Isolation and Characterization of Avirulence Genes in *Magnaporthe oryzae*

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ABSTRACT

Magnaporthe oryzae is a fungal pathogen contributing to rice blast diseases globally via their *Avr* (avirulence) gene. Although the occurrence of *M. oryzae* has been reported in Sarawak since several decades ago, however, none has focused specifically on *Avr* genes, which confer resistance against pathogen-associated molecular pattern-triggered immunity (PTI) in host. The objective of this study is to isolate *Avr* genes from *M. oryzae* 7' (a Sarawak isolate) that may contribute to susceptibility of rice towards diseases. In this study, *AvrPiz-t*, *AVR-Pik*, *Avr-Pi54*, and *AVR-Pita1* genes were isolated via PCR and cloning approaches. The genes were then compared with set of similar genes from related isolates derived from NCBI. Results revealed that all eight *Avr* genes (including four other global isolates) shared similar N-myristoylation site and a novel motif. 3D modeling revealed similar β-sandwich structure in *AvrPiz-t* and *AVR-Pik* despite sequence dissimilarities. In conclusion, it is confirmed of the presence of these genes in the Sarawak (*M. oryzae*) isolate. This study implies that Sarawak isolate may confer similar avirulence properties as their counterparts worldwide. Further R/Avr gene-for-gene relationship studies may aid in strategic control of rice blast diseases in future.

Keywords: Plant disease, rice blast, Sarawak, *Magnaporthe oryzae*

INTRODUCTION

Magnaporthe oryzae is a rice blast pathogen causing major harvest loss globally. It belongs to hemibiotrophic fungus which grows biotrophically on living plant tissue during initial life cycle and subsequently into necrotrophic mode causing the death of infected plant tissues (Horbach *et al*., 2011). It is known that *M. oryzae* encodes a variety of effector molecules to confer virulence (Li *et al*., 2009). Effectors deployed by pathogens interfere with PTI (pathogen-associated molecular pattern-triggered immunity), given that the PTI is overcome, the effectors will be recognized by specific R genes and followed by the action of ETI (effector-triggered immunity). The recognized effectors are described as AVR (avirulence) protein (Jones & Dangl, 2006). Hence, ETI brings about the development of disease resistance and often causes HR (hypersensitive response) at the site of infection. HR is a rapid and localized tissue necrosis at the penetration site and involves transcriptional activation of various defense genes of the plant which subsequently avoid further spread of pathogen through the plant tissue (Lamb, 1994; Joosten *et al*., 1997; Agrios, 2005). To date, several effector proteins encoded by *Avr* genes from *M. oryzae* have been cloned molecularly, namely *PWL2* (Sweigard *et al*., 1995), *AVR-Pita* (Orbach *et al*., 2000), *AVR-Pia* (Miki *et al*., 2009; Yoshida *et al*., 2009), *AVR-Pii* (Yoshida *et al*., 2009), *AVR-Pik* (Yoshida *et al*., 2009), *AvrPiz-t* (Li *et al*., 2009), *AVR1-CO39* (Ribot *et al*., 2013), *Avr-Pi9* (Wu *et al*., 2015), *AvrPib* (Zhang *et al*., 2015), and *Avr-Pi54* (Ray *et al*., 2016).

Malaysia is granted with temperature regime and rainfall distribution that favour year round rice cultivation, even if in the conditions of rainfed (Food and Agriculture Organization of the United Nations, 2002). According to Valera & Lee (2016), Malaysia achieved 63% self-sufficiency in rice production and relied the rest of it on rice import to compensate domestic production. With the ever increasing domestic demand in the country, ensuring a sustainable food supply is vital for the continuous development of the nation.