Transmission of Rice Wilted Stunt Virus By Brown Planthopper (*Nilaparvata lugens* Stål)[1]

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ABSTRACT

Rice wilted stunt (RWS) was first found in central Taiwan in 1977. The disease, transmissible by the brown planthopper *Nilaparvata lugens* (Stål) in a persistent manner. *N. lugens* transmitted RWSV with a incubation period of 6.7 days (range 3-14 days) at 28-30℃. Allowed an acquisition access of 24 hr, about 41% (22 to 64%) of the test insects became transmitters. The minimal acquisition and inoculation access periods were detected as 1 hr and 30 min, respectively. The insects were able to acquire RWSV at temperature range of 15-35℃ and after acquisition feeding, the incubation period was shorten as increase in temperature, through temperature up to 30℃, mortality of the test insect was higher. Neither transovarial passage nor mechanical transmission were observed. Under 25±1℃ constant temperature, bioassay and ELISA were used to detect the initial transmission time and the changes of concentration of the RWSV. Results indicated ELISA started to detect RWSV in the insect vector at 5 days after acquisition and bioassay first identified the virus transmitter at 7 days after acquisition feeding, therefore ELISA might be a more sensitive method to detect RWSV in the insect vector.

INTRODUCTION

Rice grassy stunt (RGS) was originally reported from South and South East Asia in 1966(18), causing damaging the rice plant in wide areas(10,16). In 1970, RGS was first observed in Taiwan(13), with no substantial importance to rice productions(20). In 1977, rice plants with viruslike symptoms were collected in central Taiwan during the 2nd crop season. The newly found disease was characterized by extreme plant stunting and leaf wilting, and was shown to be transmissible by the rice brown planthopper, *Nilaparvata lugens* Stål. For the reasons that it caused severe stunting and leaf yellowing in rice with a lethal effect, it was tentatively named as rice wilted stunt (RWS)(2,3). Chen and Chiu(2) considered RGS and RWS to be closely related, because of the similarities in symptom, vector relationship and cellular inclusions produced in diseased rice plants.

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In 1982-83, a new strain of RGSV was observed in the Philippines[1]. The new strain can overcome the resistance gene derived from *O. nivara* and all the improved varieties with this type of the resistance become susceptible to the new strain[12]. The new strain of RGSV resembled RWSV both in the symptomatology and transmission pattern[2,12].

Recently, a study to deal with the transmission, purification, serology and other properties of RWSV was initiated at our laboratory. We report here the transmission characteristics of RWSV.

MATERIALS AND METHODS

The agent inciting wilted stunt was First obtained from a 1977 collection from a rice field in the Tungshih area. It was maintained in rice plants by periodical transfers to new plants uning *N. lugens* as the vector.

Rice seeds of test varieties were germinated in vermiculite in the greenhouse and seedlings were inoculated at the 2-leaf stage by confining the test insects individually on the plants enclosed in test tubes (2×10 cm). Usually, each test tube contained one insect and one plant. Inoculated plants were then planted in soil and kept in the greenhouse at 20-38℃ until symptoms appeared.

The rice brown planthoppers (BPH) were maintained on Tainan 5 rice plants in insect cages (55×55×90 cm) under continuous fluorescent lighting. The insect rearing temperature was 26-30℃. To establish healthy cultures, adult females were picked and confined on *Echinochloa crus-galli* Beauv. var. *oryzicola*. Ohwi which immune from rice wilted stunt virus and the eggs hatched in 7-10 days at 28-30℃ and the desired instars were transfered to rice plants. To assure that these cultures were free from accidental infection, the insects were penriodically sampled at random and tested on healthy rice seedlings. The healthy progenies thus obtained were used experimentally. Other test insects were similarly maintained on Tainan 5 rice plants.

Antiserum production and ELISA assay. RWSV was purified from infected rice plants by differential and sucrose density gradient centrifugations. About 0.5mg purified RWSV in 1.0ml of 0.05M phosphate buffer (pH 7.2) was emulsified with 1.0ml of Freund's complete adjuvant and the emulsion was musculaneously injected into a white domestic rabbit. Three additional injectional injections were performed weekly with purified virus emulsified by Freund's irncomplete adjuvant. The rabbit was bled 10 days after the last injection. Antiserum obtained from this rabbit had a titer of 1024 by the precipitin ring test and a positive ELISA reaction at 10^4 dilution. The double-antibody sandwich method of Clark and Adams was used for ELISA[8]. The microtiter plates were initially coated with r-globulin at 1.0 μg/1 ml and the conjugate used was at 500 fold dilution. For preparing antigen to react individual brown planthoppers were ground in 250 μl of PBS-Tween (pH 7.4) in a small glass tube and 150μ1 of

the crude extract was used for each test well. Absorbance was measured at 405nm with a Titertek Uniskan ELISA reader. Samples were considered positive when a reaction became visual or their absorbance values were at least three times that of known virus-free planthoppers included in each plate. To determine the concentration increment of RWSV in the insect vector after acquisition feeding, brown planthoppers were individuany confines in test tubes with seedlings of rice cultivar Tainan 5 for one day at 3, 5, 7, 9, 11, 13 days after they had acquired virus. The insects were then ground and ELISA test performed for the insect extracts individually.

RESULTS

Transmission experiments with *N. lugens*

In a series of transmission experiments, nymphs were fed on diseased Tainan 5 plants for 24 hr before they were serially transferred to test seedlings of Tainan 5. For 168 insects which became infective, an average incubation period of 6.7 days was observed, with a range of 3-14 days. The highest frequency of transmission was found to occur at. 6-8 days after virus acquisition. Once the insects became infective, they usually infected most of the test plants on which they fed. Thus, experimentally 197 insects infected a total of 1374 plants, leaving another 157 plants uninfected. Failure to cause infection occurred only occassionally in serial transmission.

N. lugens appeared to be a fairly efficient vector tor RWS. In 23 separate transmission experiments involving 1512 insects, 41% were transmitters. The infective insects ranged from 22.2 to 64.4% in different experiments.

Effect of the length of acquisition access period on transmission

Second to 3rd instar nymphs of BPH were allowed to remain on diseased plants from 0.5 to 24 hr. The proportion of the insect which acquired virus increased with the length of an acquisition access period. A period shorter than 1 hr could not result in virus transmission.

Effect of the length of inoculation access period on transmission

To determine the effect of inoculation access period on virus transmission, viruliferous insects were selected and confined on rice seedlings in test tubes. Each tube contained one insect and one young seedling. At the end of a desired inoculation access period, the rice seedlings were removed and transplanted to pots for observing the development of symptoms. The resulting rates of infection were: 5 min, 0/18; 10 min, 0/20; 30 min, 2/18; 60 min, 3/17; 12 hr, 5/17; and 24 hr, 13/15.

Effect of temperature on virus acquisition

To determine the effect of temperature on the ability of BPH to acquire RWSV, 3rd to 4^{th} instar nymphs were exposed to different temperatures. The infection percentages were 6/49 at 15℃; 7/46 at 20℃; 18/40 at 25℃; 20/45 at 30℃; 17/44 at 35℃. All 50 test insects died during the 24 hr acquisition access period at 10℃.

Effect of post-acquisition temperature on virus incubation peroid in insect

In these experiments the test insects were confined on diseased plants for 24 hr at 28℃ and were then transferred daily to healthy rice plants in series. The average incubation period ranged from 5.8 to 28.5 days. depending on the test temperatures. Table 2 summarizes the results.

Table 1. The effect of acquisition access period on the transmission of rice wilted stunt by *N. lugens*

Acquisition access	No. insects tested	No. insects transmitting	% Infective insects
0.5 hr	76	0	0
1	66	0	0
2	96	4	4.2
4	40	8	20.0
12	40	10	25.0
24	48	22	45.8

Table 2. The effect of post-acquisition temperature on the length of the incubation period of rice wilted stunt virus in *N. lugens*

Temp. (℃)	No. insects tested	No. viruliferous insects	Incubation period (days)	
			Average	Range
15	35	4	28.5	22-36
20	60	8	19.5	13-28
24	46	18	11.9	6-17
27	51	19	8.6	5-11
30	37	13	7.0	4-11
35	40	12	5.8	3-7

Test for transovarial transmission

The brown planthoppers were allowed to acquire virus by feeding on diseased plants as 1st or 2nd instar nymphs. Subsequently they were individually tested on rice seedlings and those which transmitted were paired as adults. The progenies of 17 pairs were individually confined on rice seedlings. Of 234 newly hatched nymphs taken from these progenies and used to inoculate 1543 rice seedlings, none of them caused infection. Therefore, the RWS agent was not transovarially transmitted. *Nephofettix nigropictus*, *Inazuma dorsalis*, *Laodelphax striatellus* and *Sogatella furcifera* were tested for their ability to transmit RWSV. They were allowed a 72 hrs acquisition feeding period on diseased plants before being enclosed on the test plants. None of the seedlings developed disease symptoms, indicating that none of these insects were vectors of RWS.

Other possible means of virus transmission

In mechanical transmission tests, 10 g of diseased rice leaves was ground in 30 ml of 0.5 M phosphate buffer, pH 6.8. After filtering through four layers of cheesecloth, the sap was centrifuged at 3000 rpm for 30 min. Healthy rice seedlings at the 4-leaf stage were rubbed with the leaf extract to which carborundum was added as abrasives. Other seedlings were inoculated by pin pricking. None of the 100 and 93 seedlings inoculated, respectively, by the rubbing and pin-pricking mehtods developed disease symptoms.

In an attempt to transmit the disease through soil, seedlings were transplanted to pots filled with soil taken from around the diseased plants in the field. None of 105 test seedlings was infected.

The possibility of seed transmission was tested by collecting mature seeds from diseased plants of Tainan 5 and germinating them in an incubator, with healthy seeds serving as control. Neither the 493 seedlings from diseased seeds nor the 194 seedlings from healthy ones produced symptoms.

The detection of RWSV in insect vector by bioassay and ELISA.

For detecting the concentration changes and the incubation period of RWSV in BPH, insects were sampled at the 3rd, 5, 7, 9, 11 and 13th days after acquisition feeding. About 60-70 BPH were used in each test and almost same number of healthy individuals served as control. After allowing the test BPH fed on healthy seedlings, the insects were tested by ELISA for viral antigens. Test were replicated four times. The tested plants were transplanted and kept in the net house for symptoms.

The experiments employed 1624 BPH which had been allowed feeding on RWSV-infected rice plants and 1322 were used as control. The results showed that ELISA was highly sensitive to probe the RWSV in BPH. RWSV was first detected in the test insects sampled at 5th day after acquisition feeding, whereas the insects were first proved as RWSV-transmitter at the 7th day after acquisition under constant temperature of 25±1℃. Among the 122 RWSV-transmitters, 91 (about 75%) were positive by ELISA .test. Another 92 BPH reacted positively by ELISA but did not transmit the virus during the test period. No control insects were found to be transmitter by bioassay or reacted positively by ELISA (Table 3).

Table 3. Comparative detection of RWSV in vector insect host after access acquisition feeding by bioassay and ELISA.

Days after acquisition feeding	No. insects tested	No. insects transmitting virus	Positive ELISA reaction	Virus transmitters in ELISA positive insects
3	269	0	0	
ck	224	0	0	
5	262	0	6	
ck	223	0	0	
7	270	14	34	92.9 (13/14)
ck	221	0	0	
9	277	30	47	83.3 (25/30)
ck	221	0	0	
11	272	34	45	70.6 (24/34)
ck	215	0	0	
13	274	44	51	65.9 (29/44)
ck	218	0	0	

DISCUSSION

Two virus diseases of rice transmissible by the brown planthopper, *N. lugens* have been known to occur in Taiwan. They are grassy stunt[13] and ragged stunt[4]. The characteristics of rice ragged stunt includes ragged leaves and the formation of vein galls on leaf sheaths. These symptoms are quite distinct from rice wilted stunt dealt with in this study. Rice plants infected with RWS generally had fewer tillers than healthy in many test varieties[2]. However, TN1 and Taichung 65, the tillering ability was markedly stimulated, and in this respect RWS rsesmbles grassy stunt[16,18]. In addition, RWS and grassy stunt have a common insect vector in *N. lugens* which transmits both diseases in a persistent manner. The average incubation period of RWS as determined in this study was 6.7 days, a value which agrees fairly well with the reported incubation period of 8.4 days for RGSV-2[12]. Cellular inclusions in the form of bundles and fibrillar structure 1500 nm in length were foun in rice plants affected with RWSV[5,6]. These inclusions closely resemble those in rice plants affected with grassy stunt[9,17]. Based on symtomatological evidence, inscet-vectorship and the inclusions in both the nucleus and the cytoplasm, Chen and Chiu[2] grouped the two diseases together.

Both infectivity bioassay and ELISA test have been employed for identifying GSV in infected plant leaves and in the vector brown planthoppers[14]. Our results indicated that ELISA seemed to be more sensitive for detection of RGSV in the planthopper than infectivity test. With the former, the test insects first showed positive reaction at 4-5th day after acquisition feeding, a time when they failed to transmit virus to test plants. Some test insects started to transmit the cause agent at 6-7th day after acquistion feeding. In the whole experiment, a total of 122 insects transmitted the virus, and among them, only 91 gave a positive reaction in ELISA test. On the other hand, 86 insects which gave a positve reaction by ELISA did not transmit virus (Table 3). Similar phenomena have been observed with rice grassy stunt or other rice virus diseases[11,14].

In 1982-3, Hibino and co-workers[1,12] reported a severe strain of rice grassy stunt virus, designated as GSW-strain 2, from the Philippines. Thailand isolates similar to strain 2 in symptomatology were also reported in India and Indonesia[7,15,19]. The symptoms and vector relationship of RGSV-strain 2 are very much similar with rice wilted stunt occurring in Taiwan[2,3,12]. Recently, the causal agent of GSV was found to be filamentous particles of about 950-1350 nm in length and 6-8 nm in width[10]. RWSV has a similar morphology with GSV (Chen et al., unptibished data). GSV and RWSV also show a serological relationship (Chen et al. unpublished). More reliable bases for identification might have to derive from parallel studies on the RNA and protein compositions of RGSV and its strains from different sources.

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褐飛蝨傳播水稻萎凋矮化病病毒之研究[1]

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摘　要

水稻凋矮化病於民國66年10月在台中縣東勢鎮首次發現。本病由褐飛蝨(*Nilaparvata lugens* Stål)以持續性方式傳播。在28~30℃下，病毒在蟲體內之潛伏期平均爲6.7日(3~14日)。供試蟲群獲毒率爲41% (22.2~64.4%)。最短獲毒及接吸食時間分別爲60及30分鐘。在15~35℃溫度範圍內，媒介昆蟲均能獲毒，獲毒後之潛伏期隨溫度增高而縮短，但當溫度高於30℃以上時，媒介昆蟲死亡亦隨之增加。本病毒不能經卵或以機械方式傳播。在25±1℃定溫下，利用生物檢定法及酵素聯結法同時檢定吸食病株後之褐飛蝨，發現酵素聯結法最早於吸毒後第五日偵測到病毒；而生物檢定法則於第7日檢定到帶毒病蟲。顯然酵素聯結法比生物檢定法能較早期於帶毒蟲體內偵測到病毒。

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