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Review Article

A Brief Review on Present Status of Rice Tungro Disease: Types of Viruses, Vectors, Occurrence, Symptoms, Control and Resistant Rice Varieties

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Abstract

One of the biggest threats to the world's sustainable yearly rice supply is Rice Tungro Disease (RTD), which is currently the most damaging viral disease of rice in South and Southeast Asia. The growing global population has led to a rise in the demand for food, making it necessary to discover the causes, symptoms, and management strategies of diseases in order to lessen the economic harm that rice pathogens inflict. According to statistics, this viral illness of rice can result in a 2% national loss in India. In susceptible rice growers, rice tungro manifests as severe yellowing and stunting composite illness caused by combined infection of two unrelated viruses, Rice Tungro Bacilliform Virus (RTBV) & Rice Tungro Spherical Virus (RTSV). The Green Leafhopper (GLH) *Nephotettix virescens* Distant serves as the vector for both viruses. One of the most destructive rice pests in Asia's rice-growing regions is the green leafhopper (GLH). The green leafhopper nymphs and adults suck the plant sap and block the vascular bundles with Stylet sheaths to feed on rice. This review describes the different types of viruses, their genomic architectures, the types of vectors that carry them, when they arise, the symptoms that affect sick plants exhibit, how to control and eradicate the disease, and rice varieties that are resistant to them.

Keywords: *Resistance, Rice, RTBV, RTD, RTSV, Tungro, Virus, Yield*

Introduction

One third of the world's population depends on Asian cultivated rice, *Oryza sativa* L., which is a member of the Poaceae (Gramineae) family and one of the most important staple crops worldwide. 2.9 billion people in Asia eat rice, which is the staple diet in the majority of Asian nations. Over 90% of the world's rice is produced in Asia, with China being the primary producer, followed by Indonesia, Bangladesh, Vietnam, and India. Insects, pests, diseases, and weeds are examples of biotic stressors that cause the loss of more than 40% of the world's rice crop each year. The main virus that affects rice is called Rice Tungro Disease (RTD). It is common in South and Southeast Asian nations that cause a major obstacle to rice production, and is thought to cause yearly losses of around 109 US dollars globally (Nur *et al.*, 2020; Pangga & Cruz, 2024). Early in the 1950s, the illness was thought to be a nutritional condition of rice, and the devastating outbreaks severely damaged the rice-producing sector. According to more statistics, the disease reduces rice production in India by roughly 2% on average, while losses can be higher at the regional level (Muralidharan *et al*., 2003; Nur *et al.*, 2020). Some workers are of the opinion that stable resistance of cultivars to tungro disease could solve this

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devastation (Hibino *et al.,* 1990). Identification of tolerant/resistant rice varieties among the traditional is necessary to complement conventional breeding method by using transgenic method for genetic improvement of disease resistance and reduction of pesticide usage.

The disease has been referred to as "Penyakit Merah" in Malaysia, "Yellow-Orange Leaf" in Thailand, "Mentek" or "Habang" in Indonesia, and "Accepha Pula" in the Philippines (Truong *et al.,* 1998). The word "Tungro" in the Filipino vernacular denotes “Degenerated Growth”. Numerous nations that produce rice, including Bangladesh, Malaysia, the Philippines, China, Thailand, and India, have reported a number of outbreaks. Between 1944 and 1968, this destructive virus devastated roughly 1,99,000 acres of rice fields in Indonesia (Ling, 1979). An overview has been prepared about these plant viruses.

The Rice Tungro Virus

The Rice Tungro Bacilliform Virus (RTBV) and the Rice Tungro Spherical Virus (RTSV) are the two main viruses that cause rice tungro disease. RTBV is a double stranded (dS) DNA genomic virus that belongs to the Tungro virus genus in the Caulimo Viridae family. Its particle sizes range from 30-35 nm in width and 100-300 nm in length (Qu *et al*. 1991; Bao & Hull, 1993; Laco & Beachy, 1994). On the other hand, RTSV is a polyhedral-shaped single-stranded (SS) RNA virus that is about 30 nm in diameter and is a member of the Sequiviridae family's Wai Ka virus genus. Depending on whether one or both viruses are present in the plant, there are different symptoms (Bhakta *et al.,* 2009).

When both RTSV and RTBV are present in a plant, the former causes no discernible symptoms, the latter causes mild stunting and mild yellowing of the leaves, and the latter results in mottled leaves, severe stunting, and yellow to orange discoloration of the leaves, which significantly reduces yield (more than 85%) (Kumar & Dasgupta, 2020). RTBV is spread by RTSV, which serves as an assist virus. The Green Leafhopper *Nephotettix virescens* is the only vector by which these viruses can spread. inside the Cicadellidae family's subfamily Deltocephalinae. The bug typically feeds on the leaf blade's adaxial surface, while it occasionally solely eats the leaf sheath. By sucking the sap from the vascular tissues of the rice plant, the insect directly damages the plant and reduces the vigour, number of tillers and yield of rice. Both nymphs and adults of the Green Leafhopper feed on rice by sucking the plant sap and plugging the vascular bundles with Stylet sheaths. They cause damage to the rice crop by directly sucking the sap and during sucking, transmit the virus (Varma *et al.,* 1999).

Basic Genomic structure and strains of RTV

The polyadenylated single-stranded RNA genome of RTSV, a member of the sequiviridae family, is encapsulated within isometric particles and is around 12.5 kb (Hull, 1996). The genome contains two brief open reading frames at the 3'end and encodes polypeptides with a molecular weight of over 390 K Da. The large polypeptide chain is made up of a putative leader protein (72 K Da), three coat proteins (CPI, CP2, and CP3), a 3C-like protease, a nucleotide polymerase, and a polymerase (Tangkananond *et al.,* 2005).

Structure of RTSV

Encased in isometric particles, the polyadenylated single-stranded RNA genome of RTSV, a member of the sequiviridae family, is around 12.5 kb in size. The genome encodes polypeptides with a molecular weight of more than 390 K Da and has two short open reading frames at the 3'end. A suspected leader protein (72 K Da), three coat proteins (CPI, CP2, and CP3), a 3C-like protease, a nucleotide polymerase, and a polymerase comprise the long polypeptide chain. Upstream of CP1, a leader protein (P1) is seen. A protease (Pro), an RNA-dependent RNA polymerase (Rep), and a nucleotide triphosphate (NTP) binding protein are also included in the poly-protein. It was first discovered that two tiny ORFs (SORF-2 and SORF-3), expressed from sub-genomic mRNAs, are located at the 3'end of the RTSV genome (Kannan *et al.,* 2020).

Analysis Genomic Sequence of RTSV

An analysis of the genome of the RTSV-SP isolate that was collected in 2018 from a rice field in Seberang Perai, Malaysia. The isolate's genome sequence spanned 12,173 nucleotides. a tail that contains 45.8% GC. A single massive ORF-1, measuring 10,413 nucleotides, was followed by a 514 nucleotides un-translated region (UTR) in the 5th region of the genome. The AUG start codon in position 515 of ORF-1 codes for a possible 3,471 amino acid residue polypeptide. The poly-protein encodes a 5' to 3' RNA polymerase, a nucleotide triphosphate binding domain (NTP), three coat proteins (CPs)—CP1, CP2, and CP 3—a leader protein (P1), and a proteinase (Pro)(Kannan *et al.,* 2020; Saha *et al.*, 2023b).

Two putative short ORFs (ORF-2, comprising 72 amino acids, and ORF-3, comprising 83 amino acids) make up the 3'terminus of RTSV-SP. Located 89 nucleotides after ORF-2's stop codon, at position 11433 nucleotides, is the AUG start codon of ORF-3. RTSV-SP Five other isolates that were available were found to share 89.54-95.73% nucleotide identities and 96.51%-97.85% amino acid identities with ORF-1. Even though both RTSV-PhilA and RTSV-VT6 originated in the Philippines, RTSV-SP's ORF-1 exhibited a greater percentage of identity with Phil A isolates at the amino acid level than with Vt6 isolates (Kannan *et al.,* 2020; Saha *et al.*, 2023b).

Genome of RTBV

The bacilliform particles that make up RTBV are elongated icosahedrons measuring roughly 130 x 30 nm. The virus isolate affects the size differently. The particles have a genome made up of around 8000 base pairs of circular double-stranded DNA, with two site-specific discontinuities brought about by reverse transcription replication. As a pararetrovirus, RTBV is a member of the Caulimoviridae family, which also includes two other genera of calumo viruses. It has only lately been suggested that RTBV is the sole member of the genus known as "RTBV-like Viruses," which includes only RTBV (Rongda *et al.,* 1991). For this reason, RTBV is still classified as a badnavirus. Animal retroviruses and retroviruses in general are very similar. The distribution of the gag-pol functions, reverse transcriptase's role in the replication cycle, a transcript longer than the genome with terminal repeats, and the use of pregenomic RNA as Poly-cistronic mRNA are all similar characteristics. Pararetro viruses encapsidate DNA and transcribe their RNA from an episomal form of DNA that is not integrated into the host genome, in contrast to retroviruses, which do the same from a genome copy integrated into the host DNA. The Pararetroviral Pregenomic RNA is synthesized under the direction of a single promoter. This RNA functions as a template for both the production of the virus-encoded proteins and reverse transcription by the reverse transcriptase encoded in the virus (Dai *et al.*, 2008; Saha *et al.*, 2023a).

The genome of RTBV, a plant retrovirus, is circular and contains 8 kb of DNA. A promoter situated in the intergenic region between ORF IV and ORF I controls the transcription of the RTBV DNA genome. Vascular tissues are where RTBV accumulates, and this is also where the RTBV Promoter is mostly active. A unique box II element that is located directly upstream of the TATA box and two basic Leucine Zipper (bzip)-type rich proteins, RF2a and RF2b, have been shown to interact with Box II and activate transcription from the RTBV promoter both *in vitro* and *in vivo.* These several cis acting regulatory elements have been identified as contributing to the regulation of expression of this promoter. The development of rice likewise depends on RF2a and RF2b, and transgenic rice lines whose levels were lowered by (-) sense RNA had abnormalities that somewhat mirrored RTD symptoms. Currently, it has been discovered that the RTBV genome sequences are divided into two separate groups: Southeast Asian and South Asian groups (Rahayu *et al.,* 2024; Naresh *et al.,* 2024).

Genome Organization of RTBV

The circular, double-stranded DNA of RTBV is transcribed beginning at nucleotide 7404 or 7405 and terminating at nucleotide 7620, where it produces a primary transcript that is longer than the genome and has a terminal redundancy of 215 or 216 nucleotides. RTBV has four huge open reading frames (ORFs) in its coding potential. The interface of the first three densely packed ORFs is ATGA, with

ATG (which codes for the amino acid methionine) serving as the downstream ORF's start codon and TGA (UGA on mRNA) as the upstream ORF's stop codon. A brief noncoding area divides ORF TV from ORF III, and a longer intergenic region with multiple short ORFs lies between ORF IV and ORF I. The production of all four RTBV ORFs is possible despite eukaryotic ribosomes' limited ability to translate Polycistronic mRNAs due to specialised translation processes (Praptana *et al.,* 2021).

Strains of Tungro Virus

Furthermore, reports of many Tungro virus strains have been made. Two strains, dubbed "S" and "M," were found in the Philippines. When it came to the diseased leaves, strain 'S' was distinguished by severe inter-venial chlorosis or stripes, whereas strain 'M' merely caused minor dispersed mottling. In the Philippines, strain "S" was more prevalent and caused more serious symptoms. Later, a third strain, called "T," was reported from the Philippines to have caused stunting, yellowing, and narrow leaves on Taichung Native-I (IRRI, 1971). Ten isolates were gathered by researchers from various locations in Andhra Pradesh, Bihar, and West Bengal (Sutrawati *et al.*, 2021). Of these, four unique strains were identified and given the names RTV1, RTV2A, RTV2B, and RTV3. In Taichung Native-1, RTV1 causes moderate symptoms, while RTV2A and RTV 2B cause extremely severe symptoms. The RTV3-infected plants first displayed severe symptoms before eventually recovering from the illness (Bhakta *et al.,* 2009).

Transmission of Virus

In 1965, it was determined that Tungro was a virus spread by Leafhoppers. An outbreak of a disease is caused by the virus inoculums being available, a high level of GLH, and the crop being in its early growth stage. Different Leafhopper species can spread the illness semi-persistently like *Nephotettix nigropictus* and Zigzag leafhopper (ZLH) *Recilia dorsalis*, with *Nephotettix virescens* Distant being the most common vector (Azgar & Yonzone, 2018). It was found that while both nymphs and adults aid in secondary dissemination, adult GLH actively contributes to the introduction of the primary inoculums into the field. In certain locations, a direct relationship between the vector population and illness incidence has been noted. A significant percentage of viruliferous vectors and high vector populations are also factors (Patel *et al.*, 2018) disease outbreaks. In addition, temperature has an impact on transmission to some degree. *Nephotettix virescens* retains the virus for a significantly longer period of time at 13°C than it does at 32°C. Consequently, rather of being categorized as a non-persistent virus. Tungro is considered a transitory virus spread by Leafhoppers. A single insect can infect up to 40 seedlings every day. Under controlled circumstances, the impact of ambient temperature on the adult green leafhopper's ability to transmit the tungro virus was investigated. The findings show that the bug may pick up the virus from sick plants and inoculate rice seedlings that subsequently become infected, provided that the temperature is between 10°C and 38°C. Therefore, under natural circumstances, the tropical region's temperature might not be a factor limiting the spread of the tungro virus. On the other hand, the insect's capacity to spread the virus tends to rise from 10°C to 31°C degrees Celsius and then to decline significantly from 31°C to 38°C degrees Celsius. The spread of tungro, as indicated by the percentage of infected seedlings, increases with day-night temperatures within the 24°C -16°C to 30°C - 22°C temperature range. The adult tungro viruliferous bug has a longer life span when the temperature drops from 34 °C to 13 °C. In experiments with 6,895 insects, the longest retention at 13°C was 22 days, and the longest retention at 32°C was 6 days, following an acquisition feeding at ambient temperature. The bug lost less infectiousness at 70 °C than it did at ambient temperature (Anjaneyulu *et al.,* 1994, Jyotsna *et al.*, 2013).

For RTSV and RTBV, the GLH has a virus retention time of two to four days and four to five days, respectively. When source plants are infected with RTSV alone, the GLH acquires RTSV with ease; however, it does not acquire RTBV when plants are exclusively infected with RTBV. Since RTSV is necessary for the transmission of RTBV, GLH that had previously consumed contaminated plants and later acquired RTSV are capable of acquiring RTBV from an infected plant (Anjaneyulu *et al.,* 1994).

Nowadays mathematical models are used for the analysis of the model of the spread of tungro virus disease in rice plants taking into account the characteristics of the rice tungro spherical virus (RTSV)

and rice tungro bacilliform virus (RTBV), as well as control in the form of roguing processes and application of pesticides(Amelia *et al.*, 2023).

Factors favoring RTV

Non-synchronous planting is one of the main contributing variables that encourage the emergence of RTV disease. Since there will always be hosts—rice plants—in an asynchronous rice area, the vector will continue to feed, spread the virus, lay eggs, and grow. Additional reasons include high temperatures, fertilizers containing nitrogen applied excessively, and cultivars that are vulnerable. Both rain-fed and irrigated wetland habitats are susceptible to the illness (Sharma *et al.,* 2017).

Occurrence

In 1968, an epidemic outbreak in the eastern regions of Uttar Pradesh and Bihar, India, brought the disease to the notice of the general public for the first time. Three significant outbreaks in farmers' fields in India in 1984, 1988, and 1990 resulted in significant financial losses, both immediate and long-term. In Bihar and West Bengal, the illness occurrence rates in 1981 were 40–100% and 60–100%, respectively. An epidemic of tungro that struck three districts in West Bengal in 2001 resulted in a 0.5 million ton loss in rice crop, valued at Rs. 2911 million(Banerjee *et al.,* 2009). Nonetheless, a widespread yellowing and stunting of the rice crop was noted in the Punjabi regions of Gurdaspur and Amritsar in August 1998. Almost all the cultivars of rice grown in these districts in an area of about 0.45 million hectares were severely affected. At early stage, the infection may result in 100% yield loss.

Outside of India, major outbreaks of RTD were documented in Peninsula Malaysia in 1982–1983, affecting about 20,300 hectares of rice fields in Kedah and Perlis and resulting in output losses estimated at US$10 million. Aside from that, five Sarawakian regions have RTD prevalence in 2012. It's interesting to note that native rice growers in the impacted areas demonstrated a vulnerability to tungro viruses (Bunawan *et al.,* 2014).

The first reports of the tungro illness in Indonesia date back to 1983–1984. The provinces of West Java, East Java, Central Java, Bali, South Sulawesi, Lampung, Banten, Central Sulawesi, Noth Sumatra, South Kalimantan, and Irian Jaya are the principal distribution areas of Tungro. First identified in 1999, the tungro was discovered in Jayapura and Nabire, Papua New-Guinea (Azzam & Chancellor, 2002).

The rice tungro virus was found at Parwanipur, in the Bara district of Nepal. The early to mid-tillering phases of the rice plant were when the illness's symptoms first manifested. Later on, an IR20 rice cultivator in Janakpur's Dhanusha district experienced indications of this illness (Bhusal *et al.*, 2019).

Symptoms of Rice Tungro Disease

One of the greatest challenges of RTD management is the identification of the disease from the symptoms. Often there is an overlap of symptoms caused due to other biotic and abiotic causes. The disease's symptoms differ depending on the strains, variety, and age of the plant. Young leaves with inter-venal chlorosis and chlorotic mottling turn yellow first. When plants are afflicted in the early stages of growth, there are few tillers, poor root development, and limited crop growth. Old leaf displays rust-colored, variably-sized specks; When plants are badly impacted, flowering is delayed. Panicles become small with deformed seed sett and non-viable, frequently with dark brown specks. While the Rice tungro spherical virus is present in phloem tissue, the Rice tungro bacillus virus is restricted to the vascular bundles. Stunting, yellow or yellow to orange discoloration of infected leaves, decreased tillering, sterile panicles, and frequently irregularly shaped dark brown specks visible on the leaves are the typical signs of rice infected with RTBV and RTSV (Anjaneyulu *et al.,*1994).

Diagnosis of the Virus in Rice plants

Many diagnostic techniques have been employed for the diagnosis of RTBV and RTSV since symptoms seen in the field to identify the viruses are not always accurate and can be highly confounding (Bunawan *et al.*, 2014). Both RTSV and RTBV have been detected using techniques such the Passive Hemagglutination Test (PHA), Latex flocculation (LF) test, Simplified ELISA, and Enzyme-Linked Immunosorbent Assay (ELISA)(Jagram *et al.*, 2022). The most sensitive detection method is ELISA, which is followed by LF, PHA, and simplified ELISA (Uda, 2015). Polymerase Chain Reaction (PCR) is the most sensitive and quick method yet discovered for detecting trace amounts of extracted RTBV DNA from leaf samples (Hamdayanty *et al.,* 2021).

Introduction of resistant rice varieties against Tungro

It is extremely difficult to control the disease after it has spread throughout the field. Control mechanisms are discovered less successful in preventing tungro than direct disease control methods (Ireneo *et al.,* 2024). Although insecticides are utilized, their effectiveness in controlling the tungro vector is deemed low due to the vector's constant movement to nearby fields, which contributes to the disease's spread. Since insecticides like carbofuran have a lengthy half-life and quick action, they are thought to be the most effective against tungro. The most effective absorption and a significantly slower rate of breakdown are obtained from insecticides sprayed on plant roots. Additionally, a number of actions can be taken to manage the illness (Nas *et al.,* 2011) as follows.

a. Cultivator cultivars resistant to tungro can be employed.

b. The planting date needs to align with that of other neighbouring farms. Plantations that are planted later than usual will be more susceptible to this disease.

c. The planting period for rice seedlings should be changed to reduce the amount of disease vector populations.

d. It is best to get rid of crop waste from the field as soon as feasible. The locations where the vector thrives should be eliminated in order to lessen the vector’s source of inoculum eggs.

In the Philippines, there have been reports of multiple rice types being resistant to RTSV. The rice varieties that resulted from a cross including Utri Merah (IRGC 16682), Kataribhog, MR81, Pan Khari203 (IRGC 5999), Basmati 370 etc. It is reported that the resistance to Pankhari 203 and Kataribhog is allelic, but Utri Merah’s sole recessive gene is nonallelic to the genes in both types. Whereas three complementary recessive genes are thought to regulate resistance in Kataribhog and Pan Khari 203, resistance in Utri Merah is governed by a single recessive gene. Y1036 and Rajapan (IRGC 16684) are two well-known examples of resistant varieties. The two accessions produced from Utri Merah, IR 69705-1-1-3-2-1 and IR 69726-116-1-3, which have tolerance to RTBV and resistance to RTSV, make up Y1036, one of the three most promising advanced breeding lines. These lines demonstrated consistently minimal RTBV and RTSV infections in every site.

A number of varieties are found to be resistant against tungro in India (Habibuddin *et al*., 1997; Dey, 2016). In a study on the identification of Rice Tungro Disease (RTD) tolerant traditional varieties of rice (*Oryza sativa* L.) of West Bengal using forced inoculation methods with insect vector, green leafhopper (GLH), Nephotettix virescens (Distant) it was found that traditional rice varieties Latasail, Sonajhuli and Tulsibhog were found to be moderately tolerant with only 6%, 7% and 9% yield reduction respectively. Dumursail, Radhunipagol, Raghusail and Tulaipanja were tolerant varieties with zero yield loss. Rupsail and Gobindobhog varieties which are commonly consumed in West Bengal showed a yield reduction of 13% (Dey & De, 2016). The susceptible check was the rice variety Taichung

Native 1 (TN 1) and tolerant check was IR-36 rice variety. The list of varieties used in the study are shown in Table 1. A cross between Radhunipagol and Pusa Basmati 1 has also been studies to understand the mechanism of inheritance in Indian cultivars (Dey & De, 2019). It is found that, in Radhunipagol, Gobindobhog and IR 68305 the resistance is controlled by a single recessive gene.

Table 1. List of traditional rice varieties of West Bengal used for the identification of Rice Tungro Disease (RTD) tolerant traditional varieties of rice (*Oryza sativa* L.) of West Bengal using forced inoculation methods with insect vector, green leafhopper (GLH), Nephotettix virescens (Distant); (Dey & De, 2016).

Sl. No.	Name of the traditional rice variety of West Bengal	Variety Type	Reduction in yield (in %)
1	Balam	TV	14
2	Chandrakanta	TV	13
3	Dumursail	TV	0
4	Gobindobhog	TV	13
5	Jugal	TV	17
6	Latasail	TV	6
7	Radhunipagol	TV	0
8	Raghusail	TV	0
9	Rupsail	TV	13
10	Sonajhuli	TV	7
11	Tulaipanja	TV	0
12	Tulsibhog	TV	9
13	Pusa Bsamati 1	BV	63
14	Taichung Native 1 (TN 1)	SC	100
15	IR 36	TC	0

Conclusion

Rice tungro disease (RTD) caused by the co-infection of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) is a devastating viral disease of rice prevalent in Southeast Asia with outbreaks affecting thousands of hectares. RTD has grown to be a serious menace to farmers and rice crops. Global food security is greatly threatened by two RTV strains and their vectors. The scope of study on this research area is immense and needs to encompass different subjects. For better control of this disease multidisciplinary research seems to be the only answer. 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. However, thanks to increased knowledge and investigation, research on tungro disease is now more prevalent and important in different disciplines like Plant Virology, Molecular Biology, Biotechnology and Entomology. So, the scope of study spreads across different subjects. To reduce the loss of rice output worldwide, disease-resistant rice cultivars need to be developed by ongoing surveillance against the virus. This paper is intended to provide an overview for future research workers working on rice viruses, about structure, function, epidemiology & damage of Rice Tungro Disease (RTD).

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this work.

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