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Leafhopper Transmission of Rice Tungro Bacilliform and Rice Tungro Spherical Viruses in Leafhopper Resistant and Susceptible Rice Cultivars

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Virus vector interaction of rice tungro virus complex significantly differ with the susceptibility of the host of its vector *Nephotettix* virescens (Distant.). Retention of rice tungro bacilliform virus (RTBV) in the vector was maximum for 2 days in resistant cultivars, IR50, IR56 or IR72 while 5 days in susceptible TN1 (Taichung Native 1). Retention period of Rice Tungro spherical virus (RTSV) was much shorter. During sequential feeding on resistant and susceptible cultivars a viruliferous leafhopper could retain the infectivity although it slightly reduced as compared to successive feeding on susceptible TN1 variety. An individual vector infective of RTBV and RTSV could infect maximum of 4 seedlings in TN1, 3 in IR22, IR36 and 1 or 2 in cultivars like ARC11554, Utrimerah, Utrirajapan, Habiganja DW8, Jhingasail, Ptb18 and IR74 under caged conditions. Cultivars showed resistant reaction were predominantly infected with RTBV alone when exposed to RTBV and RTSV viruliferous insect. Movement of leafhopper on resistant cultivars were considerably higher than in susceptible cultivars, but movement not associated with high infection rate.

Key Words : Rice tungro virus, *Nephotettix virescens* Transmission, Host resistance, Retention, Movement, Infective capacity

Introduction

Tungro is one of the most important virus diseases of rice (*Oryza sativa* L.) in South and Southeast Asia. It is associated with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) (Hibino et al. 1991, Omura et al. 1983). Both viruses are efficiently transmitted in a semipersistent manner by the green leafhopper (GLH) *Nephotettix virescens* (Distant) and inefficiently by some other leafhopper species (Hibino et al. 1978). RTSV can be

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transmitted independently but RTBV transmission from singly infected plants occurs only by GLH that have fed on RTSV infected plants (Cabauatan & Hibino 1985, Hibino 1983). Generally rice plants infected with RTBV and RTSV together develop typical tungro symptoms, Infected plants with RTBV alone develop mild tungro symptoms and RTSV alone infected plants develop no clear symptoms (Hibino et al. 1990). Rice cultivars resistant to GLH are predominently infected with RTBV alone when exposed to vectors viruliferous of RTBV and RTSV (Hibino 1983, Daquioag et al. 1984), although the same cultivars have infection with RTSV when exposed to vectors viruliferous of RTSV alone (Hibino et al. 1988).

Inspite of having the qualities of GLH resistant many of the rice cultivars are infected with RTV disease and causes severe loss in production. Intensity of loss is further related on the association of causal virus and host relations, (Hibino 1989). Spread of RTV in the field depends on host resistance, vector efficiency and type of tungro associated viruses (Chowdhury et al. 1993, 1994). In view of the composite nature of tungro disease agents, it further demands additional informations on virus vector host relationship on both tungro resistant and susceptible cultivars. In Asian countries most of the irrigated areas are planted with high yielding varieties and many of which have resistance to GLH vector. Considering the complex nature of tungro disease and its host reaction it further demands additional information on virus vector relationship and this studies were conducted with the objectives to demonstrate the interaction of RTBV and RTSV with their leafhopper vector *N. virescens* in different rice cultivars with different resistance to GLH.

Materials and Methods

Viruses and Insects

A colony of *N. virescens* used in this study was collected originally at Los Banos, Philippines and was maintained on a susceptible rice cultivar Taichung Native 1 (TN1) in the greenhouse for several years. A tungro isolate has been maintained for about 15 years under greenhouse in the International Rice Research Institute (IRRI), Philippines was used by regular transfer using GLH. Infected TN1 plants showing typical 'tungro' symptoms were tested by Latex Serology (Omura et al. 1983) for the presence of RTBV and RTSV to select source plants. To get sufficient number of source plants infected by both RTBV and RTSV or single by RTSV seven-day-old TN1 seedlings were inoculated in test tube by confining 4-5 GLH for one day that had fed for 3 days on plants infected with both RTBV and RTSV or RTSV alone. Seedlings were transplanted in pots and one month later indexed by ELISA. Plants infected with either both viruses or RTBV or RTSV alone were separated and served as virus source. All tungro cultures have been maintained on TN1 by regular transfers using GLH.

Host Plants

Rice cultivars used in this experiment include ARC11554, IR50, IR56, IR72 and IR74 have resistance to GLH, IR 36 has moderate resistance and TN1 is susceptible. ARC11554 also has resistance to RTSV infection (Hasannudin et al. 1987).

Daily Serial Transmission

GLH adults were allowed a 4-day acquisition access time on doubly or only RTSV infected plants. Each GLH was given 5 serial daily inoculation access feedings on 7-day old

seedlings of GLH resistant cvs IR50, IR56, IR72 or in susceptible TN1. Approximately 80 GLH were used for each cv for the transmission of RTBV and RTSV combined and same number of GLH for the transmission RTSV alone. Three wk after inoculation seedlings were indexed by ELISA.

Sequential Transmission on GLH Resistant and Susceptible Cultivars

GLH adults that had fed on RTBV and RTSV infected source plants for 4 days were individually given 6 sequential 3-hr inoculation feedings on TN1 seedlings, or alternatively on ARC11554, IR74 or IR36 seedlings and solely on TN1 seedlings at an interval of 30 min in test tubes. After inoculation, seedlings were raised in the glasshouse and indexed after three weeks for the presence of virus by ELISA.

Retention of Virus in GLH

Two-wk-old 369 seelings each of IR50 and TN1 separately transplanted one per hill in 20 × 20 cm spacing in large compartments of a screenhouse. Ten days after transplanting, 738 adults GLH viruliferous with both RTBV and RTSV were released at the four corners in each compartment. Following day after release 80-90 leafhoppers were recovered by using an aspirator from each compartment and it continued upto five day; Recovered insect tested individually for their RTV infectivity by confining them separately in a test tube with a 7-day-old TN1 seedling for one day. After recovery, on fifth day compartments were sprayed with an insecticide. Both inoculated TN1 seedlings and all plants of the compartment were tested for the presence of virus by ELISA.

Transmission Ability of Single GLH

A total of 14 rice cvs with varying degree of resistance to GLH and RTV were used to determine the infective capacity of tungro vector on such cvs. Pre-germinated seeds of each cv were placed 10 each in 12 cm pots. At 7 days age of the seedlings, one GLH adult viruliferous of both RTBV and RTSV was confined in each pot with a myler cage and kept for 5 days for inoculation feeding. Each cv had 12 replications. Four-wk-after the confinement, seedlings were indexed by ELISA. Number of seedlings infected with RTBV and RTSV either together or separately was considered transmission ability of GLH to the particular cultivar.

Monitoring GLH Movements

Seven-day-old seedlings of some GLH resistant and susceptible cultivars were transplanted 10 each in a pot. Seedlings were confined in a cage with one GLH adult viruliferous of RTBV and RTSV for 8 hr. Pots were placed in a room under natural light. Seedlings in each pot were marked 1 to 10 and presence of insect in the seedlngs within the cage was recorded at a halfen hour interval for a period of 8 hr. Thereafter, seedlings were sprayed with an insecticide. Twenty days after the test, all the seedlings were indexed for the presence of virus.

ELISA

Antisera to RTBV and RTSV had liters of 1/2,560 and 1/640, respectively, in the ring interface precipitin test. ELISA followed the procedures described earlier (Bajet et al. 1985). Leaf samples were homogenized separately using a leaf and bud press with 0.02 M sodium phosphate buffer (*p*H 7.4) containing 0.14% NaCl, 0.05% Tween20. For

each test sample, one well of microtitre plate was used to detect either RTBV or RTSV on each plate, four wells were reserved for extract of healthy TN1 leaves, two wells for extract of TN1 leaves infected with RTBV and RTSV and four wells for the buffer solution. Samples that gave absorbence at 405 nm four times higher than the mean absorbence of healthy control were considered positive for the tested viruses.

Results

Virus Retention of GLH with Serial and Sequential Transfer

GLH that have acquired both RTBV and RTSV combined retained both RTBV and RTSV for one day and RTSV alone upto two days on GLH resistant IR50, IR56 and IR72 (table 1) whereas on susceptible TN1 GLH retained RTBV upto five days and RTSV for two days. On the contrary GLH that have acquired RTSV retained the virus only for one day on the resistant cultivars and two days on susceptible TN1 (table 2).

On TN1 seedlings, GLH adults viruliferous of both RTBV and RTSV transmitted either both viruses together or RTBV alone in decreasing rates with advance of transfers in sequential feeding of 3 hr (figure 1). When given sequential feedings alternatively on ARC11554 and TN1, GLH transmitted RTBV alone on ARC11554 but either both viruses or RTBV alone on TN1. Percentage of GLH that transmitted viruses on ARC11554 in each feeding was lower than that of TN1 in the following feeding. Similar results were also obtained in the sequential feeding alternatively on IR72 and TN1. In the alternative sequential feeding on IR36 and TN1, GLH transmitted the two viruses either together or separately on IR36 as efficient as on TN1.

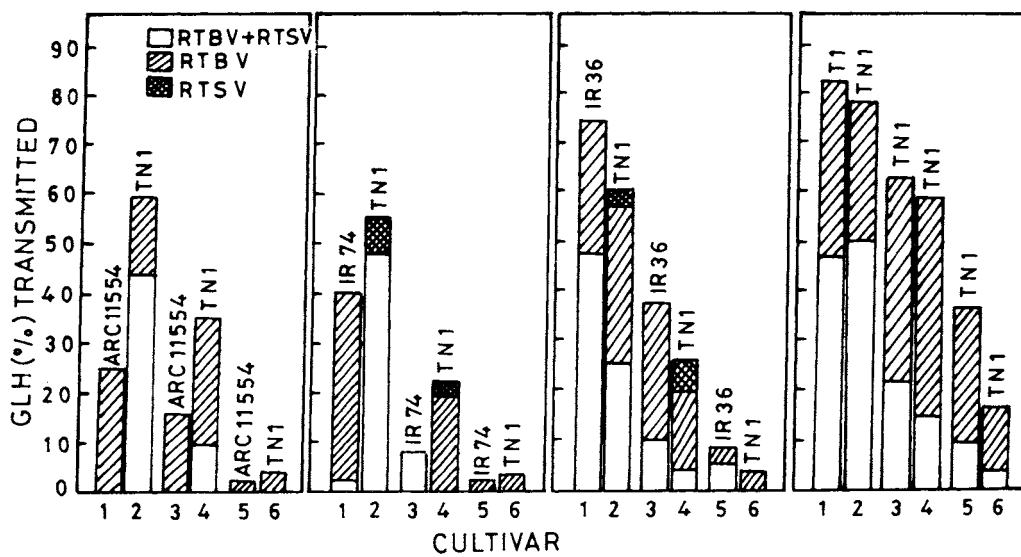

Figure 1 Percentage leafhopper transmitted RTBV and RTSV either jointly or singly in 6-sequential 3-hr inoculation feeding alternatively in seedlings of ARC11554, IR74 or IR36 and TN1 or continually on TN1

Table 1 *Percentage of leafhoppers transmitted RTBV and RTSV either jointly or singly in 5 serial daily inoculation feeding on 4 rice cultivars after acquisition feeding on RTBV + RTSV infected source plant*

Cultivar	Virus	Leafhoppers transmitted RTBV and/or RTSV				
		Number of transfer (in days)				
		1	2	3	4	5
IR50	RTBV + RTSV	5	0	0	0	0
	RTBV	27	12	0	0	0
	RTSV	0	0	0	0	0
		(80)[a]	(74)	(52)	(49)	(37)
IR56	RTBV + RTSV	9	0	0	0	0
	RTBV	25	14	0	0	0
	RTSV	5	0	0	0	0
		(80)	(73)	(66)	(58)	(49)
IR72	RTBV + RTSV	5	0	0	0	0
	RTBV	22	12	0	0	0
	RTSV	0	0	0	0	0
		(80)	(68)	(48)	(37)	(21)
TN1	RTBV + RTSV	22	11	0	0	0
	RTBV	43	35	29	20	9
	RTSV	6	1	0	0	0
		(78)	(72)	(69)	(61)	(52)

[a]Number of leafhoppers tested

Table 2 *Percentage leafhoppers transmitted RTSV in 5 serial daily inoculation feedings on seedlings of four cultivars after an acquisition feeding on RTSV infected plants*

Cultivar	Leafhoppers transmitted RTSV (in %) Number of transfer (in days)				
	1	2	3	4	5
IR50	34	0	0	0	0
	(118)[a]	(101)	(91)	(83)	(70)
IR56	48	0	0	0	0
	(112)	(109)	(101)	(95)	(81)
IR72	26	0	0	0	0
	(119)	(107)	(95)	(87)	(73)
TN1	62	23	0	0	0
	(133)	(103)	(98)	(87)	(83)

[a]Numbr of leafhoppers tested

Virus Retention of GLH with Confinement in Screenhouse

Adult GLH viruliferous of both RTBV and RTSV were released in screenhouse compartments planted with TN1 seedlings, percentage GLH infective of RTBV and RTSV either together or separately decreased gradually for third days, thereafter increased again (figure 2). When GLH were released

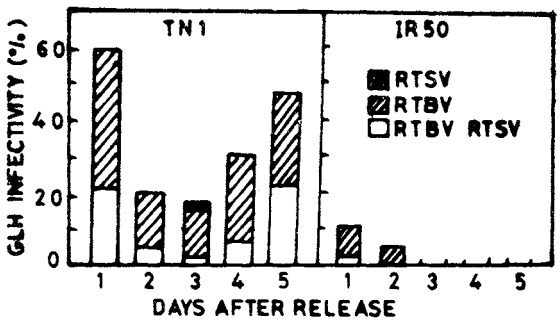

Figure 2 Retentivity of tungro agents by leafhoppers that have acquired both RTBV and RTSV when released in compartments of screenhouse planted with TN1 or IR50 seedlings separately.

in compartments planted with IR50 seedlings, percentage of infective GLH decreased to a very low level both in the first and second days and afterwards virus transmission was zero. During the confinement with viruliferous GLH, TN1 seedlings had infection upto 59% on the first day of recovery and decreased upto 17% on third day after which it started increasing to 30 and 48% by the end of fourth and fifth days respectively. On IR50 percentage of infective GLH was very low as compared to TN1 and GLH had lost the ability to transmit the viruses after two days of confinements. Transmission of RTBV and RTSV combined or RTBV alone was much higher in TN1 but in IR50, GLH transmitted mostly RTBV alone.

Transmission Ability of a Single Leafhopper

Infection rate or transmission ability of a single GLH viruliferous with both RTBV

Table 3 *Frequency distribution of seedling number infected with RTV disease when seedlings each of 14 cultivars were separately confined for 5-days with single leafhopper viruliferous of both RTBV and RTSV*

Cultivar	Average seedling* infection (no.) per pot	Frequency				
		Number of seedling infected per pot				
		4	3	2	1	0
TN1	2.4a	1	4	4	3	0
IR22	1.8ab	0	3	4	2	3
IR36	1.1 b	0	3	0	5	5
IR50	0.5 c	0	0	1	3	8
IR56	0.6 c	0	0	1	4	5
IR72	0.9c	0	0	3	4	5
IR74	0.3 c	0	0	0	4	8
Ptbl8	0.4 cd	0	0	0	4	8
ASD7	0.3 c	0	0	0	3	9
Jhinga-sail	0.4 cd	0	0	0	4	9
Habi-ganja DW8	1.1 b	0	0	3	5	4
Utri-Rajapan	0.9 b	0	0	2	6	4
Utri-Merah	0.4 cd	0	0	1	2	9
ARC 11554	0.3 c	0	0	0	3	9

*Means followed by common leter not significantly different at 5% level by DMRT

and RTSV, ranged from one to four seedlings when confined in a cage with a pot planted with 10 seedlings separately of 14 rice cultivars. The number of seedlings infected within the pot was 4 in TN1, 0 to 3 in IR22 and IR36, 0 to 1 or 2 in other cvs having resistance to GLH. The transmission ability of single GLH was considered four seedlings in TN1, three seedlings in IR22 and IR36, two or one seedlings in other cvs tested (table 3). Among the test varieties RTV infection rate varied from 0.3 to 2.4 when 10 seedlings of each cv were inoculated by a single GLH. Within the infected seedlings had either both RTBV and RTSV or RTBV or RTSV alone in TN1, IR22 and IR36; while infected seedlings in IR50, IR56 and IR72 had either both viruses or RTBV alone and all the infected seedlings in rest of the cvs tested had RTBV alone (table 4).

Table 4 *Number of seedlings infected with RTBV and RTSV either jointly or singly when seedlings each of 14 cultivars were separately confined for 5 days in a cage with single leafhopper viruliferous of both RTBV and RTSV*[a]

Cultivar	Total seedlings tested	Total seedlings infected with[a]		
		RTBV + RTSV	RTBV	RTSV
TN1	114	14	10	3
IR22	108	5	13	1
IR36	105	2	7	2
IR50	107	2	3	-
IR56	106	2	4	-
IR72	115	2	8	-
IR74	116	-	4	-
Ptb18	95	-	4	-
ASD7	108	-	3	-
Jhingasail	102	-	4	-
Habiganja DW8	103	-	11	-
Utri Rajapan	110	-	9	-
Utri Merah	107	-	4	-
ARC11554	108	-	3	-

[a]Seedlings were indexed by ELISA

Movement of Leafhopper

During the confinement of single GLH adult viruliferous of both RTBV and RTSV with

Table 5 *Settlement pattern and virus transmission of single leafhopper viruliferous of RTBV and RTSV observed at halfen hour interval during 8 hr confinement with 10 seedlings of cultivar IR36, IR74, ARC11554 or TN1*

Observation during 8 hr confinements	Cultivar			
	IR36	IR74	ARC 11554	TN1
No. of seedling visited	5	6	7	2
No. of GLH stay out of seedlings	2	7	7	0
Longest stay in one seedling (hr)	$2^1/_2$	1	1	$3^1/_2$
No. of seedlings visited before prolonged settlement	4	2	5	1
Seedling (%) infected during confinement (8 hr)	10[a]	0	0	20

[a]Seedlings were indexed by ELISA

10 seedlings in pots, GLH visited average 5, 6, 7 and 2 number of seedlings in IR36, IR74 and ARC11554 and TN1 respectively. GLH in all observations stayed more on TN1 seedlings while in IR74 and ARC11554 GLH recorded 7 times stay out of seedlings (table 5).

Discussion

Tungro virus is mostly adopted to cultivated rice (*O. sativa*) plants and it has a very minimum number of wild alternate host. Infected plants and stubbles left after harvesting are the primary sources of tungro disease under field conditions in most of the rice growing countries of the south and southeast Asia (Chancellor & Cook 1995, Anjaneyulu et al. 1994). Overlapping cropping sequence may accelerate the changes of perpetuation of the disease. Dispersal of tungro viruses by vectors in a population are further influenced by the presence of tungro associated viruses (Chowdhury et al. 1994). Generally GLH resistant cultivars have antibiosis and/or antixenosis (nonpreference) to GLH. In the resistant cvs GLH has high mortality, short lifespan and less population build up (Heinrichs & Rapusas 1983, Dahal et al. 1990). GLH resistant cvs usually have less seedling infection when exposed to tungro viruliferous GLH and the levels of resistance to GLH is related to the infection rates of the cvs (Heinrichs & Rapusas 1983).

Because of the complex interactions between RTBV or RTSV, its host and GLH resistance a comprehensive information on the host resistance is very much essential to breed resistant varieties to both virus and vector. In these experiments, we compared GLH transmission characteristics of RTBV and RTSV in GLH resistant and susceptible cultivars to elucidate the differences in the dispersal of the viruses. Resistant cvs had less overall seedling infections when exposed to GLH viruliferous with both RTBV and RTSV as having been reported earlier (Hibino et al. 1987). Infections in resistant cvs were predominant for RTBV alone, while infections in susceptible ones were predominant for with both viruses together. These characteristics of resistant cvs are unfavourable for the dispersal of tungro as RTBV is unable to be transmitted independently by the leafhopper vectors. Moreover viruliferous GLH transmitted RTBV and RTSV jointly for 1 day and RTBV alone for 2 days in resistant cvs. while it retained RTBV for 5 days and RTSV for 2 days in susceptible cvs. When the viruliferous GLH were confined for 5 days with seedlings, single GLH infected one or two seedlings in resistant cultivars, while three or four seedlings in susceptible cultivars. During the confinement, GLH moved more frequently on resistant cultivars and stayed frequently out of seedlings. On the contrary, in susceptible cvs GLH moved infrequently and stayed longer period on seedlings.

The serial inoculation feedings alternatively on seedlings, GLH resistant and susceptible cvs demonstrated no apparent reduction in infection after feeding on resistant cvs. GLH transmitted predominantly RTBV alone on the resistant cultivars but no apparent change in transmission profile of the two viruses was observed in the subsequent inoculation feeding after the feeding on resistant cvs. In cultivar susceptible to tungro and GLH, a higher percentage of seedlings were infected jointly by RTBV and RTSV and also transmission rate decreased gradually with increasing the number of serial transmission. These results indicate GLH retained the infectivity for some

period even after feeding on resistant cvs.

Because of antixenosis and antibiosis, GLH density in GLH resistant cultivars is generally low in the fields. So low levels of tungro incidence in resistant cultivars was attributed merely to the low vector population. Alternatively higher rate of RTBV infection will minimise the spread of tungro disease in GLH resistant varieties. These observations also indicate that low tungro incidence was partly because of the characteristics features of in GLH transmission of RTBV in resistant cultivars.

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