



Understanding the Molecular Mechanism of Instability of the Avirulence Gene *AVR-Pita1* in Field Isolates of *Magnaporthe oryzae*



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Abstract

The avirulence gene *AVR-Pita1* in *Magnaporthe oryzae* triggers a resistance response in rice plants that contain the resistance gene *Pi-ta*. To understand the molecular mechanism of its instability, a total of 187 field isolates of *M. oryzae* collected from the U.S., China, Columbia, Egypt, India, and the Philippines over a 40-year period were studied. Among them, 151 were determined to be avirulent toward *Pi-ta* while 36 were virulent. *AVR-Pita1* was amplified and sequenced from the 151 avirulent isolates as well as 4 virulent isolates. Based on DNA sequences, 38 haplotypes of *AVR-Pita1* were identified in avirulent isolates and the gene was found to be under diversifying selection in nature. A two-base-pair insertion leading to frame-shift mutation, 5' portion deletion and complete deletion were detected in the *AVR-Pita1* locus in virulent isolates demonstrating that these three mechanisms are responsible for "defeating" *Pi-ta*-mediated resistance in commercial rice cultivars. Finally, virulent field isolates from Arkansas became avirulent after *AVR-Pita1* was incorporated. These findings should impact the development of novel strategies for crop protection in the future.

Introduction

Resistance to blast occurs when an avirulence (*AVR*) gene in the pathogen is recognized by a resistance (*R*) gene in rice (Flor, 1971). Genetic analysis demonstrated that *AVR-Pita1* determines the efficacy of the rice blast *R* gene *Pi-ta* (Orbach *et al.*, 2000); therefore, understanding the evolution of the *AVR-Pita1* gene in field isolates should benefit the deployment of *Pi-ta* for the control of rice blast disease. The objective of the present study was to investigate how sequence variations in the *AVR-Pita1* allele impact the efficacy of *Pi-ta*-mediated resistance responses and the potential alternative by which a stable resistance could be achieved with *AVR* genes in the future.

Materials and Methods

A total of 187 field isolates were used in this study. Pathogenicity assay (Fig.1) was used to characterize the race identity of the pathogen isolates. MEGA, DNasp, Vector NTI and TCS were used for sequence and evolutionary analyses. Southern blot was used to detect genomic variations in most virulent isolates. Homologous recombination technique was applied to validate the function of *AVR-Pita1* by introducing the gene into virulent isolates collected from Arkansas rice fields.

Results

Pathogenicity assay (Fig.1), evolutionary study (Fig.2), statistical analyses (Fig.3), mutation analyses (Fig.4, Fig.5, Fig.6) and functional analyses (Fig.7) are presented below.

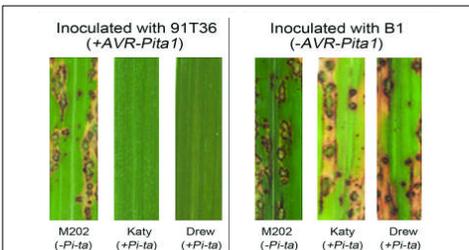


Fig 1. Disease reaction of U.S. rice cultivars inoculated with isolates with or without functional *AVR-Pita1*.

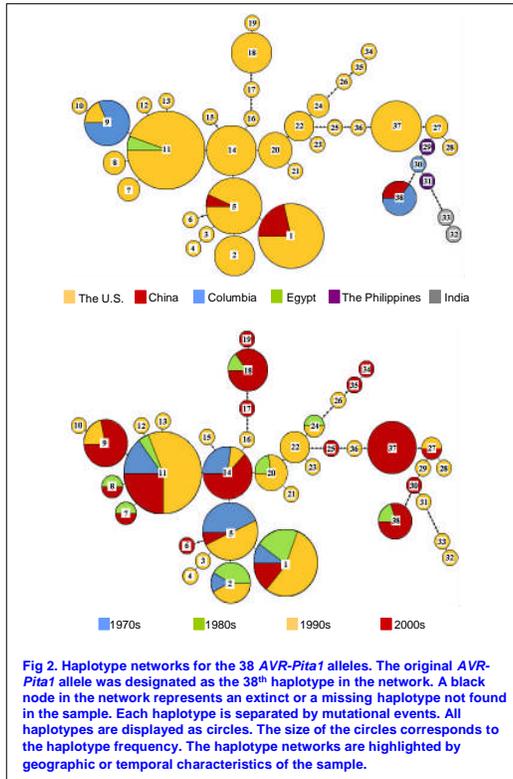


Fig 2. Haplotype networks for the 38 *AVR-Pita1* alleles. The original *AVR-Pita1* allele was designated as the 38th haplotype in the network. A black node in the network represents an extinct or a missing haplotype not found in the sample. Each haplotype is separated by mutational events. All haplotypes are displayed as circles. The size of the circles corresponds to the haplotype frequency. The haplotype networks are highlighted by geographic or temporal characteristics of the sample.

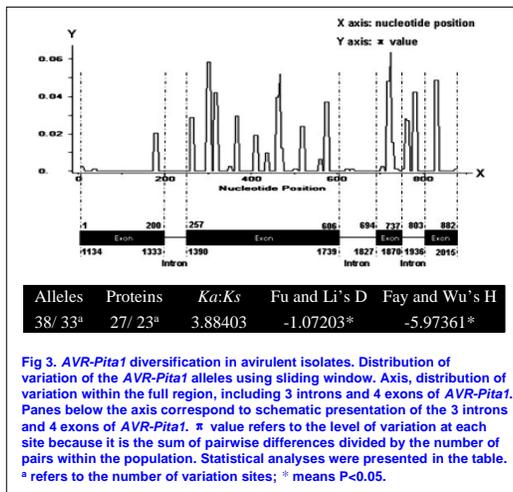


Fig 3. *AVR-Pita1* diversification in avirulent isolates. Distribution of variation of the *AVR-Pita1* alleles using sliding window. Axis, distribution of variation within the full region, including 3 introns and 4 exons of *AVR-Pita1*. Panes below the axis correspond to schematic presentation of the 3 introns and 4 exons of *AVR-Pita1*. π value refers to the level of variation at each site because it is the sum of pairwise differences divided by the number of pairs within the population. Statistical analyses were presented in the table. ^a refers to the number of variation sites; * means $P < 0.05$.

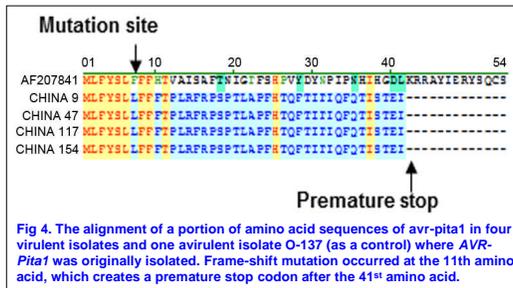


Fig 4. The alignment of a portion of amino acid sequences of *avr-pita1* in four virulent isolates and one avirulent isolate O-137 (as a control) where *AVR-Pita1* was originally isolated. Frame-shift mutation occurred at the 11th amino acid, which creates a premature stop codon after the 41st amino acid.

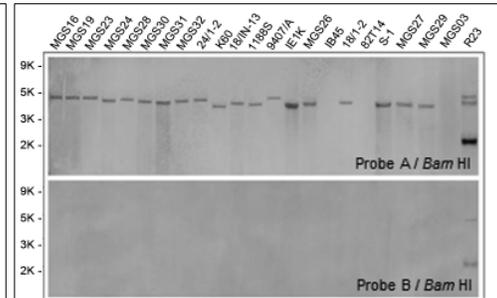


Fig 5. Partial and complete deletions of *AVR-Pita1* detected with Southern blot. Genomic DNA of each isolate was digested with *Bam*HI, and hybridized with probes A: *AVR-Pita1* coding region and B: 5' portion of *AVR-Pita1*, respectively.

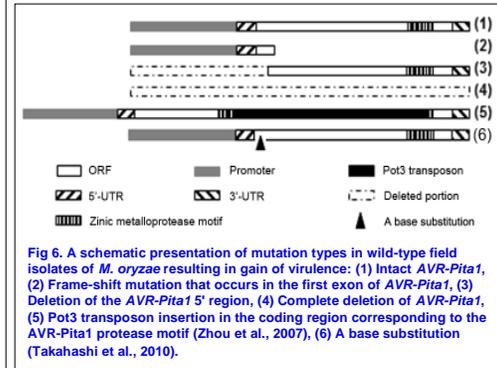


Fig 6. A schematic presentation of mutation types in wild-type field isolates of *M. oryzae* resulting in gain of virulence: (1) Intact *AVR-Pita1*, (2) Frame-shift mutation that occurs in the first exon of *AVR-Pita1*, (3) Deletion of the *AVR-Pita1* 5' region, (4) Complete deletion of *AVR-Pita1*, (5) Pot3 transposon insertion in the coding region corresponding to the *AVR-Pita1* protease motif (Zhou *et al.*, 2007), (6) A base substitution (Takahashi *et al.*, 2010).

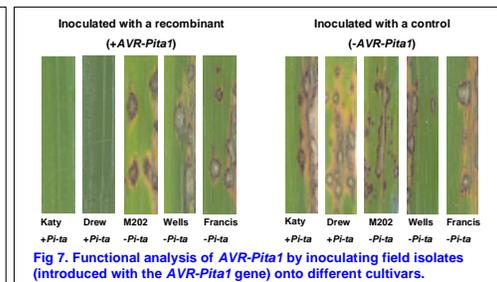


Fig 7. Functional analysis of *AVR-Pita1* by inoculating field isolates (introduced with the *AVR-Pita1* gene) onto different cultivars.

Conclusion

We provide evidence to support a hypothesis that *AVR-Pita1* is under diversifying selection and mutations in *AVR-Pita1* are responsible for defeating race-specific resistance in nature. We demonstrated that lost resistance can be restored by reintroducing *AVR-Pita1* into virulent field isolates of *M. oryzae*. In the future, we will identify cellular targets of *AVR-Pita1* for a better understanding of co-evolutionary dynamics of host and pathogen.

Reference:

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