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Rice Tungro Disease: From Identification to Disease Control

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Abstract: As the most devastating viral disease of rice in South and Southeast Asia, rice tungro disease remains one of the significant fears to sustainable annual rice productions in the world. Due to the increasing world population and subsequent increase in demand for food, identifying the causal agents and symptoms of disease and the methods of disease management are key to understanding how to reduce the economic damage caused by rice pathogens. In this review, we described the current state of knowledge of rice tungro disease caused by two morphologically and genomically dissimilar viruses: *Rice tungro bacilliform virus* and *Rice tungro spherical virus*. This includes genome structure, transmission, symptoms, diagnostic methods and biological control of the disease.

Key words: *Oryza sativa* · Rice Tungro · Rice tungro bacilliform virus · Rice tungro spherical virus

INTRODUCTION

Asian cultivated rice, *Oryza sativa* L., belongs to the family of Poaceae (Gramineae) and is one of the world's most important staple crops for a third of the human population. As rice is the primary food for most Asian countries and is consumed by 2.9 billion Asians, Asia produces more than 90% of world's rice, with China as the largest producer followed by India, Indonesia, Bangladesh and Vietnam [1]. In major rice-growing countries, the outbreaks of rice disease remain the major threat to sustainable rice production.

Rice tungro disease (RTD) is widely distributed in South and South-east Asian countries and has been recognised as a serious constraint to rice production. This disease was considered as a nutritional disorder of rice in the early 1950's and the catastrophic epidemics significantly devastated the rice production industry. In the Filipino dialect, the word "Tungro" means degenerated growth and the disease has been called "Penyakit merah" in Malaysia, "yellow-orange leaf" in Thailand, "mentek" or "habang" in Indonesia and "accepna pula" in the Philippines. A series of outbreaks were recorded in several rice production countries such as India, Indonesia, Malaysia, Philippines, China, Thailand and Bangladesh. As one of the most destructive disease, RTD was found to cause a worldwide annual loss in rice production of approximately US $1.5 billion and 5% to 10% reduction in rice yields in South and Southeast Asia [2]. In Indonesia, this devastating virus affected about 199 000 ha of rice crops between 1968 and 1994 [3].

Rice tungro disease was found to be associated with two distinct viruses: *Rice tungro bacilliform virus* (RTBV) and *Rice tungro spherical virus* (RTSV) [4]. RTBV is a double stranded (ds) DNA genome virus and a member of the *Tungrovirus* genus in the *Caulimoviridae* family with particles sizes of 100-300 nm in length and 30-35 nm in width. On the other hand, RTSV is a single-stranded (ss) RNA virus and a member in the genus of *Waikavirus* (*Sequiviridae*). Virus particles are polyhedral and about 30 nm in diameter [5]. Interestingly, tungro disease can be caused either by a single or mixed infection of RTBV and RTSV, however, rice (varieties TN1 and FK-135) infected with RTBV alone or infected with both RTSV and RTBV produced significant yield reductions (more than 85%) but these yield reductions were not see when infected with RTSV alone [6]. RTSV acts as a helper virus for transmission of RTBV and

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simultaneous infection with these two viruses allows disease development and full symptom expression to occur.

Basic Genome of the RTBV and RTSV: RTSV is a single-stranded, positive-sense RNA virus with genome of approximately 12.5 kb. Its genome encodes polypeptides of more than 390 kDa and has two short open reading frames at the 3' end [5, 7]. A putative leader protein (72kDa), three coat proteins (CP1, CP2 and CP3), a 3C-like protease, a nucleotide polymerase and a polymerase are all contained in the large polypeptide [5, 7]. Conversely, RTBV has a circular double-stranded genome of approximately 8 Kb and this virus is named after its bacilliform particles [5, 8]. As the only member of the genus *Tungrovirus*, the RTBV genome is transcribed asymmetrically, with its coding capacity on a single strand, which contains four open reading frames (ORFs) and two site-specific discontinuities in comparison with other *Badnaviruses* [9]. Genus *Badnavirus* differs to *Tungrovirus* by only having three ORFs and having mealy bugs as transmission vectors. The corresponding proteins from the four ORFs are P1 (24kDa), P2 (12 kDa), P3 (194 kDa) and P4 (46 kDa). These frames encode a CP, protease, movement protein and a reverse transcriptease/RnaseH, as well as proteins of unknown function [5, 8, 10-12]. At present, the genome sequences of RTBV have been found to be clustered into two distinct groups, South East Asian and South Asian groups [13]. Further recent developments in rice tungro virus genomes have focused on understanding the RNA recombination mechanism, the molecular biology of interaction between the two viruses as well as analysis of significant motif and domains indicating their biological importance [2, 13].

Transmission: Tungro disease was discovered as a leafhopper transmitted virus in 1965 [14]. The tungro disease is transmitted by six leafhopper species in a semi-persistent manner and *Nephotettix virescens* (dist.) is known as the predominant vector [15]. The virus retention period for the green leafhopper (GLH) is two to four days for RTSV and four to five days in the case of RTBV. The GLH readily gains RTSV on source plants infected with RTSV alone, but does not gain RTBV on plants infected only by RTBV [16]. The transmission of RTBV is dependent on the presence of RTSV, thus GLH that previously fed on plants infected by RTSV and subsequently obtained RTSV can then acquire RTBV from a plant infected with RTBV [17-18].

Symptoms and Diagnostic Methods: Generally tungro diseases are not easy to identify in the field due to confusion with disorders induced by abiotic and biotic factors as well as different symptoms that are being expressed depending upon the rice cultivars, the presence of virus particle(s), rice growth stages and growth conditions of the rice plants [19]. The typical symptoms of rice infected with RTBV and RTSV are stunting, yellow or yellow to orange discoloration of infected leaves, reduced tillering, sterile panicles and often irregular-shaped darkbrown specks are visible on the leaves (Figure 1). The young infected leaves may have a mottled appearance and interveinal chlorosis, whilst old leaves will show rust-coloured specks of varying size.

Fig. 1: Rice infected with tungro in Kedah, Malaysia.

RTBV infection alone produces similar but milder tungro symptoms in the infected plants. RTSV alone causes indistinct symptoms such as mild stunting. As the plant ages, the percentage of infection decreases at the time of infection but the latent period of virus infection increases [20-21]. Resistant cultivars infected with either virus exhibit delayed flowering whereas susceptible rice plants do not produce flowers. Visual observations of typical rice tungro disease symptoms is a common practical method for virus detection in the field, although sometimes the disease is misdiagnosed and misidentified as a non-pathogenic disorder, because the symptoms produced are similar to symptoms exhibited following overwatering, nutritional deficiencies or insect damage.

As symptom observation in the field to detect the viruses is not always reliable, several diagnostic methods have been developed for the detection of RTBV and/or RTSV. The most common, specific and relatively reliable method is based on a serological method of detection [22-23]. The four serological methods: enzyme-linked immunosorbent assay (ELISA), simplified ELISA, the latex flocculation test (LF) and the passive hemagglutination test (PHA) have been used for detection of RTBV and RTSV in both rice plants and insect vectors [23]. In the study, ELISA was found to have highest detection sensitivity followed by simplified ELISA, LF and PHA. However, none of the methods used in the study are able to detect RTSV and RTBV antigens in their respective vector insects. It might be that the sensitivity of ELISA is not high enough to detect the rice viruses in insect vectors with low virus concentration.

The rapid and the most sensitive technique that has been developed to detect low levels of extracted RTBV DNA from leaf samples is polymerase chain reaction (PCR) [24]. Takahashi *et al*. [24] demonstrated the effectiveness of PCR for evaluating rice cultivars that were tolerant to RTBV and this technique was found to be 10^3-10^4 more sensitive than ELISA. Dasgupta *et al*. [25] later simplified the methods of detection of field infection of RTBV directly from leaf extracts without preparation of DNA. Reverse transcriptase PCR and RFLP analysis have been carried out with viral RNA for differentiation of two RTSV variants [26].

Periasamy *et al*. [27] demonstrated the detection of both viruses in a single multiplex RT-PCR amplification using first strand cDNA as a template. A reverse transcription-loop mediated isothermal amplification (RT-LAMP) assay was established by Le *at al*. [28] for detection of both RTBV and RTSV as well as in viruliferous insect vectors. In a RT-LAMP assay, two pairs of primers that recognize six regions were designed based on the conserved regions of the nucleotide sequence for each virus. ORF3 and ORF1 regions were used as the target segment for RTBV and RTSV for detection using RT-LAMP, respectively. Recently, SYBR Green 1-based real-time PCR was developed for quantitative determination of RTBV and RTSV in an infected plant [29].

Control

Insecticides: One of the significant protections against tungro disease is the use of insecticides, although the efficiency of insecticides against tungro through vector control is considered to be low. In addition, the use of insecticides is non-specific, killing non-target organisms and causing environmental pollution as well as emergent vector resistance [30-32]. Despite of these disadvantages, use of insecticides is seen to be essential in order to successfully control *N. virescens,* especially after the outbreak of another grass plant hopper, *Nilaparvata lugens* (Stal), following which regular spraying in most of the countries was recommended in huge quantities, with up to six applications per year suggested, regardless of the level of pest infestation [33].

The use of systemic granular insecticides such as Carbofuran is considered to be most effective against tungro disease as they are long lasting and have rapid activity [34-35]. Insecticide applied to the roots of plants provides the most efficient uptake and also much slower degradation rate [36]. Oils and plant extracts have also been evaluated in order to avoid the toxic effects of compounds including neem (*Azadirachta indica*) and custard apple (*Annona squamosal*). Neem is reported to have antifeedant and insecticidal properties and works well against tungro disease if applied directly into the soil [37]. Natural products are not currently being used in the fight against tungro disease due to cost effectiveness and slow pest killing effect.

Conventional Resistance Breeding: Besides suggesting the use of insecticide to control the vector, the main strategy for control of tungro disease is to grow tungro resistant cultivars [38-39]. More than 80,000 accessions of rice are maintained at the International Rice Research Institute (IRRI). At least a third of these have been tested since 1963 for resistance to tungro using various methods [40-41]. As vector resistance was plentiful and easy to identify in the germplasm, a lot of the IR varieties released after 1969, with exception of IR22, were evaluated as leafhopper resistant rather than resistant to the tungro

viruses. However, this type of resistance was not sustainable due to high disease pressure and very often varieties with vector resistance were defeated by tungro as the vector population become adapted after their release [42-43]. As a result of this, many genetic studies have been focused on understanding the inheritance virus resistance [44-47].

Genetically Engineered Resistance against RTSV, RTBV and Green Leaf Hopper (GLH): Several efforts have demonstrated acquired resistance against RTBV, RTSV and GLH using transgenic approaches [2, 48]. Interestingly, the discovery of two rice transcription factors, RF2a and RF2b, that have been shown to play a role in virus replication and symptom development, implies that this could provide a new source of candidate genes for engineered resistance [2, 49]. No drastic phenotypic changes were observed in production of overexpressed RF2a and RF2b transcription factors in transgenic lines [50-52]. Surprisingly, if transgenic lines that overexpressed one of these two transcription factors were inoculated with RTBV through *Agrobacterium*-mediated infection, or by both RTBV and RTSV through GLH transmission, RTBV titers were considerably reduced and substantial resistance to RTD was apparent in transgenic lines [49] and this could be a promising result towards a world without rice tungro disease. This result may suggest more research is needed to elucidate the interaction components between the host and pathogens that can be used to engineer disease resistance [53].

CONCLUSIONS

Rice tungro disease has the potential to cause massive losses in rice production and the lack of any completely effective method to control the disease makes tungro a big threat to world food security. In previous decades, our understanding of rice tungro disease has led researchers to focus on more targeted strategies to overcome this disease and this has been possible with use of multidisciplinary research that may point towards new strategies for their management. Enhanced understanding of the identification, genome type, transmission and biological control of these viruses makes tungro disease very significant in terms of plant virology, molecular biology and entomology, with the focus on achieving the ultimate goal of improved management strategies for control of rice tungro disease in order to reduce the economic damage to global rice production.

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