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Microtomy Studies of Rice Tungro Disease Infected Leaves

P. Valarmathi[1*] and D. Ladhalakshmi[2]

[1]Department of Plant Pathology, ICAR-Central Institute for Cotton Research (CICR), Coimbatore-641 003, India
[2]Department of Plant Pathology, Department of Plant Pathology, ICAR-Indian Institute of Rice Research (IIRR), Hyderabad-500 030, India

**Corresponding author*

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A B S T R A C T

In our study, cross section of the healthy and Tungro infected leaf sample of rice exhibited wide differences among the cells. The area of vascular bundles was reduced in the Tungro infected rice samples and lower epidermis cells got damaged wherein the area was large in the healthy rice leaf. In case of healthy leaves of rice, the cells were normal in size with properly distributed intercellular spaces and evenly present bundle sheath cells. The xylem and phloem tissues showed no damage in healthy sample. Bundle sheath cells remained undisturbed above the phloem tissues. In the infected rice leaf section, due to the invasion by both the RTBV and RTSV viruses, bundle sheath cells and phloem got distorted.

Introduction

Rice, *Oryza sativa* (Graminae) was domesticated earlier than 5000 B.C. in North East of India. Its cultivation then spread throughout Southeast Asia and Southern China. Rice is the most important staple or subsistence food of world and India. China, India, Indonesia, Bangladesh, Vietnam, Thailand and Myanmar are the biggest rice producers and account for over 75 per cent of the production (Thottapalli, 2003). Annually, more than 40 per cent of the world's rice crop is lost owing to biotic stresses like insects, pests, pathogens and weeds (Hossain and Fischer, 1995). Several diseases caused by bacterial, fungal and viral pathogens devastate rice yields all over the world. Among the diseases blast, bacterial blight and rice Tungro are important because of economic yield loss which they cause.

Viruses cause several important diseases of rice. The epidemics tend to be periodic but very severe. Understanding disease complexes like Rice Tungro has been difficult but has led to a better understanding in permitting a range of strategies to be developed in order to

control the disease. Tungro infected plants with both Rice Tungro Bacilliform Virus (RTBV) and Rice Tungro Spherical Virus (RTSV) are stunted and the leaves become discoloured turning yellow or yellow-orange. RTD is known to be transmitted by the vector green leaf hopper, *Nephotettix* sp. Tungro was first detected in the experimental fields of IRRI in 1963 and identified as a distinct virus disease (Rivera and Ou, 1965). The occurrence of disease was observed seven years after the introduction of world's first semi dwarf variety TN1 (Taiching Native) to India from China. In 1969, RTD incidence was confirmed from Andhra Pradesh, West Bengal, Uttar Pradesh and Mysore (Anonymous, 1980). This disease is one of the economically important viral diseases of rice in South and South East Asia (Cabautan and Hibino, 1984). Presence of Tungro in India was confirmed by John (1968) based on the symptom, vector acquisition, inoculation feeding period and non-persistence nature of the virus in the vector. During the year 1984-85, Hibino (1987) reported that about 80,000 ha of rice crop was affected by Rice Tungro disease in Tamil Nadu and Andhra Pradesh. Similarly, the reports published by Dasgupta, (1999) revealed that tungro which was confined to eastern parts of Bihar, West Bengal, East Uttar Pradesh, emerged latter in south, threatening rabi rice production in Andhra Pradesh and Tamil Nadu**.**

Electron micrograph of RTBV and RTSV are depicted. Both RTSV and RTBV multiply independently in rice plants. In rice plants, RTSV particles are restricted to the phloem tissues, while RTBV particles are found in both phloem and xylem cells. RTSV particles are isometric and 30 nm in diameter. RTSV belongs to the genus Waikavirus within the family Sequiviridae. 'Waika' means stunting in Japanese, and susceptible japonica varieties infected with RTSV are stunted. RTBV particles contain a circular double stranded DNA of 8 kb with site-specific discontinuities. It is classified in the group of "rice tungro-bacilliform-like viruses" within the family Caulimoviridae. The genomes of both RTSV and RTBV have been recently characterized (Hay *et al.,* 1991; Qu *et al.,* 1969; Shen *et al.,* 1993; Zhang *et al.,* 1993).

Rice Tungro disease is caused by the joint infection of two separate viruses, Rice Tungro Bacilliform Virus (RTBV) and Rice Tungro Spherical Virus (RTSV) (Hibino *et al.,* 1978). Plants infected with RTBV alone show similar but milder symptoms, while those infected with RTSV alone show no prominent symptoms except very mild stunting (Sta Cruz *et al.,* 1993). RTSV is mainly responsible for transmission by the leafhopper vector while RTBV cannot be leafhopper transmitted independently, confirming that tungro disease in the field conditions often confuses with symptoms produced by the various nutrient deficiency (Anjaneyulu *et al.,* 1994).

An epidemic outbreak of Tungro during 2001 in three districts of West Bengal caused an unmilled rice production loss of 0.5 mt valued at Rs 2911 million at current prices (Muralidharan *et al.,* 2003). Tungro viruses are transmitted by six leafhopper species, five of which are in the genus *Nephotettix*. Due to its close biological relationship with rice, the rice green leafhopper *N. virescens* is by far the most important vector species (Hibino and Cabunagan, 1986). *N. virescens* has higher transmission efficiency than other vector species and is usually more abundant in irrigated rice fields. *N. virescens* lays its eggs in batches of upto 44 in the tissues of the leaf sheath of rice tillers (Cheng and Pathak, 1971).

Tungro disease is not always easy to identify in the field, as characteristic symptoms are not expressed in all varieties. Tungro may be detected by the iodine/starch test or through

the use of insect transmission tests to assay plants. Identification of this disease in the early stage is difficult and the management is a daunting task. The yield loss can be reduced substantially, if the disease was diagnosed at correct stage. Various improved detection methods have been standardized and among them molecular method of detection seems simple, ingenious method to detect RTD from the suspected samples. This research paper emphasis on the microtomy studies of healthy and Rice Tungro disease infected rice leaf samples.

Materials and Methods

Microtomy studies of infected leaves

Microtome sectioning of healthy as well as diseased leaves was carried out as described by Johanson (1940). For this purpose, rice leaves from healthy and tungro infected plants were taken.

Fixation and washing

The leaf bits were fixed in 5 parts of 30 per cent formalin, 5 parts of glacial acetic acid and 90 parts of 70 per cent ethanol for 29 hours at 4 °C. The leaf bits were then transferred successively to 50, 60 and 70 per cent ethanol for one hour.

Dehydration

The leaf bits were gradually dehydrated in tertiary butyl alcohol (TBA) series as furnished in the following table.

Alcohol (%)	TBA (ml)	Ethanol (95 %) (ml)	Distilled water (ml)	Duration (h)
50	1.0	4.0	5.0	2
70	2.0	5.0	3.0	12
85	3.0	5.0	1.5	12
95	4.5	5.0	1.0	12
100	7.5	2.5	0.0	12

Later the samples were washed in running tap water for 10-15 min followed by washing in ethanol 10 %, 50 %, 70 % and 100 % for 5 min respectively. Then the samples were transferred to xylene I, xylene II for 5 min each and stained with toluidine blue (1g/100 ml).The slides were mounted in DPX and left undisturbed for 24 h and photographed in an image analyzer.

Results and Discussion

Cross section of the healthy and Tungro infected leaf sample of rice exhibited wide differences among the cells. The area of vascular bundles was reduced in the Tungro infected rice samples and lower epidermis cells get damaged wherein the area was large in the healthy rice leaf (Plate 1a). In case of healthy leaves of rice, the cells were normal in size with properly distributed intercellular spaces and evenly present bundle sheath cells.

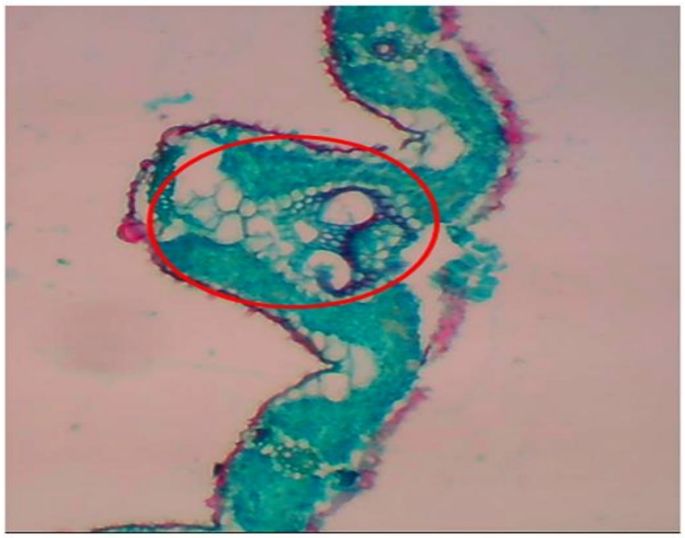
The xylem and phloem tissues showed no damage in healthy sample. Bundle sheath cells remain undisturbed above the phloem tissues. In the infected rice leaf section, the bundle sheath cells and phloem get distorted by the invasion of Tungro viruses (Plate 1b).

Microtomy studies of Tungro infected Rice leaves

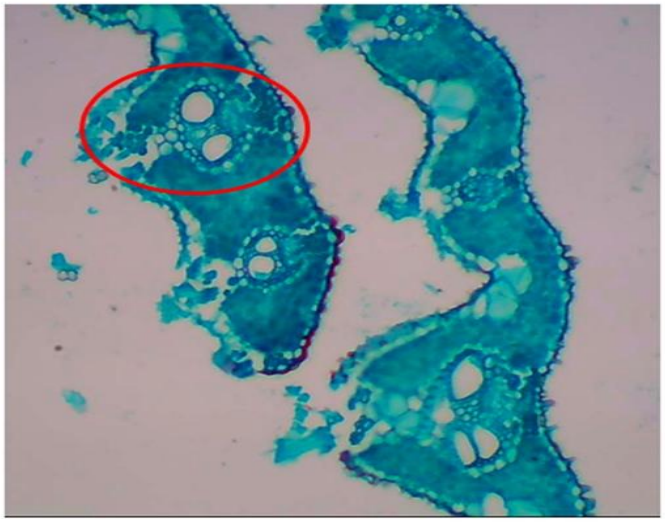
In infected plants, RTBV is localized in the vascular bundles and RTSV in the phloem tissues. Sta Cruz *et al.*, (1993) demonstrated that RTBV was present in both the xylem and phloem of the infected plants.

In infected cells, both RTBV and RTSV particles are scattered or aggregated in the cytoplasm. RTSV particles also occur in vacuoles. Viroplasm-like inclusions and membraneous masses occur in the cytoplasm of RTSV-infected cells.

Fig.1a Microtomy of healthy and Tungro infected rice leaves

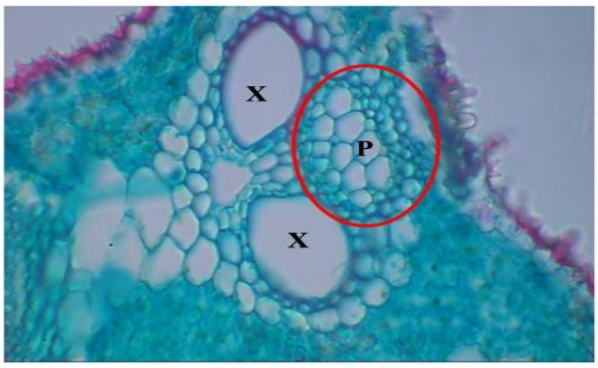


C.S. of healthy leaf

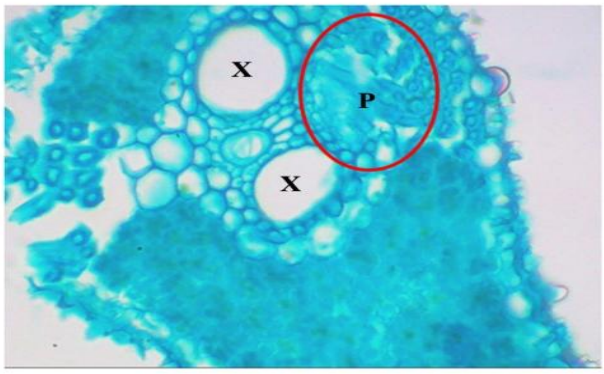


C.S. of infected leaf

Fig.1b Microtomy of healthy and Tungro infected rice leaves



C.S. of healthy leaf



C.S. of infected leaf

X- Xylem

P- Phloem

In our study, cross section of the healthy and Tungro infected leaf sample of rice exhibited wide differences among the cells. The area of vascular bundles was reduced in the Tungro infected rice samples and lower epidermis cells got damaged wherein the area was large in the healthy rice leaf. In case of healthy leaves of rice, the cells were normal in size with properly distributed intercellular spaces and evenly present bundle sheath cells.

The xylem and phloem tissues showed no damage in healthy sample. Bundle sheath cells remained undisturbed above the phloem tissues.

In the infected rice leaf section, due to the invasion by both the RTBV and RTSV viruses, bundle sheath cells and phloem got

distorted. These results are in agreement with the studies by Sta Cruz *et al.*, (1993).

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