

Rice tungro bacilliform virus および rice tungro spherical virus と媒介虫 *Nephotettix virescens* の関係

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Relations of Rice Tungro Bacilliform and Rice Tungro Spherical Viruses with their Vector *Nephotettix virescens*

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日比野啓行*: Rice tungro bacilliform virus および rice tungro spherical virus と媒介虫 *Nephotettix virescens* の関係

Abstract

Rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) were isolated from rice plant infected with a Philippines isolate of tungro. RTBV was transmitted alone or together with RTSV by the leafhopper *Nephotettix virescens* from rice plants infected with both RTBV and RTSV, but not from plants with RTBV alone, while RTSV was transmitted alone. RTBV transmission by the leafhopper also occurred when the leafhoppers were previously fed on RTSV diseased plants. The minimum acquisition access period of RTSV and hypothetical "helper factor" for RTBV transmission by the leafhopper was 10 min, and RTBV was acquired in a 30 min access. The "helper factor" was retained for 7 days by the leafhopper, whereas RTSV was retained for 2 days. The "helper factor" was not retained in the leafhopper after the moulting.

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Key Words: vector relations, rice tungro bacilliform virus, rice tungro spherical virus, *Nephotettix virescens*.

Introduction

Rice tungro and tungro-like diseases are distributed in South and Southeast Asia, and have been designated with different names¹³⁾. They are transmitted in a semi-persistent manner by *Nephotettix virescens* and also by several leafhopper species in the laboratory^{13,19)}. Recent findings that they are a complex caused by small bacilliform, and spherical or isometric virus particles^{8,9,17)} gave further evidence that tungro-like diseases are the same as or caused by a strain of tungro. The names rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) were recently given to these two viruses^{15,18)}.

Relations of RTBV and RTSV with *N. virescens* have been studied using a tungro isolate called penyakit habang in Indonesia^{8,9)}. *N. virescens* exposed to a penyakit habang diseased plant containing both RTBV and RTSV transmitted both viruses together, or RTBV or RTSV alone. The leafhopper transmitted RTSV from a plant infected with RTSV alone, but did not transmit RTBV from a plant infected with

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RTBV alone. RTBV was transmitted from a plant infected with RTBV alone by the leafhopper carrying RTSV.

Here, the role of RTSV in the transmission of RTBV by the leafhopper is confirmed using a tungro isolate in the Philippines and the "helper" function of RTSV in the transmission of RTBV is further elucidated. A preliminary report was made⁶⁾.

Materials and Methods

Viruses, insect and plant. RTBV and RTSV were isolated from a tungro isolate which was kindly supplied by K. C. Ling, The International Rice Research Institute, Los Baños, Philippines in 1977⁷⁾. Virus-free *N. virescens* was kindly supplied by J. Hirao, The Kyushu National Agricultural Experimental Station, Japan and reared on rice plant, *Oryza sativa* L., seedlings in a plastic cage. If not otherwise specified, two male adult leafhoppers exposed to a diseased rice plant for 1 day were given an inoculation access period of 8 hr on a 7-day old rice seedling (cultivar Taichung Native 1) in a test tube. The acquisition and inoculation accesses were performed at room temperature (24–28°C) under continuous fluorescent illumination. Inoculated seedlings were grown in a greenhouse for 1–2 month and tested for the presence of virus particles by the leaf-dip method. A leaf tip about 5 cm long was collected from each plant. The edge of the tip was cut off by a razor blade and the new edge was immediately dipped for a few seconds into a small drop of 1% neutralized phosphotungstate on a grid covered with a collodion membrane. The tip was cut again and dipped into the same drop. After excess solution on the grid was removed by a filter paper, the grid was observed under a Hitachi H-500 electron microscope. RTSV particles were abundant in the dip preparation but RTBV particles were scarce. Therefore, samples were considered positive for RTBV after at least 3 typical particles were observed in one preparation.

Results

Transmission ability of individual leafhoppers

Individual leafhoppers exposed to both RTBV and RTSV were allowed an 8 hr inoculation access on a seedling. Each leafhopper was again allowed an overnight acquisition access on a plant containing both viruses and then allowed a second inoculation access period of 8 hr on a seedling. The acquisition and inoculation accesses were repeated six times. Twenty-three out of 25 leafhoppers tested transmitted at least one virus through the six inoculation accesses. Most leafhoppers transmitted both viruses together or a single virus, or did not transmit in each access (Table 1). Only four leafhoppers transmitted both viruses together in some of the six accesses but did not transmit a single virus at all. No leafhoppers transmitted either RTBV or RTSV alone through the six transfers. These results indicate that each leafhopper tested had the ability to transmit both RTBV and RTSV, but did not always transmit both viruses together.

Table 1. Transmission of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) in repeated inoculation access period of 8 hr by *Nephotettix virescens* which are given an acquisition access period of 2 days to RTBV and RTSV, followed by overnight acquisition accesses to both viruses after every inoculation accesses

Insect number	Virus particles in inoculated seedlings					
	Number of inoculation access					
	1	2	3	4	5	6
1	BS ^{a)}	B	—	—	—	—
2	BS	BS	BS	B	B	B
3	BS	S	BS	BS	—	B
4	BS	—	—	—	BS	—
5	BS	S	—	B	—	—
6	BS	BS	BS	B	B	S
7	BS	BS	BS	BS	—	BS
8	BS	BS	—	B	BS	BS

a) B and S indicate RTBV and RTSV, respectively.

Serial hourly transmission by the leafhopper

Individual leafhoppers exposed to both RTBV and RTSV, or RTSV alone were transferred serially 10 times, at hourly intervals, to a fresh seedling for inoculation access. Thirty-two out of fifty-six leafhoppers carrying both viruses transmitted at least one virus in the serial ten transfers. Twenty-eight out of the 32 leafhoppers transmitted both RTBV and RTSV in the ten transfers. Results obtained with 10 leafhoppers are shown in Table 2. Only two leafhoppers transmitted RTBV alone in the ten transfers and two transmitted RTSV alone. The latter two leafhoppers transmitted RTSV only in one transfer and did not transmit virus in any other transfers. Individual leafhopper

Table 2. Serial transmission of rice tungro bacilliform virus (B) and rice tungro spherical virus (S) by single *Nephotettix virescens* given an acquisition access period of 1 day to both viruses and transferred at hourly intervals to test seedlings

Insect number	Virus particles in inoculated seedlings									
	Number of transfers									
	1	2	3	4	5	6	7	8	9	10
1	—	SB	SB	SB	—	SB	SB	—	SB	—
2	—	SB	SB	B	SB	SB	SB	B	SB	SB
3	—	—	SB	—	—	SB	—	—	—	SB
4	—	—	SB	—	B	—	—	—	SB	SB
5	—	SB	B	B	—	B	—	B	S	—
6	B	—	B	—	S	—	—	B	—	—
7	B	B	B	—	B	B	—	—	—	B
8	—	B	B	—	—	—	—	—	—	—
9	—	—	—	S	—	—	—	—	—	—
10	—	S	—	—	—	—	—	—	—	—

Table 3. Serial transmission of rice tungro spherical virus by single *Nephotettix virescens* given an acquisition access period of 1 day to the virus and transferred at hourly intervals to test seedlings

Insect number	Number of transfers									
	1	2	3	4	5	6	7	8	9	10
1	— ^{a)}	+	+	—	—	+	—	—	—	—
2	—	+	—	—	+	—	—	—	—	—
3	—	—	+	+	—	+	+	—	+	—
4	—	—	—	+	—	+	+	—	+	—
5	+	+	—	+	—	—	+	—	—	—
6	—	—	—	+	—	—	—	—	—	—
7	—	—	—	+	—	—	+	—	—	+
8	—	+	+	—	—	—	+	—	—	—
9	—	+	+	+	+	—	—	—	+	—
10	—	—	+	—	—	—	+	—	—	—

a) + and — indicate transfers resulted in transmission and not resulted in transmission, respectively.

carrying both viruses did not randomly transmit viruses in each transfer but had a tendency to transmit both viruses together or a single virus alone in the serial transfers. This result indicates that individual leafhoppers may be conditioned to a preferential transmission.

Seventeen out of twenty leafhoppers exposed to RTSV alone transmitted RTSV at least once in a serial transfers. Transmission of RTSV occurred intermittently in the serial transfers as shown in Table 3. A few leafhoppers transmitted RTSV in only one transfer and other leafhoppers transmitted RTSV in more than one transfer.

Relations between RTBV and RTSV in host Plant

Rice seedlings infected either with RTBV or RTSV were inoculated again with RTSV or RTBV, respectively, by means of leafhoppers two weeks after the first inoculation. Ten leafhoppers exposed either to RTBV or RTSV were used to inoculate each seedling for the first and the challenge inoculation. None of the seedlings which were infected with RTSV and challenged with RTBV contained RTBV and developed symptoms, but all of them contained RTSV. On the other hand, seedlings infected with RTBV and challenged with RTSV developed severe symptoms and contained both viruses. These results indicate that RTSV in rice plants did not assist super-infection of the rice plant with RTBV.

In another test, leafhoppers were given an acquisition access to either RTBV or RTSV. Two leafhoppers exposed to RTBV and two exposed to RTSV were combined and allowed an inoculation access together on a seedling. None out of twenty seedlings inoculated developed symptoms and contained RTBV, while 18 seedlings contained RTSV.

Interaction between RTBV and RTSV in the leafhopper

Leafhoppers were allowed sequential acquisition accesses first to RTBV or RTSV, and then alternatively to RTSV or RTBV. Leafhoppers which acquired RTSV first

Table 4. Transmission of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) by *Nephotettix virescens* allowed sequential acquisition accesses to plants infected with RTBV or RTSV alone

Acquisition access		Virus transmission ^{a)} (number of plants with virus)			
1st for 1 day	2nd for 8 hr	RTBV+RTSV	RTBV	RTSV	None
RTSV	—	0	0	19	4
—	RTSV	0	0	11	10
RTSV	RTBV	17	15	0	10
RTBV	—	0	0	0	20
—	RTBV	0	0	0	19
RTBV	RTSV	0	0	6	13

a) Two leafhoppers were allowed an overnight inoculation access on a seedling.

and then RTBV transmitted both viruses together, or RTBV alone, while leafhoppers which acquired RTBV first and then RTSV did not transmit RTBV but transmitted RTSV alone (Table 4).

Minimum acquisition access period of RTBV and RTSV

Effects of acquisition access period on the transmission of RTBV and RTSV were examined in sequential accesses. Leafhoppers were given an acquisition access period of various times to RTSV and then an additional access period of 8 hr or overnight to RTBV. RTSV was acquired in as little as 10 min acquisition access (Table 5). The leafhopper acquiring RTSV in a 10 min access transmitted both RTBV and RTSV. An acquisition access period of RTSV longer than 4 hr was required for efficient transmission of RTBV and RTSV.

Leafhoppers exposed to RTSV were allowed sequentially an acquisition access period of various times to RTBV. RTBV was acquired in an access period of at least 30 min (Table 6).

Table 5. Effect of various acquisition access periods on rice tungro spherical virus (RTSV) infected plants followed by an additional acquisition access period on rice tungro bacilliform virus (RTBV) infected plants on transmission of viruses by *Nephotettix virescens*

Acquisition access period		Virus transmission ^{a)} (number of plants with virus)			
First to RTSV	Second to RTBV	RTBV+RTSV	RTBV	RTSV	None
0	8 hr	0	0	0	21
10 min	8 hr	1	0	0	21
30 min	8 hr	0	0	0	22
1 hr	8 hr	0	0	0	22
4 hr	8 hr	2	2	1	19
10 hr	overnight	9	3	2	12
36 hr	8 hr	10	4	4	5

a) Two leafhoppers were allowed an inoculation access period of 8 hr or through night on a seedling.

Table 6. Effect of various acquisition access period on rice tungro bacilliform virus (RTBV) infected plants following an acquisition access on rice tungro spherical virus (RTSV) infected plants on transmission of viruses by *Nephotettix virescens*

Acquisition access period		Virus transmission ^{a)} (number of plants with virus)			
First to RTSV	Second to RTBV	RTBV+RTSV	RTBV	RTSV	None
1 day	0	0	0	14	6
1 day	10min	0	0	11	9
1 day	30min	1	1	14	4
1 day	1 hr	7	1	9	3
1 day	4 hr	12	5	1	2
1 day	8 hr	8	7	0	8

a) Two leafhoppers were allowed an inoculation access period of 8 hr or through night on a seedling.

Persistence of "helper factor" in the leafhopper

Leafhoppers exposed to RTSV were fed on virus-free seedlings for various periods and then given a second acquisition access period of 4 hr to RTBV. Two leafhoppers exposed to RTSV and then RTBV were allowed an inoculation access on a seedling. Leafhoppers held on virus-free seedlings for 0-2 days between the first and second accesses transmitted both RTBV and RTSV, while leafhoppers held longer than 3 days transmitted RTBV alone (Table 7). Leafhoppers held longer than 8 days transmitted neither RTBV nor RTSV. As a control, leafhoppers exposed to RTSV were fed on virus-free seedlings for various periods and then allowed an inoculation access on seedlings. Leafhoppers lost ability to transmit RTSV 3 days after the termination of

Table 7. Effect of intervals between a first acquisition access to rice tungro spherical virus (RTSV) for 1 day and a second access to rice tungro bacilliform virus (RTBV) for 4 hr on the transmission by *Nephotettix virescens*

Days between removal of insects from RTSV source plant and transfer to RTBV source	Virus transmission ^{a)} (number of plants with virus)					
	Transferred to RTBV				Not transferred to RTBV	
	RTBV+RTSV	RTBV	RTSV	None	RTSV	None
0	14	1	0	7	15	5
1	2	12	0	9	14	6
2	2	18	0	4	6	14
3	0	15	0	11	0	20
4	0	10	0	15	0	20
5	0	5	0	17	0	20
6	0	2	0	20	0	20
7	0	4	0	18	0	20
8	0	0	0	16	0	20

a) Two leafhoppers were allowed an overnight inoculation access period of 8 hr on a seedling.

the acquisition access. These results indicate that retention period of "helper factor" in the leafhopper was 7 days, whereas that of RTSV was 2 days.

Fifth instar nymphs of the leafhopper were given a first acquisition access period of 2 days on a RTSV infected plant. Nymphs which had not emerged during the access period were collected and fed on healthy seedlings for one day. Leafhopper emerging during the one day feeding period were collected and given a second acquisition access period of 8 hr on a RTBV infected plant. Two leafhoppers thus exposed to RTSV and then RTBV were allowed an overnight inoculation access on a seedling. As a control, adult leafhoppers exposed to RTSV were fed on virus-free seedlings for one day and then given a second acquisition access. Ninety-two leafhoppers emerging during the one day feeding period were used to inoculate 46 seedlings. None of the seedlings exposed to emerging leafhoppers developed symptoms, while 6 out of 21 seedlings exposed to adult leafhoppers developed symptoms and contained RTBV alone. This result indicates that the retention period of "helper factor" by the leafhopper ends after moulting.

Discussion

Relations of RTBV and RTSV with *N. virescens* and other leafhopper vectors have been studied using a tungro isolate, penyakit habang, in Indonesia^{8,9)}. In our recent investigation using the Philippines isolate, RTBV and RTSV were transmitted similarly by the leafhopper vectors⁷⁾. Relations of RTBV and RTSV with *N. virescens* for the Philippines isolate in the present experiments were shown to be similar to those for the Indonesian isolate^{8,9)}. Other tungro-like diseases occurring in Asia are surely a complex of the two viruses. Saito reported association of spherical and small bacilliform particles with tungro-like diseases in several countries by thin sectioning¹⁷⁾.

Several plant viruses are known to require a "helper" virus or a helper component produced in a plant as a result of infection with another virus for the transmission by insect vectors¹⁶⁾. These viruses are aphid-borne and the RTBV-RTSV complex is the first example of a pair of a dependent and a "helper" virus transmitted by leafhopper vectors. Retention period of RTSV in the leafhopper was 2 or 3 days, while that of "helper factor" was 7 days. The evidence for the "helper factor" being something other than the RTSV particles may be explained as following. After the retention period is over, RTSV may be no longer infectious, but is still able to function in the "helper" role, or the "helper factor" may not be RTSV itself but may be produced in rice plants as a result of RTSV infection. Several combinations of dependent virus and helper component had been investigated precisely and the "helper" virus itself has been shown not to be the helper component^{3,4,14)}. The role of "helper" viruses has not been studied in detail in other cases. These dependent viruses are transmitted by aphid vectors which are fed on "helper" virus sources or helper component first and then allowed to acquire the dependent viruses¹⁶⁾. RTBV was transmissible following similar sequential leafhopper acquisition from virus infected plants. These facts may indicate the possibility that RTSV itself is not the "helper factor". It was not examined in the present experiments whether the "helper factor" was RTSV itself or protein as reported previously for the helper component in PVY infected plants^{4,5)}. Transmission

of RTBV and RTSV by the leafhoppers fed on purified viruses or sap from infected plants through membranes have been attempted. However, the leafhoppers were unable to acquire the viruses and also "helper factor" (Hibino, unpublished data). Further improvement of the method is required to elucidate the properties of the "helper factor".

The plant viruses known to require a "helper" virus or a helper component are mostly transmitted in a non-persistent manner by aphid vectors¹⁶⁾. Only parsnip yellow fleck virus and anthriscus yellows virus complex are known to be transmitted in a semi-persistent manner^{1,2)}. The relations of the two viruses with the aphid vector are similar to those of RTBV and RTSV with the leafhopper vector. However, "helper" viruses and hypothetical helper factor could not be distinguished from each other on maximum retention period in the case of that complex²⁾, unlike in the case of RTBV-RTSV complex.

It is known that the ability to transmit tungro by the leafhopper ends after the moulting^{10,12)}. Retention of rice waika virus, which is related closely or identical to RTSV¹⁷⁾ also ends after the emergence or moulting¹¹⁾. In these experiments, retention period of the "helper factor" by the leafhopper ended after the moulting. These results indicate that specific adsorption of RTSV and RTBV to the mouth or intestinal surface is essential for the transmission of the viruses. The specific adsorption of RTBV may be completed under the presence of RTSV or the "helper factor". When the leafhopper acquires RTSV or the "helper factor" from a RTSV infected plant, the mouth or intestinal surface may be modified to provide specific receptor sites for RTBV.

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和文摘要

Rice tungro bacilliform virus および Rice tungro spherical virus と媒介虫 *Nephotettix virescens* の関係

日比野啓行

イネ tungro 病の Philippine isolate を接種したイネ苗から rice tungro bacilliform virus (RTBV) および rice tungro spherical virus (RTSV) 単独感染株を得た。RTBV は媒介虫タイワンツマグロヨコバエにより RTBV 単独感染株からは伝搬されず、RTSV との重複感染株からは伝搬された。RTSV はその単独感染株からも伝搬された。RTBV はまた RTSV を前もって獲得吸汁した媒介虫により RTBV 単独感染株からも伝搬された。最短獲得吸汁時間は RTSV および RTBV の伝搬を助ける “helper factor” ではそれぞれ10分、RTBV では30分であった。媒介虫は RTSV 獲得吸汁後3日目には RTSV を伝搬しなくなったが、3日目以後も RTBV を伝搬する能力を保持しており、獲得された “helper factor” は7日間保持された。媒介虫は RTSV 獲得後脱皮すると “helper factor” を失い、RTBV を伝搬できなくなった。