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TRANSMISSION OF RAGGED STUNT VIRUS DISEASE OF RICE IN MALAYSIA

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RINGKASAN

Penyakit virus padi, ragged stunt telah dijumpai di Malaysia dan penyebarannya hanyalah melalui serangga pembawa bena perang, *Nilaparvata lugens* Stal. (Homoptera: Delphacidae) secara persistan, dan tidak oleh *Nephotettix virescens, Sogatella furcifera* atau melalui biji benih. Tempoh penyerapan virus minima adalah 30 minit dan 10 minit bagi tempoh inokulasi virus. Tempoh pengeraman virus ditubuh pembawa adalah kurang dari 30 jam. Terdapat perbezaan keupayaan pemindahan virus di antara dua bentuk sayap pembawa pada $P < 0.05$ dengan bentuk sayap besar lebih aktif. Jangka hidup pembawa tidak ditentukan oleh sama ada perumah dijangkiti ataupun sihat.

INTRODUCTION

Ragged stunt and grassy stunt virus diseases of rice were recorded earlier in Malaysia (HABIBUDDIN, 1978; HABIBUDDIN, RUBIAH, NG and ZAKARIA, 1978). Both diseases are transmitted by the brown planthopper, *Nilaparvata lugens* Stal. (Homoptera: Delphacidae). Ragged stunt disease was first reported in 1977 in the Philippines (LING, TIONGCO, AQUIERO and CABAUATAN, 1978) and Indonesia (HIBINO, ROECHAN, SUDARISMAN and TANTERA, 1977). Its occurrences in Thailand and India were reported in 1978 and 1979 respectively (WEERAPAT and PONGPRASERT, 1978; GHOSH, JOHN and REDDY, 1979). SINGH (1978) speculated that this disease is similar to the previously reported witches broom disease of rice in Malaysia.

The disease is readily recognised by its symptoms viz. severe stunting, ragged and twisted leaves, incomplete emergence of the panicles and occasional branching at the nodal region of the tillers. Vein swellings on the lower leaf surface could also be detected. This paper presents some aspects of the studies on the relationship of rice ragged stunt virus (RRSV), rice plant and its vector, *N. lugens*.

MATERIALS AND METHODS

All the experiments were conducted in the insect-proof houses at MARDI in Bumbong Lima and Serdang. Temperature during the experiments varied between 24°C – 34°C. The original RRSV-infected plant was obtained from a field in Bumbong Lima. The insects used were laboratory culture, collected from the surrounding fields of Bumbong Lima and maintained on rice variety TN1 for more than two years. Rice plants of variety TN1 were used as test plants. These were inoculated at the seedling age of 8–14 days. Recording of infected plants was made a month after inoculation and based on the symptoms as described earlier.

1. Seed transmission

A total of 1 000 seeds collected from RRSV-inoculated plants were sown and kept insect-free for disease development and observation was made one month after sowing.

2. Insect transmission

a) Vector determination

Brown planthopper *(Nilaparvata lugens),* white-backed planthopper *(Sogatella furcifera)* and green leafhopper *(Nephotettix virescens)* were used in this study. Third instar nymphs of these insects were caged on RRSV-infected plants for 10 days and then transferred individually to each TN1 seedling for another five days.

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b) Transmission efficiency of two forms of *N. lugens*

In nature, *N. lugens* exists either as the long-winged (macropterous) or as the short-winged (brachypterous) form . Nymphs of similar age were caged on virus-infected plants until they became adults. Three days after adult emergence, they were sorted out according to their wing forms and then transferred singly onto healthy rice seedlings for inoculation access period of five days.

c) Acquisition access period

(i) Using nymphs

First instar nymphs of RRSV-free brown planthoppers were allowed to feed on virus-infected rice plants for 10, 20 and 30 minutes. They were then transferred singly onto healthy test seedlings as soon as the required acquisition periods were over.

(ii) Using adults

Macropterous adults of *N. lugens* were allowed to feed on the infected plants for 0.5, 1, 6 and 12 hours; and 1, 4, 8 and 12 days. They were then individually transferred onto healthy TN1 seedlings for five days.

d) Inoculation access period

N. lugens nymphs were left on the infected plants until they became adults. They were then individually transferred onto RRSV-free test seedlings for 5, 10, and 30 minutes; 1 and 12 hours; and 1, 3 and 5 days for the assessment of inoculation assess period. At the end of the inoculation access period, all planthoppers were killed with 0.1% a.i. of Bassa 50 EC. (2-sec-butylphenyl methylcarbamate).

e) Serial transmission

Twelve male and thirty female macropterous adults of *N. lugens* were given six hours acquisition access period and then transferred singly onto healthy TN1 seedling for 24 hours. The planthoppers were serially transferred to different seedlings every 24 hours until they died.

3. Life span of *N. lugens* on virus infected plants

Female adults of *N. lugens* of similar age and from the same colony were caged on healthy and RRSV-infected plants for six hours of oviposition period. Newly emerged nymphs were then removed to healthy and RRSV-infected plants. The number of dead insects, their sexes and their wing forms were recorded daily.

RESULTS

1. Seed transmission

All the seedlings remained healthy until maturity. Hence, RRSV was not transmitted through seeds collected from infected rice plants.

2. Insect transmission

a) Vector determination

As indicated in *Table 1*, only *N. lugens* transmitted the virus. *S. furcifera* and *N. virescens* failed to do so under the present condition.

b) Transmission efficiency of macropterous and brachypterous forms

Results in *Table 2* show that both macropterous and brachypterous *N. lugens* were able to transmit RRSV. However, the macropterous form were the better transmitters.

c) Acquisition access period

The minimum acquisition access period of the virus differed according to the age of the insects. Using the first instar nymphs, the minimum acquisition access period was 30 minutes. However, with macropterous adults, the period was six hours *(Table 3)*. The percentage of infective planthoppers increased with increasing acquisition access period.

Table 1. Transmission of rice ragged stunt virus by three species of homopterans

Vector species	No. infected / No. of inoculated seedlings
Nephotettix virescens	0/320 (0)
Sogatella furcifera	0/385 (0)
Nilaparvata lugens	144/295 (49)

Each seedling is inoculated individually by a single hopper.
The number in bracket represents the percentage of infection.

Table 2. Transmission of ragged stunt by two forms of *Nilaparvata lugens*

Wingform	Percentage transmission
Macropterous	49*
Brachypterous	34

*Significantly different at $P<0.05$.
Each transmission study is the result of 100 seedlings inoculated individually by a single hopper.

Table 3. Effects of acquisition access period on transmission of rice ragged stunt virus by nymphs and adults of *Nilaparvata lugens*

Acquisition access period	ADULTS	NYMPHS
	No. infected / No. inoculated seedlings	No. infected / No. inoculated seedlings
10 min	–	0/20 (0)
20 min	–	0/20 (0)
30 min	0/50 (0)	6/20 (30)
1 hr	0/50 (0)	–
6 hr	2/50 (4)	–
12 hr	2/50 (4)	–
1 day	2/50 (4)	–
4 days	1/50 (2)	–
8 days	22/50 (44)	–
12 days	37/50 (74)	–

The number in the bracket represent the percentage of infection.

d) Inoculation access period

Table 4 shows that upon completion of the latent period, viruliferous brown planthoppers could transmit RRSV after ten minutes of inoculation access period. However, the probability of obtaining positive transmission increased with longer inoculation access period. For instance, 40% infection was obtained after the viruliferous planthoppers were allowed to feed for five days on healthy plants.

e) Serial transmission

Only 13 of the 50 planthoppers transmitted RRSV *(Table 5)*. Data obtained also indicated that the incubation of the virus in adult insects occurred within 30 hours. The highest transmission percentage occurred in the first two days after acquisition feeding began. The transmission pattern was intermittent, although few of the planthoppers transmitted the virus daily until they died. The maximum virus retention in the adult planthoppers was eight days (insect No. 50) indicating that RRSV is transmitted by brown planthoppers in the persistent manner.

3. Life span of *N. lugens* on virus infected and healthy plants

Results show that the differences in average life span of *N. lugens* of both sexes and wing-forms when reared on RRSV-infected and healthy rice plants were not significant at the 5% probability level *(Table 6)*. However those reared on virus-free plants survived for two to three days longer than those on RRSV-infected plants.

DISCUSSION

The rice ragged stunt virus disease is present in various rice growing areas in Malaysia (HABIBUDDIN, 1978; OOI, 1980; ANON, 1978). The disease is reported to be devastating in the Philippines where 90% of rice hills in a field were infected resulting in total yield loss (LING *et al.*, 1978). Similarly OKA (1982) reported that it was the most important disease of rice in Indonesia. However the incidence of the disease in Malaysia

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

Acquisition access period	BUMBONG LIMA	SERDANG
	No. infected / No. inoculated seedlings	No. infected / No. inoculated seedlings
5 min	0/50 (0)	–
10 min	–	1/40 (2.5)
20 min	–	5/40 (12.5)
30 min	1/50 (2)	5/40 (12.5)
1 hr	1/50 (2)	9/40 (25.5)
12 hr	4/50 (8)	–
1 day	10/50 (20)	11/40 (27.5)
3 days	11/50 (22)	–
5 days	20/50 (40)	–

The number in the bracket represent the percentage of infection.

Table 5. Serial transmission of rice ragged stunt virus by *Nilaparvata lugens*

Insect[1] No.	Sex	Results of serially inoculated seedlings[2]												
		1	2	3	4	5	6	7	8	9	10	11	12	13 (days)[3]
9	Male	*	*	D										
10	Male	*	*	*	*	*	*	*	D					
14	Female	*	o	*	D									
15	Female	*	o	D										
17	Female	o	*	*	D									
18	Female	o	*	o	D									
29	Female	*	D											
35	Female	o	o	o	*	*	o	o	o	o	o	D		
37	Female	*	*	D										
41	Female	*	*	*	*	D								
42	Female	*	*	D										
45	Female	*	*	*	*	D								
50	Female	o	o	o	o	o	o	*	*	o	o	o	o	D

o = Healthy seedling
* = Infected seedling
D = Death of insect
1 = Each insect was given 6 hr of acquisition access period prior to inoculating seedling No. 1.
2 = Each seedling was inoculated for 24 hr. Thereafter, the insect was transferred to next seedling.
3 = Each number of days represents different seedling number after 6 hr of acquisition assess period.

Table 6. Life span of *Nilaparvata lugens* on healthy and rice ragged stunt virus-infected plants

Wing form	Sex	Status of host plant		Life-span range (days)	Average life-span (days)	X^2 –test
Macropterous	Female	healthy	(20)	19 – 42	35	NS
		diseased	(17)	21 – 45	32	
	Male	healthy	(14)	17 – 38	25	NS
		diseased	(18)	18 – 30	22	
Brachypterous	Female	healthy	(75)	16 – 40	24	NS
		diseased	(67)	16 – 29	22	
	Male	healthy	(61)	16 – 38	25	NS
		diseased	(69)	16 – 40	22	

Number in bracket indicates number of *N. lugens* observed.
NS = Not significantly different at P = 0.05

has remained low. For example in Tanjung Karang (Selangor) where there have been several outbreaks of brown planthopper, the incidence of the disease has continued to be very low. This could be attributed to the low level of inoculum source, the degree of

susceptibility of the rice varieties planted, degree of staggering of the crop and possibly to better field management. However, these factors still need to be investigated.

Present studies show that the RRSV disease in Malaysia is very similar symptomatically to that reported in the Philippines, Thailand, Indonesia and India. RRSV was not transmitted through rice seeds and its only insect vector is the brown planthopper, *N. lugens*, which transmits the virus in the persistent manner. It is not transmitted by *N. virescens* or *S. furcifera*.

Both forms of *N. lugens* transmitted RRSV. The percentage transmission in the present studies was found to be 49% for the macropterous adults and 34% for brachypterous form. In IRRI, LING *et al.*, (1978) reported 0% – 100% and 16% – 100% for macropterous and brachypterous forms respectively. In India, only 6% of the macropterous adults and 15% – 17% of the brachypterous adults transmitted the virus (GHOSH and JOHN, 1980).

N. lugens required a minimum of 30 minutes acquisition access feeding to become viruliferous and a minimum of 10 minutes inoculation access period to transmit the RRSV to healthy rice seedlings. HIBINO *et al.*, (1977) however reported longer periods of eight hours and one hour respectively. These differences in transmission ability and virus-vector relationships could probably be explained by the variability in the brown planthopper populations and in the number of insects used (SINHA, 1968).

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SUMMARY

Rice ragged stunt virus in Malaysia could only be transmitted by the brown planthopper, *N. lugens* (Homoptera: Delphacidae) in a persistent manner but not by *Nephotettix virescens* nor *Sogatella furcifera*. It is also not seed-transmitted. The insect needed a minimum of 30 minutes for acquisition feeding and 10 minutes of inoculation feeding period. The latent period of the virus in the insect body was less than 30 hours, and the virus persisted inside the planthopper until the vector died. The transmission efficiency between the two wing-forms of planthopper was statistically significant at $P<0.05$. There was no significant difference in the life-span of brown planthoppers caged on virus-infected and healthy rice plants.

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