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A Review of False Smut Disease in Rice

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ABSTRACT

False smut disease is caused by Ustilaginoidea virens (Cooke) Takahashi on rice. It has become a serious pathogen in almost all rice-growing areas in the world . The fungus over winters in soil by means of sclerotia and chlamydospores. Sclerotia produce ascospores, which are primary source of infection to rice plants, whereas secondary infection may come from air-borne chlamydospores. a precise assessment method to evaluate the severity of the disease was developed. The 'yield representative' (YR) based on 'mean floret wt.' and 'filled grain %' was simulated for the precise disease severity assessment of rice false smut disease. Different fungicides or bio-control agents against false smut were applied at different times before heading on a susceptible rice variety. three dsRNA segments from the rice false smut fungus Ustilaginoidea virens,the causal agent of a serious disease in rice, with molecular size ranging from 1.3 to 5 Kb, were isolated and named as dsRNA-L, dsRNA-M, and dsRNA-S. To investigate population genetics, it is necessary to find markers that are polymorphic, such as multilocus DNA fingerprinting, random amplified polymorphic DNA (RAPD) or amplified fragment length polymorphism (AFLP).
Keywords: False Smut Disease, Ustilaginoidea Virens, Chlamydospores, Precise Assessment

I. INTRODUCTION

False smut pathogen, Ustilaginoidea virens (Cooke) Takahashi (teleomorph Villosiclava virensTanaka) on rice was first described by Cooke in 1878 from Tirunelveli, Tamil Nadu in India. Earlier it was regarded as a minor disease, occurring sporadically in certain regions, but now epidemics of the disease are also being reported (Rush et al., 2000; Singh and Pophaly, 2010). It is an important devastating disease causing yield losses from 1.01 to 10.91% (Atia, 2004). Disease incidence of 10-20% and 5-85% respectively has been reported from Punjab and Tamil Nadu on different rice cultivars (Ladhalakshmi et al., 2012). In recent years, its outbreak might be possibly due to high input cultivation, increased use of hybrid varieties, and climate change (Lu et al., 2009). The fungus over winters in soil by means of sclerotia and chlamydospores. Sclerotia produce ascospores, which are primary source of infection to rice plants, whereas secondary infection may come from air-borne chlamydospores (Ashizawa et al., 2010). Infection results in one or more kernels on mature heads of plants being replaced by globose, yellowish-green, velvety smut balls. When smut balls burst open, powdery dark green spores are released (Atia, 2004). The rice false smut balls are then formed in the infected rice spikelets (Hu et al., 2014; Tang et al., 2013). The recent widespread cultivation of hybrid rice and heavy application of nitrogenous fertilizer have been considered as being responsible for the increased rice false smut disease (Guo et al., 2012; Zhang et al., 2014). This disease results in yield loss, polluted rice grains, and even more important, generating toxins poisoning to humans and domestic animals (Koiso et al., 1994; Zhou

et al., 2012). Two kinds of mycotoxins, namely ustiloxins and ustilaginoidins, have been isolated and identified from rice false smut pathogen (Lu et al., 2014; Zhou et al., 2012). To analyze ustiloxins, some methods have been developed,which includes high-performance liquid chromatography (HPLC)and liquid chromatography–mass spectrometry (LC–MS) (Ji, Cao,Xu, Yin, & Shi, 2012; Miyazaki, Matsumoto, Uchihara, &Morimoto, 2009; Shan et al., 2012). Mycoviruses are widespread among a variety of fungi throughout the major fungus taxonomic groups, including Ascomycetes, Basidiomycetes, and Deuteromycetes (Nuss, 2005), Recently, with the increasing discovery of novel mycoviruses, the classification of the mycovirus has been updated (Chiba, 2009; Liu, 2009). The dsRNA segment diversity presented in one fungal host is very common, and the various sizes of the dsRNA segments may be the result of multipartite viral genomes of coinfecting viruses, or the defective products of virus replication and satellite RNA (Herrero, 2012).

II. METHODS AND MATERIAL

A. Isolation technique and culture conditions of false smut pathogen

In recent years, its outbreak might be possibly due to high input cultivation, increased use of hybrid varieties, and climate change (Lu et al., 2009). The fungus overwinters in soil by means of sclerotia and chlamydospores. Sclerotia produce ascospores, which are primary source of infection to rice plants, whereas econdary infection may come from air-borne chlamydospores (Ashizawa et al., 2010). Infection results in one or more kernels on mature heads of plants being replaced by globose, yellowish-green, velvety smut balls. When smut balls burst open, powdery dark green spores are released (Atia, 2004).he conditions for successful isolation of pathogen in axenic cultures have been standardized and the growth conditions were optimized. The fastest growth rate was achieved with potato sucrose agar medium having a pH of 6.0 at an incubation temperature of 27°C. Dark incubation was also found highly conducive for the growth of fungus compared to incubation in light. These conditions could be useful for the best isolation of the pathogen for different studies. Beside morphological identification, the identity of the fungal pathogen was confirmed through ITS sequencing which showed up to 98 % identity with U. virens in NCBI-BLAST analysis (Mathew et al., 2015).

B. Precise Disease Severity Assessment for False Smut Disease

Standard Evaluation System (SES) of IRRI (2002), is the most referred disease severity assessment system. Recently, six scales for evaluation of the rice cultivars against false smut in the field were built based on average diseased grains per panicle (ADGPP), which agreed with SES for Rice of International Rice Research Institute (Li et al. 2011). a precise assessment method to evaluate the severity of the disease was developed. The ‘yield representative’ (YR) based on ‘mean floret wt.’ and ‘filled grain %’ was simulated for the precise disease severity assessment of rice false smut disease (Urmila et al., 2015).

C. Integrated Approach to Control False Smut in Hybrid Rice

It has become one of the most important rice diseases in China due to the large-scale expansion of high-yielding rice varieties, over use of chemical fertilizers and more frequent irrigation (Zhou et al., 2004; Lu et al., 2009). Chemical fungicides such as simeconazole (Tsuda et al, 2006) and bio-control agents, for example, Bacillus subtilis (Liu et al., 2007) were reported to be effectively against the disease. Conventional field trials are the major method for evaluating resistance (Sonoda et al., 1992; Kurauchi et al., 2006), and quantitative resistance to false smut disease has been observed among rice varieties (Fujita et al., 1990; Sonoda et al., 1992; Kurauchi et al., 2006; Li et al., 2008a, b, 2011). In China. When Neixiangyou 8156 and Nei5you 317 were sprayed with 2.5% Wenquning at 4.5 L/hm 2for two times at 6 d before and 1 d after heading, respectively, the control efficiencies of Nei5you 317 and Neixiangyou 8156 were respectively 100% and 82.24% compared to that of Gangyou 725. Satisfactory control effects had also obtained by single spray of 2.5% Wenquning at 4.5 L/hm 2 at 5–6 d before heading. Therefore, less susceptible hybrid rice in combination with spraying Wenquning at 5–6 d before heading was suggested for the disease control (Liang 2014).

D. Simple and rapid detection of rice false smut pathogen Ustilaginoidea virens

It is crucial to detect the RFS pathogenU. virensrapidly in rice seeds before export because the presence of the spore on the rice grain may affect the quality. Isolations on nutrient media and morphological examinations are the conventional methods for detection and identification ofU. Virens (Zhou et al., 2003). These methods are time-consuming and complicated and therefore there is a need to develop a simple and rapid detection tecA method for simple and rapid detection of Ustilaginoidea virensin rice seeds was developed based on specific polymerase chain reaction (PCR). To design the specific primers for detection of U. virens, the comparison was made on 5.8S rDNA intra- and interspecific variations in nucleotide sequences of U. Virens and other pathogenshnique to identify the pathogen in rice seeds. The development of the simple and rapid detection technique using specific primers will be applicable for direct identification of U. virensin rice seeds to screen large numbers of samples .(Yu et al., 2014).

E. Morphological and molecular characterization of Ustilaginoidea virens isolates

The fungus forms chlamydospores and sclerotia late in the season which fall in the soil and may survive the winter. Sclerotia germinate to produce ascocarp containing ascospores, which are the primary source of infection to rice plants, whereas secondary infection may come from airborne chlamydospores. In infected plants mature head is replaced by velvety smut balls which are globose and yellowish green. When the smut balls burst open, powdery dark green spores are released (Atia,2004). Based on colony characters isolates of U. virens were grouped into three groups. The maximum colony diameter was observed in isolate UV3 (40 mm) while minimum was in UV7 (25 mm). The width of the hyphae in different isolates varied from 1.26-2.81 µm. The maximum width of the hypha was 2.81 µm in UV4 isolate and minimum in UV3 (1.26 µm). The genetic diversity of the eight isolates of U. virens, by random amplified polymorphic DNA (RAPD) marker using nine primers, revealed a considerable level of genetic variation. The dendrogram showed two main clusters; cluster I comprised of five isolates (UV1, UV5, UV8, UV6 and UV7), while Cluster II consisted of three isolates (UV2, UV3 and UV4) (Mathew, 2014).

III. RESULT AND DISCUSSION

Complete genome sequence and organization of a novel virus from the rice false smut fungus

Ustilaginoidea virens causing false smut disease in rice, is a destructive pathogenic fungus of rice, which was first reported in the Tirunelveli district of Tamil Nadu state, India (Cooke, 1978). three dsRNA segments from the rice false smut fungus Ustilaginoidea virens,the causal agent of a serious disease in rice, with molecular size ranging from 1.3 to 5 Kb, were isolated and named as dsRNA-L, dsRNA-M, and dsRNA-S. The complete nucleotide sequences of dsRNA-M and dsRNA-S were determined and analyzed. The dsRNA-M putatively encodes an RNA-dependent RNA polymerase, which is similar to that of the partitiviruses in the family Partitiviridae. Although the protein encoded by dsRNA-S showed less similarity to the typical coat protein of the virus in the family Partitiviridae, the structural analysis results indicated that the dsRNA-S might function as the capsid protein. the virus is Ustilaginoidea virens partitivirus 2-Uv0901, a new member, but distantly related to the newly proposed genus Gammapartitivirus with a distinct sequence pattern of capsid protein (Jie et al., 2014).

Genetic analysis of the population structure of the rice false smut fungus

To investigate population genetics, it is necessary to find markers that are polymorphic, such as multilocus DNA fingerprinting, random amplified polymorphic DNA (RAPD) or amplified fragment length polymorphism (AFLP). Genetic diversity research on V. virens based on Rep-PCR, RAPD and AFLP technology has found no significant genetic variation between populations from different geographical regions (Zhou et al., 2008) or from different hosts (Li et al., 2012). Species-specific markers such as simple sequence repeat (SSR) or single nucleotide polymorphism (SNP) are considered robust for detailed genotypic assessment of fungi and have become more commonly used to assess genetic diversity (Dutech et al., 2007). These markers are highly reproducible (Santana, 2009), and they allow genotyping

directly with DNA extracted from diseased plant tissue, by passing the requirement for isolation of the pathogen from diseased plant tissues, and avoiding the problem of inaccurate identification results from DNA of mixed species extracts (Rouxel, 2012). Currently, three SNP markers hav ebeen developed and used to assess genetic diversity of V. virens (Sun et al., 2013), and researchers found significant genetic diversity and differentiation among populations of the pathogen in five major rice-growing regions of China. By use of the three SNP markers, Wang et al. (2014) further speculated that geographical factors influence V. virenspopulations more than rice cultivars. However, because the development of SNP markers is costly and time-consuming, SSR markers may be a more economical and rapid method for genetic diversity studies using species specific marker (Dutech et al., 2007). Because of the complexity and the requirement that genome sequences are known in advance, there have been no SSR markers to data reported for V. virens.

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