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e-Xtra*

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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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. 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.