Gene Reports 40 (2025) 102242

Contents lists available at ScienceDirect

Gene Reports

journal homepage: www.elsevier.com/locate/genrep

New haplotypes of *AvrPi-54* gene detected in *Pyricularia oryzae* from Malaysia, India and China

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ARTICLE INFO

Edited by Jormay Lim

Keywords:
Avirulence gene
Rice blast fungus
AvrPi-54 haplotypes
Neutrality test

ABSTRACT

Pyricularia oryzae, the causal agent of rice blast disease, poses a significant threat to rice production. Limited information exists regarding the genetic diversity of *Avr* genes and the resistance profile of Sarawak rice against *P. oryzae* isolates in Malaysia. This study aimed to investigate the sequence variation of the *AvrPi-54* gene in *P. oryzae* isolates from Sarawak rice, in comparison with sequences available in the National Centre for Biotechnology Information (NCBI) database. A total of 20 *P. oryzae* isolates were analyzed together with 98 *AvrPi-54* gene sequences from NCBI. Nine distinct haplotypes were identified, including six novel haplotypes. The *AvrPi-54* gene was found to be under positive selection pressure. Notably, Sarawak rice exhibited varying levels of susceptibility to *P. oryzae* isolates carrying the H1 haplotype. Understanding the genetic diversity of *AvrPi-54* together with other *Avr* genes is crucial for developing effective strategies to manage *P. oryzae* in Sarawak, Malaysia.

1. Introduction

Rice blast is a devastating fungal disease caused by *Pyricularia oryzae* (teleomorph: *Magnaporthe oryzae*), can lead to significant yield losses. Globally, this disease can reduce yields by 10–30 % (Mandal et al., 2023) and up to 70 % yield loss in Malaysia (Gianessi, 2014). One effective strategy to control rice blast disease is to develop resistant rice varieties through the integration of resistance (R) genes into susceptible cultivars. This approach aligns with the gene-for-gene interaction model proposed by Flor (1971), where plant R genes recognize specific avirulence (*Avr*) genes in the pathogen, triggering a resistance response. To overcome the *R*-gene mediated resistance of the host, *P. oryzae* population will evolve rapidly through sexual recombination and genetic mutation. This co-arms evolution results in new variance of *Avr* gene that cause resistance breakdown (Huang et al., 2014; Yeo, 2014). As of now, 40 *Avr* genes have been identified (Ning et al., 2020), with 12 of them successfully cloned and characterized. These include *Avr-Pita* (Orbach et al., 2000), *Avr-CO39* (Farman and Leong, 1998), *PWL1* (Kang et al., 1995), *PWL2* (Sweigard et al., 1995), *ACE1* (Fudal et al., 2005), *AvrPiz-t* (Li et al., 2009), *AvrPia*, *AvrPii*, *AvrPik/km/kp* (Yoshida et al., 2009), *AvrPib* (Zhang et al., 2015), *AvrPi9* (Wu et al., 2015) and *AvrPi-54* (Ray et al., 2016).

In Malaysia, *P. oryzae* is diverse morphologically and physiologically (Hussin et al., 2020; Rafael et al., 2023). The information on the variation of *Avr* genes of *P. oryzae* isolate is very limited. Thus far, *AvrPiz-t*, *Avr-Pik*, *AvrPi-54* and *AvrPita1* were isolated from *P. oryzae* isolate 7′ of Sarawak, Malaysia (Jee et al., 2017). Recently, Yeo et al. (2024) reported the haplotypes of *AvrPiz-t* found in *P. oryzae* isolates of Sarawak and declared a total of 19 haplotypes of *AvrPiz-t*. It is the interest of this study to report the finding for another *Avr* gene, *AvrPi-54* isolated from *P. oryzae* isolates of Sarawak, Malaysia, together with isolates from India and China.

AvrPi-54 gene directly interacts with the *Pi-54* gene (Wang et al., 2017). This gene encodes a predicted secreted protein consisting of 462 bp, featuring a signal peptide at the N-terminal region. The 3D structure analysis of *AvrPi-54* reveals the presence of six β-sheets, while the mature SP truncated protein lacks α-helices, as demonstrated by Ray et al. (2016).

2. Methodology

A total of 20 *P. oryzae* isolates from Malaysia (Sarawak) were

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https://doi.org/10.1016/j.genrep.2025.102242
Received 12 December 2024; Received in revised form 2 April 2025; Accepted 29 April 2025
Available online 1 May 2025
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