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RICE GRASSY STUNT VIRUS 2: A NEW STRAIN OF RICE GRASSY STUNT IN THE PHILIPPINES

P. Q. CABAUATAN, H. HIBINO, D. B. LAPIS, T. OMURA, and T. TSUCHIZAKI

The International Rice Research Institute
P.O. Box 933, Manila, Philippines

RICE GRASSY STUNT VIRUS 2: A NEW STRAIN OF RICE GRASSY STUNT IN THE PHILIPPINES[1]

ABSTRACT

A virus disease of rice, characterized by stunting and yellowing of the plant, was observed at IRRI and in Koronadal, South Cotabato, Philippines. The disease is transmitted by the brown planthopper *Nilaparvata lugens* (Stal). Based on symptomatology, virus-vector interactions, serological reaction, and particle morphology, the virus was identified as a strain of rice grassy stunt virus. The new strain was designated as rice grassy stunt virus 2 (RGSV 2) and the type strain (ordinary grassy stunt) was designated as rice grassy stunt virus 1 (RGSV 1).

Aside from causing tungro-like symptoms, RGSV 2 differs from RGSV 1 in pathogenicity to some rice varieties. *Oryza nivara* Sharma et Shastry, highly resistant to RGSV 1, is susceptible to RGSV 2. Consequently, IR varieties possessing *O. nivara* genes for resistance to RGSV 1 are susceptible to RGSV 2 in greenhouse tests.

[1] By Pepito Q. Cabauatan, Hiroyuki Hibino, and Delfin B. Lapis, senior research assistant, plant pathologist, and former senior research fellow, Department of Plant Pathology, International Rice Research Institute, P. O. Box 933, Manila, Philippines; and Toshihiro Omura and Tsuneo Tsuchizaki, plant pathologists, Agricultural Research Center, Tsukuba, Ibaraki 305, Japan.

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RICE GRASSY STUNT VIRUS 2: A NEW STRAIN OF RICE GRASSY STUNT IN THE PHILIPPINES

An unknown disease of rice, characterized by stunting and yellowing of the plant, was observed in some experimental plots at IRRI in 1979 and 1980. Because of its low incidence and similarity to rice tungro virus disease, not much attention was given to it. However, in 1982, the disease was frequently observed at IRRI. IR36, IR19735-5-2-3-2-1, IR19743-25-2-2-3-1, and IR19743-46-2-3-3-2 were among the varieties that showed resistance to the disease, and IR8, Dee-geo-woo-gen, CR143-2-2, Carreon, Milbuen 5-3, and Zhu-Xi 26 seemed very susceptible to the disease. In the same year, a disease of similar nature was observed in Koronadal, South Cotabato, Philippines.

Preliminary studies showed that the disease is neither caused by fungus, bacteria, nutritional disorder, insect toxin (toxemia), nor insect damage. Ratooned plants showed mottling or striping symptom on the emerging leaves. Later, the leaves yellowed, indicating that the disease is systemic. Initial results showed that the disease is not transmissible by the green leafhopper *Nephotettix virescens* (Distant), a vector of tungro and yellow dwarf diseases.

This paper reports on the identification of the unknown disease based on studies of transmission, symptomatology, host range, particle morphology, and serological reaction of the causal virus. The studies were conducted at IRRI and at the Institute for Plant Virus Research, Tsukuba Science City, Japan.

TRANSMISSION

Attempts to transmit the disease by sap, soil, and seed failed. The disease was also not transmitted by other insects, including *Sogatella furcifera* (Horvath), *Recilia dorsalis* (Motschulsky), *Nephotettix virescens* (Distant), and *N. nigropictus* (Stal). However, the disease was transmissible by the brown planthopper *Nilaparvata lugens* (Stal) (Table 1).

The causal virus persisted in the insect, with an incubation period ranging from 5 to 21 d (av 8.4 d). Of the insects used in separate tests 5-20% were active transmitters. The virus was not transmitted through the eggs. The shortest acquisition time was about 1 h (Table 2). The percentage of infective insects increased as the acquisition access time was increased up to 4 d. On the other hand, the shortest inoculation time was 15 min (Table 3). About 10% of the plants exposed to viruliferous insects for 15 min became infected.

Table 1. Transmission of the unknown virus by insects and other means.

Transmission method	Plants inoculated or observed (no.)	Insects used (no.)	Transmission
Mechanical (sap)	100		–
Seed	350		–
Soil	100		–
Insects[a]			
N. virescens	2993	368	–
N. nigropictus	255	37	–
R. dorsalis	474	62	–
S. furcifera	302	43	–
N. lugens	12000	800	+

[a]Serially transferred until death after 4 d acquisition access time. (–) no transmission, (+) = transmission.

Table 2. Effect of acquisition access time on percentage of active transmitters.

Acquisition access time	Infective insects[a]	
	No.	%
15 min	0	0
30 min	0	0
1 h	2	1.67
2 h	3	2.50
1 d	4	3.33
2 d	10	8.33
4 d	24	20.00
8 d	17	14.17
12 d	6	5.83

[a]No. of insects tested = 120.

Table 3. Effect of inoculation access time on percentage of infected seedlings.

Inoculation[a] access time	Seedlings infected[b]	
	No.	%
5 min	0 (349)	0
10 min	0 (403)	0
15 min	38 (380)	10.0
30 min	48 (281)	17.1
1 h	66 (311)	21.2
2 h	85 (295)	28.8
4 h	69 (212)	32.5
8 h	187 (426)	43.9
16 h	196 (343)	57.1
24 h	195 (247)	78.9

[a]7-9 insects/seedling. [b]Figures in parentheses are number of seedlings inoculated.

SYMPTOMATOLOGY

The symptoms of the disease were observed 7-14 d after inoculation of 7-d-old Taichung Native 1 (TN1) seedlings. The early symptoms consisted of stunting, yellowing of lower leaves, and narrowing of leaf blades. The young leaves were pale green and sometimes, almost entirely chlorotic. Newly emerged leaves were striped or mottled. Severely infected seedlings produced few tillers and died 3-4 wk after inoculation (Fig. 1). However, mildly infected plants tillered profusely similar to plants infected by rice grassy stunt virus (RGSV) 3-6 wk after inoculation (Fig. 2). These plants were difficult to distinguish from plants infected with RGSV. However, plants infected at 30 d old or older showed symptoms similar to those caused by infection of rice tungro virus complex. Plants infected at later stages of growth produced small and incompletely emerged panicles bearing mostly empty and discolored grains.

HOST RANGE

Weeds commonly found in rice paddies were not infected by the unknown virus. They were: *Digitaria ciliaris* Presl., *Fimbristylis littoralis* (Gaudich), *Echinochloa colona* (L.) Link, *Cyperus rotundus* L., *C. difformis* L., and *Eleusine indica* (L.) Gaerth. However, several accessions of wild rices were infected (Table 4). *Oryza nivara,* resistant to grassy stunt, was susceptible to the new virus (Table 5).

PURIFICATION AND PARTICLE MORPHOLOGY OF THE VIRUS

To purify the unknown virus, the procedure for purifying rice grassy stunt in Japan (13) was followed (Fig. 3). The purified virus fraction had a maximum absorbance at 260 nm and minimum at 246-247 nm, typical of a nucleoprotein (Fig. 4). The average ratio of UV absorption at 260 nm and 280 nm (A260/280) for purified virus was about 1.29. Virus yields from 300 to 400 g of infected plants ranged from 0.85 to 2.0 OD_{260} units.

Electron microscopic examination of the purified virus particles negatively stained with uranyl acetate (1%) revealed filamentous particles (6-8 diam) of various lengths (Fig. 5).

1. TN1 seedlings severely infected with the unknown virus 14 d after inoculation.

2. TN1 plants mildly infected with the unknown virus (A), rice grassy stunt virus (B), and healthy check (C).

Table 4. Reaction of wild rices to the unknown virus disease by mass screening method.

Accession no.	Species	Infected plants[a]	
		No.	%
101395	*Oryza alta*	1 (4)	20.0
100161	*Oryza alta*	10 (24)	41.7
102154	*Oryza officinalis*	0 (10)	0
101154	*Oryza officinalis*	0 (8)	0
101166	*Oryza officinalis*	0 (12)	0
101089	*Oryza minuta*	15 (16)	93.8
101387	*Oryza minuta*	1 (28)	3.6
101125	*Oryza minuta*	3 (19)	15.8
101087	*Oryza minuta*	3 (8)	37.5
101443	*Oryza latifolia*	5 (11)	45.5
100168	*Oryza latifolia*	4 (15)	26.7
100966	*Oryza latifolia*	0 (1)	0
101940	*Oryza perennis*	18 (19)	94.7
102178	*Oryza perennis*	33 (33)	100.0
100588	*Oryza perennis*	5 (10)	50.0
101988	*Oryza* spp.	6 (7)	85.7
101408	*Oryza punctata*	1 (12)	8.3
101439	*Oryza punctata*	0 (26)	0
101998	*Oryza rufipogon*	5 (5)	100.0
	O. sativa × *O. rufipogon*	4 (4)	100.0

[a] Figures in parentheses are number of inoculated plants.

SEROLOGY AND CROSS PROTECTION

A rabbit was immunized against the purified virus by 3 injections at 2-wk intervals: 2 intramuscular injections of the purified virus emulsified with equal volumes of Freund's complete adjuvant, followed by 1 intraveinal injection of the purified virus. For each injection, 1 ml of

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Table 5. Reaction of *Oryza nivara* to grassy stunt (RGSV) and to the unknown virus.

Trial	Infected plants[a]			
	RGSV		Unknown virus	
	No.	%	No.	%
I[b]	0 (10)	0	14 (14)	100
II[b]	0 (11)	0	9 (11)	82
III[c]	2 (84)	2.4	29 (31)	93.5

[a]Figures in parentheses are number of inoculated plants. [b]Inoculated at 3 wk old at 15-20 insects/seedling. [c]Inoculated by the mass screening method for RGSV.

300 g infected tissue
- Homogenized – 0.1 M PB (pH 7.2), 0.01 M Na_2SO_3, 0.01 M Sod. DIECA; filtered through cheese cloth
- 9,000 rpm, 20 min

Supernatant
- 1.25 mg Magnesium bentonite; stir for 5 min
- 9,000 rpm, 20 min

Supernatant
- CC14, 20% (V/V); stir for 3 min
- 9,000 rpm, 20 min

Aqueous layer
- PEG (5%); Triton X-100 (1%); 0.2 M NaCl; stir for 40 min
- 9,000 rpm, 20 min

Pellet
- 30 ml 1:10 extraction buffer (no. 1 above); homogenize
- 8,000 rpm, 15 min

Supernatant
- 40,000 rpm, 90 min

Pellet
- 1 ml 0.01 M PB
- 8,000 rpm, 15 min

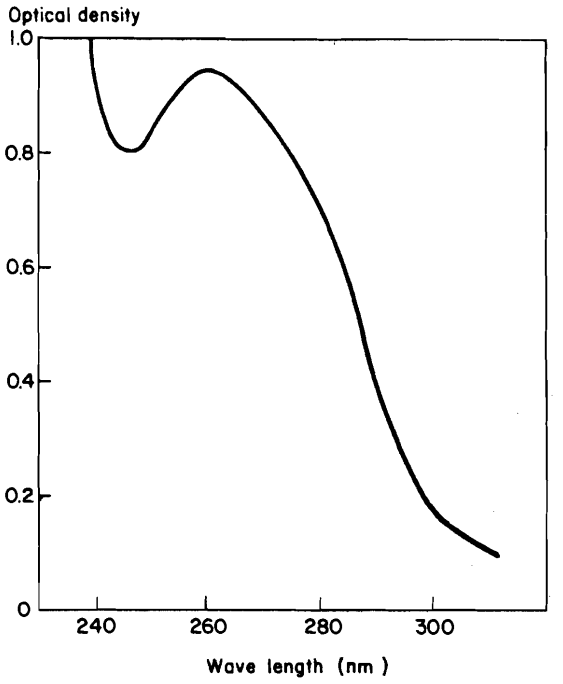
Supernatant
- 10-45% sucrose in 0.01 M PB
- 26,000 rpm, 180 min

Virus zone
- 40,000 rpm, 90 min

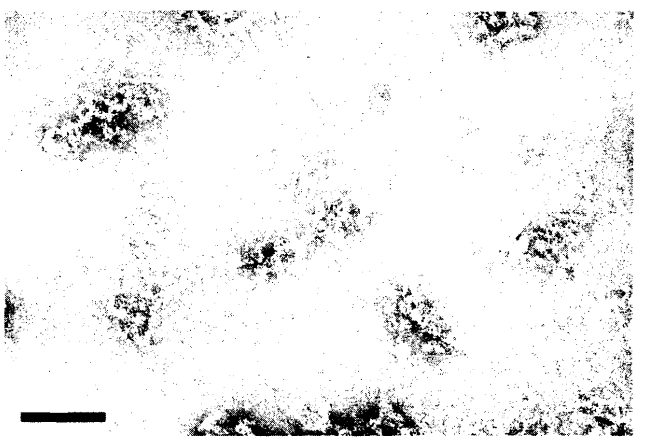
Pellet
- 1 ml 0.01 M PB
- 10,000 rpm, 15 min

Supernatant (virus suspension)

3. Procedure for purifying the unknown virus.



4. UV absorption spectrum of the purified unknown virus.



5. Filamentous particles associated with the unknown disease of rice. The bar represents 100 nm.

virus suspension with OD_{260} at 1.0 was made for each rabbit. The antiserum was collected about 1 wk after the third injection. Serological tests were performed by precipitin ring interface and double gel diffusion tests.

In the ring interface test, the antiserum was diluted stepwise and reaction of the diluted antiserum was tested against purified virus suspension with OD_{260} adjusted to 0.1. Gel diffusion was conducted in 0.8% agar containing 0.1% SDS and 0.005 M NaN_3 using undiluted antiserum and purified virus suspension with OD_{260} adjusted to 0.5 or 1.0.

In the ring interface test, the antiserum against the unknown virus reacted strongly with RGSV. Similar results were obtained in reciprocal tests using antiserum against RGSV in Japan (13). The antiserum had a titer of 1:640 (Table 6). In the double gel diffusion tests, a single band was developed between antiserum to RGSV in Japan, and both the unknown virus and RGSV in Japan (Fig. 6a). The reaction bands fused each other. Reaction bands between the antiserum to the unknown virus, and RGSV in the Philippines and to unknown virus were also fused (Fig. 6b). These results indicate that the unknown virus and RGSV are serologically very close or indistinguishable.

On the other hand, plants infected with the unknown virus could be dually infected with tungro and ragged stunt. However, when RGSV-infected plants were cross-inoculated with the unknown virus, or vice versa, the doubly infected plants did not show any striking difference from plants

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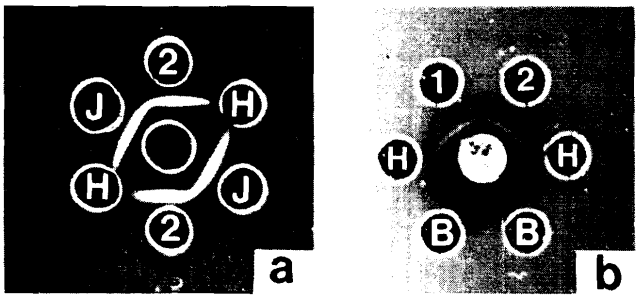
Table 6. Reaction of RGSV and the unknown virus to antisera against purified RGSV and unknown virus and vice versa by the precipitin ring interface test.

Antisera against	Antigen (virus)	Dilution of antiserum								
		20	40	80	160	320	640	1280	2560	5120
Unknown	Unknown	+	+	+	+	+	+	–	–	–
	RGSV in Japan	+	+	+	+	+	+	±	–	–
RGSV in Japan	Unknown	+	+	+	+	+	+	–	–	–
	RGSV in Japan	+	+	+	+	+	+	+	±	–



6. Serological reactivity of the unknown virus, RGSV in the Philippines, and RGSV in Japan. a) The center well contains antiserum to RGSV in Japan and the peripheral wells contain RGSV in Japan (J), unknown virus (2), and clarified healthy sap (H). b) The center well contains antiserum to the unknown virus and the peripheral wells contain RGSV in the Philippines (1), unknown virus (2), clarified healthy sap (H), and buffer (B).

singly infected by each virus. The two viruses may cross-protect each other.

DISCUSSION

Identification of viruses that are not mechanically transmissible must necessarily be based largely on symptomatology, host range, and if the vector is known, the identity of the vector and virus-vector relations, serology, and particle morphology (24). Hence, these criteria were used to identify the unknown virus under study.

As for other plant viruses, the symptom is a widely used criterion in determining the identity of rice virus diseases. Each virus disease has its own characteristic symptoms which are usually diagnostic. For this reason, almost all rice virus diseases are named according to their characteristic symptoms on susceptible plants.

Three rice virus diseases had been reported to be transmitted by the brown planthopper. They are grassy stunt (23), ragged stunt (3, 11, 17), and wilted stunt (7, 9). Rice ragged stunt has symptoms distinct from the other two diseases. Also, its virus particle morphology greatly differs from that of rice grassy stunt (12, 19, 25). On the other hand, RGSV and rice wilted stunt virus (RWSV) show similar symptoms particularly during summer (8). The unknown virus shows similarity in symptoms to both RGSV and RWSV. However, since quarantine regulations preclude the possibility of comparing the three diseases, the unknown virus was compared only with RGSV. Nevertheless, Chen and Chiu (8) grouped RWSV with RGSV based on similarities in symptomatology, virus-vector relations, and cellular inclusions found in RWSV- and RGSV-infected plants (8, 20).

The unknown virus differed from RGSV in severity of symptoms produced particularly in the field. It induced tungro-like symptoms in the field and in susceptible plants artificially inoculated at later stages of growth. These symptoms had not been described for RGSV before. Moreover, the unknown virus induced mottling and/or striping symptoms in most of naturally infected varieties and caused premature death of infected plants. Except for these differences, the unknown virus and RGSV are very similar in symptomatology, particularly when the plants are inoculated at the seedling stage and even more so when the plants are mildly infected with the unknown virus. In this case, the viruses could not be distinguished from each other by symptomatology alone. However, the two viruses could be separated by their ability to infect *Oryza nivara* and other *O. nivara*-derived varieties (differential varietal reaction). *O. nivara* is highly resistant to RGSV but susceptible to the unknown virus. Consequently IR varieties with *O. nivara* as RGSV-resistant gene source were also susceptible to the unknown virus in greenhouse tests.

On the other hand, the virus-vector relations of the two diseases (RGSV and unknown virus) agree fairly well with RWSV. The three viruses are transmitted by the same vector, *N. lugens*, persistently without transovarial passage. The other transmission characteristics do not differ markedly from each other (Table 7). The minor differences could be due to different colonies of insects used. Hence, the virus-vector interaction could not be used to distinguish the three viruses from each other.

Several plant viruses have been identified or their presence has been detected based on serological reactions, and many plant virologists have concluded that serology provides some of the most reliable evidence of virus identity. This conclusion is based largely on the premise that most

Table 7. Transmission characteristics and virus-vector relations of RGSV, RWSV, and the unknown virus.

Transmission characteristic	RGSV[a]	RWSV[b]	Unknown
Vector	*N. lugens*	*N. lugens*	*N. lugens*
Virus retention	Persistent	Persistent	Persistent
Incubation in the insect (d)	10.6	7.0	8.4
Transovarial passage	None	–	None
Transstadial blockage	None	None	None
Transmission pattern	Intermittent	–	Intermittent
Minimum acquisition access time	30 min	2 h	1 h
Minimum inoculation access time	9 min	30 min	15 min

[a]Sources: (16, 23). [b]Sources: (7, 9).

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virus strains are serologically related and will react with the antiserum prepared against the *type* virus. Results of serological tests showed that the unknown virus is very closely related to RGSV. Besides, cross-inoculation test showed that the two viruses did exhibit cross protection.

The unknown virus differs from RGSV in severity of symptoms and differential reaction of *O. nivara* (Table 8). However, it is not uncommon for plant virus strains to differ in symptom severity and/or virulence. Differences in virulence and severity of symptoms have also been observed among strains of plant viruses like sugar beet curly top virus (6), TMV (18), potato virus X (26), potato virus Y (5), potato yellow dwarf virus (4), apple mosaic virus (21), and many others. Among rice virus diseases, however, only stripe virus (curly and opening type) and tungro virus have known strains (14, 22). Different strains of RTV (S, M, and T) differ only in severity of symptoms produced in some differential varieties but not in varietal reaction (16). However, in India, RTV strains differ in both severity of symptoms and varietal reaction. Some varieties are susceptible to one strain but resistant to another (1, 2). The same characteristic was observed in the unknown virus. Varieties resistant to RGSV were susceptible to the unknown virus.

Table 8. Comparison between the unknown virus and RGSV using Knight's (15) criteria for establishing relationship between virus strains.

Criteria	RGSV	Unknown virus
Host range	*O. sativa* *Oryza* spp. (wild rice)	*O. sativa* *Oryza* spp. (wild rice)
Particle morphology	Filamentous circular (6-8 nm dm; 1,000-3,000 nm long)	Filamentous circular
Cross protection	Cross-protected	
Serological reaction	Closely related	
Response to genetic change in host	*O. nivara*-resistant Mild symptom	*O. nivara*-susceptible Severe symptom

Particle morphology appears to be a constant characteristic feature of a given virus and its strains. Hence, strains of the same virus vary little in size and shape. Even mutation of a virus generally does not involve an apparent change in particle morphology (15, 27). Electron microscopic study of the purified virus isolated from the unknown disease revealed filamentous particles of various length. Similar particles were observed from RGSV-infected plants in Japan (10, 13). The unknown virus was serologically very close or equivalent to RGSV in Japan and in the Philippines. These observations are strong evidence that the unknown virus is a strain of RGSV. Therefore, the unknown virus was designated as RGSV 2 and the former type as RGSV 1.

On the other hand, although RGSV 2 is very similar to rice wilted stunt in symptomatology and virus-vector interaction, the relationship between the two diseases could not be well ascertained. The important data on varietal reaction (reaction of *O. nivara*), serology, and particle morphology are not available. Hence, further studies are needed to establish the relationship of RWSV to RGSV 1 and RGSV 2.

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The International Rice Research Institute
P.O. Box 933, Manila, Philippines

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