

# Experimental Host Range of Rice Tungro Virus and Its Vectors

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## ABSTRACT

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Of 15 rice cultivars tested, Bala, IR 8, Krishna, Padma, and Taichung (Native) 1 were the best sources of rice tungro virus in both standing crop and stubbles. *Oryza sativa* and the following wild species of rice were susceptible to the virus: *O. australiensis*, *O. barthii*, *O. brachyantha*, *O. eichengeri*, *O. glaberrima*, *O. nivara*, *O. perennis*, and *O. punctata*. The vector *Nephotettix nigropictus* reproduced on *O. eichengeri*, *O. glaberrima*, *O. malampuzhaensis*, *O. minuta*, *O. nivara*, *O. officinalis*, *O. perennis*, *O. punctata*, *O. sativa*, *Echinochloa colonum*, *Ischaemum indicum*, *Leersia hexandra*, and *Paspalum orbiculare*; *N. virescens* reproduced only on *O. glaberrima*, *O. nivara*, *O. perennis*, *O. sativa*, *E. colonum*, and *P. orbiculare*.

Rice tungro virus (RTV) was epidemic in northeast India during 1969 (8) and 1973 (2). It has been found in the states of Andhra Pradesh, Assam, Bihar, Karnataka, Kerala, Manipur, Orissa, Tamil Nadu, Tripura, Uttar Pradesh, and West Bengal (2,5,6,8,9,12,15). The mode of survival of RTV and its vectors, *Nephotettix virescens* (Distant) and *N. nigropictus* (Stål), between crops is not well understood. Mishra et al (11) reported five natural weed hosts and nine other weed hosts susceptible in artificial inoculation. Several researchers found other weeds and wild species of rice susceptible to RTV in artificial inoculation but did not report any natural hosts (1,7,13,14,16,18,19). Rao and Anjaneyulu (13) suggested that RTV may survive between crops in *Oryza* species.

Where the overlapping of crops is common, we believe that RTV may survive in rice cultivars, stubbles, and in

wild rice. We thus tested various commercial rice cultivars and wild species for their reaction to RTV and their ability to serve as virus sources. Stubbles of rice cultivars were also indexed for the presence of the virus.

Because RTV is exclusively transmitted by the leafhoppers *N. virescens* (17) and *N. nigropictus* (10), perpetuation of the virus—especially between rice crops—also depends on the availability of vectors. We tested several cereal crops, weeds, and wild rice species as potential reservoir hosts of the leafhopper vectors.

## MATERIALS AND METHODS

Strain 2A of RTV (3) was used to inoculate rice cultivars and wild rice species. The strain was maintained on the susceptible rice, Taichung (Native) 1 (TN 1). Nonviruliferous leafhoppers (*N. virescens* and *N. nigropictus*) were reared in insect-proof cages on cultivar IR 20, which is resistant to the virus but susceptible to the vector. *N. virescens* was allowed to feed for 48 hr on 45-day-old, diseased TN 1 plants and immediately transferred to test plants for a 24-hr inoculation feeding.

Fifteen plants of each of 15 high-yielding, semidwarf, commercial rice cultivars were inoculated with RTV using

five viruliferous leafhoppers (*N. virescens*) per plant. Twenty and 40 days after inoculation, 10 nonviruliferous leafhoppers (*N. virescens*) were allowed to feed on each of the inoculated plants for 48 hr (a total of 150 leafhoppers on 15 plants) and then transferred to 10-day-old TN 1 seedlings at the rate of one insect per seedling for 24 hr. Stubbles of 15 rice cultivars were also indexed for the virus 10 and 20 days after harvesting as described above.

Twenty plants of each of 17 species of *Oryza* were infested during the seedling stage with 10 viruliferous *N. virescens* leafhoppers per plant. An equal number of healthy plants, on which nonviruliferous leafhoppers fed (10 per plant), served as controls. Starting the fifth day after inoculation, the plants were observed periodically for symptom development. Heights and tiller numbers were recorded 90 days after inoculation.

Between 15 and 90 days after inoculation, we indexed RTV in inoculated plants by confining 150 nonviruliferous *N. virescens* leafhoppers (10 insects per plant) on 15 selected plants for 48 hr at 15-day intervals. After the acquisition feeding period, we transferred individual leafhoppers to 10-day-old TN 1 plants to estimate the percentage of infective leafhoppers.

Six cereal crops, 10 weed species in the Graminae and Cyperaceae, and 17 species of *Oryza* were tested for suitability as food or reproductive hosts for *N. virescens* and *N. nigropictus*. Twenty nymphs were caged on each of 15 plants of the different plant species using galvanized iron screen cages; 15 healthy TN 1 plants, each caged with 20 nymphs, served as checks. By daily observation, we determined the survival period of the leafhoppers. We tested leafhopper reproduction by confining 10 male and 10

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female young adults on each of 15 plants of each species for 30 days. The nymph population was observed. Plant species supporting leafhoppers for 10 or more days were considered food hosts, and those supporting one full life cycle were considered reproductive hosts.

## RESULTS

Rice cultivars Bala, IR 8, Krishna,

Padma, and TN 1 were the best sources of RTV both in standing crop and stubbles (Table 1). Annapurna, CR 138-802-10, CR 138-994-27, IR 20, IR 26, IR 30, and Ratna were poor sources.

We recovered more RTV from standing crop than from stubbles with IR 20, Pankaj, and Vijaya, whereas the reverse was true with Bala, CR 138-802-10, CR 138-994-27, IR 30, and TN 1. The virus

was recovered from standing crop but not from stubbles with Annapurna, IR 20, and IR 26.

*Oryza* species susceptible to RTV were *O. australiensis*, *O. barthii*, *O. brachyantha*, *O. eichengeri*, *O. glaberrima*, *O. nivara*, *O. perennis*, *O. punctata*, and *O. sativa* var. TN 1. Species not susceptible were *O. alta*, *O. grandiglumis*, *O. latifolia*, *O. malampuzhaensis*, *O. minuta*, *O. officinalis*, *O. perrieri*, and *O. schweinfurthiana*. Table 2 shows the

**Table 1.** Recovery of rice tungro virus by *Nephotettix virescens* from standing crop and stubbles of rice cultivars.

Cultivar	Viruliferous leafhoppers, % <sup>a</sup>			
	Standing crop		Stubbles	
	Days after inoculation		Days after harvesting	
	20	40	10	20
Annapurna	13.3	0.0	0.0	0.0
Bala	44.4	53.3	70.8	35.0
CR 138-802-10	17.4	0.0	13.6	23.3
CR 138-994-27	20.0	0.0	17.8	11.1
IR 8	53.8	50.0	42.8	34.5
IR 20	15.0	4.3	0.0	0.0
IR 26	26.7	0.0	0.0	0.0
IR 30	10.0	0.0	14.3	10.3
Krishna	68.0	43.3	76.5	24.1
Padma	75.9	28.0	61.9	10.0
Pankaj	25.0	68.4	42.8	0.0
Pusa 2-21	10.0	4.5	23.0	54.2
Ratna	20.7	3.3	13.8	14.3
Taichung (Native) 1	34.5	56.7	92.8	25.0
Vijaya	64.3	0.0	21.4	5.3

<sup>a</sup>Based on positive transmissions by *N. virescens* to indicator host Taichung (Native) 1 from a total of 150 leafhoppers caged on 15 inoculated rice plants (10 leafhoppers per plant) for 48 hr.

**Table 2.** Susceptibility of *Oryza* species to rice tungro virus

Species	No. of plants infected/inoculated	Incubation period, days	Stunting, %	Reduction or increase in tiller number, %
<i>O. australiensis</i>	12/20	9	13.8	+ 4.7
<i>O. barthii</i>	8/20	8	44.9	-66.2
<i>O. brachyantha</i>	4/20	10	40.0	-54.0
<i>O. eichengeri</i>	10/20	7	24.8	-63.4
<i>O. glaberrima</i>	20/20	6	41.2	-81.0
<i>O. nivara</i>	20/20	7	46.3	-80.0
<i>O. perennis</i>	15/20	8	3.9	-28.8
<i>O. punctata</i>	16/20	9	15.0	- 7.0
<i>O. sativa</i> 'Taichung (Native) 1'	20/20	6	76.8	-80.0

**Table 3.** Recovery of rice tungro virus by *Nephotettix virescens* from inoculated plants of susceptible *Oryza* species

Species	Viruliferous insects, % <sup>a</sup>					
	Days after inoculation of the donor host					
	15	30	45	60	75	90
<i>O. australiensis</i>	0.0	6.7	10.0	0.0	0.0	0.0
<i>O. barthii</i>	8.3	10.0	20.0	50.0	35.0	14.3
<i>O. brachyantha</i>	0.0	7.6	0.0	0.0	0.0	0.0
<i>O. eichengeri</i>	0.0	0.0	0.0	13.8	5.2	0.0
<i>O. glaberrima</i>	43.5	61.9	66.7	54.5	79.3	38.9
<i>O. nivara</i>	55.0	33.3	46.7	65.4	39.1	60.0
<i>O. perennis</i>	0.0	6.6	0.0	0.0	0.0	0.0
<i>O. punctata</i>	0.0	11.1	0.0	0.0	0.0	0.0
<i>O. sativa</i> 'Taichung (Native) 1'	73.1	68.2	63.3	71.4	47.4	53.3

<sup>a</sup>Based on positive transmissions by *N. virescens* to indicator host Taichung (Native) 1 from a total of 150 leafhoppers caged on 15 inoculated plants of *Oryza* species (10 leafhoppers per plant) for 48 hr.

**Table 4.** Hosts of *Nephotettix virescens* and *N. nigropictus*

Plant type Species	Days of survival	
	<i>N. virescens</i>	<i>N. nigropictus</i>
<b>Gramineae</b>		
<i>Oryza</i> spp.		
<i>Oryza alta</i>	3	10
<i>O. australiensis</i>	2	3
<i>O. barthii</i>	12	15
<i>O. brachyantha</i>	5	8
<i>O. eichengeri</i>	5	14 <sup>a</sup>
<i>O. glaberrima</i>	30 <sup>a</sup>	30 <sup>a</sup>
<i>O. grandiglumis</i>	6	3
<i>O. latifolia</i>	3	3
<i>O. malampuzhaensis</i>	5	26 <sup>a</sup>
<i>O. minuta</i>	3	12 <sup>a</sup>
<i>O. nivara</i>	30 <sup>a</sup>	25 <sup>a</sup>
<i>O. officinalis</i>	3	15 <sup>a</sup>
<i>O. perennis</i>	25 <sup>a</sup>	24 <sup>a</sup>
<i>O. perrieri</i>	10	12
<i>O. punctata</i>	3	22 <sup>a</sup>
<i>O. sativa</i> 'Taichung (Native) 1'	45 <sup>a</sup>	30 <sup>a</sup>
<i>O. schweinfurthiana</i>	4	7
<b>Cereal crops</b>		
<i>Eleusine coracana</i>	3	14
<i>Hordeum vulgare</i> 'Ratna'	6	13
<i>Pennisetum typhoides</i> 'PHB 10'	6	5
<i>Sorghum vulgare</i> '148'	6	5
<i>Triticum aestivum</i> 'Kalyana Sona'	6	13
<i>Zea mays</i> 'Vijaya'	7	13
<b>Weeds</b>		
<i>Cynodon dactylon</i>	3	8
<i>Digitaria ciliaris</i>	4	6
<i>Echinochloa colonum</i>	20 <sup>a</sup>	28 <sup>a</sup>
<i>Eleusine indica</i>	10	12
<i>Eragrostis pilosa</i>	5	6
<i>E. tenella</i>	3	4
<i>E. unioloides</i>	3	7
<i>Eriochloa procera</i>	2	5
<i>E. ramosa</i>	3	4
<i>Ischaemum indicum</i>	5	15 <sup>a</sup>
<i>Leersia hexandra</i>	5	30 <sup>a</sup>
<i>Leptochloa chinensis</i>	3	8
<i>Panicum repens</i>	3	5
<i>Paspalum orbiculare</i>	20 <sup>a</sup>	28 <sup>a</sup>
<b>Cyperaceae</b>		
<b>Weeds</b>		
<i>Cyperus difformis</i>	2	2
<i>C. iria</i>	2	2
<i>C. rotundus</i>	2	2
<i>Fimbristylis bis-umbellata</i>	2	2
<i>F. cymosa</i>	2	2
<i>Kyllinga monocephala</i>	3	4

<sup>a</sup>Oviposition and nymphal hatching occurred.

incidence of infection, incubation period, percentage of stunting, and reduction in tiller number in the susceptible species.

*O. glaberrima*, *O. nivara*, and *O. sativa* exhibited chlorosis of newly emerged leaves and orange discoloration of older leaves. Symptoms in *O. australiensis* were less severe. *O. barthii*, *O. perennis*, and *O. punctata* showed yellowing, interveinal chlorosis, and veinal necrosis, especially in boot leaves. Although infected *O. brachyantha* plants were greatly reduced in height and tiller number, the leaves were not discolored. Leaves of infected *O. eichengeri* plants were twisted spirally and were darker green than those of healthy plants.

RTV was recovered from *O. barthii*, *O. glaberrima*, *O. nivara*, and *O. sativa* at all five test periods after inoculation (Table 3). The virus was recovered from the other species only at certain times after inoculation.

*N. nigropictus* reproduced on *O. eichengeri*, *O. glaberrima*, *O. malam-puzhaensis*, *O. minuta*, *O. nivara*, *O. officinalis*, *O. perennis*, *O. punctata*, *O. sativa*, *Echinochloa colonum*, *Ishaemum indicum*, *Leersia hexandra*, and *Paspalum orbiculare*. *N. virescens* reproduced on *O. glaberrima*, *O. nivara*, *O. perennis*, *O. sativa*, *E. colonum*, and *P. orbiculare* (Table 4). Other species acted as food hosts. *N. nigropictus* survived on *O. barthii*, *Eleusine coracana*, *Hordeum vulgare*, *Triticum aestivum*, *Zea mays*, *O. perrieri*, *Eleusine indica*, and *O. alta* for 15, 14, 13, 13, 13, 12, 12, and 10 days, respectively; *N. virescens* survived on *O. barthii*, *O. perrieri*, and *E. indica* for 12, 10, and 10 days, respectively.

## DISCUSSION

The possibility of weed and wild rice species acting as alternate hosts for RTV has been investigated by several workers from India (4,11,13-15), Malaysia (18,19), Philippines (16,20), and Thailand (7). Although several weed and wild rice species have been reported as hosts, many workers (7,14-16,18,19) failed to recover the virus from some of the infected plants. The susceptibility of these species is thus in doubt. Moreover, these species have no practical role in perpetuating RTV between crops.

We confirmed our previous finding

that *O. barthii*, *O. glaberrima*, *O. nivara*, and *O. perennis* are susceptible to RTV (13). Species reported as susceptible from other countries are *O. barthii*, *O. officinalis*, *O. ridleyi*, and *O. rufipogon* from the Philippines (16); *O. fatua* from Malaysia (19); and *O. rufipogon* from Thailand (16). Our study found that *O. australiensis*, *O. brachyantha*, *O. eichengeri*, and *O. punctata* were also susceptible to RTV, and these species thus represent new hosts.

Because the high-yielding, dwarf rice cultivars are intensively cultivated, it has become common practice to overlap two crops of rice. In these circumstances, RTV might survive in the rice crop. This is especially likely with such high-yielding, semidwarf cultivars as Bala, IR 8, Krishna, and Padma, which are potential reservoir hosts of the virus both in standing crop and stubbles (Table 1). The occurrence of RTV throughout the year in West Bengal and Tamil Nadu, where the overlapping of crops is common, strongly supports this assumption. Some weed hosts (11), including wild rice species, might also act as a link between crops in locations where a single crop is grown in a year.

*N. virescens*, an efficient vector of RTV (17), appeared to have a more limited host range than the relatively inefficient vector *N. nigropictus* (10). Although both species are found together in the same paddy ecosystem, they prefer different plant species for food and reproduction. We observed repeatedly that *N. virescens* preferred rice over grassy weeds, whereas *N. nigropictus* preferred grassy weeds, especially *L. hexandra*, over rice. In areas where crops are overlapped, *N. virescens* may thus play a greater role than *N. nigropictus* in the propagation of RTV. In areas where single cropping is practiced, *N. nigropictus* may contribute substantially to the survival of RTV by transmitting it among grassy weeds during the fallow season.

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