

SSR marker analysis

SSR marker analysis was performed by capillary electrophoresis. For each marker, forward primers were labeled with fluorescent dyes 6FAM, NED, or Hex from Applied Biosystems (Foster City, CA, USA) or Integrated DNA Technologies (Coralville, IA, USA). Reverse primers were not labeled. DNA was amplified with MJ Research Tetrad thermocyclers (Waltham, MA, USA) under the following PCR conditions: (1) initial denaturation at 94 ^{0}C for 5 min; (2) 35 cycles of 94 ^{0}C for 1 min, 55-67 ^{0}C (marker dependent) for 1 min, and 72 ^{0}C for 2 min; (3) 5 min final extension at 72 ^{0}C. PCR products were pooled based on color and size range of the amplified PCR products and the DNA was denatured by heating at 94 ^{0}C for 5 min.

PCR products were diluted between 500 and 1000X, and 2 ul of the diluted product were added to 9 ul of formamide-containing ROX-labeled size standards (Applied Biosystems, Foster City, CA). PCR products from different primer pairs having different size ranges and labels were combined for simultaneous analysis using a Mini Prep75 (Tecan Group Ltd., Männedorf, Switzerland) instrument based on the manufacturer protocols, and analyzed to determine the size of the SSR alleles. The reaction was run on an ABI Prism 3730 DNA Analyzer (Applied Biosystems) following the manufacturer instructions. Fragment size and SSR marker genotype analysis were performed with Gene Mapper® software version 3.7 (Applied Biosystems). Analyzed alleles were exported into a Microsoft Excel spreadsheet.

Results and Discussion

In the present study, we relied on previously identified markers closely linked with the *Pi- z* locus and differential blast races to identify accessions with *Pi-z* . The germplasm accessions selected in this study were from 1700 rice germplasm accessions initially analyzed for several R genes (Yan et al. 2007). Our data further supported the findings of previously identified markers for *Pi-z* (Fjellstrom et al. 2006) and blast races. The gene-for-gene theory predicts that a germplasm accession is resistant due to *Pi-z* when this germplasm is (i) resistant to an *AVR* race IE1k (ii) and susceptible to a virulent (*VIR*) race, IB33 or IB49. As expected, the cultivar 'Bengal' carrying *Pi-z* was resistant to IE1k and susceptible to both IB33 and IB49. The cultivar 'Wells' lacking *Pi-z* was susceptible to all three races. The cultivar 'Zhe733' carrying *Pi42*(t) and *Pi43*(t) was resistant to all three *M. oryzae* races (Lee et al. 2009). The cultivar 'Jefferson' carrying *Pi-z* was susceptible to IB33 and IB49 but resistant to IE1k (Table 1). Using these *M. oryzae* differential races, we identified 77 germplasm accessions that were resistant to IE1k but susceptible to IB33 and IB49, indicating the possible presence of *Pi-z* (Fig.3). Of these 77 accessions, 40 had identical marker alleles for all four SSR markers (Table 1). The presence of the same marker alleles in these accessions suggests the possibility that they contain a single *Pi-z* haplotype. This finding is important because these 40 accessions were collected from several geographic regions of the world: the United States, South America, Europe, Asia and Africa (Table 1). One possibility for this haplotype similarity is that the original donor parent for the *Pi-z* gene may contain the same genomic fragment for all these cultivars. In contrast, 33 accessions showed 1-3 of the *Pi-z* allele (haplotype) markers, suggesting that these rice germplasm accessions contain different *Pi-z* haplotypes, presumably inherited from different donors. Although

pathogenicity data supported the presence of *Pi-z*, no expected marker (null) alleles were found in the remaining four accessions. The existence of the *Pi-z* gene in these four accessions could not be verified with the available present markers and differential blast races that were studied. There could be the possibility of a gene that conferred resistance to IE1k but resistance was not due to Pi-z since marker alleles for *Pi-z* were not detected. This further validates the importance of MAS (Jia 2003).

According to the gene for gene theory, if resistance in these accessions is due to *Pi-z* only, susceptibility to IB49 and IB33 would be expected. Using pathogenicity assays, a total of 16 germplasm accessions were found to be resistant to all three races (Fig. 3 and Table 1). These findings suggest the presence of additional *R* genes in these germplasm accessions. There were 9 germplasm accessions that were susceptible to all isolates tested, 8 of which showed the presence of 2-4 expected marker alleles. 'Montakcl' from Egypt was the only cultivar susceptible to all isolates evaluated but that did not show any expected marker allele. There were 5 germplasm accessions that were resistant to IB49, and 4 (3+1, Fig.3) that were resistant to IB33 in addition to being resistant to IE1k, indicating the presence of additional *R* genes. There was 1 germplasm accession that was resistant to both IB33 and IB 49 but susceptible to IE1k suggesting *Pi-z* was absent or non functional.

Race IB49 versus IB33

In the rice blast system, a pair of blast races is adequate to identify the corresponding *R* gene (Silue et al. 1992). An additional blast race will increase the complexity for *R* gene identification since it may contain a different avirulence gene. Each *AVR* gene is sufficient to trigger the corresponding *R* gene mediated resistance.

IB33 was a laboratory-generated strain (F. Lee, unpublished data) and IB49 was a field isolate. Both these isolates were similar in DNA fingerprinting analysis (Correll et al. 2000; Zhou et al. 2007). If *AVR/VIR* interaction cannot be observed to detect an *R* gene, it may suggest that there are *R* genes in rice that interfere with expected disease reactions. In the present study, 16 accessions were found to be resistant to all three blast races. The presence of the *Pi-z* gene in these accessions could not be verified, although 12 contained one to four expected alleles using differential blast races. Since it was difficult to determine the presence or absence of the gene based on the pathogenicity assay, the marker allele linked with the gene was used to indicate its presence. The assumption was that the linked allele associates with the gene and/or quantitative trait locus (QTL) of interest. Hence MAS should be useful for traits that are difficult to measure, exhibit low heritability, and/or are expressed late in development. MAS has been previously used with success in rice breeding (Jia 2003).

_R_ gene modifier

R genes can have different phenotypic effects in different germplasm accessions or in different genetic backgrounds (Jia and Martin 2008). These differences are often conditioned by *R* gene modifiers, some critical for complete resistance (Jia and Martin 2008). In the present study, we found nine germplasm accessions that may contain 0-4 *Pi-z* marker alleles that were susceptible to all three races tested. Although it is possible that mutations in the coding region of *Pi-z* can result in the loss of resistant function, these findings suggest that some of these germplasm accessions may have at least one nonfunctional critical modifier rendering susceptibility. A similar study was recently reported for the *Pi-ta* gene where a component *Ptr(t)* in a mutant M2354 was disrupted

by fast neutrons. As a result, M2354 lost *Pi-ta* mediated resistance although the *Pi-ta* gene in M2354 was intact and expressed (Jia and Martin 2008).

Identification of additional *R* genes

In this study, five germplasm accessions, Chao Puak Deng and Assaw from China, Biribra from Ghana, CA902/b/2/2 from Chad, and Agami Mont-1 from Egypt, were resistant to both IE-1k and IB49 and susceptible to IB33, suggesting these accessions contain additional *R* genes. In addition, three accessions, Perititovo 1417 from Madagascar, R 100/2 from Zaire and Ku Mun Do No. 84 from Korea, were also determined to carry additional *R* genes to IB33. The cultivar Wanni Dahanala from Sri Lanka was known to be resistant to IB49 and IB33 but susceptible to IE1K, indicating the absence of *Pi-z* yet indicating the presence of additional *R* genes. The cultivar Shimla Early from Iraq was susceptible to IE1k and IB49 resistant to IB33, suggesting the presence of other genes but not *Pi-z*. Three accessions (PI 184675-4 from Iran; Ken Yen from China, and GPNO 22232 from Germany) did not show any *Pi-z* haplotype alleles for the SSR markers, yet showed resistance to all three races. Despite the presence of *R* genes it is unknown if these materials could be useful as resistant donors. This suggests that MAS would not work for these five germplasm accessions. If a germplasm is selected as an *R* gene donor, marker analysis should be performed and the results verified using pathogenicity assays on local differential races. Despite MAS use as a promising new tool that can overcome some disadvantages of pathogenicity assays for monitoring *R* genes, the power of MAS is often limited by the availability of a near-isogenic pair of *AVR/VIR* genes. In our study, the existence of *Pi-z*(t) could not be verified because the differential races used were not near-isogenic. This is a typical situation in the rice blast

system because one isolate may contain different *AVR* genes as mentioned previously. Furthermore, the presence of all marker alleles does not always indicate the presence of functional *R* genes. Hence, MAS is not a silver bullet.

Geographic origin of accessions carrying *Pi-z*

Rice accessions predicted to contain *Pi-z* were noted from 42 countries on six continents, Asia, Europe, North America, South America, Africa and Australia (Table 1). U.S. and Puerto Rico, 5 + 4 of 76 respectively, had most of the germplasm accessions with *Pi-z*. Cote D' Ivoire of West Africa had 7 of 77 germplasm accessions with *Pi-z* in the present study. Interestingly enough, we did not identify any accessions predicted to contain *Pi-z* from India or China, major rice producing nations.

In conclusion, we not only verified the *Pi-z* gene in 73 of 77 rice germplasm accessions utilizing previously identified DNA markers (Fjellstrom et al 2006), but also demonstrated the usefulness of DNA markers and pathogenicity assays with differential blast races for germplasm characterization. IE1k proved to be a better AVR race than IB49 for detecting the presence of the *Pi-z* gene in rice germplasm. Differential blast races, IE1k, IB33 and IB49 further verified the predicted existence of the *Pi-z* gene for conventional breeding for blast resistance. Previous work identified the *Pi-z* gene in germplasm accessions utilizing only two DNA markers, RM 527 and AP4791, and no pathogenicity assays were included. All results presented were summarized in Table 1. For germplasm requests, please visit (www.ars.usda.gov/spa/dbnrrc/gsor) at GSOR of DB NRRC.

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Table 1. Summary of disease reaction, pathogenicity assays, SSR marker profile for the analysis of the *Pi-z* gene in rice germplasm.

Accession Number	Name	Origin	Disease Reaction			SSR Markers				No. of Expected *Pi-z* Alleles	*Pi-z**
			IB49 (ZN61)	IE1k (TM2)	IB33 (FL9)	AP5659-1	AP5659-5	AP4791	RM527		
GSOR310003	WC3398	Mexico	S 3	R 1	S 4	220	279	290	217	4	+
GSOR310010	Coray 4	Honduras	S 3	R 2	S 3	204	299	Null	238	0	-
GSOR310013	WC 4431	Panama	S 3	R 1	S 3	220	279	287	217	3	+
GSOR310021	PR325	Puerto Rico	S 3	R 1	S 3	220	279	287	217	3	+
GSOR310022	PR358	Puerto Rico	S 3	R 0	S 3	222	279	287	217	2	+
GSOR310035	Stg58-2158	United States, Arkansas	S 3	R 0	S 3	220	279	Null	217	3	+
GSOR310037	Stg625377	United States, Arkansas	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310046	Clor11009	United States, Louisiana	S 3	R 0	S 5	220	279	290	217	4	+
GSOR310051	15	Iran	S 3	R 0	S 3	220	279	290	217	3	+
GSOR310064	PR433	Puerto Rico	S 3	R 0	S 3	220	279	290	217	3	+
GSOR310073	Fortuna Negro	Peru, Lima	S 4	R 2	S 3	Null	297	300	217	1	+
GSOR310074	Mejicano	Peru, Lima	S 3	R 1	S 3	220	279	290	217	4	+
GSOR310078	Saku	Mongolia	S 3	R 0	S 3	220	279	290	217	4	+

GSOR310107	1021	Guatemala, Jalapa	S 3	R 2	S 4	220	279	290	217	4	+
GSOR310146	WC3396	Jamaica	S 3	R 2	S 4	220	279	287	217	4	+
GSOR310177	AP439	Venezuela	R 2	R 0	R 1	220	279	292	233	4	-
GSOR310201	WC 1909	Japan	S 3	R 2	S 3	203	290	Null	233	0	-
GSOR310214	Campino	Portugal	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310222	Baraggia	Italy, Piedmont	S 3	R 0	S 3	220	279	300	217	2	+
GSOR310265	Nilo 48A	El Salvador	S 3	S 4	S 3	220	279	290	217	4	-
GSOR310301	H57-3-1	Argentina, Buenos Aires	S 4	R 0	S 3	220	279	290	217	4	+
GSOR310302	H62-3-1	Argentina, Buenos Aires	S 5	S 3	S 5	220	276	290	217	3	-
GSOR310303	H71-11-1	Argentina, Buenos Aires	S 3	R 2	S 4	220	297	300	217	2	+
GSOR310321	Bankoram	Ghana, Ashanti	S 3	R 0	S 3	220	279	287	217	3	+
GSOR310322	PindeGogo Wiere	Suriname	S 3	R 0	S 4	220	279	287	217	3	+
GSOR310327	52/16-0-2	Papua, New Guinea	S 4	R 0	S 5	220	279	290	217	4	+
GSOR310329	GPNO 15007	Senegal	S 4	S 3	S 4	220	279	290	217	4	–
GSOR310336	Chao Puak	Laos	R 0	R 0	S 3	220	279	300	221	2	–

	Deng										
GSOR310391	Assaw	China, Sichuan	R 0	R 0	S 3	220	279	290	217	4	−
GSOR310404	Ken Yen	China	R 1	R 0	R 0	Null	295	296	221	0	−
GSOR310477	BIRIBRA	Ghana	R 0	R 1	S 4	220	279	300	221	2	−
GSOR310480	Djimoron	Guinea	S 3	R 2	S 4	220	279	300	221	2	+
GSOR310500	VARY LAVA 9	Madagascar	S 4	R 0	S 5	220	279	287	217	3	+
GSOR310502	Perititovo 1417	Madagascar	S 3	R 0	R 1	220	279	287	238	2	−
GSOR310503	Manga Kely 694	Madagascar	S 3	R 0	S 3	220	279	287	217	3	+
GSOR310512	CA497/V/7	Chad	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310518	Gaza	Mozambique	S 4	R 1	S 5	220	279	290	238	3	+
GSOR310525	India Pa Lil 92	Sierra Leone	S 3	R 2	S 5	220	279	287	238	2	+
GSOR310533	MAKALIOKA 752	Madagascar	S 3	R 1	S 4	220	279	290	238	3	+
GSOR310535	CA435/B/5/1	Chad	S 3	R 0	S 4	220	279	290	217	4	+
GSOR310538	PI 184675-4	Iran	R 0	R 0	R 0	202	290	304	235	0	−
GSOR310562	Higueyano	Dominican Republic, La Alt	S 3	R 0	S 5	220	279	290	217	4	+
GSOR310577	GPNO22232	Germany,	R 0	R 0	R 0	Null	294	296	221	0	−

		Saarland									
GSOR310586	Baluola 11	Zaire	R 0	R 1	R 0	Null	297	293	217	1	–
GSOR310591	R 89	Zaire	R 0	R 0	R 0	220	279	290	217	4	–
GSOR310596	R 100/2	Zaire	S 3	R 1	R 0	220	279	290	217	4	–
GSOR310602	P 817	Russian Federation	S 4	R 0	S 5	220	279	290	238	3	+
GSOR310635	IM 16	Nigeria	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310638	IRAT 104	Cote D'Ivoire	R 0	R 0	R 0	220	279	290	217	4	–
GSOR310642	IRAT132	Cote D'Ivoire	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310643	IRAT 134	Cote D'Ivoire	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310644	IRAT139	Cote D’Ivoire	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310683	IITA130	Nigeria, Oyo	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310692	Tox177-1-2-B	Nigeria, Oyo	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310704	CT7378-2-1-3-1-4	Colombia, Valle	S 5	R 0	S 3	220	279	290	217	4	+
GSOR310707	Medusa	Italy, Lombardy	S 3	R 0	S 4	220	279	290	217	4	+
GSOR310712	IRAT13	Cote D'Ivoire	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310734	CNAX 5072-2-1-2	Colombia	S 3	R 0	S 3	220	279	290	217	4	+

GSOR310762	IRAT44	Burkina Faso	S	3	R	0	S	3	220	279	290	217	4	+
GSOR310774	WAB502-13-4-1	Cote D'Ivoire	S	3	R	0	S	3	220	279	290	217	4	+
GSOR310775	WAB 501-11-5-1	Cote D'Ivoire	S	3	R	0	S	3	220	279	290	217	4	+
GSOR310777	WC3532	Peru	S	3	R	0	S	3	220	279	290	217	4	+
GSOR310805	Chivacia-1	Venezuela, Aragua	S	3	R	0	S	3	220	279	290	217	4	+
GSOR310815	PR 147	Puerto Rico	R	1	R	0	R	0	220	279	287	217	3	–
GSOR310829	Stg 64M3390	United States, Arkansas	S	5	R	0	S	3	220	279	290	217	4	+
GSOR310831	Zenith	Puerto Rico	S	5	R	0	S	4	220	279	290	217	4	+
GSOR310833	71Cr-308	United States, Louisiana	S	3	R	0	S	5	220	279	290	217	4	+
GSOR310886	WC2656	Zaire	S	3	R	0	S	3	220	279	290	217	4	+
GSOR310889	Brazilero Perla	El Salvador, La Libertad	R	1	R	0	R	0	220	279	287	217	3	–
GSOR310892	Mamoriaka	Madagascar	S	3	R	0	S	4	220	279	287	238	2	+
GSOR310915	Zale	Myanmar, Rangoon	S	3	R	0	S	3	Null	295	293	221	0	-
GSOR310965	Vary Tarva Osla	Portugal	S	3	R	0	S	4	220	297	300	217	2	+

GSOR310966	Perlita Jalapa	Guatemala	S	5	R	0	S 3	220	279	287	217	3	+
GSOR311012	WC 5015	Mexico, Federal District	S	4	R	0	S 3	220	279	290	217	4	+
GSOR311025	IR647-PDI-C1	Philippines, Luzon	S	3	R	0	S 3	220	295	296	221	1	+
GSOR311037	H75-23-1	Argentina, Buenos Aires	S	4	R	0	S 3	220	279	290	217	4	+
GSOR311049	Blakka Tere Thelma	Suriname	S	3	R	0	S 4	220	279	287	217	3	+
GSOR311060	Hal Suduwi	Sri Lanka	S	4	R	0	S 4	220	279	287	223	2	+
GSOR311069	Lay Sort	Laos	S	3	R	0	S 3	220	279	290	217	4	+
GSOR311080	YRL-1	Australia	S	3	R	0	S 3	220	279	290	217	4	+
GSOR311099	Ku Mun Do No.84	Korea	S	4	R	0	R 0	220	279	290	217	4	–
GSOR311104	EEA 406	Brazil, Rio Grande do Sul	S	3	R	0	S 3	220	276	290	217	3	+
GSOR311118	Cadung Ket	Vietnam	S	3	R	0	S 3	220	279	296	221	2	+
GSOR311150	Five months	Guyana	S	3	R	0	S 4	220	279	290	217	4	+
GSOR311180	Sapundali Local	India	S	3	R	2	S 3	220	279	290	238	3	+
GSOR311186	CA 902/8/2/2	Chad	R	0	R	0	R 0	220	279	290	217	4	–
GSOR311198	WC6570	Spain	R	1	R	0	R 0	220	279	287	217	3	–

GSOR311200	Kalila 50	Madagascar	R 2	R 0	R 0	220	279	287	217	3	–
GSOR311204	CA 902/b/2/2	Chad	R 1	R 0	S 3	220	279	290	217	4	–
GSOR311222	Agami Mont-1	Egypt	R 0	R 0	S 3	220	276/279	287	217	3	–
GSOR311257	Basala BaatkaS-R	Zaire	R 0	R 0	R 0	220	279	290	217	4	–
GSOR311269	Shimla Early	Iraq	S 4	S 3	S 5	220	279	290	238	3	+
GSOR311272	Sadri Dum Sufaid	Iran	S 5	R 0	S 3	220	279	300	221	2	+
GSOR311277	Ghoal Champa	Iran	S 4	R 0	S 3	220	279	300	221	2	+
GSOR311278	Montakcl	Egypt	S 3	S 4	S 5	Null	300	270	223	0	–
GSOR311305	IB 94	Nigeria	S 3	R 0	S 3	220	279	290	217	4	+
GSOR311306	Mange 2	Nigeria	R 0	R 0	R 0	Null	295	296	221	0	–
GSOR311309	IRAT 142	Cote D'Ivoire	S 3	R 0	S 3	220	279	290	217	4	+
GSOR311341	63-83	Cote D'Ivoire	S 3	R 0	S 3	220	279	290	217	4	+
GSOR311349	Tox 782-20-1	Nigeria, Oyo	R 0	R 1	R 0	220	279	290	217	4	–
GSOR311371	Estrela	Colombia, Valle	S 4	R 0	S 3	220	279	287	217	3	+
GSOR311403	Panama 1048	Colombia, Valle	R 0	R 0	R 0	220	279	287	238	2	–
GSOR311565	517	Uruguay,Treinta y Tres	S 3	R 0	S 3	Null	295	296	221	0	-

GSOR311583	Bakula	Sierra Leone, Southern	S 3	R 0	S 3	220	279	287	217	3	+
GSOR311590	Sadri Siah Dum	Iran	S 3	S 5	S 3	220	279	287	240	2	−
GSOR311624	Suduwi 305	Sri Lanka	S 3	R 0	S 3	220	279	287	238	2	+
GSOR311630	Hatadawee	Sri Lanka	S 3	S 5	S 3	220	279	306	221	2	−
GSOR311632	Wanni Dahanala	Sri Lanka	R 2	S 5	R 1	220	279	287	238	2	−
GSOR311634	Patchaiperumal	Sri Lanka, Kurunegala	S 3	S 4	S 3	220	279	306	221	2	−
GSOR311635	AMANE	Sri Lanka, Matale	S 3	S 5	S 3	220	279	306	221	2	−
GSOR311673	Sadri Ter Misri	Iran	S 3	R 0	S 3	220	279	287	240	2	+
PI561735	Bengal (Control)		S 3	R 0	S 3	220	279	290	217	4	+
PI612439	Wells (Control)		S 5	S 3	S 4	203	290	290	233	1	−
PI629016	Zhe 733 (Control)		R 0	R 0	R 2	205	290	301	223	2	−
PI593892	Jefferson (Control)		S 3	R 0	S 3	220	279	293	217	3	+

* + indicates the presence of *Pi-z*; - indicates the presence of *Pi-z* could not be determined and verified.

Table 2. Summary of marker sizes, chromosomal locations, annealing temperature, dilution and sequences of simple sequence repeat markers at the *Pi-z (t)* locus.

Markers	Size (bases)	Chrom. Location	Chrom. Distance (cM)	Annealing Temp (°C)	Dilution	Forward	Reverse	Start	Stop
RM527	210- 247	6	62.1+ /59	61	1:500	5'-GGCTCGATCTAGAAAATCCG-3'	5'-GGCTCGATCTAGAAAATCCG-3'	9862290	9862522
AP4791	270-320	6		55	1: 1000	5'-AAACGGAGGGAGTACATTG-3'	5'-GGATCGTCGATTTGATTTG-3'	10093246	10093556
AP5659-1	190-220	6	62 +/60	61	1:500	5'-TGCTGAGATAGCCGAGAAATC-3'	5'-ACTAGCTGCCCACCTAAGC-3'	10414829	10415031
AP5659-5	250-330	6		55	1:500	5'-CTCCTTCAGCTGCTCCTC-3'	5'-TGATGACTTCCAAACGGTAG-3'	10357166	10357453

Physical locations were determined from Release 3, TIGR Rice Pseudomolecules http://www.tigr.org/tdb/e2k1/osa1/pseudomolecules/info.shtml. Approximate nucleotide (nt) sizes were based on Nipponbare sequence determined from Gene Bank database information. Chromosome distance was based on IRMI Map 2003; Chromosome distance was based on Cornell Map 2001 at www.gramene.org.

Figure Legends

Fig. 1. Evaluation standard for determining disease reactions of rice germplasm. Resistant (0-2): No lesion formation- 0; Lesions covering less than 5% of total leaf area, lesions restricted at the site of infection-1; Lesions covering between 5% to 10% of the total leaf area; restricted spindle lesions at diameter less than 2 mm - 2; Susceptible (3-5): Lesions in several locations on the leaf to form a large eye-shaped brown area (diameter greater than 2mm) - 3; Lesions covering greater than 50% of the leaf area, diseased area with lesion greater than 30% of the total leaf area - 4; Lesions covering greater than 70% of the total leaf area - 5.

Fig. 2. Genetic and physical maps of the *Pi-z* gene as defined by SSR markers. Genetic map showing indicated SSR markers spanning the *Pi-z* locus (A) and physical map of the *Pi-z* locus as delimited by indicated SSR markers. Modified from Fjellstrom et al. 2006.

Fig.3. Analysis of the *Pi-z* gene in rice germplasm using disease reaction and SSR marker. The diagram shows results of disease reactions and expected SSR marker alleles for germplasm in different categories.

Fig. 1

Fig. 2

RM527
AP4791
AP5659-1
AP5659-5
IB49
IB33
IE1k
111
77 → IB49(S), IB33(S), IE1k(R)
(40 had 4 expected alleles, 15 had 3 expected alleles,16 had 2 expected alleles and 2 had 1 expected allele and 4 had null alleles)
16 → IB49(R), IB33(R), IE1k(R)
(6 had 4 expected alleles, 4 had 3 expected alleles, 1 had 2 expected alleles,1 had 1 expected allele and 4 had null alleles)
5 → IB49(R), IB33(S), IE1k(R)
(2 had 4 expected alleles, 1had 3 expected alleles and 2 had 2 expected alleles)
9 → IB49(S), IB33(S), IE1k(S)
(2 had 4 expected alleles, 2 had 3expected alleles 4 had 2 expected allele and 1 had null allele)
3 → IB49(S), IB33(R), IE1k(R)
(2 had 4 expected alleles and 1 had 2 expected alleles)
1 → IB49(R), IB33(R), IE1k(S)
(1 had 2 expected alleles)
→ Confirms presence of Pi-z in 73 although showing different haplotypes
→ Other disease resistance gene(s) associated with Pi-z which provides resistance to IB49 and IB33
→ Other disease resistance gene(s) associated with Pi-z which provides resistance to IB49
→ No R genes, or components of Pi-z is altered , or changed making it non functional
→ Other disease resistance gene(s) associated with Pi-z provides resistance to IB33
→ Other disease resistance gene(s) provides resistance to IB33 and IB49 while Pi-z is either absent or non functional

Fig. 3

Characterization of the *Pi-b* Rice Blast Resistance Gene in the National Small Grains Collection

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Additional Keywords IB54• IE1k• IB1• *Magnaporthe oryzae*• *Pi-b* •SSR markers

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Abbreviations

Acc Accession

AVR Avirulent

D Day

MAS Marker Assisted Selection

R Resistance

SSR Simple Sequence Repeat

VIR Virulent

Abstract

The *Pi-b* gene in rice confers resistance to a wide range of races of the rice blast pathogen, *Magnaporthe oryzae*, including race IE1k that overcomes *Pi-ta*. In this study, the presence of *Pi-b* in 164 rice germplasm accessions from the National Small Grains Collection was determined utilizing DNA markers and pathogenicity assays. The presence of *Pi-b* was evaluated with two simple sequence repeat markers (SSRs) and a dominant marker Pibdom derived from the *Pi-b* gene sequence. Pathogenicity assays using two AVR races (IE1k and IB1) and a virulent race (IB54) were performed to verify the resistance responses of accessions. Of the 164 accessions evaluated, 129 contained the *Pi-b* gene as determined using both SSR markers and pathogenicity assays, albeit different haplotypes were detected. The remaining 35 germplasm accessions were different in their responses to the blast races IB54, IE1k, and IB1, thus indicating the presence of *R* gene(s) other than *Pi-b*. The accessions characterized in this study could be used for marker-assisted breeding to improve blast resistance in *indica* and *japonica* cultivars worldwide.

Introduction

Rice blast is the most destructive disease affecting rice production worldwide. The use of resistant cultivars has been the most economical and efficient method for controlling this disease. However, the lifespan of many resistant cultivars is only a few years, due to the breakdown of resistance in the face of hypervariability of the pathogen (12). The inheritance of major-gene-mediated resistance to the blast pathogen has been studied extensively worldwide. A major resistance gene is effective in preventing infection by races of *Magnaporthe oryzae* B. Couch containing the corresponding avirulence gene (4, 22). Presently, more than 70 blast *R* genes have been identified, and

13 of them have also been characterized using molecular markers and subsequently used to develop resistant cultivars (1,2,3,7,9,10,13,14,15,20,21,24,28).

Molecular markers tightly linked to major *R* genes are important for MAS, particularly during the early stages of plant growth, and when the molecular markers are used to circumvent the association with undesirable agronomical traits (linkage drag). Although a large number of blast *R* genes have been fine mapped based on closely linked markers and/or some of them cloned based on marker information, there are only a few examples where the markers have had a direct impact in plant breeding, e.g. DNA markers derived from two cloned blast *R* genes (*Pi-b* and *Pi-ta)* and currently used in several rice breeding programs (11). In addition, PCR-based SNP markers for genes at the *Pi-z* locus are also known to be used in breeding programs (8).

Pi-b has been used extensively in rice breeding programs in Japan, China, and Indonesia (27, 18). *Pi-b*, encoding a cytoplasmic protein with nucleotide binding sites and leucine rich repeat (NBS-LRR), was the first cloned blast *R* gene (24). The availability of a high-density linkage map (7) and DNA markers in the *Pi-b* region (19) have facilitated the identification of additional molecular markers more closely linked to *Pi-b* (5). In the US, *Pi-b* has been identified in rice cultivars resistant to blast races IA45, IB1, IH1, IB45, IG1, IE1k, IC17 and IE1k (Fig.1). *Pi-b* was introduced into the US rice cultivar 'Saber' from the Chinese cultivar 'Teqing' (17). Race IE1k overcomes *Pi-ta* resistance, and was subsequently used to predict the existence of *Pi-b* in rice cultivars utilizing classical pathogenicity assays (16) (Fig. 1). The AVR race IB1 was also used to ensure that resistance is not due to *Pi-z*. Although *Pi-z* was also resistant to IE1k (Fig.1), *Pi-z* was susceptible to IB1 but *Pi-b* was not.

The objectives of this study were to identify the *Pi-b* gene in a core collection of 1711 rice germplasm accessions from the National Small Grains Collection using previously identified SSR markers closely linked to the *Pi-b* gene, a dominant marker derived from *Pi-b*, and to determine disease reactions of the accessions containing *Pi-b* to differential U.S. blast races.

Materials and Methods

Plant Materials

A USDA core collection consisting of 1711 accessions from 113 countries representing an estimated 70% of the genetic diversity of the entire USDA rice collection (26) was used for this study. Four grams of seed from each accession was provided by the Genetic Stock Collections of *Oryza* at Dale Bumpers National Rice Research Center (DB NRRC). Rice cultivar 'Saber' (PI 633624) [+*Pi-b*], was used as the positive control. Twelve seeds of each accession were germinated in 96 well inserts (10 x 20 x 2 cm), (Hummert International, Missouri USA). Prior to seeding, the inserts were placed in trays (26.67 x 53.34 x 6.35[in cm], Model # INT0804, Hummert International, Missouri, USA) and filled with silt loam soil (pH 5.5 – 5.8) fertilized with Osmocote Pro 15-9-12 (Scotts-Sierra Horticultural Products Company, OH), autoclaved and stored at -20 ^{0}C for three days. The trays were completely filled with water. Seedlings were grown for 4 weeks in the greenhouse maintained at 23 -29 ^{0}C during the day in winter (November to December) and 22-25 ^{0}C during the night until the 3 to 4 leaf stage, in preparation for pathogenicity assays and subsequent DNA extraction.

Pathogenicity assays

Pathogenicity assays were performed on 164 experimental germplasm accessions confirmed by SSR markers as having Pi-b, and a positive control 'Saber' (17). A virulent (VIR) isolate (unnamed-race IB54); avirulent (AVR) isolates TM2 (race IE1k) and (unnamed-race IB1) of *M. oryzae* were selected for pathogenicity tests. There were four replicates for each germplasm accession. The presence of the *Pi-b* gene in each accession was verified by the pattern of resistance or susceptibility to a pair of *AVR* and *VIR* isolates. Pathogen inoculation was performed using a modified procedure based on Valent and colleagues (23) (Fig.2). Briefly, plants were inoculated with 40 mL of a spore suspension (5 X 10^5 spores/ml, 0.25% gelatin) using a hand atomizer connected to an air compressor (100 kPa). Inoculated plants were maintained at approximately 95% relative humidity in a clear polyethylene autoclave bag 24 x 36 [in cm] and 1.5 mm thick at room temperature (Product code 018143 Fisher Scientific, USA). Approximately 24 h after inoculation, plants were moved to the greenhouse for an additional 6 d. Disease reactions were assessed 7 d after inoculation using a visual rating scale (Fig.2). For each accession, 7 to 8 seedlings were evaluated and each pathogenicity assay was conducted three times.

DNA extraction

DNA was extracted from bulked leaves from each of four replicates for further analysis by the rapid DNA extraction procedure (25). After extraction, sample DNAs were prepared for PCR through a Biomek 2000 Lab Automation Work Station (Beckman and Coulter, Brea, CA) using manufacturer protocols.

DNA markers and analysis

Two simple sequence repeat markers (SSRs) RM208 and RM166 closely linked to *Pi-b* were used for this study (Fig. 3; Table 2) (5). Germplasm accessions with these markers

were examined utilizing a marker Pib-dom derived from a portion of *Pi-b* utilizing a method described previously (5). Fluorescently labeled SSR markers were analyzed by capillary electrophoresis based on the methods previously described (5). For each marker, forward primers were labeled with fluorescent dyes (6FAM, NED, and Hex) from Applied Biosystems (Foster City, CA, USA) or Integrated DNA Technologies (Coralville, IA, USA). Reverse primers were not labeled. DNA was amplified using MJ Research Tetrad thermocyclers (Waltham, MA, USA) under the following PCR conditions: (1) initial denaturation at 94 ^{0}C for 5 min; (2) 35 cycles of 94 ^{0}C for 1 min, 55-61 ^{0}C (marker dependent) for 1 min, and 72 ^{0}C for 2 min; (3) 5 min final extension at 72 ^{0}C. PCR products were pooled based on color and size range of the amplified PCR products and the DNA was denatured by heating at 94 ^{0}C for 5 min. PCR products were diluted between 200, 500 and 2000X, and 2 ul of the diluted product were added to 9 ul of formamide-containing ROX/LIZ (dependent on the size of the product) labeled size standards (Applied Biosystems, Foster City, CA). PCR products from different primer pairs having different size ranges and labels were combined for simultaneous analysis using a Mini Prep75 (Tecan Group Ltd., Männedorf, Switzerland) instrument based on the manufacturer protocols, and analyzed to determine the size of the SSR alleles. The reaction was run on an ABI Prism 3730 DNA Analyzer (Applied Biosystems) following manufacturer instructions. Fragment size and SSR marker genotype analysis were performed with Gene Mapper® software version 3.7 (Applied Biosystems). Analyzed alleles were exported into a Microsoft Excel spreadsheet. Allele sizes for all SSR markers used in the present study are listed in Table 1.

Results and Discussion

In the present study, there were only two accessions - Daudzai Field mix from Pakistan and ARC 10378 from India – that did not have any expected marker alleles for the *Pi-b* gene and were susceptible to IBI, IE-1k and IB54. Both marker and pathogenicity assays suggested that these two germplasm accessions did not contain *Pi-b*. Hence, these served as ideal negative controls. A total of 178 rice accessions were initially identified by utilizing a dominant marker for *Pi-b*. Later, 164 out of these 178 accessions were verified by SSR markers RM166 and RM208. The remaining 14 accessions were removed due to suspected seed mix. The gene-for-gene theory predicts that a germplasm accession contains *Pi-b* only if the germplasm accession is (i) resistant to *AVR* races, such as IE1k and IB1, and (ii) susceptible to a virulent (*VIR*) race, such as IB54. The cultivar 'Saber' carrying *Pi-b* was resistant to IE1k and IB1and susceptible to IB54 as predicted (Table 1). Utilizing these differential races, *Pi-b* was verified in 130 of 164 germplasm accessions since they were resistant to IE1k and IB1 but susceptible to IB54 (Fig.4) and followed the gene for gene relationship pattern. Out of the 130 accessions with *Pi-b*, 88 had the expected alleles for all three markers examined (Table 1; Fig. 4). The presence of all three *Pi-b* marker alleles in these germplasm accessions suggests that they contain the same *Pi-b* haplotype. This was unexpected because these 88 germplasm accessions were collected from several geographic regions of the world, including the United States, South America, Europe, Asia and Africa (Fig 4; Table 1). Regardless, it is still possible that these germplasm accessions may have inherited *Pi-b* from the same donor. In contrast, 19 germplasm accessions contained two of the expected marker alleles, suggesting that these accessions contain different *Pi-b* haplotypes, presumably having inherited them from different donors. Additionally, 16

germplasm accessions had followed the gene for gene concept but had no expected marker alleles for the gene in question. Thus, the presence of *Pi-b* utilizing our present markers and differential blast races could not be verified. In this study, 28 germplasm accessions were found to be resistant to both IE-1k and IB54, suggesting these accessions contain *Pi-b* independent *R* genes that are responsible for resistance to IB54.

There were 24 of 28 accessions with all three expected alleles for *Pi-b,* indicating the possibility of receiving *Pi-b* from the same donor. In contrast, one accession had two expected alleles, one had one expected allele, and two had no expected alleles, indicating that they received *Pi-b* from different donors. The presence of a different avirulence gene can mask the ability to identify the other *R* gene. Finally, a total of 5 germplasm accessions (BR-IRGA-410 from Brazil, R647 from China and RP2199-16-2-2-1 from India, 17465-4 and Bilo from Fiji) had all expected marker alleles for the *Pi-b* gene but were susceptible to both IE1k and IB54 (Fig.4;Table1). One hypothesis is that the modifiers of *Pi-b* resistance are non-functional in these five accessions. Exact reasons for the inconsistency of marker and phenotype analysis in this collection needs to be further investigated.

Geographical Distribution

Rice germplasm with *Pi-b* was found in 39 countries (Table 1). Germplasm accessions containing *Pi-b* in our study were mostly from China with 20 out of 114 tested. Germplasm accessions from the Philippines was second, with 16 of 114 accessions containing *Pi-b*. Accessions from Columbia and India followed with 9 and 8 germplasm accessions out of 114, respectively.

Although *Pi-b* is not as well studied as *Pi-ta*, this gene is a major blast resistant gene in the US. The gene confers resistance to 7 US blast races (Fig.1). *Pi-b* is particularly useful because it also offers resistance to IE1k, a virulent race that has overcome resistance mediated by *Pi-ta*. We not only verified the *Pi-b* gene in 114 rice germplasm accessions, but also demonstrated the usefulness of combining DNA markers and pathogenicity assays to confirm the presence of resistance genes in rice germplasm accessions. The pathogenicity assays helped verify the accuracy of DNA markers and also identified additional blast *R* genes. For germplasm requests, please visit (www.ars.usda.gov/spa/dbnrrc/gsor) at GSOR of DB NRRC.

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Table 1. Summary of disease reaction, pathogenicity assays, SSR marker profile for the analysis of the *Pi-b* gene in rice germplasm.

Sample	Country	Name	SSR Markers			Disease Reactions			Presence of *Pi-b*
			Pibdom	RM208	RM166	IB54	IE1k	IB1	
GSOR310164	Mexico	C1-6-5-3		164		R 0	R 0	R 0	?
GSOR310278	Iraq	Amber 33		168	318	S 3	R 0	R 2	*
GSOR310285	Philippines	IR 532-1-47	360	179	316	S 3	R 0	R 0	+
GSOR310298	Guyana	51779	360	179	316	S 3	R 2	R 0	+
GSOR310319	India	BC5-55		164	318	S 3	R 2	R 0	*
GSOR310326	Philippines	IR 1103-15-8-5-3-3-3	360	179	316	S 3	R 2	R 0	+
GSOR310340	Laos	Chao Hay b	360	179	316	S 4	R 0	R 0	+
GSOR310350	Papua, New Guinea	C 8435	360	179	316	R 1	R 0	R 0	?
GSOR310352	Malaysia	Padi Bangka		164	418	S 3	R 0	R 0	*
GSOR310363	Colombia	P773-44-3-1	360	179	316	S 3	R 2	R 0	+
GSOR310367	Colombia	P 738-97-3-1	360	179	316	S 3	R 0	R 0	+
GSOR310368	Colombia	P 761-40-2-1		179	316	S 3	R 0	R 0	+

GSOR310436	Cuba	Zayas Bazan		176	318	S 3	R 0	R 0	*
GSOR310481	India	Anandi	360	179	316	S 3	R 0	R 0	+
GSOR310485	Sri Lanka	Perum Karuppan	360	164	318	S 4	R 2	R 0	+
GSOR310487	Indonesia	SIGADIS	360	179	316	S 4	R 0	R 0	+
GSOR310517	Hong Kong	Fa Loh Pak		164	316	S 3	R 2	R 0	+
GSOR310528	United States	J 312	360	179	316	S 3	R 0	R 0	+
GSOR310539	Mali	Segadis	360	179	316	S 4	R 0	R 0	+
GSOR310540	Thailand	T442-57		176	316	R 1	R 0	R 0	?
GSOR310542	Bangladesh	BR51-319-9	360	179	316	S 3	R 0	R 0	+
GSOR310543	Costa Rica	CR 1113	360	179	316	R 1	R 2	R 0	?
GSOR310545	Indonesia	B462B-PN-31-2	360	179	316	S 4	R 0	R 0	+
GSOR310547	Peru	HUALLAGA	360	179	316	S 3	R 2	R 0	+
GSOR310548	Thailand	BKN 6820-6-3-2	360	179	316	S 3	R 2	R 0	+
GSOR310549	Sri Lanka	BG 90-2	360	179	316	S 3	R 0	R 0	+
GSOR310553	Iran	205		170	318	S 3	R 2	R 0	*

GSOR310555	Colombia	COLOMBIA 1		176	318	S 5	R 1	R 0	*
GSOR310566	Ecuador	INIAP 7	360	179	316	R 1	R 2	R 0	?
GSOR310567	Guatemala	TIKAL2	360	179	316	S 3	R 2	R 0	+
GSOR310574	Malaysia	SM II	360	179	316	S 3	R 2	R 0	+
GSOR310575	Haiti	Gros Riz	360	176	316	S 3	R 2	R 0	+
GSOR310576	India	PUSA 33	360	179	316	S 3	R 1	R 0	+
GSOR310583	Fiji	17465-4	360	179	316	S 3	S 4	S 3	-
GSOR310612	Uzbekistan	Uz Begohef 2	360	179	316	S 3	R 2	R 0	+
GSOR310630	Thailand	BKN 6987-68-14	360	179	316	S 4	R 2	R 0	+
GSOR310631	Guinea	GPNO 22236	360	179	316	S 4	R 2	R 0	+
GSOR310632	Philippines	IR 4482-5-3-9-5	360	179	316	S 3	R 2	R 0	+
GSOR310636	Cote D’Ivoire	IRAT 8	360	179	316	S 3	R 0	R 0	+
GSOR310648	Zimbabwe	IR 400	360	179	316	R 0	R 0	R 0	?
GSOR310650	India	PR 106	360	179	316	S 3	R 0	R 0	+
GSOR310655	Chile	CH 272-132	360	179	316	S 3	R 0	R 0	+

GSOR310657	Egypt	CR418-3-12	360	179	316	S 3	R 0	R 0	+
GSOR310658	Egypt	CR 561-4-2-1	360	179	318	S 4	R 2	R 0	+
GSOR310659	Egypt	YNA 223		168	418	S 3	R 0	R 0	*
GSOR310663	Kazakhstan	Kasakstanica	360	164	318	S 3	R 0	R 0	+
GSOR310668	Azerbaijan	Bak Saly Mestnyj		164	418	S 3	S 3	S 4	-
GSOR310683	Nigeria	IITA 130		164	421	S 3	R 0	R 0	*
GSOR310685	Brazil	BR-IRGA-410	360	179	316	S 3	S 3	S 3	-
GSOR310686	Brazil	Pratao		164	418	S 3	R 0	R 0	*
GSOR310687	Philippines	IR 9660-48-1-1-2	360	179	316	R 1	R 2	R 0	?
GSOR310688	Korea, South	MILYANG 56	360	179	316	R 1	R 0	R 0	?
GSOR310689	Korea, South	RAEGYEONG	360	179	316	R 1	R 0	R 0	?
GSOR310690	Korea, South		360	179	316	S 3	R 0	R 0	+
GSOR310709	Bangladesh	BR19	360	179	316	S 3	R 0	R 0	+
GSOR310730	Dominican Republic	JUMA 61	360		316	R 0	R 0	R 0	?

GSOR310732	Colombia	C 3CU77-1CU-2CU-2CU-2CU-SMCU2	360	179	316	S 3	R 0	R 0	+
GSOR310735	Panama	ANAYANSI	360	179	316	S 3	R 0	R 0	+
GSOR310741	Cuba	PERLA	360	179	316	S 3	R 0	R 0	+
GSOR310746	Cambodia	376	360	179	316	S 4	R 2	R 0	+
GSOR310748	Nepal	IR-44595	360	176	316	S 3	R 0	R 0	+
GSOR310750	Nigeria	FARO 37	360	179	316	R 0	R 0	R 0	?
GSOR310751	India	RP1821-5-17-2	360	179	316	R 0	R 0	R 0	?
GSOR310752	Cuba	ECIA 128	360	179	316	R 0	R 0	R 0	?
GSOR310753	Egypt	GZ1368-5-4	360	179	316	R 0	R 0	R 0	?
GSOR310754	Argentina	H232-44-1-1		164	418	S 3	R 2	R 0	*
GSOR310756	Dominican Republic	J355-6-2-1-1	360	179	316	R 0	R 0	R 0	?
GSOR310757	India	RP2151-173-1-8	360	179	316	S 3	R 2	R 0	+
GSOR310770	China	MIYANG	360	179	316	S 4	R 0	R 0	+
GSOR310772	Brazil	CL SELECCION 56	360	179	316	R 0	R 1	R 0	?

GSOR310773	Cuba	ECIA76-S89-1	360	179	316	S 3	R 0	R 0	+
GSOR310856	China	WC 521		164	418	S 3	R 1	R 0	*
GSOR311005	Philippines	IR 8-296-2-1	360	179	316	S 3	R 0	R 0	+
GSOR311024	India	RP1 332	360	179	316	S 3	R 2	R 0	+
GSOR311032	Guyana	50638	360	179	316	S 3	R 0	R 0	+
GSOR311033	Argentina	FORTUNA CORRIENTES SEL INTA	360	179	421	S 3	R 0	R 0	+
GSOR311039	Philippines	IR 1321-19	360	179	316	R 0	R 0	R 0	?
GSOR311042	Philippines	IR 1314-28-1-2	360	179	316	S 3	R 0	R 0	+
GSOR311044	Philippines	IR 773A1-36-2-1-3	360	179	316	R 0	R 0	R 0	?
GSOR311059	Philippines	IR 1103-49-4-1-3-3-2		164	418	S 3	R 0	R 0	*
GSOR311061	Philippines	Siryan	360	179	316	S 4	R 0	R 0	+
GSOR311066	Laos	Kh. Mack Fay	360	179	316	S 3	R 0	R 0	+
GSOR311073	Indonesia	Tukan Tuna	360	179		S 3	R 0	R 0	+
GSOR311076	Bulgaria	Sesilla	360	179	316	S 3	R 0	R 0	+
GSOR311082	Pakistan	Hansraj		164		R 0	R 0	R 0	?

GSOR311097	Portugal	Indo Yiaia Lonica	360	179	316	R 0	R 0	R 0	?
GSOR311113	Hong Kong	Shui Ya Jien	360	179	316	S 3	R 0	R 0	+
GSOR311152	Fiji	Rani	360	179	316	S 3	R 0	R 0	+
GSOR311153	Philippines	IR 2061-214-2-3	360	176	316	S 3	R 0	R 0	+
GSOR311154	Philippines	IR2151-598-3-5	360	179	316	S 3	R 0	R 0	+
GSOR311162	Guyana	60-283	360	179	318	S 3	R 2	R 0	+
GSOR311168	Philippines	IR9-60	360	179	316	S 3	R 0	R 0	+
GSOR311184	Thailand	Bang Tuey	360	176	316	S 4	R 0	R 0	+
GSOR311207	India	NP 97		164	316	S 3	R 2	R 0	+
GSOR311210	Philippines	IR 2151-745-3-1	360	179	316	S 3	R 2	R 0	+
GSOR311213	Bangladesh	BIPLAB	360	179	316	S 3	R 2	R 0	+
GSOR311214	Philippines	IR 1514A-E597	360	176	318	S 3	R 1	R 0	+
GSOR311217	Pakistan	Sella Manzkhora	360	179	316	S 3	R 0	R 0	+
GSOR311219	Korea, South	SUWEON 258	360	179	316	R 1	R 0	R 0	?
GSOR311223	Indonesia	KN-1 B-361-BLK-2	360	172	316	S 3	R 0	R 0	+

GSOR311238	Sierra Leone	Chen Chu Ai	360	179		S 3	R 1	R 0	+
GSOR311239	Brazil	Pratao Tipo Guedes		164	421	S 4	R 0	R 0	*
GSOR311244	Peru	INTI	360	179	316	R 0	R 0	R 0	?
GSOR311248	Dominican Republic	Mingolo	360	179	316	S 4	R 0	R 0	+
GSOR311249	Dominican Republic	TONO BREA 439	360	179	316	S 5	R 0	R 0	+
GSOR311253	Fiji	BILO	360	179	316	S 3	S 3	S 3	-
GSOR311262	Zaire	R 46/3		164	421	S 3	R 0	R 0	*
GSOR311264	Zaire	Sechele		164	421	S 3	R 2	R 0	*
GSOR311294	Senegal	CAS 209	360	179	316	S 3	R 2	R 0	+
GSOR311298	Thailand	Jek Chuey 159	360	179	316	S 3	R 2	R 0	+
GSOR311302	Sierra Leone	SL 22-613	360	179	421	S 4	R 2	R 0	+
GSOR311304	Nigeria	ADNY 11	360	179	316	R 0	R 0	R 0	?
GSOR311306	Nigeria	Mange2	360	179	316	S 3	R 0	R 0	+

GSOR311310	India	Archana	360	179	318	S 4	R 0	R 0	+
GSOR311317	Philippines	IR 1615-246	360	179	316	S 3	R 0	R 0	+
GSOR311325	Italy	Bajang Allorio	360	179	316	S 3	R 0	R 0	+
GSOR311344	Philippines	IR 9209-26-2	360	179	316	S 4	R 2	R 0	+
GSOR311348	Korea. South	SEOGWANGBYEO	360	179	316	S 3	R 0	R 0	+
GSOR311359	Colombia	17632	360	179	316	S 3	R 0	R 0	+
GSOR311360	Colombia	19965	360	179	316	S 3	R 0	R 0	+
GSOR311366	China	Te Qing	360	179	316	S 3	R 0	R 0	+
GSOR311380	Bangladesh	BR24	360	179	316	S 3	R 0	R 0	+
GSOR311399	Colombia	AMISTAD 82	360	179	316	S 3	R 0	R 0	+
GSOR311402	Ecuador	INIAP 11	360	179	316	S 4	R 0	R 0	+
GSOR311403	Colombia	PANAMA 1048	360	179	316	S 3	R 0	R 0	+
GSOR311405	Colombia	HURI 282	360	179	316	S 3	R 0	R 0	+
GSOR311409	Mexico	CAMPECHE A 80	360	179	316	R 1	R 0	R 0	?
GSOR311411	Peru	SAN MARTIN 86	360	179	316	R 1	R 0	R 0	?

GSOR311421	Philippines	C2764-10-2	360	179	316	R 1	R 0	R 0	?
GSOR311423	Philippines	IR 58614-B-B-8-2	360	176	316	S 3	R 0	R 0	+
GSOR311424	Japan	BL 1	360	179	418	S 3	R 0	R 0	+
GSOR311430	Cuba	ECIA 66	360	179	316	S 3	R 2	R 0	+
GSOR311433	Philippines	IR 54055-142-2-1-2-3	360	179	316	S 4	R 0	R 0	+
GSOR311435	Vietnam	CM1, HAIPONG	360	179	316	S 3	R 0	R 0	+
GSOR311436	China	ZHONGYU NO.1		172	318	S 3	R 0	R 0	*
GSOR311438	Liberia	2071-621-2	360	179	316	S 3	R 2	R 0	+
GSOR311439	China	4582	360	172	316	S 3	R 0	R 0	+
GSOR311441	China	GP-2	360	179	316	S 3	R 0	R 0	+
GSOR311442	Philippines	IR58025 B	360	179	316	S 3	R 0	R 0	+
GSOR311443	China	GUI 99	360	179	316	S 3	R 0	R 0	+
GSOR311445	China	Z 535	360	179	316	S 4	R 0	R 0	+
GSOR311447	China	XIANGZHAOXIAN NO. 15	360	179	316	S 4	R 0	R 0	+
GSOR311448	China	HUNANRUANMI	360	179	316	S 3	R 0	R 0	+

GSOR311449	China	ZHONGYU NO. 6	360	179	316	S 3	R 0	R 0	+
GSOR311456	China	ERXI NO. 149	360	179	316	S 3	R 0	R 0	+
GSOR311459	China	71198	360	179	316	S 3	R 0	R 0	+
GSOR311467	China	JINNUO NO. 6	360	176	316	S 3	R 2	R 0	+
GSOR311468	China	DIAN NO. 01		172	316	S 3	R 0	R 0	+
GSOR311471	China	YOU NO. 51	360	179	316	S 3	R 0	R 0	+
GSOR311477	China	H 323	360	179	316	S 3	R 0	R 0	+
GSOR311478	China	CDR 22	360	179		S 3	R 0	R 0	+
GSOR311481	China	SHUFENG 121	360	179	316	S 3	R 0	R 0	+
GSOR311494	China	R 647	360	179	316	S 5	S 3	S 3	_
GSOR311503	China	ZHONG 413	360	179	316	R 0	R 0	R 0	+
GSOR311511	China	MPH 501	360	179	316	S 3	R 0	R 0	+
GSOR311513	China	ZAO 402	360	179	316	R 0	R 0	R 0	+
GSOR311518	Bangladesh	Bhujon Kolpo	360	179	316	S 3	R 0	R 0	+
GSOR311519	Bangladesh	Khoia	360	179	316	S 3	R 0	R 0	+

GSOR311520	Bangladesh	Bogra	360	179	316	S 3	R 0	R 0	+
GSOR311521	Philippines	IR 56450-28-2-2	360	179	316	S 3	R 0	R 0	+
GSOR311524	India	RP2199-16-2-2-1	360	179	316	S 3	S 3	R 0	+
GSOR311525	Indonesia	S972B-22-1-3-1-1	360	179	316	S 4	R 0	R 0	+
GSOR311640	India	ARC 10378		176	418	S 5	S 3	S 4	-
GSOR311668	Pakistan	Daudzai Field Mix		164	318	S 5	S 3	S 4	-
		Saber	360	179	316	R 0	S 0	R 0	+

+ Accessions containing *Pi-b* with different haplotypes
? Accessions containing additional R genes
- Accessions which do not contain *Pi-b*
* Accessions with no marker alleles with pathogenicity response similar to accessions containing *Pi-b*

Table 2. Summary of marker sizes, chromosomal locations, annealing temperature, dilution and sequences of simple sequence repeat markers at the *Pi-b* locus.

Markers	**Size (bases)**	**Chromosome Location**	**Chromosome Distance**	**Annealing Temperature(^{0}C)**	**Dilution**	**Forward Primer**	**Reverse Primer**
Pibdom	360	2	0.0	55	1:2000	GAACAATGCCCAAACTTGAGA	GGGTCCACATGTCAGTGAGC
RM208	179	2	0.0	55	1:500	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC
RM166	316	2	2.3	61	1:200	GGTCCTGGGTCAATAATTGGGTTACC	TTGCTGCATGATCCTAAACCGG

Approximate nucleotide (nt) sizes were based on 'Nipponbare' sequence determined from Gene Bank database information.

Figure Legends

Fig.1. Major blast resistance genes and their response to various US blast races. *Pi-b* has a broader spectrum of resistance than other genes shown and is resistant to IE1k.

Fig. 2. Pathogenicity Assay

A-D. Different stages of seedling development until the third leaf stage; E. AVR race IE1k ; F. Virulent race IB54; G. Seedlings sprayed with 40 ml of spore suspension/tray using a concentration of $5X10^5$ spores/ml. The spores were sprayed by placing the seedling tray in plastic bags making sure that inoculum was evenly distributed; H. The seedlings were left for incubation at room temperature; I. Plastic bags were opened and the seedlings allowed to grow for approximately 7 days for disease symptoms to appear; J. Improved evaluation standard for determining disease reactions of rice germplasm. Resistant (0-2); No lesion formation- 0; Lesions covering less than 5% of total leaf area, lesions restricted at the site of infection-1; Lesions covering between 5% to 10% of the total leaf area; restricted spindle lesions at diameter less than 2 mm - 2; Susceptible (3-5): Lesions in several locations on the leaf to form a large eye-shaped brown area (diameter greater than 2mm) - 3; Lesions covering greater than 50% of the leaf area, diseased area with lesion greater than 30% of the total leaf area - 4; Lesions covering greater than 70% of the total leaf area - 5. Note: Improvement was based on disease reactions of both *indica* and *japonica* cultivars to blast. Plants at the three to four leaf stage were inoculated and the second youngest leaf was evaluated one week after inoculation.

Fig. 3. SSR marker genotype analysis

A. Allele maker for a Pib dominant marker; B. Allele marker for RM208; and C. Allele marker for RM166. The DNA used was from the different cultivars. The SSR marker genotype analysis was performed using Gene Mapper® software version 3.7, Applied Biosystems, Foster City, CA.

Fig.4. Analysis of the *Pi-b* gene in rice germplasm using disease reaction and SSR markers. The diagram shows results of disease reactions and expected SSR marker alleles for germplasm in different categories.

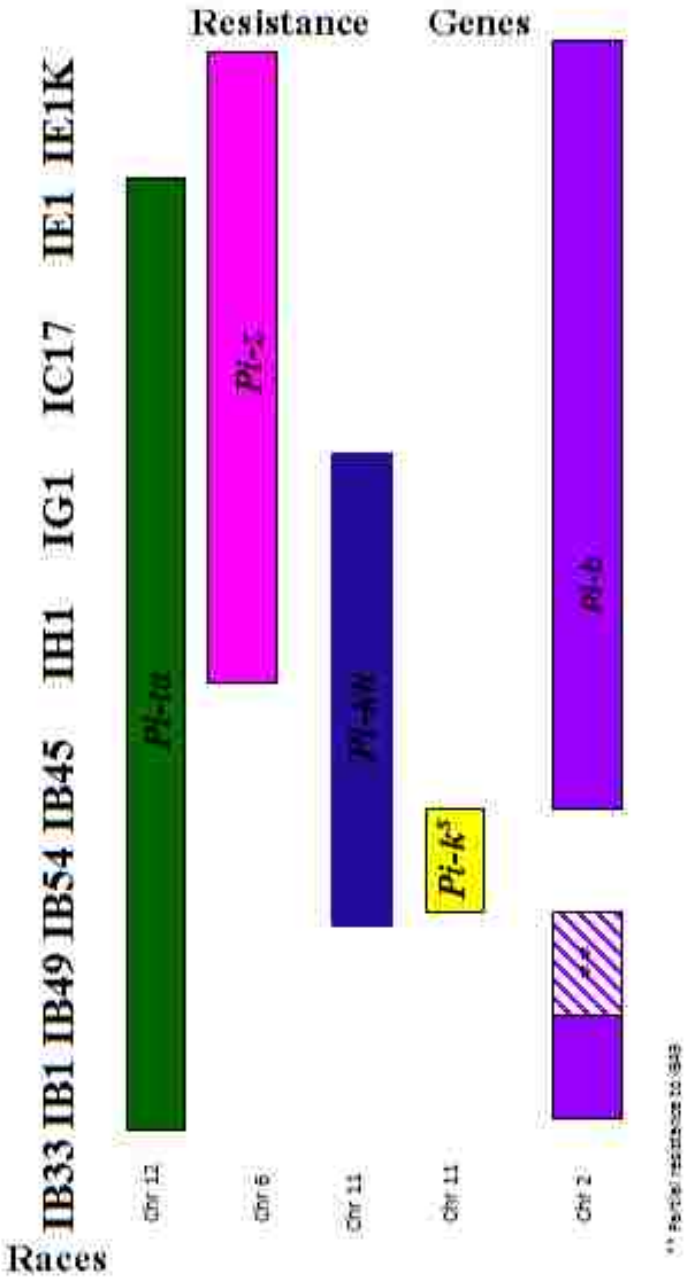
Resistance Genes
IE1K
IE1
IC17
IG1
IH1
IB45
IB54
IB49
IB1
IB33
Races
Pi-ta
Pi-2
Pi-kh
Pi-k^s
Pi-b
Chr 12
Chr 6
Chr 11
Chr 11
Chr 2

Fig. 1

A
Seeds germinated (Day 0)
B
Germinated seedlings 4th leaf stage(3 days old seedlings shown)
C
Germinated seedlings 4th leaf stage(3 days old seedlings shown)
D
Germinated seedlings 4th leaf stage(4 days old seedlings shown)
E
IE1k (TM2)
F
IB54 (laminarase isolate)
G
Seedling sprayed with 40ml of spore suspension 5X10⁵ spores/ml, 0.3% gelatin
H
Isolates were grown in oatmeal media 10 days prior to spraying (Petri dish plate shown)
I
24hr after inoculation, plants were moved to the green house and maintained for a additional days before rating
J
Resistant 0-2
Susceptible 3-5
Leaf rating showing after evaluating virulence
Incubation at 21°C,95% relative humidity for 24 hr

Fig. 2

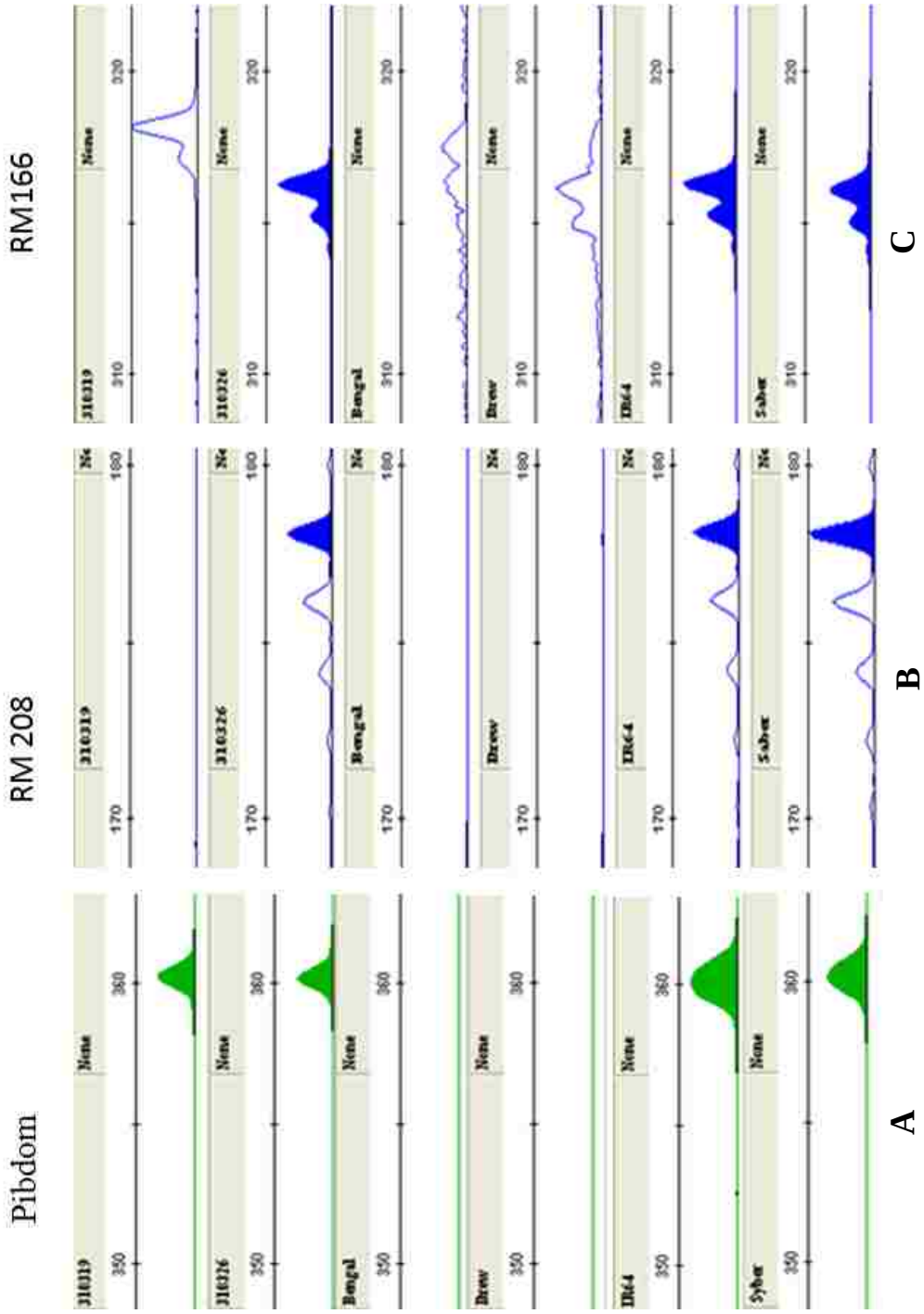

Fig. 3

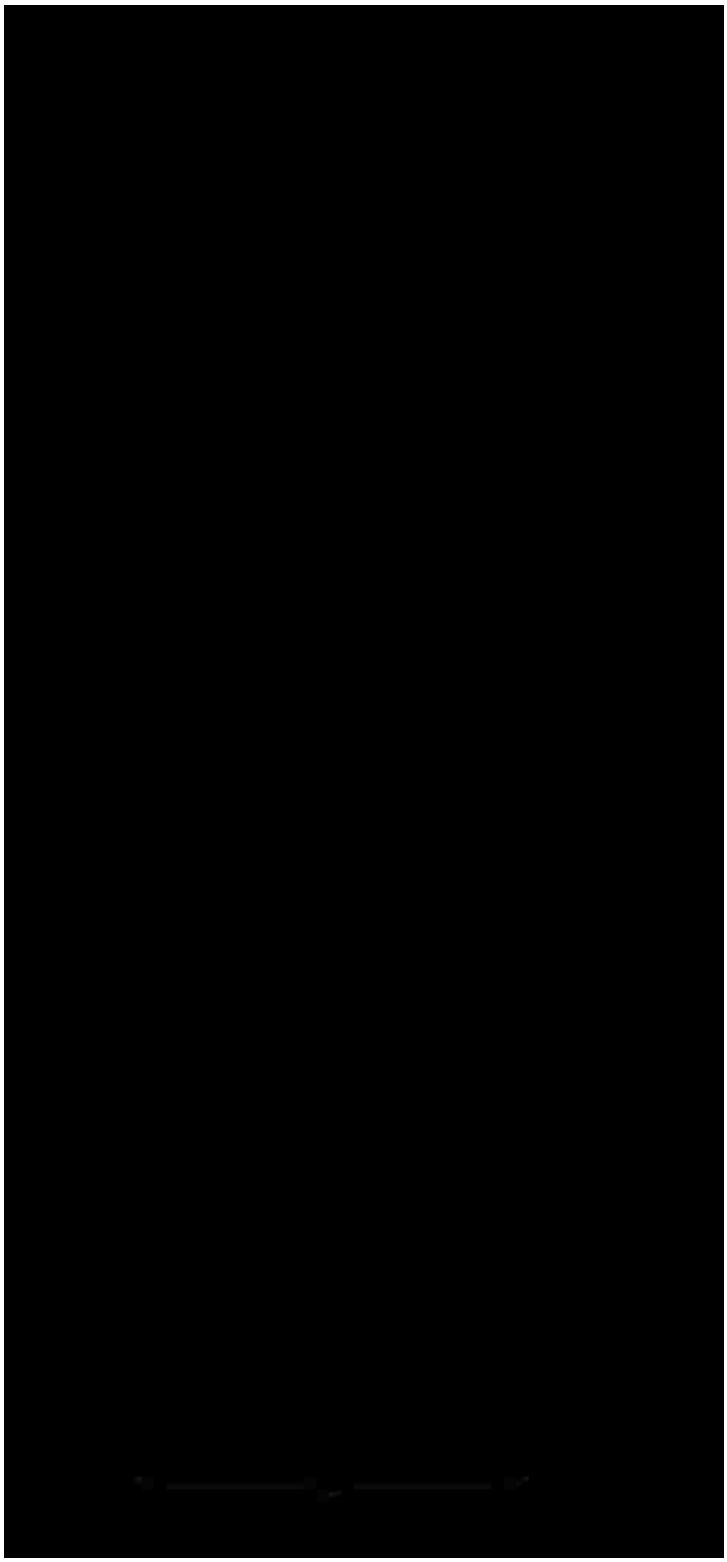

Fig. 4

Summary

Pi-z and the *Pi-b* genes are two widely studied blast resistant genes in the Southern US. These genes confer resistance to the blast race IE1k, known to overcome rice cultivars containing the widely used resistant gene *Pi-ta*, making these genes of interest to researchers and rice breeders. In 2004, IE1k resulted in significant economic loss in several rice fields planted to the rice cultivar 'Banks', known to contain *Pi-ta*. Subsequently, 'Banks' was discontinued by the rice industry.

The overall objective of our studies were to identify germplasm accessions from the worldwide Oryzae collection at the Dale Bumpers National Rice Germplasm Center containing *Pi-z* and *Pi-b* for use by rice breeders.

Today, marker-assisted selection (MAS) is one of the most widely used methods in rice breeding for improved resistance to blast disease. MAS selections are based on DNA markers closely linked to a blast *R* gene that confers resistance to a particular race of the pathogen. MAS can be used to screen seeds or seedlings under laboratory conditions, which is much faster than traditional pathogenicity assays in which accurate selection can only be made during later stages of plant growth. In addition, MAS can avoid the overlapping effects of other matched pairs of *R* and *AVR* genes. Since it is more difficult and time-consuming to determine the presence or absence of the gene based solely on pathogenicity assays, the marker allele which is linked with gene is used to determine the presence of the gene. The assumption is that the linked allele associates with the gene and/or quantitative trait locus (QTL) of interest. Hence MAS can be useful for traits that are difficult to measure, exhibit low heritability, and/or are expressed late in development.

This research used a non SNP type of marker approach to characterize the *Pi-z* gene which was not reported previously. Also, the markers used were more closely linked than the SNP markers developed previously, hence minimizing chances of recombination. This research also confirmed marker analysis by using targeted pathogenicity assays.

Pi-b characterization utilized a dominant marker (Pibdom),a fragment from the *Pi-b* cloned gene. In addition, SSR markers and pathogenicity assays were also utilized to characterize this gene.

The germplasm accessions identified can be utilized as donors for breeding and as reference accessions by inoculating with additional isolates of *M. oryzae.* Germplasm accessions identified as lacking *Pi-z* and *Pi-b* alleles can be used as recurrent breeding parents for receiving the genes to develop improved resistance to current blast races.

The *Pi-z* gene was confirmed in 81 germplasm accessions with different haplotypes out of 117 germplasm accessions which were marker tested previously at DBNRRC. The findings also matched the pathogenicity data. The study also confirmed *Pi-b* in 88 of 164 germplasm accessions from around the world, and previously marker tested at DBNRRC. Characterization was accomplished utilizing previously identified DNA markers and pathogenicity assays, resulting in improved results.

For germplasm requests, please visit (www.ars.usda.gov/spa/dbnrrc/gsor) at GSOR of DB NRRC.