TARC Report

Transmission of Rice Ragged Stunt Disease in Thailand

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Introduction

A new rice virus disease was found in Indonesia in 1976.[6] Symptoms of the diseased rice plants were stunting, twisted and ragged leaves, empty panicles and galls on the outer surface of flag leaves. The disease has been called "kerdil hampa" in Indonesian, and it was transmitted by the brown planthopper, *Nilaparvata lugens*, in a persistent manner.

At about the same time, a new rice disease also occurred in the Philippines in 1977.[9] The disease was named "ragged stunt" and the symptoms were similar to those of kerdil hampa described by Hibino et al.[6]

At present, kerdil hampa and ragged stunt of rice are thought to be the same disease, and the name "rice ragged stunt (RRS)" is adopted to describe the disease. In addition to the occurrence in Indonesia and the Philippines, the disease has been reported in Thailand,[15] India and Sri Lanka,[5] Taiwan,[1,2] China,[17] and Japan.[14]

In Thailand, ragged stunt symptoms on rice were observed for the first time in a farmer's field at Chachoengsao Province, 60 km east of Bangkok in 1977.[3,15] The disease is called "rok bai ngik" (literally means twisted leaf disease) by Thai plant pathologists, and "rok joo" (literally means short or stunt disease) by Thai farmers. The area affected by rice ragged stunt disease has increased year after year, and the disease has become one of the most important rice diseases in Thailand.

This report deals with the experiments on transmission of the disease conducted at the Department of Agriculture, Thailand, from September, 1978 to April, 1981.

Material and methods

Rice variety Taichung Native 1 was used as a test plant. Colonies of brown planthopper collected from Chainat Province had been maintained in screened cages for 4 years at Division of Plant Pathology and Microbiology, Department of Agriculture, Thailand. The 3rd to 4th instar nymphs were given an acquisition access of 1 to 2 days on diseased rice plants and the transmission ability of the insects was tested by serial transmission. In some experiments, the insects were reared for 4 to 10 days after acquisition access on the healthy feeding materials to allow an incu-

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Table 1. Serial daily transmission of rice ragged stunt virus by *Nilaparvata lugens*

Insect No.	Days after acquisition access																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
2	○	○	○	◎	○	○	●	●	●	●	●	●	●	●	○	○	●	○		
3	○	◎	○	○	◎	○	●	○	●	●	○	○	○	○	○	○	○	○	○	○
10	○	○	◎	○	○	●														
13	○	○	○	○	○	◎	○	●	●	●										
15	◎	○	○	◎	○	○	○	○	○	●	●	●	●	●	○	○				
18	○	○	○	○	◎	○	○	●	○											
23	○	○	◎	○	○	○	◎	○	●											
28	○	○	◎	○	○	◎	●	●	●	●	●	●	●	●	●	○	●			
29	○	○	◎	○	◎	○	○	○	○	○	○	○	○	○	○	○	○	●	●	○
31	◎	○	○	◎	○	○	○	●	●	●	●	●	●	●	●					
34	○	○	○	○	○	◎	○	●	●	○	●	●								
41	○	○	○	◎	○	●	●	●	●	●	●	●	●	●	●	○	●	●	●	●
42	○	○	○	○	◎	○	●	○	●	●	●	○	●							
44	○	○	○	◎	○	◎	○	○	○	○	○	●								
45	○	○	○	○	◎	○	○	○	○	○	●	●	●	●	●	●	●	●	●	●
46	○	○	◎	○	○	○	●	●	●											
48	◎	○	○	○	◎	○	○	○	○	○	●	●	●	●	●	●	●	●	●	
50	○	○	○	○	○	○	○	●	●	○	○	●	○	○	○	○				

● Transmitted the disease
○ Failed to transmit the disease
○ Insect molted and transmitted the disease
◎ Insect molted but failed to transmit the disease

bation period in the insects, before using for inoculation. The inoculated test plants were transplanted to seedling boxes in a screened house. Symptoms of the disease were observed about 4 weeks after inoculation.

In order to check the virus particles in the inoculated rice plants, electron microscopic grids were prepared by the leaf dip method with neutral 2% sodium phosphotungstate as a negative stain. Grids were examined under a Hitachi Model H–300 electron microscope.

Experimental results

1) Retention of the virus in the insect vector

Fourth instar nymphs of *N. lugens* were fed on a diseased rice plant for 1 day. Each insect was then serially transferred at 1-day intervals on a healthy test plant in a test tube for 20 days. The seedlings inoculated were transplanted to seedling boxes. Fifty individuals were tested in this experiment and 18 insects transmitted the disease. The transmission patterns of the transmitters are shown in Table 1. Incubation periods in the insects ranged from 4 to 17 days with an average of 8 days. The vectors transmitted the virus in a persistent manner. Some active transmitters were able to transmit the virus for 10 continuous days. But some individuals transmitted it intermittently.

2) Acquisition access period

Nymphs of *N. lugens* were given an acquisition access on a diseased rice plant for 30 min, 1 hr, 3 hr, 6 hr, 1 day, and 2 days in Experiment 1, and 30 min, 1 hr, 2 hr, 4 hr, 8 hr, and 16 hr in Experiment 2. After an acquisition access, the insects were transferred to healthy rice seedlings for a 7-day incubation period. And then, the transmission ability was tested by inoculation to the test plants. As shown in Table 2, the insects ac-

quired the virus by 3 hr of acquisition access, but failed to acquire by a shorter acquisition access period. The rate of transmission increased with longer acquisition access period.

3) Acquisition of the virus by different instar of nymphs and adult of the insect vector

Brown planthopper nymphs at the 1st to 5th instar stages and adults were allowed acquisition access on diseased rice plants for 1 day. After acquisition access, the number of transmitters was determined by daily serial transmission (one insect per plant). As shown in Table 3, the 5th instar nymphs showed the highest percentage of transmitters followed by the 4th instar as the second highest. There was no significant difference in incubation period in the insects at different growth stages of the insects tested.

Table 2. Effect of acquisition access period on the transmission of rice ragged stunt virus by *Nilaparvata lugens*

Acquisition access period	Infected plants / Inoculated plants	Percentage of infected plants
30 min	0/16	0
1 hr	0/16	0
3 hr	2/15	13
6 hr	2/18	11
1 day	3/19	16
2 days	4/18	22
control	0/17	0

Inoculation access for 1 day (5 insects/seedling)
Result of the Experiment 1.

Table 3. Acquisition of rice ragged stunt virus by nymphs and adult of *Nilaparvata lugens*

Instar of nymphs	Transmitted/tested					Percentage of transmission	Average incubation period (days)
	1	2	3	4	Total		
1 st	—	—	7/50	5/50	12/100	12	8
2 nd	4/46	7/50	7/50	4/50	32/196	16	8
3 rd	9/50	12/50	9/50	6/50	36/200	18	8
4 th	18/50	12/50	13/50	8/50	51/200	26	8
5 th	13/50	—	12/50	15/50	42/150	28	10
Adult	3/35	10/50	—	4/50	17/135	13	9

Acquisition access: 1 day
Transmission ability was tested by serial transmission (1 insect per plant).

4) Inoculation access period for the transmission

Nymphs of *N. lugens* were given an acquisition access of 1 day and 3 days, in Experiment 1 and Experiment 2, respectively. The vectors were then allowed to feed on healthy seedlings for 7 days, as an incubation period in the insect before inoculation access on the test plants. Results are shown in Table 4. Minimum inoculation period was 1 hr and

Table 4. Effect of inoculation access period on the transmission of rice ragged stunt virus by *Nilaparvata lugens*

Inoculation access period	Infected plants / Inoculated plants	Percentage of infected plants
30 min	0/19	0
1 hr	1/19	5
3 hr	3/17	18
6 hr	7/19	37
1 day	5/20	25

Acquisition access: 3 days, Incubation: 7 days
Inoculation access: 1 insect/plant

Table 5. Effect of insect number on the transmission of rice ragged stunt virus

Number of insects per plant	Infected plants / Inoculated plants	Percentage of infected plants
1	3/20	15
2	4/20	20
3	2/15	13
4	9/19	48
5	11/18	61

Acquisition access for 3 days, inoculation for 1 day

the rate of transmission increased with the increasing inoculation period.

5) Effects of the insect number on the transmission

Nymphs of brown planthopper were given an acquisition access of 3 days. After 8 days of incubation on healthy rice plants, the insects were given an inoculation access of 1 day on the test plants. The number of insects per test plant was varied from 1 to 5. As shown in Table 5, the rate of transmission increased with the increasing number of insects per plant.

6) Transmission by leafhoppers and planthoppers

Rice ragged stunt virus transmission ability of *Nephotettix virescens*, *Recilia dorsalis*, *Sogatella furcifera*, and *Nilaparvata bakeri* was tested. Nymphs of *N. virescens* and adult of *R. dorsalis* and *S. furcifera* were given an acquisition access of 1 day on diseased rice plants. Each insect was then tested for its transmission ability by serial daily transfers on a test plant in a test tube. *N. bakeri* nymphs were given an acquisition access of 17 hr (overnight) on diseased plants, and they were reared on a host plant, *Leersia hexandra*, for 7 days as an incubation period. Transmission ability of *N. bakeri* was then tested by a series of alternate transfers of 16 hr on a rice test plant and 8 hr on *L. hexandra*. *N. bakeri* would soon die, if they were fed on rice plant for a long time. As shown in Table 6, *N. virescens*, *R. dorsalis* and *S. furcifera* did not transmit rice ragged stunt virus but *N. bakeri* did.

Table 6. Transmission of rice ragged stunt virus by leafhoppers and planthoppers

Insect tested	Transmitted/tested
*Nephotettix virescens**	0/69
*Recilia dorsalis**	0/64
*Sogatella frucifera**	0/98
*Nilaparvata bakeri***	4/75

* Acquisition: 1 day, Inoculation: serial transmission (1 insect per plant)

** Acquisition: 17 hr, Inoculation: alternate transfers of 16 hr on a rice seedling and 8 hr on *Leersia hexandra*

7) Dual transmission of ragged stunt and grassy stunt viruses

The 4th instar nymphs of *N. lugens* were fed successively on rice plants affected by ragged stunt and on those affected by grassy stunt for 2 days each. Serial transmission test was done 10 days after acquisition of viruses. Each insect was transferred to a new test plant every other day. As shown in Table 7, brown planthopper was able to acquire both ragged stunt and grassy stunt viruses and was able to transmit them at the same time or each one of them separately in

Table 7. Dual transmission of rice ragged stunt and grassy stunt viruses by *Nilaparvata lugens*

Transmission	Number of insects
Simultaneous transmission of RRS and RGS	10
Separate transmission of RRS and RGS*	9
RRS	7
RGS	6
No transmission	23

RRS: rice ragged stunt
RGS: rice grassy stunt

* Individual insect transmits each one of RRS and RGS separately in the course of serial transmission test.

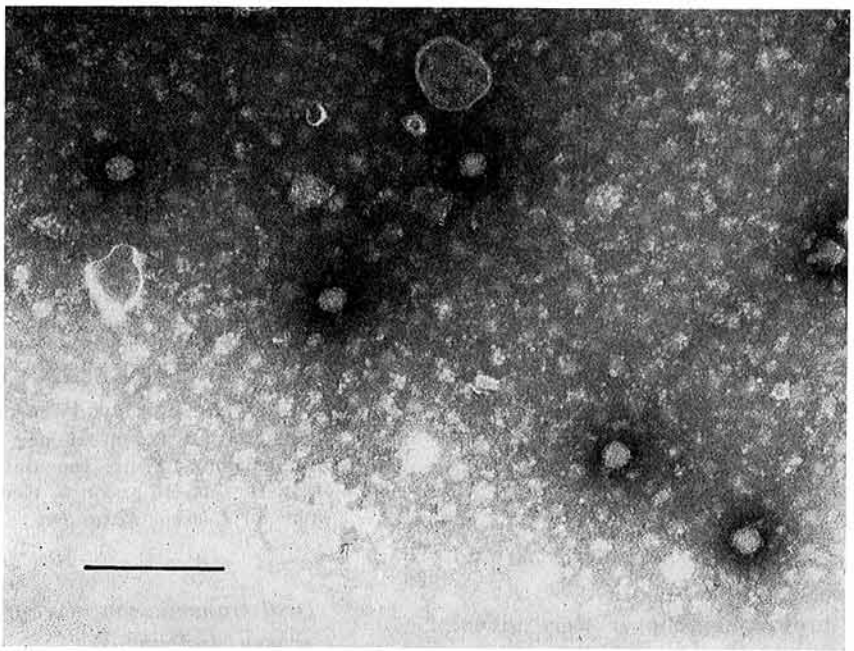

Plate 1. Electron micrograph of rice ragged stunt virus in a dip preparation stained with 2% PTA from vein swelling of a rice plant infected with rice ragged stunt disease
Bar represents 300 nm.

the course of serial transmission test. Some individuals transmitted only either ragged stunt virus or grassy stunt virus.

8) Electron microscopy

In dip preparations, spherical particles about 60 nm in diameter were observed. They were more abundant in preparations from galls than from other leaf tissues. The electron micrograph of the particles is shown in Plate 1.

Discussion

Rice ragged stunt virus was transmitted by brown planthopper in a persistent manner with a 8-day incubation period on an average. Some individuals transmitted the virus intermittently in the daily transfer to the test plants (Table 1). These data coincide with the result so far reported that the incubation period in the insect ranged from 7.6 to 10.7 days with the peak of around 8 days, and the pattern of transmission was intermittent.[2,4,6,9,12,16]

The minimum acquisition access period was 3 hr in the present study, whereas it was 8 hr,[6] 2 hr,[2] and 30 min[16] in other reports. These difference might be due to the different origin of insects and/or the experimental conditions.

The minimum inoculation access period was 1 hr in this study and the period was the same in other papers.[2,6]

Ghosh and John[4] reported that nymphs were more efficient than adults in transmitting rice ragged stunt virus. Our data (Table 3) also show that brown planthopper nymphs of the 5th and 4th instar transmitted the virus more efficiently than younger instar nymphs and adults.

The rate of transmission increased with the increasing numbers of insect per plant and the transmission efficiency of a single insect was 15%. Hibino et al.[6] reported the same tendency and the transmission efficiency by

a single insect was 12%.

We showed that *N. lugens* transmitted rice ragged stunt virus and rice grassy stunt virus at the same time or on some occasions the insect transmitted one of them. This result indicates that the two viruses multiply independently of each other in the brown planthopper.

Some rice plants inoculated by brown planthoppers which had been successively fed on the plants infected with ragged stunt and on those infected with grassy stunt showed symptoms of both diseases. The dual infection indicates the absence of cross protection between ragged stunt and grassy stunt. Absence of cross protection was also reported between ragged stunt and grassy stunt,[6,9] ragged stunt and tungro,[9] ragged stunt and rice bunchy stunt,[16] ragged stunt and rice dwarf,[16] ragged stunt and rice transitory yellowing[16] and ragged stunt and rice yellow dwarf.[16] These phenomena show that ragged stunt virus is not closely related to the other viruses mentioned above.

In this study *N. bakeri* was shown to be an additional insect vector of rice ragged stunt virus, but *N. virescens*, *R. dorsalis*, and *S. furcifera* did not transmit the virus. *Nephotettix cincticeps*,[2,16] *Nephotettix nigropictus*,[2,10] *Laodelphax striatellus*,[2,13,16] *R. dorsalis*,[2,10] *S. furcifera*,[2,10,16] and *Sogatella longifurcifera*[2] were reported as non-vectors. Iwasaki et al.[8] reported that both *N. bakeri* and *Nilaparvata muiri* transmitted rice grassy stunt. Therefore, *N. lugens* and *N. bakeri* are common insect vectors for rice ragged stunt and rice grassy stunt viruses.

Spherical particles about 60 nm in diameter were observed in dip preparations from the diseased leaf samples obtained by transmission tests. According to Hibino et al.,[6,7] and Shikata et al.,[13] particles 55–60 nm or 44–63 nm in diameter were observed in dip preparations of ragged stunt infected tissues and particles about 65 nm or 50–70 nm in diameter were observed in ultrathin sections, respectively. Our observation coincides with the results of the other reports in particle size of dip preparations.

All these results of our transmission tests as well as disease symptoms[3,15] were similar to those reported in Indonesia,[6] the Philippines,[9] and other countries.[2,4,16] Therefore, it can be concluded that "rok bai ngik" in Thailand is the same as "kerdil hampa" in Indonesia and ragged stunt disease in the Philippines.

Summary

Transmission tests of rice ragged stunt virus in Thailand were conducted. *Nilaparvata lugens* transmitted the disease in a persistent manner and the incubation period in the insect ranged from 4 to 17 days with an average of 8 days. The minimum acquisition access period was 3 hr and the minimum inoculation access period was 1 hr. The 5th and 4th instar nymphs of *N. lugens* showed higher transmission efficiency than other instar nymphs and adults. *Nilaparvata bakeri* transmitted the ragged stunt virus but *Nephotettix virescens*, *Recilia dorsalis*, and *Sogatella furcifera* did not. Dual infection of ragged stunt and grassy stunt viruses in rice plant occurred by inoculation through *N. lugens* which dually acquired the viruses, suggesting the absence of cross protection between ragged stunt virus and grassy stunt virus. Spherical particles about 60 nm in diameter were observed in the dip preparations from the diseased leaves.

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