New Insights Into Rice Blast Resistance Provide Novel Strategies For Developing Durable Resistance to *Magnaporthe oryzae*

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Abstract

Blast, caused by the filamentous ascomycete fungus Magnaporthe oryzae, is a destructive and widespread disease of rice. The planting of resistant cultivars is the most economic and environmentally sound way to control the disease. In the last two decades, significant progress has been made in elucidating the molecular mechanism of host resistance and fungal pathogenesis. Over 85 resistance (R) genes have been mapped in the rice genome, and 23 have been cloned. Similarly, about 40 avirulence (Avr) genes have been mapped in the M. oryzae genome, and nine have been cloned. Based on deep understanding of the molecular basis of host resistance and fungal pathogenesis and rapid advancement of new genomics technologies, we propose four novel strategies for breeding next-generation rice cultivars that are resistant to M. oryzae.

Keywords: Rice, blast, *Magnaporthe oryzae,* durable resistance, avirulence genes, gene silencing

Introduction

Rice is the primary staple for more than half of the world's population and is crucial for food security and social stability in many developing countries in Asia and Africa. Rice production, however, can be greatly reduced by blast, a destructive disease in almost all rice-growing regions. Rice blast is caused by the filamentous ascomycete fungus *Magnaporthe oryzae*, which was recently listed as one of the ten most important fungal pathogens of crop plants (Dean et al 2012).Blast reduces rice yield 10 to 30% and by even more in epidemic years (Skamnioti and Gurr 2009). The most effective and economic method for the control of this disease is the planting of blast-resistant cultivars. Because of the high variability of the *M. oryzae* genome, however, the resistance of new cultivars is often overcome within a few years. Therefore, developing innovative strategies for breeding next-generation rice cultivars with broad-spectrum and durable resistance has becomea high priority in rice breeding programs. This mini-review discusses recent research concerning the molecular basis of host resistance and fungal pathogenicity, and also proposes novel approaches for blast control.

Recent advances in understanding host resistance

Rice blast resistance can be classified as qualitative and quantitative. Qualitative resistance is usually race-specific and complete, and is often associated with an early and strong hypersensitive reaction (HR) at the infection site. Qualitative resistance is often controlled by a single dominant or recessive resistance (*R*) gene. In contrast, quantitative resistance is partial (pathogen infection is reduced but not stopped) and is generally controlled by multiple genes or quantitative trait loci (QTLs), each of which has minor effects. During the last two decades, 85 major *R* genes and 49 meta-QTLs on the rice linkage map have been mapped (Ballini et al 2008; Liu et al 2010). Among them, 21 blast *R* genes and two blast QTLs have been cloned. The isolation of these genes has not only provided new insights into the molecular mechanism of host resistance in rice, but has also accelerated the breeding of new rice cultivars with broad-spectrum resistance by marker-assisted selection (MAS).

The predicted proteins encoded by cloned *R*genes in rice can be classified into three types: nucleotide binding sites (NBS)-leucine rich repeat (LRR) proteins (NLRs), receptor-like kinase proteins (RLKs), and proline-rich proteins. The *R* genes that have been determined to encode NLRs are: *Pib, Pita, Pi9, Pi2, Piz-t, Pi36, Pi37, Pikm, Pit, Pi5, Pid3, Pi54^{kh}, Pish, Pik, Pik-p, Pia, Pi25, Pb1, Pi1,Piks*, and *Pi7* (Liu et al 2007). Notably, several of the cloned *R*genes are allelic to each other and clustered in a specific chromosome region. For example,

at least 10 alleles (*Pi9*, *Pi2*, *Piz-t*, *Piz*, *Pigm*, *Pi26*, *Pi40*, *Pi50*, *qBR6*, and *Pi2-1*) are located at the Pi*2/9* locus in a 100-kb interval on chromosome 6 (Liu et al 2010; Wang et al 2012). Sequence analysis demonstrated that Pi*2/9*is an ancient and conserved locus in both cultivated and wild species (Dai et al 2010; Liu et al 2011).

In the long arm of chromosome 11, the *Pik* locus also contains at least 11 allelic genes (*Pi-k*, *Pikm*, *Pikg*, *Pikh*, *Pik-p*, *Piks*, *Pikh*, *Pi-1*, *Pi-7*, *Pi46*, and *Pi47*), six of which have been isolated: *Pikm* (Ashikawa et al 2008), *Pik* (Zhai et al 2011), *Pik-p* (Yuan et al 2011), *Pi1* (Hua et al 2012), *Piks* (PanQ et al, personal comm), and *Pi7*(Gan L et al personal comm). Interestingly, all six genes require two NLR genes to generate resistance. Similarly, two other genes, *Pi5* and *Pia*, also require two NLR members to function together (Lee et al 2009; Okuyama et al 2011). Further understanding of these two-component NLRs may provide novel insight about the interaction between the two NLRs and their cognate avirulence effectors from *M. oryzae*.

Pid2 is the only member in the second group, and *pi21* is the sole one in the third group. *Pid2* encodes a novel plasma membrane-localized RLK protein with an extracellular domain of a bulb-type mannose-specific binding lectin (B-lectin) (Chen et al 2006). *pi21* is a recessive gene and the first isolated blast QTL (Fukuoka et al 2009). The dominant *Pi21* gene encodes a proline-rich protein that includes a putative heavy metal-binding domain and protein–protein interaction motifs. A deletion of the proline-rich motif in the wild-type *Pi21* leads to the gain of resistance against the infection, suggesting that the *pi21*-mediated partial resistance might represent a novel defense mechanism in plants.

Function of fungal effect or proteins in pathogenesis and their interaction with host targets

Plant pathogens secret numerous effector proteins into host cells that interfere with plant defense (Hogenhout et al 2009). Based on genome-wide analysis and depending on the threshold and program used, 7% (739) (Dean et al 2005), 12% (1546) (Soanes et al 2008), or 22% (2470) (Choi et al 2010) of *M. oryzae* genes were predicted to encode putative secreted proteins. Most avirulence (*Avr*) genes encode effector proteins. More than 40 *Avr* genes in *M. oryzae* have been identified (Ma et al 2006), and nine have been molecularly characterized: *PWL1*, *PWL2*, *AvrPi-ta*, *AvrPiz-t*, *AvrPia*, *AvrPii*, *AvrPik/km/kp*, *Avr-CO39*, and *ACE1*. *ACE1* encodes a hybrid polyketide synthase-nonribosomal peptide synthetase (PKS-NRPS) (Collemare et al 2008), but the other eight genes are putative secreted proteins and have no highly homologous proteins in other fungal species.

To interfere with host defense and cellular processes, blast effector proteins must be delivered into host cells. Analysis of the biotrophy-associated secreted (BAS) protein 1 (BAS1), which was found to preferentially accumulate in biotrophic interfacial complexes (BIC) along with the Avr effector PWL2 (Mosquera et al 2009), demonstrated that blast effectors are delivered into rice cytoplasm by biotrophic invasive hyphae (IH). Furthermore, PWL2 and BAS1 are translocated into rice cytoplasm and even moved into un-invaded neighbor cells (Khang et al 2010). Recently, a secreted LysM protein 1 (SLP1) was shown to be translocated into rice cells and to accumulate at the interface between the fungal cell wall and the rice plasma membrane. SLP1 can bind to chitin to suppress pathogen-associated molecular patterns (PAMPs) chitin-triggered immune responses by competing with the chitin-elicitor binding protein (CEBiP) for binding of chitin oligosaccharides (Mentlak et al 2012).

Innovative strategies for rice blast control

Because of the rapid development of new genomics technologies and the deeper understanding of host defense and fungal pathogenicity, several new approaches are being developed for breeding of next-generation rice cultivars that confer broad-spectrum resistant to blast.

1. Identification of functional, single nucleotide polymorphism (SNP) markers for genomics-based breeding of blast-resistant cultivars

With the application of high throughput sequencing technologies, genome-wide association study (GWAS) is becoming a highly efficient gene mapping strategy in rice. For example, Huang et al (2010; 2012) identified 80 QTLs for 14 rice agronomic traits in a collection of 517 rice landraces and 32 new loci in 950 rice cultivars. Similarly, SNP-chip-based GWAS has been used to map complex traits in 400 rice lines (Zhao et al 2011). These GWAS methods can be easily adapted for the mapping of both qualitative and quantitative resistance genes in order to identify functional SNPs against local *M. oryzae* populations. The availability of SNP markers will accelerate genomics-based breeding for rice blast resistance.

2. Deployment of *R* genes according to *Avr* gene frequency in the field

Breakdown of blast resistance in rice cultivars is mainly due to the shift of the avirulence composition in the *M. oryzae* populations. Resistant cultivars with known *R* genes can be deployed strategically in a region based on the frequency of their corresponding *Avr* genes in the fungal populations. With more than 40 *Avr* genes

mapped and nine of them cloned, PCR can now be used to monitor the frequency of *Avr* genes that correspond to the *R* genes in the specific rice cultivars currently planted in a region. This information can be used to guide the rotation of elite rice lines with different *R* gene combinations.

3. Host-induced gene silencing (HIGS)

Research demonstrated that host resistance to *Fusarium verticillioides*, *Blumeria graminis* and *Puccinia striiformis f. sp. tritici* can be enhanced by the expression of fungal genes in transgenic host plants (see review by Nunes and Dean 2012). If genes essential for the growth and development of invasive hyphae of *M. oryzae* inside rice cells are efficiently silenced by their corresponding HIGS transgenes, this approach should be effective in reducing blast infection.

4. Modification of host genes targeted by *M. oryzae* effectors

Li et al (2012) recently used transcription activator-like (TAL) effector nucleases (TALEN) technology to modify a specific target gene recognized by the *Xanthomonas oryzae* pv. *oryzae* TAL effectors. The method causes precise editing of the disease-susceptibility elements in *Os11N3* and leads to resistance to the pathogen in transgenic rice. As more host targets of blast effectors are identified, modifications of these genes in transgenic rice using TALEN technology should facilitate the engineering of rice plants that are highly resistant to *M. oryzae*.

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