RICE GRASSY STUNT VIRUS

Hiroyuki Hibino*

ABSTRACT

Rice grassy stunt (RGS) was associated with the presence of virus-like filamentous and small isometric particles. Filamentous particles were 6–8 nm in diameter, 950–1350 nm long, and often circular. The filamentous particles consisted of ribonucleoprotein. Antiserum to the nucleoprotein specifically reacted with extracts of RGS-affected plants and the vector brown planthopper *Nilaparvata lugens* exposed to RGS-affected plants. Serologically, the nucleoprotein was distantly related to rice stripe virus. The name rice grassy stunt virus (RGSV) is proposed for the filamentous nucleoprotein. RGSV was efficiently detected in RGSV-infected rice leaves and RGSV-exposed planthoppers by ELISA and latex agglutination test. ELISA has been applied to monitor RGSV carriers in migrating planthoppers. A new RGSV strain causing tungro-like symptoms occurred in the Philippines. The strain shows a pathogenicity to rice cultivars with a resistance gene. Similar strains also occurred in India and Thailand.

Introduction

Rice grassy stunt (RGS) (Ling, 1972) occurs widely in rice-growing areas in South and Southeast Asia, and also in Taiwan and Japan (Chen and Chiu, 1982; Iwasaki and Shinkai, 1979). The grassy stunt agent is transmitted by the brown planthopper, *Nilaparvata lugens* Stal., in a persistent manner. From time to time, it has caused severe damage to rice production in the last 15 years. In 1974–1977 in Indonesia, combined yield losses caused by grassy stunt and the vector brown planthopper were well in excess of 3 million metric tons of paddy rice with a value of more than US$510 million (Palmer *et al.,* 1978).

So far *Oryza* species are the known hosts of RGS (Ling, 1972). RGS-affected plants show severe stunting, excess tillering, and develop pale green or pale yellow, narrow and erect leaves.

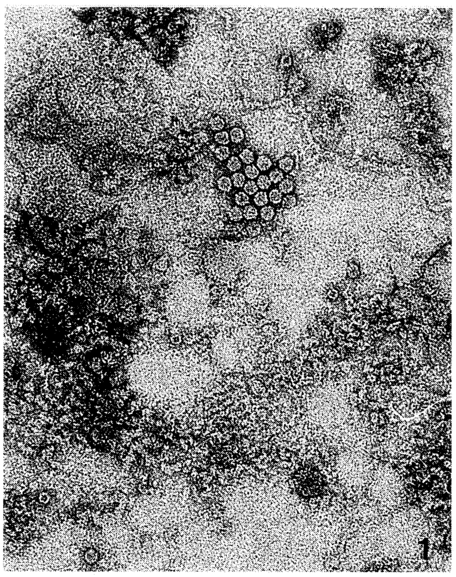
Purification and properties

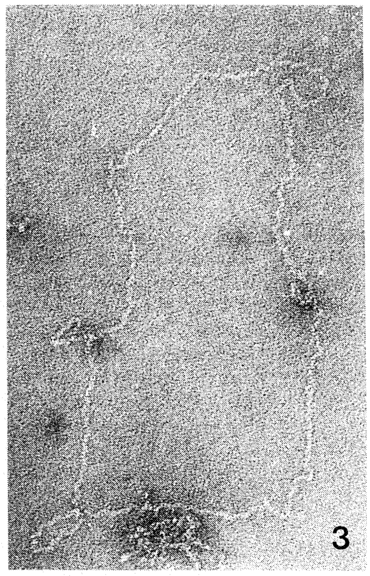
RGS has been long suspected to be a virus or mycoplasma disease because of its relations to the vector brown planthopper, but only a limited number of detailed studies on the virus agent were available. Recently, the viral nature of the RGS-infective agent was demonstrated by micro-injection techniques (Hibino *et al.,* 1985b; Shikata *et al.,* 1980). In 1978, Pellegrini and Bassi reported the occurrence of small osmiophilic particles, 25 nm in diameter, in RGS-affected sieve elements. The particles were associated with tubular structures. Shikata *et al.,* (1980) also reported the occurrence of similar small particles arranged within tubules in the phloem cells of RGS-affected rice plants. Similar particles were also found in a crystalline arrangement in cells of the fat body and trachea of viruliferous planthoppers. Fractions with numerous small particles obtained from RGS-affected rice extracts were infective (Shikata *et al.,* 1980).

On the other hand, Chen *et al.* (1979) reported the occurrence of inclusion bodies consisting of filaments of varying length in rice cells infected with rice wilted stunt, a strain of grassy stunt. Beside small isometric particles, thread-like filaments were found in extracts of RGS-affected rice plants (Hibino *et al.,* 1985a; Hibino *et al.,* 1985b; Iwasaki, *et al.,* 1985). There were numerous filamentous particles 6–20 nm in diameter, often aligned in tubules, and scarce in the extracts Hibino *et al.,* 1985b) (Fig. 1).

The filamentous particles were purified from RGS-affected rice plants (Figs. 2 and 3) (Hibino

* Plant Pathologist, International Rice Research Institute, P.O. Box 933, Manila, Philippines.





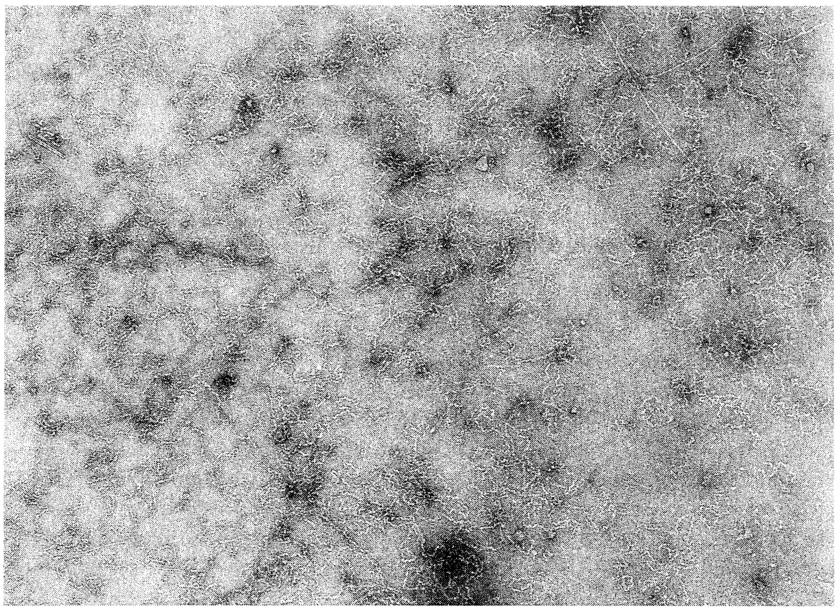


Fig. 1 **Electron micrograph of isometric (I) and filamentous particles (F) in clarified extracts from grassy stunt-infected rice plants stained with 1% uranyl acetate.** X 150,000.

Fig. 2 **Purified preparation of filamentous particles from rice grassy stunt-infected rice plants stained in 1% uranyl acetate.** X 60,000.

Fig. 3 **A circular filamentous particle prepared as in Fig. 2.** X 150,000.

et al., 1985a; Hibino *et al.,* 1985b; Iwasaki *et al.,* 1985). The purified filaments were often circular and 200-2,400 nm long. Length distribution of the circular filaments showed a peak in the 950-1,350 nm range. The filaments contained RNA and a single protein species of a molecular mass of 31,000 daltons. The purified filaments were not infective, though infectivity was recovered from the fractions obtained during the purification procedure. An antiserum to the filamentous nucleoprotein specifically reacted with extracts of RGS-affected plants and the planthoppers exposed to RGS-affected plants (Hibino *et al.,* 1985b). The infectivity in the RGS-affected leaf extracts was neutralized by an antiserum to the RGS-associated filamentous particles (Iwasaki, M., personal communication). These results indicate that the filamentous nucleoprotein is the virus agent of grassy stunt. The name "rice grassy stunt virus" (RGSV) was proposed for the filamentous nucleoprotein (Hibino *et al.,* 1985b). The role of the isometirc particles in RGSV infection remains to be clarified.

Relation to other viruses

So far rice stripe (RSV), maize stripe (MStpV) and rice hoja blanca (RHBV) viruses are the known planthopper-borne viruses which are filamentous in morphology (Table 1) (Gingery *et al.,* 1982; Koganezawa *et al.,* 1975; Morales and Niessen, 1983). The three viruses contain RNA and a single coat protein with a molecular mass of 32,000-34,000 daltons and are transmitted by planthoppers in a persistent manner. Serologically, the RSV and MStpV are related (Gingery *et al.,* 1983), and RGSV is distantly related to RSV (Table 2) (Hibino *et al.,* 1985b). RSV also consists

Table 1 Some properties of rice grassy stunt virus (RGSV), rice stripe virus (RSV), maize stripe virus (MStpV) and rice hoja blance virus (RHBV)

Virus	Nucleic acid	Morphology	Protein MW × 10^4	Planthopper transmission: Major vector	Planthopper transmission: Through egg	Host range
RGSV	RNA	6×950-1350 nm	3.1	*Nilaparvata lugens*	No	Rice
RSV	RNA	12×150- 650 nm	3.2	*Laodelphax striatellus*	Yes	Gramineae
MStpV	RNA	3 nm wide filament	3.27	*Peregrinus maidis*	Yes	Gramineae
RHBV	RNA	3 nm wide filament	3.4	*Sogatodes oryzicola*	Yes	Gramineae

Table 2 Serological reactions of rice grassy stunt virus (RGSV), rice stripe virus (RSV) and healthy antigens with antisera to RGSV, RSV, maize stripe virus (MStpV) and rice hoja blanca virus (RHBV) and normal serum in the precipitin ring test (Hibino *et al.,* Phytopathology 75, In press, 1985)

Antigen[a]	Reaction to antiserum[b]				
	RGSV	RSV	MStpV	RHBV	Normal
RGSV	1280	80	0	0	0
RSV	20	1280	160	0	0
Healthy rice	<10	0	0	0	0
Healthy maize	<10	0	0	0	0

a RGSV was purified from rice plants infected with grassy stunt.
RSV was purified from RSV-infected maize plants.

b Reciprocal of dilution end point of the antiserum. 0 indicates no reaction.

of circular filaments, 11–13 nm in diameter, with a helical configuration (Hibino *et al.,* 1985b). The RSV helix was constructed on the basis of a filament similar to RGSV. Circular form of the filamentous virus particles had not been detected for MStpV and RHBV. The three planthopper-borne viruses and RGSV may be members of the same virus group.

Strains

Three grassy stunt strains were reported in Taiwan (Chen and Chiu, 1982). A severe strain designated as wilted stunt was often lethal to rice plants, while strain B and strain Y caused milder symptoms. Strain B consistently caused excess tillering and strain Y did not affect tillering in winter, but promoted it significantly under summer greenhouse conditions. Extracts of rice plants infected with wilted stunt strain or strain B reacted to the RGSV antiserum (Chen *et al.,* personal communication).

In 1981, a new RGS strain (RGSV 2) which causes tungro-like symptoms occurred in the Philippines (Cabauatan *et al.,* 1985; Hibino *et al.,* 1985a). Plants infected with RGSV 2 at the seedling stage showed RGS-like symptoms and usually died prematurely, while plants infected at later stages developed symptoms similar to those caused by tungro infection. Virus particles purified from RGSV 2-infected plants consisted of circular filaments. RGSV 2 was serologically closely related to or undistinguishable from ordinary RGSV strain (RGSV 1) (Fig. 4). Strains of grassy stunt similar to RGSV 2 were also reported in Thailand and India (Disthaporn *et al.,* 1983; Mariappan, 1984), and observed in Indonesia. RGSV 2 and the similar grassy stunt strains seem to be closely related to wilted stunt virus.

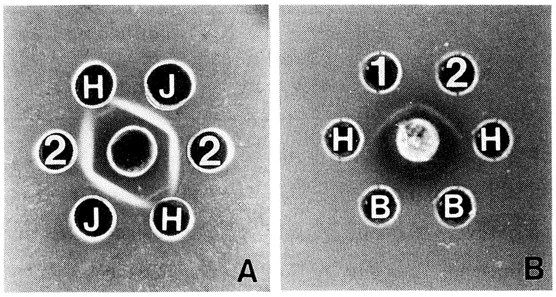


Fig. 4 Serological reactivity of rice grassy stunt virus strain 1, strain 2 and Japan isolate (Hibino *et al.,* 1985).
A The central well contains antiserum to Japan isolate and the peripheral wells contain Japan isolate (J), strain 2 (2) and clarified healthy sap (H).
B The central well contains antiserum to strain 2 and the peripheral wells contain strain 1 (1), strain 2 (2), clarified healthy sap (H) and buffer (B).

Transmission

RGSV is transmitted by *N. lugens* (Ling, 1972), *N. muiri* and *N. bakeri* (Iwasaki *et al.,* 1980) in a persistent manner. Incubation period in the *N. lugens* is 5–28 days (average 10.6 days) (Ling, 1972). The planthoppers exposed to RGSV-infected plants intermittently transmit virus until they die. Proportion of active transmitters is 10–40% for *N. lugens* and is higher for *N. muiri.* The average life span of virus-free *N. lugens* is longer than that of virus exposed ones (Ling, 1972).

Diagnosis and epidemiology

RGSV has been generally diagnosed based on the characteristic symptoms it induces. However, RGSV 2 and related strains caused symptoms similar to tungro in the field. Transmission test is used to identify RGSV, but it takes one month and requires insect-proof greenhouse facilities and virus-free vector colonies.

RGSV is a good immunogen and antisera can be easily obtained by injecting purified RGSV fraction into rabbits (Hibino *et al.,* 1985a; Hibino *et al.,* 1985b; Iwasaki *et al.,* 1985). Two serological tests, latex test and ELISA, have been applied to diagnose grassy stunt. By the latex test, RGSV was detected from crude sap of infected rice leaves and virus-exposed brown planthoppers diluted up to 1/5120 and 1/1024-1/2400, respectively (Hibino *et al.,* 1985b; Omura *et al.,* 1984). In ELISA, RGSV was detected in leaf- and planthopper extracts diluted up to 1/5120-1/100000 and 1/5120, respectively (Hibino *et al.,* 1985b; Iwasaki *et al.,* 1985).

The brown planthopper is known as a long distance migrator and immigrant planthoppers carrying virus are thought to be the major source of RGSV and another brown planthopper-borne rice ragged stunt virus. ELISA has been applied to monitor RGSV carriers in the migrating brown planthopper populations in Japan and Philippines. In Japan, the planthoppers which had landed in Kyushu across the East China Sea or in a boat on the sea were tested (Iwasaki *et al.,* 1985). Percentage of RGSV carriers was about 0.1% in 1982. In the Philippines, migrating planthoppers were collected daily by a high beam trap on the top of a three-story building and individually tested for the presence of RGSV as well as rice ragged stunt virus. In the 1983 monitoring test, about 1.2% of the planthoppers were found to be carriers of RGSV and 4.0% of rice ragged stunt virus from which 0.4% carried both viruses (Fig. 5) (Flores and Hibino, unpublished data).

N. lugens fed on RGSV-infected plants were tested for infectivity and then for the presence of RGSV. All planthoppers that transmitted the virus gave positive reactions in the latex test and about half of the planthoppers that reacted in the latex test transmitted the virus (Table 3) (Omura *et al.,* 1985).

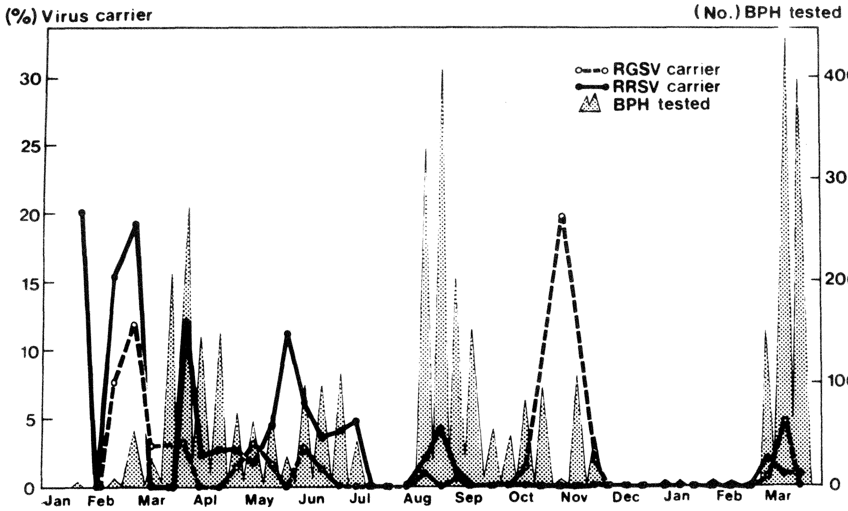


Fig. 5 Percentage of rice grassy stunt (RGSV) and rice ragged stunt (RRSV) virus-carriers in brown planthopper (BPH) catches of a high beam light trap at IRRI (1983—84) (Z.M. Flores and H. Hibino, unpublished).

Table 3 Relationship between infectivity of *N. lugens* and the ability to detect rice grassy stunt virus antigen in their extracts by the latex test.[a] (Omura *et al.*, Plant Disease, 68:374-378, 1984)

Sex of vector	Tested	Number of vectors			
		Latex text		Transmission test	
		Positive	Negative	Positive	Negative
Female	60	11		6	5
			49	0	49
Male	60	15		8	9
			45	0	45

a Thirty healthy *N. lugens* were used as controls and all produced negative reactions in the latex and transmission tests.

Resistance

So far, only *Oryza* species are known as hosts of RGS. They are *O. arta, O. australiensis, O. breviligulata, O. glaberrima, O. granulata, O. latifolia, O. minuta, O. nivara, O. officinalis, O. punctata, O. perenis, O. perenis* subsp. *balunga, O. rufipogon, O. sativa, O. sativa.* f. *spontanea,* and *O. spontanea.* A line of *O. nivara* was found to be highly resistant to RGSV (Ling, 1972). The line has a single dominant gene for resistance and the gene has been incorporated into many lines and cultivars. These cultivars were released in the early 1970s and RGS has been successfully controlled by the resistant cultivars at least in Indonesia, Philippines and Vietnam. On the other hand, cultivars having the resistance gene were susceptible in India (Ghosh *et al.*, 1979).

A new strain RGSV 2 which occurred in the Philippines has a pathogenicity to *O. nivara* and to cultivars with the resistance gene (Table 4) (Cabauatan *et al.*, 1985; Hibino *et al.*, 1985a). RGS strains similar to RGSV 2 which occurred in Thailand and India were also pathogenic to *O. nivara* (Disthaporn *et al.*, 1983; Mariappan *et al.*, 1984). Three strains reported in Taiwan (Chen and Chiu, 1982) also gave a fairly high percentage of infection on *O. nivara* (Chen, C.C., personal communication). These facts suggest that RGSV strains which can withstand the resistance gene from *O. nivara* are distributed widely in Asia.

In the field, cultivars resistant to the brown planthopper also show resistance to RGS. Reactions of the planthopper resistant cultivars may vary from area to area depending on planthopper biotypes in the respective areas.

Table 4 Reaction of *Oryza nivara* (IRRI Acc. No. 101508) to rice grassy stunt virus ordinary strain (RGSV 1) and new strain (RGSV 2). (Cabauatan, P.Q. and Hibino, H., Int. Rice Res. Newsl., 8(2); 12-13, 1983)

Trial	RGSV 1		RGSV 2	
	Plants infected/ inoculated (No.)	Infection (%)	Plants infected/ inoculated (No.)	Infection (%)
1[a]	0/10	0	14.14	100
2[a]	0/11	0	9/11	82
3[b]	2/84	2.4	29/31	93.5

a Inoculated at 3 weeks old using 15-20 insects/seedling.
b Inoculated using the mass screening method for RGSV.

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Discussion

Omura, T. (Japan): What is the percentage of viruliferous insects that migrate? Do you have any data on the occurrence of the disease in fields in the Philippines?

Answer: In 1983 the monitoring test using beam traps showed that 1.2% of the planthoppers carried rice grassy stunt virus, 4% rice ragged stunt virus and 0.4% both viruses. These data refer to the traps set at the top of the IRRI farm building. In remote areas less than 1% of brown planthoppers carried rice grassy stunt virus or rice ragged stunt virus. In Japan, Iwasaki showed that there was a very low percentage of brown planthoppers carrying rice grassy stunt virus (0.1%) that migrated across the sea to Japan.

Honda, Y. (Japan): You mentioned that the pathogen of rice grassy stunt disease is presumably a filamentous nucleoprotein. An arthropod virus (Bunyaviridae) also consists of spherical particles 100 nm in size which contain 3 types of filamentous nucleoprotein. I detected a filamentous nucleoprotein in the tomato spotted wilt virus treated with Triton X 100. It appears that these particles are infective by sap inoculation. Therefore I assume that the purified filamentous nucleoprotein you described is probably the pathogen of grassy stunt disease.

Dollet, M. (CIRAD): Did you identify the round particles associated with the filamentous ones in thin sections of vector and rice samples?

Answer: No sections were made. The spherical isometric virus-like particles were detected in rice plants infected with grassy stunt.