



WEED HOST RANGE OF RICE TUNGRO VIRUS DISEASE

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Abstract

Out of twenty weed host species tested through forced feed inoculation with viruliferous leaf hoppers (*Nephotettix virescens*), thirteen weed host species were found positive reaction for tungro disease viz., *Cynodon dactylon*, *Digitaria sanguinalis*, *Echinochloa colonum*, *E. crusgalli*, *Leptochloa chinensis*, *Panicum repens*, *Cyperus rotundus*, *Fimbristylis miliaceae*, *Dinebra aratica*, *Brachiaria ramosa*, *Paspalum dilatatum*, *Leersia hexandra* and *Paspalum hydrophilum* and exhibited leaf yellowing, stunting, reduction in number of tillers and interveinal chlorosis with typical symptoms of tungro disease as expressed in rice crop. The virus was also recovered from the seven virus infected weed host species viz., *E. colonum*, *E. crusgalli*, *Panicum repens*, *C. rotundus*, *Paspalum dilatatum*, *P. hydrophilum* and *Leersia hexandra*. Weed hosts viz., *Paspalum dilatatum* and *Paspalum hydrophilum* exhibited prominent symptoms of the tungro disease.

Key words: Rice tungro virus, Leaf hopper vector (*Nephotettix virescens*), rice, weed host

Introduction

Rice (*Oryza sativa* L.) is the most important food crop of the world and is an ideal model crop plant due to its small genome size, extensive genetic resources and ease of transformation with other cereal crops. In spite of phenomenal increase in area and production of rice, its productivity is limited by various biotic constraints among which rice tungro disease (RTD) is one of the most devastating problems affecting rice crop in South-east Asia with estimated annual losses to an extent of US \$ 1.5×10^9 (Herdt, 1988). The disease came into limelight for the first time in an epidemic form in the eastern parts of Uttar Pradesh and Bihar and its widespread occurrence was confirmed by Mukhopadhyay and Chowdhury (1970). The occurrence of this disease is sporadic in nature and it can appear at any time from seedling stage to reproductive phase (Krishnaveni *et al.*, 2011).

Disease is caused by the simultaneous infection with Rice tungro bacilliform virus (RTBV, genus Tungrovirus, family Caulimoviridae), a pararetrovirus with a double-

stranded DNA genome and Rice tungro spherical virus (RTSV, genus Waikavirus, family Secoviridae), a plant picornavirus with a single-stranded (+) - sense RNA genome. The tungro virus complex is transmitted by the green leafhopper (GLH) (*Nephotettix virescens* Distant) and other leafhopper species viz., *N. nigropictus* Stal, *N. parvus* Ishihara, *N. sympatricus* Ghauri, *Recilia dorsalis* etc in a semi-persistent manner.

Generally, plants infected with both viruses shows severe tungro symptoms, including yellowing and stunting of plants, while RTBV-infected plants show mild stunting and yellowing. Rice tungro spherical virus-infected plants have indistinct symptoms. Symptoms of tungro disease in rice plants vary according to the age of the plant, rice variety, and virus strains. The most conspicuous symptoms of rice plants infected with both RTSV and RTBV are stunting and yellow to orange discoloration of the leaves.

The possibility of weed and wild rice species acting as alternate hosts for RTD has been investigated by several workers (Mukhopadhyay *et al.*, 1978; Khan *et al.*, 1991; Mallick and Chowdhury 1999). Although several weed and wild rice species have been reported as hosts, many workers failed to recover the virus from

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some of the infected host plants. Rice is the preferred host of leafhopper vector *i.e.*, *N. virescens* although it feeds on other alternate hosts like *Eleusine indica*, *Cynodon dactylon* and *Cyperus rotundus*. Green leafhopper can also feed on a number of graminaceous hosts like *Paspalum distichum*, *Eleusine indica*, *Cynodon dactylon* and *Cyperus rotundus* and *Echinochloa colona*, *E. crus-galli*. Vectors can survive and feed on a few dicotyledonous plants like *Bergia capensis* and *Ammannia bassifera* and cyperaceous hosts. The population of green leafhopper fluctuates depending on the availability of host plants, environmental conditions, natural enemies and cropping pattern. In India, many wild species such as *O. australiensis*, *O. barthii*, *O. brachyantha*, *O. eichengari*, *O. glaberrima*, *O. nivara*, *O. perennis*, and *O. punctata* were found to be susceptible to the virus (Anjaneyulu *et al.*, 1982). *N. virescens* preferred rice to grassy weeds, where as *N. nigropictus* preferred grassy weeds, especially *L. hexandra* over rice. *N. virescens* may thus play a greater role than *N. nigropictus* in the propagation of rice tungro disease (Anjaneyulu *et al.*, 1982). The present study was undertaken to understand the weed host range of rice tungro disease.

Material and Method

Twenty weed host species belonging to the families Gramineae and Cyperaceae were collected from rice fields, rice nurseries and also from research farms of Directorate of Rice Research, Hyderabad at seedling stage and tested for host range of rice tungro disease. Weeds host were collected at seedling stage and planted in 5 kg plastic pots under glasshouse conditions.

Mass multiplication of green leaf hopper (GLH) (*Nephotettix virescens* Distant) on susceptible rice cv. T (N) 1

Rice cv. T(N)1 seeds were sown in plastic trays and grown to one leaf stage. At this stage, rice seedlings were transplanted at the rate of five seedlings per each pot comprising of 5 kg soil and maintained in an insect proof cage or mylar cage (Plate 3.1) with a size of 53 × 53 × 90 cm under glasshouse conditions. This process was repeated at seven days intervals in different batches until the T(N)1 rice seedlings grows up to 45 days old plants. Then adult GLH (*Nephotettix virescens*) were collected from rice fields by using sweep net and 500-1000 adult green leafhoppers were released on rice cv. T(N)1 for 2-3 days for oviposition and emergence of nymphs and adults (plate 3.2). This process was repeated on 45 days old T(N)1 rice plants at every 2-3 days to generate new adult insect vector population.

Maintenance of rice tungro disease (RTD) on susceptible rice cv. T (N) 1

Rice tungro disease was maintained on 45 days old susceptible T(N)1 rice seedlings grown in pots @ 2 seedlings/pot and 16 pots were maintained. Adult green leaf hoppers multiplied earlier were fed on RTD infected plants for acquisition of virus by the insect vector for four days. After acquisition of the virus by green leaf hopper, T(N)1 rice seedlings grown in pots under insect proof cages were inoculated with RTD using viruliferous GLH @ 2 insects/seedling for 2-3 h by inoculating 8 pots in the morning and 8 pots in the evening hours. The inoculated T(N)1 rice plants were observed for expression of symptom development for about 2-3 weeks. This process was repeated several times to maintain RTD infection.

Inoculation of weed hosts with RTD by using insect vectors

Twenty weed host species belonging to the families Gramineae and Cyperaceae were tested by artificial inoculation for their reaction to RTD (Table 1). The vector *Nephotettix virescens* were reared in insect proof cages and used for transmission of the virus. Virus inoculum was maintained on the susceptible cv. T(N)1. Fifteen day old infected plants were used as inoculum source.

The virus free weed plants were inoculated during the seedling stage by confining 5 viruliferous leaf hopper per plant for 24 h along with weed hosts T(N)1 plant were also inoculated by the virus. After inoculation, the leafhoppers were killed by spraying of monocrotophos @ 1.6 ml/L. ten plants were tested for each species. An equal number of plants with 5 non viruliferous leafhoppers per plant served as controls. Periodical observations on symptom development were made up to 30 days after inoculation. Leaves of inoculated weed species were tested after 15 days after inoculation for the presence of virus by confining 15 non viruliferous leafhoppers for 2 days on each plant. After an acquisition feeding period, the leafhopper, were transferred singly to 10 day old healthy T(N)1 seedling for 24 h. observation of infected seedling were taken 20 days after inoculation.

Results and discussion

Twenty weed host species belonging to Gramineae and Cyperaceae family for host range studies against rice tungro disease were tested. These weeds species were inoculated by using viruliferous green leafhopper (*Nephotettix virescens*) under glasshouse conditions and maintained suitable controls. Symptoms expressed and plant height reduction was recorded in different weed host species at DRR, Rajendranagar.

Table 1: List of weed host species tested for host range and survival of rice tungro disease (RTD) at Directorate of Rice Research, Rajendranagar, Hyderabad during 2013-2014.

S. No.	Scientific Name	Common Name (English)	Vernacular Name Telugu(T)/Hindi(H)	Family	Growth habit	Mode of propagation
1.	<i>Chloris barbata</i> Sw.	Finger grass	Jargi (H) Uppugaddi (T)	Poaceae (Graminaceae)	Annual	Seeds
2.	<i>Cynodon dactylon</i> (L.) Pers.	Bermuca grass/ Bahama grass	Dhoob (H) Garika (T)	Poaceae (Graminaceae)	Perennial	Stolons and rhizomes
3.	<i>Dactyloctenium aegyptium</i> (L.) Beauv.	Crowsfoot grass/ Indian millet	Chikara/ makra grass (H) Nakshathragaddi (T)	Poaceae (Graminaceae)	Annual	Seeds
4.	<i>Digitaria sanguinalis</i> (L.) Scop.	Hairy or Large crabgrass	Pavane (H)	Poaceae (Graminaceae)	Annual	Seeds
5.	<i>Echinochloa colona</i> (L.) Link.	Jungle rice/awnless barnyard grass	Janguli/ sawak (H) Oodha (T)	Poaceae (Graminaceae)	Annual	Seeds
6.	<i>Echinochloa crus-galli</i> (L.) Beauv.	Barnyard grass	Kayada (H) Oodha(T)	Poaceae (Graminaceae)	Annual	Seeds
7.	<i>Eleusine indica</i> (L.) Gaertn	Goose grass	Bairaja (H)	Poaceae (Graminaceae)	Annual	Seeds
8.	<i>Leptochloa chinensis</i> (L.) Nees	Red sprangletop	Chanhel (H)	Poaceae (Graminaceae)	Annual	Seeds
9.	<i>Leersia oryzoides</i>	Rice cutgrass	-	Poaceae (Graminaceae)	Perennial	Seeds
10.	<i>Leersia hexandra</i>	Swamp rice grass	-	Poaceae (Graminaceae)	perennial	Rhizomes and stolons
11.	<i>Paspalum distichum</i> L.	Knotgrass	Besak (H)	Poaceae (Graminaceae)	Perennial	Stolons and rhizomes
12.	<i>Paspalum dilatatum</i> Poir.	Dallisgrass	Kodo (H)	Poaceae (Graminaceae)	perennial	Seeds/ vegetative parts
13.	<i>Paspalum hydrophilum</i>	Water paspalum	-	Poaceae (Graminaceae)	perennial	Seeds
14.	<i>Panicum repens</i> L.	Torpedo grass	Bansi/ Gunaaraa (H) Karigaddi (T)	Poaceae (Graminaceae)	Perennial	Underground Stems
15.	<i>Dinebra arabica</i> (J.)	Khare gavat	-	Poaceae (Graminaceae)	Annual	Seeds
16.	<i>Setaria verticillata</i> (L.)	British foxtail/ hooked bristle grass	Laptuna (H)	Poaceae (Graminaceae)	Annual	Seeds
17.	<i>Brachiaria ramosa</i> (L.) Stapf	Browntop millet	Indian rice grass (H)	Poaceae (Graminaceae)	Annual	seed
18.	<i>Cyperus difformis</i> L.	Umbrella sedge	Dila (H)	Cyperaceae (Sedge family)	Annual	Seeds
19.	<i>Cyperus rotundus</i> L.	Purple nutsedge	Motha/ Mutha (H) Thunga (T)	Cyperaceae (Sedge family)	Perennial	Rhizomes and Tubers
20.	<i>Fimbristylis miliacea</i> (L.) Vahl	Globe fingerush	Ghuen/ dilli (H) Rakasithunga (T)	Cyperaceae (Sedge family)	Annual	Seeds

Table 2: Testing of weed host species against rice tungro disease under glass house conditions.

S. No.	Weed species	Presence of virus	Symptoms expressed due to RTD	Virus recovery from the weeds showing positive reaction
1.	<i>Chloris barbata</i>	–	–	–
2.	<i>Cynodon dactylon</i>	+	Leaf yellowing and stunting	–
3.	<i>Dactyloctenium aegyptium</i>	–	–	–
4.	<i>Digitaria sanguinalis</i>	+	Leaf yellowing and stunting	–
5.	<i>Echinochloa colona</i>	+	Leaf yellowing and stunting	R
6.	<i>Echinochloa crusgalli</i>	+	Leaf yellowing and stunting	R
7.	<i>Eleusine indica</i>	–	–	R
8.	<i>Leptochloa chinensis</i>	+	Reduced tillers, stunting growth	–
9.	<i>Leersia oryzoides</i>	-	-	-
10.	<i>Leersia hexandra</i>	+	Leaf yellowing and stunting	R
11.	<i>Paspalum distichum</i>	–	–	–
12.	<i>Paspalum dilatatum</i>	+	Leaf yellowing, stunting and reduced tillering	R
13.	<i>Paspalum hydrophilum</i>	+	Leaf yellowing, interveinal chlorosis	R
14.	<i>Panicum repens</i>	+	Stunting, leaf yellowing	R
15.	<i>Dinebra arabica</i>	+	Leaf yellowing	–
16.	<i>Setaria verticillata</i>	–	–	–
17.	<i>Brachiaria ramosa</i>	+	Leaf yellowing	–
18.	<i>Cyperus difformis</i>	–	–	–
19.	<i>Cyperus rotundus</i>	+	Leaf yellowing and stunting	R
20.	<i>Fimbristylis miliacea</i>	+	Leaf yellowing, reduced tillers	–

*R: Recovery of the virus from weed host species

The results revealed that all the weed species tested were found virus free and maintained in insect cages for virus inoculation. Then these weed hosts were subjected to forced feed inoculation with viruliferous leaf hoppers. Some of the weed host species were found positive for tungro disease in about 10 to 15 days after inoculation. Among twenty weed species tested for presence of tungro disease, thirteen weed host species viz., *Cynodon dactylon*, *Digitaria sanguinalis*, *Echinochloa colona*, *E. crusgalli*, *Leptochloa chinensis*, *Panicum repens*, *Cyperus rotundus*, *Fimbristylis miliacea*, *Dinebra arabica*, *Brachiaria ramosa*, *Paspalum dilatatum*, *Leersia hexandra*, and *Paspalum hydrophilum* showed positive reaction when inoculated with RTD. All the weeds species identified were found positive and expressed variation in symptoms. The kind of symptoms expressed by the weed species to RTD was given in table 2.

The weed species viz., *Paspalum dilatatum* and *Paspalum hydrophilum* expressed clear symptoms like leaf yellowing, stunting, number of reduced tillers and interveinal chlorosis and resembled the typical symptoms of tungro as expressed in rice. The virus was also recovered from the seven virus infected weed host

species viz., *E. colona*, *E. crusgalli*, *Panicum repens*, *C. rotundus*, *Paspalum dilatatum*, *P. hydrophilum* and *Leersia hexandra*. Most of the weed species exhibited symptoms like reduced tillering and leaf yellowing.

The results revealed that *N. virescens* transmitted rice tungro virus from rice to rice and from rice to seven weed host species. All the infected weed host species exhibited one or other symptoms related to tungro disease. In the earlier studies, many of the weed host species were tested for its susceptibility to tungro disease (Anjaneyulu *et al.* 1988; Anjaneyulu *et al.* 1982; Mishra *et al.* 1973; Mohanty *et al.* 1987; Rao and Anjaneyulu, 1982; Tarafder and Mukhopadhyay, 1979) and many of the weed species were reported to be susceptible to virus as well as vectors (*N. virescens*). The tungro disease symptoms expressed on some weed hosts in the earlier studies were not found on the same weed hosts in some studies. Leaf hopper recovery tests of tungro viruses from infected weed host plants also had conflicting results. Some tests indicated positive recovery from infected weed hosts but others indicated no recovery from the same weed that was artificially infected with RTD. In the present study, forced feeding by viruliferous leaf hoppers

to introduce RTD in weed host species showed that virus could be introduced into *Paspalum distichum* and *Echinochloa colonum* and the results are in agreement with the findings of Rao and Anjaneyulu (1982). The data also indicated that virus could not be recovered from two of the positively identified weed species *i.e.*, *Cynodon dactylon* and *Digitaria sanguinalis*. The similar findings were also confirmed by the earlier workers of Rao and Anjaneyulu, (1978); Rao and John (1974) were failed to recover the virus from the infected plants. The variation in the results was due to lack of reliable diagnostic method and a reliable indexing method.

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