

The *Magnaporthe oryzae* Avirulence Gene *AvrPiz-t* Encodes a Predicted Secreted Protein That Triggers the Immunity in Rice Mediated by the Blast Resistance Gene *Piz-t*

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Submitted 24 September 2008. Accepted 17 November 2008.

The Magnaporthe oryzae avirulence gene AvrPiz-t activates immunity in a gene-for-gene fashion to rice mediated by the blast resistance gene Piz-t. To dissect the molecular mechanism underlying their recognition, we initiated the cloning of AvrPiz-t using a map-based cloning strategy. The AvrPiz-t gene was delimited to an approximately 21-kb genomic fragment, in which six genes were predicted. Complementation tests of each of these six candidate genes led to the final identification of AvrPiz-t, which encodes a 108-amino-acid predicted secreted protein with unknown function and no homologues in *M. oryzae* or in other sequenced fungi. We found that AvrPiz-t is present in the virulent isolate GUY11 but contains a Pot3 insertion at a position 462 bp upstream from the start codon. Complementation tests of AvrPiz-t genes driven by promoters of varying length revealed that a promoter larger than 462 bp is essential to maintain the AvrPiz-t function. These results suggest that a Pot3 insertion in GUY11 might interfere with the proper function of AvrPiz-t. Additionally, we found that AvrPiz-t can suppress the programmed cell death triggered by mouse BAX protein in Nicotiana benthamiana, identifying a mechanism by which AvrPiz-t may contribute virulence of M. oryzae.

In the long warfare between plants and phytopathogens, the plants have culminated in a highly effective immune system able to protect them from attack by a wide variety of pathogens. They have evolved two layers of immune responses referred to as pathogen-associated molecular pattern–triggered immunity (PTI) and effector-triggered immunity (ETI) (Chisholm et al.

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2006; Jones and Dangl 2006). The recognition of common features of microbial pathogens by diverse plant cell-surface receptors activates the PTI response to mount the primary defense. For example, both FLS2 and EFR can activate resistance to bacterial disease through perception of flagellin and EF-Tu, respectively (Zipfel et al. 2004, 2006). However, PTI can be effectively suppressed by so-called effector proteins encoded and delivered by phytopathogens into the plant cytosol. For example, several type III secretion system (T3SS) effectors from Pseudomonas syringae (e.g., HopM1, AvrPto, AvrRpm1, AvrRpt2, and HopAO1) were found to interfere with PTI in Arabidopsis (de Torres et al. 2006; He et al. 2006; Kim et al. 2005; Li et al. 2005; Nomura et al. 2006; Oh and Collmer 2005; Underwood et al. 2007). In order to monitor the presence of pathogen effectors, plants have developed the ETI, a more specialized immune response mediated by surveillance proteins, the so-called resistance (R) proteins. The resistance activated by this pairwise association of R and avirulence (Avr) genes was proposed as gene-for-gene resistance decades ago (Flor 1971). Only the coexistence of an R gene and its cognate Avr gene during interaction can activate the resistance, which is manifested as localized cell death (i.e., hypersensitive response [HR]). This response occurs at the site of infection to inhibit pathogen growth. The majority of R genes in plants encode nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins (Dangl and Jones 2001). Comparative genomics has demonstrated that all plants have large collections of NBS-LRR genes (McHale et al. 2006). Arabidopsis, for instance, maintains approximately 150 NBS-LRR genes (Meyers et al. 2003).

It has been found that bacterial pathogens utilize a specialized T3SS to deliver the Avr proteins into the plant cytoplasm (Alfano and Collmer 2004; Grant et al. 2006). Most Avr genes in eukaryotic pathogens encode secreted proteins, including flax rust AvrL567, AvrM, AvrP4, and AvrP123 (Catanzariti et al. 2006; Dodds et al. 2004), AvrPi-ta in Magnaporthe oryzae (Orbach et al. 2000), Avr1b-1 in Phytophthora sojae (Shan et al. 2004), Avr3a in P. infestans (Armstrong et al. 2005), and ATR13 (Allen et al. 2004) and ATR1 (Rehmany et al. 2005) in Hyaloperonospora parasitica. Furthermore, expression of these Avr genes inside the host cytoplasm was found to be

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The genomic sequence of the *AvrPiz-t* gene was deposited at GenBank database (accession no. EU837058).

^{*}The *e*-Xtra logo stands for "electronic extra" and indicates that three supplementary tables are published online.