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Diversity of dsRNA Viruses Infecting Rice Sheath Blight Fungus *Rhizoctonia solani* AG-1 IA

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Abstract: Rice sheath blight, caused by *Rhizoctonia solani* (Kühn), is a notorious soil-borne disease prevalent in many rice-growing regions. Although several sporadic studies of mycoviruses in *R. solani* AG-1 IA have been reported for single strain of *R. solani* AG-1 IA, there have been no reports describing the distribution and diversity of mycoviruses in natural populations. In this study, 43 *R. solani* AG-1 IA strains collected from different locations in China were examined for the presence of dsRNA elements to confirm the presence of viral infections. Electrophoretypes showed that 16 of the 43 fungal strains (37.2%) contained dsRNAs that can be characterized as viruses. Furthermore, the species-specific reverse transcription PCR (RT-PCR) showed dsRNA bands with similar sizes do not always contain the same virus but exist as mixed mycoviral infections. Thus, our findings indicate mycoviruses infecting *R. solani* AG-1 IA in China are diverse, widespread and universal.

Key words: mycovirus; *Rhizoctonia solani*; reverse transcription polymerase chain reaction; diversity; dsRNA; rice sheath blight

The basidiomycete fungus *Rhizoctonia solani* (Kühn) [teleomorph: *Thanatephorus cucumeris* (Frank) Donk] is an important species of soil-borne phytopathogenic pathogen, which infects a wide range of hosts including field crops, vegetable crops, fruit trees, ornamental plants and forest trees, causing considerable economic loss worldwide (Zheng et al, 2013). The fungus is a collective species consisting of at least 14 genetically isolated anastomosis groups (AGs) defined by their hyphal interactions (Ogoshi, 1987). Among them, *R. solani* (AG-1 IA) is considered as the major cause of rice sheath blight throughout tropical and subtropical rice-growing areas. *R. solani* does not produce any asexual spores, moreover, the teleomorph of *R. solani* is rarely found in nature and is extremely difficult to induce *in vitro* (Zheng et al, 2014). Although various fungicides have been used to control rice sheath blight, constant and indiscriminate use of fungicides can cause serious environmental problems, and further, present a health hazard to animals and humans, and potentially lead to the development of fungicide resistance in the pathogen population (Zhang et al, 2012). Therefore, more environmentally friendly approaches such as the biological control of rice sheath blight disease have attracted a great deal of interest in recent years.

Mycoviruses, despite their belated discovery, are common in all major filamentous fungal groups, oomycetes and yeasts (Pearson et al, 2009). Most characterized mycoviruses have the double-stranded RNA (dsRNA) genome, although recently single-stranded RNA (ssRNA) and circular ssDNA genomes have also been discovered (Ghabrial, 1998; Ghabrial and Suzuki, 2008; Yu et al, 2010; Liu et al, 2014). Some are encapsidated in rigid virus particles, whereas the others do not form typical virus particles (Ghabrial and Suzuki, 2009; Lin et al, 2012). In contrast to most of the known animal and plant viruses, mycoviruses usually have no any associated symptoms and sometimes have even demonstrated beneficial effects on the fungal host (Ghabrial, 1998). A few mycoviruses are known to cause phenotypic alterations to their fungal host, including hypovirulence and debilitation (Nuss, 2010), and therefore, they can be used as biological control agents against fungal diseases. The ssDNA mycovirus *Sclerotinia sclerotiorum* hypovirulence-associated DNA virus is one example that can be used to

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control rape sclerotinia rot caused by *S*. *sclerotiorum* (Yu et al, 2013). Moreover, the ssRNA mycovirus *Cryphonectria hypovirus* 1 has been used to control chestnut blight caused by *Cryphonectria parasitica* (Nuss, 2005). In 1978, dsRNA mycoviruses were first characterized in *R*. *solani* by Castanho and Butler (1978). Growing research suggests dsRNA mycoviruses can be commonly identified in natural populations of *R*. *solani* AG-2 to -13 (Bharathan et al, 2005; Bartholomäu et al, 2016; Das et al, 2016). With respect to rice-infecting *R*. *solani* AG-1 IA, only three viruses, RsRV1 (*R*. *solani* dsRNA virus 1) (Zheng et al, 2013), RsPV2 (*R*. *solani* partitivirus 2) (Zheng et al, 2014) and RsRV-HN008 (*R*. *solani* RNA virus HN008) (Zhong et al, 2015), have been reported, however, previous studies focused only on single strains of *R*. *solani* AG-1 IA. Thus, the main objective of this study was to determine whether the mycoviruses infecting *R*. *solani* AG-1 IA in China are diverse, widespread and universal.

MATERIALS AND METHODS

Fungal isolates and culturing

In the present study, 43 randomly selected strains of *R*. *solani* AG-1 IA were collected from different locations in China. Details of the strains are listed in Table 1. All strains were maintained in our laboratory at South China Agricultural University, Guangzhou, China. Strains were cultured on potato dextrose agar plates covered with cellophane membranes at 28 °C–30 °C for 7 d, and mycelia were harvested and stored at -80 °C until required.

Table 1. Details of *Rhizoctonia solani* AG-1 IA strains used in this study.

Isolate No.	Location [a]	Isolate No.	Location [a]
A5	Danzhou, Hainan	B315	Wuhua, Guangdong
A90	Danzhou, Hainan	B316	Wuhua, Guangdong
A92	Danzhou, Hainan	B331	Lianzhou, Guangdong
A107	Ledong, Hainan	C40	Minhou, Fujian
A132	Haikou, Hainan	D164	Hechi, Guangxi
A142	Haikou, Hainan	D168	Yangshuo, Guangxi
A154	Wenchang, Hainan	D175	Yangshuo, Guangxi
A182	Tunchang, Hainan	D181	Baise, Guangxi
B120	Dongyuan, Guangdong	D186	Baise, Guangxi
B237	Maoming, Guangdong	E51	Dali, Yunnan
B240	Maoming, Guangdong	GD-2	Lechang, Guangdong
B246	Suixi, Guangdong	GD-11	Ruyuan, Guangdong
B249	Suixi, Guangdong	GD-61	Guangzhou, Guangdong
B250	Suixi, Guangdong	GD-118	Doumen, Guangdong
B255	Suixi, Guangdong	H1	Xuancheng, Anhui
B261	Conghua, Guangdong	H2	Chaohu, Anhui
B265	Conghua, Guangdong	H3	Jingzhou, Hunan
B266	Conghua, Guangdong	N1	Hangzhou, Zhejiang
B270	Xinxing, Guangdong	X-5	Yangzhou, Jiangsu
B275	Taishan, Guangdong	Y1	Hangzhou, Zhejiang
B278	Taishan, Guangdong	ZJG-15	Zhangjiagang, Jiangsu
B297	Lufeng, Guangdong		

[a] All strains are from China.
All the hosts of the strains are rice accessions.

Extraction and detection of dsRNA

Screening for dsRNA viruses was carried out by the extraction and detection of dsRNA segments of fungal mycelia using a previously published protocol by Morris and Dodds (1979) with minor modifications (Zheng et al, 2014). To detect the presence of dsRNA, 15.0 g mycelia were ground into a fine powder using a mortar and pestle in the presence of liquid nitrogen. dsRNA was extracted by selective absorption to columns of cellulose powder CF-11 in the presence of 16% ethanol (Morris et al, 1979). Purified dsRNA fractions were further treated with DNase I and S1 nuclease to digest contaminating DNA and ssRNA, respectively. After purification, the quality and concentration of the dsRNA fractions were determined by electrophoresis in 1.0% agarose gel in Tris-Acetate-EDTA (TAE) buffer, stained with 500 ng/mL ethidium bromide staining, and visualized under ultra-violet light.

Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA samples were prepared using an E.Z.N.A.® Fungal RNA Miniprep Kit (Omega, USA), and RT-PCR was carried out according to the method described by Vainio et al (2012) with minor modifications. Species-specific primers were designed based on the conserved sequences of RNA dependent RNA polymerase (RdRp) of reported viruses in *R*. *solani* (Zheng et al, 2013, 2014; Zhong et al, 2015).

RESULTS AND DISCUSSION

Sixteen out of the 43 *R*. *solani* AG-1 IA strains tested in this study were found to contain dsRNAs (Fig. 1), and all the strains contained dsRNAs showed multiple segments (up to four). The dsRNA segments detected by agarose gel electrophoresis were estimated to range from 1.7 to 9.0 kb (Table 2). The 1.7 to 2.3 kb dsRNA bands were most commonly detected in different strains, and a segment of approximately 6.5 kb was present in strains Y1 and N1. In the case of strains A5 and B266, the larger dsRNA segments may correspond to the genome of a member of the family *Endornaviridae*, composed of only one segment greater than 9.0 kb (Okada et al, 2011). To classify

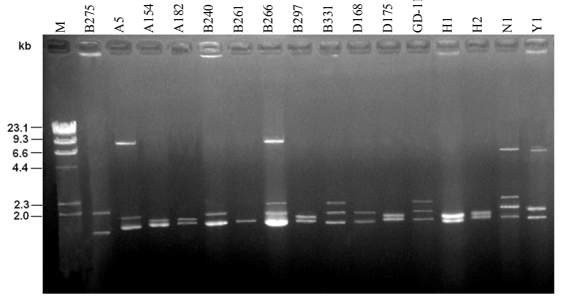

Fig. 1. Agarose gel electrophoresis of dsRNAs extracted from *Rhizoctonia solani* AG-1 IA strains.
M, Molecular marker (λ DNA digested with *Hind* III).