RICE RAGGED STUNT VIRUS

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1. Introduction

Rice ragged stunt virus (RRSV) was observed for the first time in 1976-1977 in Indonesia and the Philippines (12, 23), and soon afterwards became epidemic in several countries in South and Southeast Asia (10). RRSV was also found in Taiwan in 1977 (4), in China in 1978 (43), and in Japan in 1979 (41). It is not known whether the virus had long been present in these countries without being noticed or the virus had spread to these countries from elsewhere sometime before it was found. RRSV is transmitted by the brown planthopper, *Nilaparvata lugens* Stal., in a persistent manner (12, 23). The brown planthopper is known as a long distance migrator and it can migrate even across the ocean (20). In the temperate regions where the planthopper cannot overwinter, migrating planthoppers are the major source of RRSV.

Since the early 1970's the brown planthopper population increased in many countries in South and Southeast Asia. With the increase of the damage caused by the planthopper, the damage caused by RRSV as well as another brown planthopper-borne virus, rice grassy stunt virus, also increased in these countries. In Indonesia, estimated losses of rice crop due to the brown planthopper and the brown planthopper-borne viruses amounted to 0.7 and 1.1 million metric tons of dry unhulled rice without stalk in 1975, 1976, and 1977, respectively, corresponding to about 3, 3 and 5% of the total rice production in Indonesia, respectively (29).

RRSV incidence depends primarily on the density of the vector brown planthopper and disease source. In tropical Asia, RRSV incidence and its vector population are generally high in the areas where rice is grown throughout the year. Brown planthopper migration occurs throughout the year in the tropics. The planthoppers transfer RRSV from the hot areas to the other rice growing areas via migration. The brown planthopper can be controlled by proper application of insecticide. However, it is rather difficult to prevent RRSV and grassy stunt by controlling the vector planthopper with the application of insecticide, when the density of the planthopper in the area is high (29). Improper insecticide application may induce the resurgence of the brown planthoppers. In the areas where the planthopper density is generally high, planting the cultivars resistant to the planthopper, RRSV and grassy stunt virus has been recommended, and simultaneous cropping and eradication of rice plants for several months are proposed.

2. Symptoms and host range

Rice seedlings infected with RRSV develop symptoms 2-3 weeks after inoculation

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(10, 12, 23). New leaves' development is delayed, and leaves are short, narrower than healthy ones, and darker-green in color. They are often deformed, ragged or twisted (Fig. 1). Number of ragged or twisted leaves generally decreases as the plant grows and these symptoms may be inconspicuous on new leaves at two months after inoculation. In some cultivars, infected plants are nearly healthy except for slight stunting and occasional appearance of twisted leaves.

Tillering number of infected plants increases at early growth stages but is lower than that of healthy plants at the later tillering stage. Galls or vein swellings appear along the leaf vein on the outer surface of sheaths and leaf blades at the later tillering stage (Fig. 2). Symptoms become well defined again at heading stage (Fig. 3). The flag leaves of infected plants are often short, ragged and twisted. Panicle exsertion is delayed and incomplete, and grains are discolored and unfilled. Tillers often generate nodal branches which may bear small panicles and retain a green color after heading.

Rice plants are often infected with both RRSV and grassy stunt virus in fields. Symptoms of doubly infected plants include those of ragged stunt, i.e. ragged or twisted leaves, and those of grassy stunt, i.e. narrow, short and discolored leaves and excess tillering.

Fig. 1

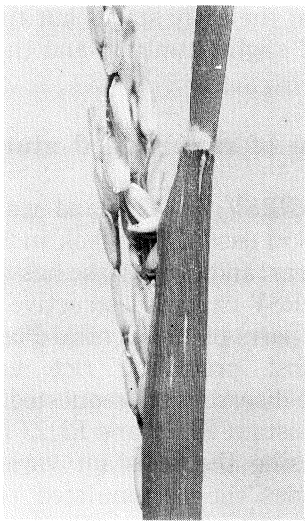

Fig. 2

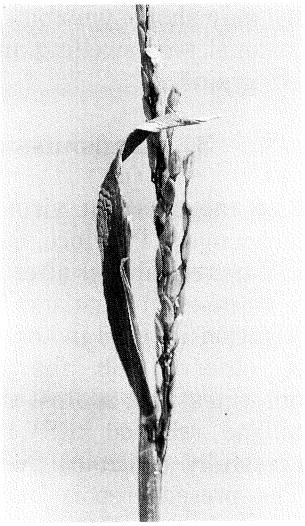

Fig. 3

Fig. 1. Short ragged or twisted leaves on a rice ragged stunt virus-infected rice plant. One month after inoculation.
Fig. 2. Vein swellings on a sheath of rice ragged stunt virus-infected plant.
Fig. 3. Incomplete panicle exsertion, and a ragged and twisted flag leaf on a rice ragged stunt virus-infected plant.

Plants of several gramineous species have been tested for RRSV infection (Table 1). Fifteen rice taxa tested showed stunting, ragged leaves, vein swelling and nodal branches, similar to those on *Oryza sativa* (14). Maize, barley, wheat, rye, oat and millet inoculated with RRSV also developed symptoms similar to those on rice (10, 17). Some weeds are also known to be host plants for RRSV (G.Z. Salamat and H. Hibino, unpublished data). Although RRSV has a wider host range, these hosts may not be

Table 1. Plant host of rice ragged stung virus.

Rice taxa[1]	*Oryza sativa, O. alta, O. australiensis, O. barthii, O. brachyantha, O. eichingeri, O. grandiglumis, O. longistaminata, O. latifolia, O. nivara, O. minuta, O. officinalis, O. perennis, O. punctata, O. ridleyi, O. rufipogon.*
Cereals[2]	*Avena sativa, Hordeum vulgare, Panicum milaceum, P. crus-galli, Secale cereals. Triticum vulgare, Zea mays.*

1 1978 IRRI Annual Report. 1979.
2 Hibino, H. Rev. Plant Protect. Res. 12:98—110, 1979; Kawano, S. et al., Ann. Phytopath. Soc. Japan 47:697—699, 1981.

important for RRSV epidemics, as those plants are non-host of the brown planthopper except for some rice taxa and recovery of RRSV from those plants by the planthopper was difficult or unsuccessful even in forced feeding.

The symptoms caused by RRSV varied depending on the cultivars. In the case of Sitopas, infected plants produced normally long flag leaves and 60% of the panicles emerged completely (15). The tolerant cultivars infected with RRSV at the seedling stage may show symptoms at the early stages but the symptoms disappear except for occasional vein swelling and slight stunting, and the cultivars develop panicles with filled grains.

3. Transmission of rice ragged stunt virus in Thailand

Rice ragged stunt virus (RRSV) in Thailand was observed for the first time at Chachoengsao Province, 60 km east of Bangkok in 1977 (5, 42). The area affected by RRSV increased year after year, and the disease has become one of the most important rice diseases in Thailand. RRSV caused destructive damage in the rainy season rice cultivation in 1980 in some parts of the Central Plain of Thailand. Severe incidence was observed on the most prevailing rice varieties RD7 and the urgent countermeasures against the disease were requested. The Department of Agriculture, Thailand, released RRSV-resistant RD21 and RD23 in 1981, and the disease has been successfully controled by using the resistant varieties.

The present report includes the accumulated results of experiments on RRSV conducted at the Department of Agriculture, Thailand, from September, 1978 to April, 1981.

Rice variety Taichung Native 1 was used as a test plant. Colonies of the vector brown planthopper, *Nilaparvata lugens,* had been maintained in cages for 4 years at the Division of Plant Pathology and Microbiology, Department of Agriculture, Thailand. Transmission tests were conducted at a temperature of 28-34°C. The inoculated seedlings were transplanted in seedling boxes in a screen house and symptoms of the disease were observed about 4 weeks after inoculation.

1) Serial transmission test

Fourth instar brown planthopper nymphs were fed on a RRSV-infected rice plant for 1 day. Each insect was then serially transferred daily to a healthy seedling in a test tube for 20 days. Eighteen insects out of fifty tested transmitted the disease. The

brown planthopper transmitted RRSV in a persistent manner and the incubation period in the insect ranged from 4 to 17 days with an average of 8 days. Some insects transmitted RRSV for 10 continuous days, but others transmitted it intermittently.

2) Effects of acquisition access period on transmission

Nymphs of *N. lugens* were given an acquisition access to a RRSV-infected rice plant for 30 min, 1 hr, 6 hr, 1 day, and 2 days. After the acquisition access, the insects were fed on healthy rice seedlings for 7 days, and then allowed an inoculation access period. The insects acquired RRSV in a 3-hr acquisition access, but failed to acquire it in a shorter acquisition access period. Totally 15 to 19 insects were tested for each acquisition access period and 11 to 22 percent of them transmitted the virus. The rate of transmission increased with a longer acquisition access period.

3) Transmission of RRSV by the brown planthoppers at different ages

Brown planthopper nymphs at the first to 5th instar stages and adults were allowed an acquisition access on RRSV-infected rice plants for 1 day. After the acquisition access, the number of transmitters was determined by daily serial transmission (one insect per seedling). One hundred to 200 nymphs were tested for each instar. Percentage of transmitters was 12, 16, 18, 26, 28, and 13 for 1st, 2nd, 3rd, 4th and 5th instar nymphs, and adults, respectively. The 5th instar nymphs accounted for the highest percentage of transmitters followed by the 4th instar nymphs as the second highest. There were no significant differences in the incubation period in the insects at different growth stages.

4) Effects of inoculation access period on the transmission

Nymphs of *N. lugens* were given an acquisition access of 3 days on RRSV-infected rice plants. The insects were allowed to feed on healthy rice seedlings for 7 days, and then individually given an inoculation access on test seedlings for 30 min, 1 hr, 6 hr, and 1 day. One insect out of 19 tested transmitted RRSV in a 1-hr inoculation access, but failed to transmit it in a 30-min inoculation access. Percentage of transmitters increased with a longer inoculation access period and 7 out of 19 insects transmitted the virus in a 6-hr inoculation access period.

5) Transmission of RRSV by Nilaparvata bakeri

RRSV transmission ability of *Nephotettix virescens, Recilia dorsalis, Sogatella furcifera,* and *Nilaparvata bakeri* was tested. Nymphs of *N. virescens* and adults of *R. dorsalis* and *S. furcifera* were given an acquisition access of 1 day on RRSV-infected rice plants. Each insect was then allowed serial daily acquisition access on test seedlings in test tubes. *N. bakeri* nymphs were given an acquisition access of 17 hr (overnight) on infected rice plants and were allowed to feed on a host plant, *N. bakeri* was then tested in a series of alternate transfers of 16 hr on rice seedlings and 8 hr on *Leersia hexandra. N. bakeri* would soon die, if they were fed on rice plants for a long time. *N. virescens, R. dorsalis* and *S. furcifera* did not transmit RRSV, whereas 4 *N. bakeri* out of 75 tested transmitted the virus.

6) Dual transmission of RRSV and rice grassy stunt virus (RGSV)

The 4th instar nymphs of *N. lugens* were successively fed on RRSV-infected and RGSV-infected rice plants for 2 days each. The insects were serially transferred 5 times to fresh seedlings every other day for inoculation accesses. A total of 55 insects were tested, and 10 transmitted RRSV and RGSV together in the transfers, 9 transmitted both RRSV and RGSV but separately in the transfers, 7 transmitted RRSV alone, 6 transmitted RGSV alone, and 23 did not transmit either.

The results of the present study are summarized in Table 2. RRSV was transmitted by the brown planthopper in a persistent manner with an average 8-day incubation period. Some of the insects transmitted RRSV intermittently in the daily transfers. According to other previous reports, the incubation period of RRSV in the insect also ranged from 7.6 to 10.7 days (average around 8 days) and the pattern of transmission was intermittent (4, 8, 12, 23, 37, 43).

Table 2. Interaction of RRSV and brown planthopper in Thailand

Property tested	Result
Transmission mode	Persistent
Transmission efficiency	36%
Incubation period in the insect	4—17 (8) days
Minimum acquisition access period	3 hr
Minimum inoculation access period	1 hr
Higher affinity for acquisition access	4- 5th instar
Dual transmission of RRSV and RGSV	yes
Transmission by leaf- and planthoppers	(Transmitted/tested)
Nilaparvata bakeri	yes (4/75)
Nephotettix virescens	no (0/69)
Recilia dorsalis	no (0/64)
Sogatella furcifera	no (0/98)

The minimum acquisition access period was 3 hr in the present study, whereas it was 8 hr (12), 2 hr (4) and 30 min (43) in other reports. These differences might be due to the difference in the origin of insects and/or the experimental conditions.

The minimum inoculation access period was 1 hr in this study as reported previously (4, 12).

Brown planthopper nymphs at the 5th and 4th instar acquired RRSV more efficiently than nymphs in younger instars and adults in these experiments. It was also reported that nymphs were more efficient than adults in transmitting the virus (8).

N. lugens fed on both RRSV and RGSV infected plants transmitted RRSV and RGSV together or separately. The rice plants infected with both RRSV and RGSV showed symptoms of both diseases. The dual infection of plants with RRSV and RGSV may

indicate the absence of cross protection between the two viruses.

In the present study *N. bakeri* was shown to be an additional vector of RRSV, but *N. virescens, R. dorsalis,* and *S. furcifera* did not transmit the virus. Iwasaki *et al.* reported that *N. bakeri* and *Nilaparvata muiri* also transmitted RGSV (16). Therefore, *N. lugens* and *N. bakeri* are common insect vectors for RRSV and RGSV.

4. Relation of rice ragged stunt virus with cells and tissues

Rice ragged stunt virus (RRSV) particles occur in the phloem tissues of infected rice leaves (5, 13, 39). They are scattered in the cytoplasm or embedded in viroplasm-like inclusions composed of filaments 7 to 10 nm in diameter in the parenchymatous cells. Virus particles are also found in the sieve tubes. Necrotic cells in the phloem tissues often contain particles.

The gall tissues occur at the abaxial side of the vascular bundles and are formed by hyperplasia of the phloem tissues (10, 13, 23). Amorphous inclusions are observed in some cells of the gall tissue under the light microscope. By electron microscopy, the gall cells are found to contain virus particles in viroplasm-like inclusions present in the cytoplasm (Fig. 4) (13). Virus particles occasionally form arrays assuming crystalline arrangements. Virus particles and filaments which might originate from inclusions are scattered in the degenerated gall cells. Parenchymatous cells

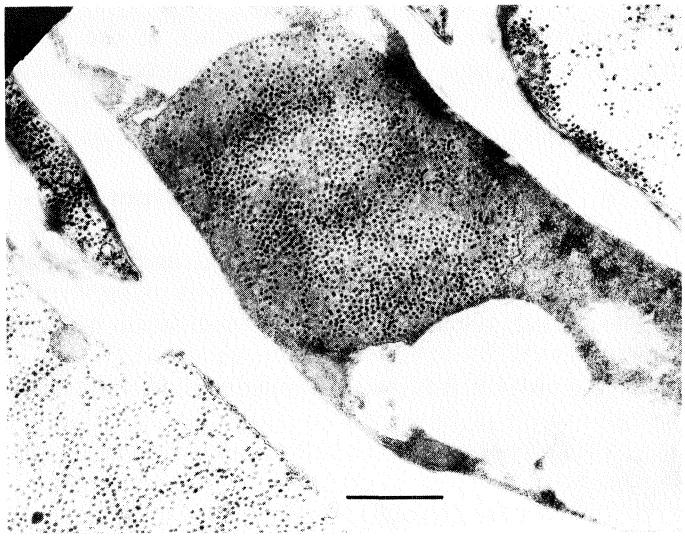

Fig. 4. Parts of gall cells showing rice ragged stunt virus particles embedded in a viroplasma-like inclusion or scattered in the degenerated cytoplasm. Bar represents 1 μm.

surrounding xylem elements in the gall tissue are free of particles.

Thin sections obtained from the infectious brown planthoppers were examined under the electron microscope (13). RRSV particles were scattered or aggregated in crystalline arrangements in viroplasm-like inclusions in the cytoplasm of the cells of salivary gland, fat body, nerve tissues, muscles and fore-gut (Fig. 5).

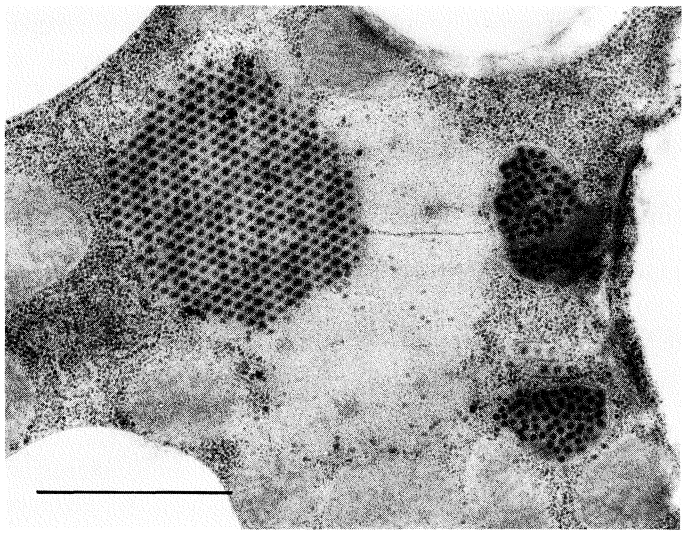

Fig. 5. Part of a fat-body cell showing crystalline aggregate of rice ragged stunt virus, and tubules and phagocytic vesicles with virus particles. Bar represents 1 μm.

Occasionally, particles were found on the surface of the secretory granules in salivary gland cells and some of these granules contained particles. Tubules containing particles occurred in the cytoplasm of the fat-body cells. Tubules were about 110 nm in diameter and their walls were 10–15 nm thick.

5. Purification of rice ragged stunt virus

Rice ragged stunt virus (RRSV) was maintained and propagated in rice plants (cultivar Taichung Native 1) by transmitting the virus using viruliferous *Nilaparvata lugens*. Fresh leaves or roots about 40 days after inoculation were ground with a meat chopper in two volumes (W/V) of 0.1 M sodium phosphate buffer, pH 7.0 containing 0.01 M $MgCl_2$ (PB-Mg) ($MgCl_2$ was added immediately before use). The homogenate was passed through a layer of finely woven cotton cloth, carbon tetrachloride (CCl_4) was added to it to a final concentration of 20%, and the homogenate was blended in a Waring Blender for 2 min. After centrifugation for 15 min at 3,000 × g, to the supernatant fluid polyethylene glycol 6,000 (PEG), NaCl and Triton X-100 were added to a final concentration of 6% (W/V), 0.3 M and 1% (W/V), respectively. The mixture was stirred for 40 min and then centrifuged for 15 min at 6,000 × g. The pellet was resuspended in 0.1 M histidine buffer containing 0.01 M $MgCl_2$ (pH 7.0) (His-Mg), incubated for 30 min and centrifuged for 15 min at 3,000 × g. The supernatant was treated with CCl_4 (10%) and then centrifuged for 40 min at 96,000 × g. The pellet was resuspended in His-Mg, incubated for 30 min, and centrifuged for 15 min at 3,000 × g. The supernatant fluid was layered on a 10–40% (W/V) linear sucrose gradient in His-Mg and centrifuged for 1 hr at 87,000 × g in a Hitachi RPS-27 rotor. The zone containing virus particles was recovered with an ISCO model UA 2 scanner, layered on a 40–60% (W/V) linear sucrose gradient in His-Mg, and centrifuged for 15 hr at 62,000 × g in a Hitachi RPS-27 rotor. The gradient was fractionated by the scanner

and viruses in the fraction were concentrated by centrifugation for 2 hr at 96,000 × g. The final pellet was resuspended in His-Mg.

Yield of virus was higher in roots than in leaves in infected plants. Addition of $MgCl_2$ to the buffer solution stabilized the virus particles and reduced particle disintegration during purification as compared to the case without using the chemical. Histidine buffer which was used at the later step of purification was also useful for the preservation of the particle structure. The use of Triton X-100 was effective in removing the host material from the virus fraction. Without the detergent treatment, the purified preparation still contained membranous host materials.

Viral preparations were stained in 2% aqueous uranyl acetate (UA) and were examined with a Hitachi H-500 electron microscope. Particles about 55 nm in diameter with spike-like projections were observed in the purified preparations (Fig. 6). Outer capsid shells of particles were not found in purified virions and their morphology was very similar to that of cytoplasmic polyhedrosis virus (9).

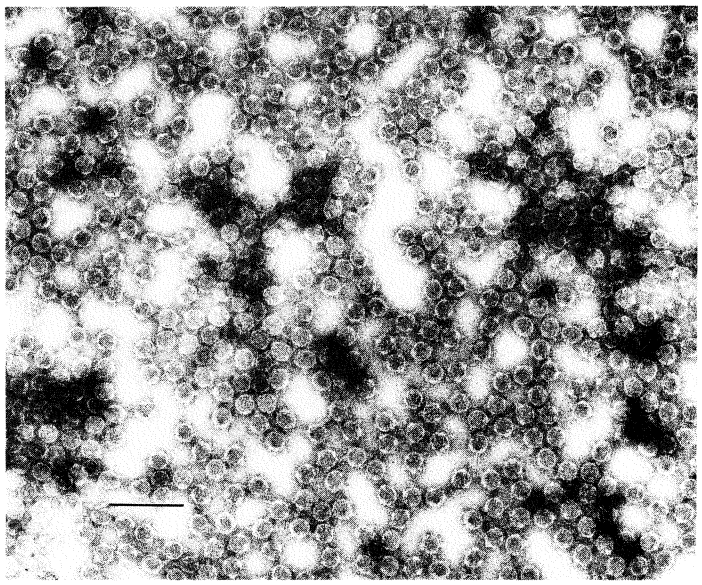

Fig. 6. Purified rice ragged stunt virus stained with 2% uranyl acetate. Bar represents 200 nm.

Purified preparations of RRSV (A_{260} = 1.0) were injected into the abdomen of third- to fourth-instar nymphs of *N. lugens* to determine the infectivity of the particles. As shown in Table 3, 71-81% of the insects given inoculation access feeding on test rice seedlings induced typical symptoms of rice ragged stunt disease in plants. These results suggest that the purified 55 nm particle is the causal virus of rice ragged stunt disease.

Purified virus and the emulsion were injected into muscles of domestic rabbits. Injection was repeated three times at 3-wk intervals. Antiserum derived from the rabbits was tested by the precipitin ring interface test and its titer against purified RRSV was 1,240.

Table 3. Infectivity of purified RRSV. (Omura *et al.*, 1983)

Exp. no.	Number of insects			Percentage of transmission
	Injected	Survived 12 days	Transmitted virus	
I	100	21	15	71
II	100	52	42	81
Control[a)]	100	46	0	0

a) Buffer-injected control.

6. Morphology of rice ragged stunt virus

Rice ragged stunt virus (RRSV) particles in sectioned plant and vector cells were spherical, about 65 nm in diameter, and had electron dense cores about 45 nm in diameter and less dense shells about 10 nm in width (Fig. 7) (10, 13). Particles often appeared to be polyhedral in sections. Distance between centers to neighboring particles in crystalline arrangements was about 68 nm.

RRSV particles in negatively stained preparations were partially degraded and smaller than those in sectioned cells. In phosphotungstate stain, particles appeared to be spherical, 55-60 nm in diameter, while in uranyl acetate stain, particles were spherical with projections (spikes) (Fig. 7) (10, 13, 18, 19, 25, 30). Body diameter of the

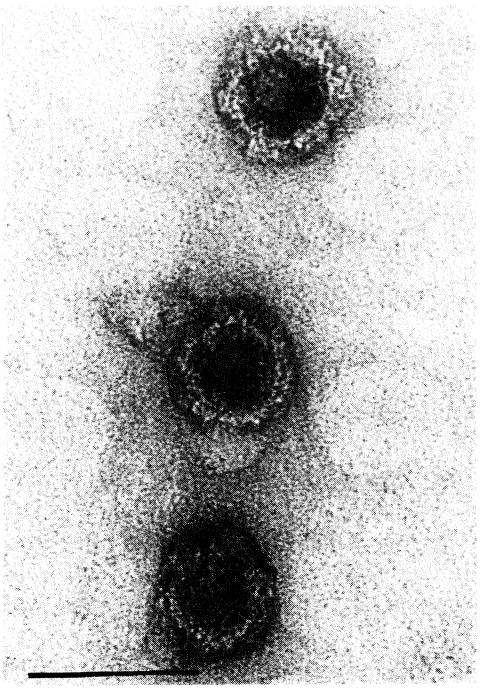

Fig. 7. Rice ragged stunt virus particles in a dip preparation of gall tissue on a infected rice plant. Bar represents 0.1 μm.

particles was about 50 nm and the spikes formed a truncated cone, about 12 nm at the top, 15-18 nm at the base, and 7-8 nm in length. Often, filamentous structures with a helical configuration extended from the particles (10, 13). Particles fixed with glutaraldehyde or paraformaldehyde prior to phosphotungstate staining also showed spikes and occasional extrusion of filamentous structures.

The spiked particles are similar in size and shape to the Fijivirus subviral particles. The existence of RRSV with a complete outer shell as in the Fijiviruses is still inconclusive. RRSV particles in thin sections often appeared to be polyhedral with outer shells (Fig. 8), and particles in uranyl acetate stain were occasionally surrounded but partly with an outer shell as in the case of the Fijiviruses (10, 13). On the other hand, Milne suggested that the broad based spikes may in some profiles gave the appearance of a continuous shell (25). So far complete double shelled virions have not been observed in RRSV preparations in the negative stains (10, 13, 18, 19, 25).

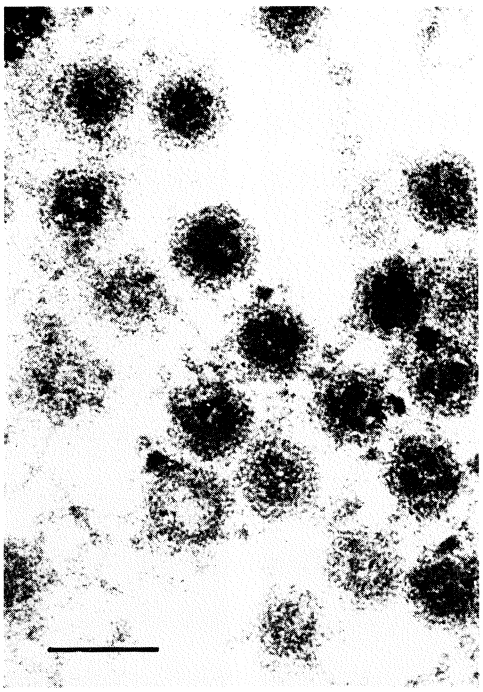

Fig. 8. Rice ragged stunt virus particles in a degenerated gall cell of a infected rice plant. Bar represents 0.1 μm.

7. Nucleic acids of rice ragged stunt virus

Rice ragged stunt virus (RRSV) was reported to contain double stranded-ribonucleic acid (ds-RNA) separated into 8 approximately equimolar bands with a total molecular weight of 11.63×10^6 using RNA purified directly from the enation of virus-infected plants (1). A plant reoviruses are classified into two subgroups: the Phytoreovirus (plant reovirus subgroup 1) with 12 segmented genomes and the Fijivirus (plant reovirus subgroup 2) with 10 segmented genomes as subgroups (24). The present study was carried out to analyze RNA genome segments of the purified virus by

electrophoresis.

The phenol-sodium dodecyl sulfate (SDS) procedure (2) was employed to extract RNA from purified RRSV. Alternatively, RNA was released by adding 1% SDS and 0.01% ethylene-diaminetetraacetic acid (EDTA) to purified virus preparations and was directly used for electrophoresis (33). Cytoplasmic polyhedrosis virus (CPV) was used as standard. Slab gels consisting of 7.5% (W/V) acrylamide and 0.2% (W/V) bisacrylamide were prepared by the method of Laemmli (20), as applied by Ramig *et al.* (32) for reovirus genome RNAs. Electrophoresis was performed at room temperature for 6 or 12 hr at 30 mA/gel in 0.025 M Tris-HCl, pH 8.3 containing 0.192 M glycine and 0.1% SDS (Tris-glycine). The gels were washed in distilled water and RNA bands were traced using the Shimadzu chromatoscanner CS-910 and were then stained with

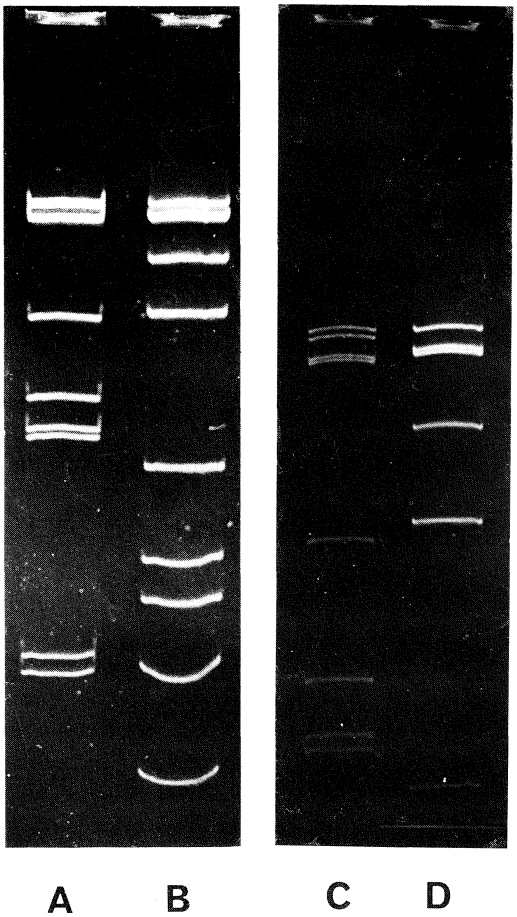

Fig. 9. Photographs of gels in which the RNA segments of RRSV and CPV genomes were separated by electrophoresis in a 7.5% slab gel. The electrophoresis was performed for 6 hr (A, B) and 12 hr (C, D) at 30 mA, and gels were stained with ethydium bromide. Migration was from top to bottom. The RNAs were: RRSV RNA (A, C) and CPV RNA (B, D). Low molecular weight segments moved away from the gel in C and D. (Omura *et al.*, 1983).

ethidium bromide in distilled water (1 μg/ml). RNA bands were visualized under U. V. light and were photographed onto the Polaroid film type 665.

RRSV genome could be resolved in 9 distinct segments in 7.5% gels (Fig. 9). The third band was thick and broad and expected to contain about twice as many molecules as the others judging from the peak height of the densitometric tracing (data not shown). As shown in Fig. 9, the band was separated into two segments by prolonged electrophoresis (12 hr) in the same system. No difference in distribution and number of RNA segments was found between RNAs extracted with SDS-phenol and those obtained by treating the virus with SDS-EDTA. Molecular weights of RRSV RNA segments were 3.65, 3.55, 3.40, 3.38, 2.38, 1.76, 1.55, 1.52, 0.88 and 0.85 × 10^6 with a total molecular weight of 22.91 × 10^6 when estimated in using the standards developed by Galinski *et al.*, (7). The present study showed that RNAs of RRSV could be separated into 10 segments confirming that the virus belongs to the Fijivirus subgroups.

8. Serological detection of rice ragged stunt virus

1) Latex agglutination test

Latex particle suspension (Difco, 0.81 μm in diameter) sensitized with antiserum to rice ragged stunt virus (RRSV) was mixed with diseased-leaf extract and RRSV antigen in the extract was detected based on aggregations formed in the mixture (31). RRSV was detected up to 1/10 dilution from diseased-leaf extracts and up to 1/40 from diseased-root extracts. Attempts to improve the detection efficiency by changing the extraction buffer were unsuccessful.

2) ELISA

Immunoglobulin (IgG) was purified from RRSV antiserum and the conjugate of alkaline phsphatase with IgG was prepared following the procedure described by Clark and Adams (6). The appropriate concentration of coating IgG was 2.5 μg/ml and the conjugate was diluted at a 1/320 dilution (11). RRSV was detected up to 1/320 dilution from RRSV-infected leaf extracts and up to 1/5,120 from RRSV viruliferous vector brown planthoppers, *Nilaparvata lugens*. However, strong nonspecific reactions occurred in extracts of virus-free female planthoppers carrying eggs. Polyvinyl-pyrolidone reduced the intensity of the non-specific reaction. The intensity of the nonspecific reaction varied remarkably with the pH of the extraction buffer. The nonspecific reaction was remarkable in the alkaline range and was negligible at pH 6.0 or 6.5 (Fig. 10). RRSV was detected in extracts of viruliferous insects diluted up to 10,240 times with phosphate buffer (pH 6.5) containing 2% polyvinylpyrolidone (Fig. 11). Planthopper extracts in this buffer showed negligible nonspecific reactions.

The planthoppers fed on RRSV-infected plants were individually tested for infectivity and homogenate of each planthoppers was tested for the presence of RRSV in ELISA. All the planthoppers which transmitted RRSV gave positive reactions in ELISA, while some of the RRSV non-infective planthoppers also gave positive reactions. Fifty percent of the planthoppers tested gave positive reactions and 27% of the virus carrying planthoppers transmitted RRSV in the test (Table 4). RRSV was also detected in dried or freeze-stored planthoppers and rice leaves in ELISA.

Luisoni *et al.*, also applied ELISA to detect RRSV from infected rice plants (26).

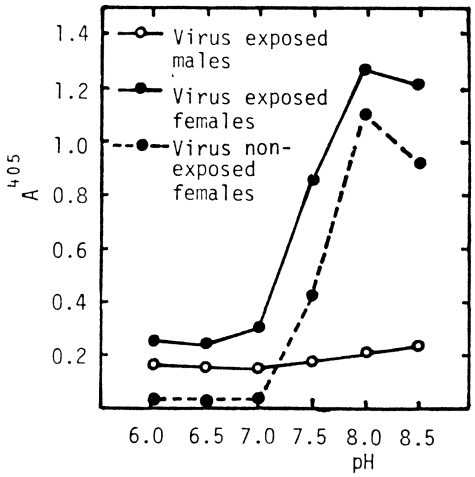

Fig. 10. Effects of pH of phosphate buffer on reaction of extracts of ragged stunt virus-exposed and unexposed planthoppers in ELISA.

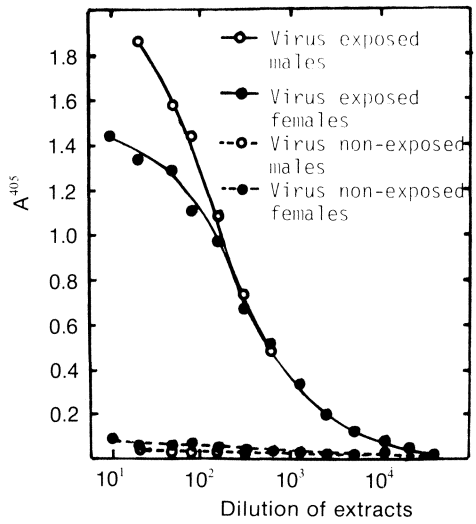

Fig. 11. Reactions of extracts of rice ragged stunt virus exposed and unexposed planthoppers in ELISA. Male and female planthoppers carrying eggs were homogenized separately and diluted with PBS-Tween (pH 6.5) containing 2% polyvinylpyrrolidone for ELISA.

RRSV was generally detected from RRSV infected rice extracts up to 1/100 dilution.

Table 4. Relation between infectivity of the brown planthoppers and reactions of their extracts in ELISA.

Test methods		Planthoppers (%)
Infectivity	ELISA	
+	+	57 (27)
+	-	0 (0)
-	+	48 (25)
-	-	106 (50)

3) Immunosorbent electron microscopy

Copper grids coated with freshly prepared carbon with membrane were incubated with RRSV antiserum and subsequently with extracts of RRSV-infected plants (26). RRSV was detected in extracts diluted to 1/1000 under an electron microscope, while on grids that were not pretreated with antiserum RRSV could only be detected to 1/10.

4) Serological relationships

RRSV is serologically unrelated to rice black-streaked dwarf (40) and all the recognized members of the fijivirus group (28).

9. Varietal resistance to ragged stunt virus in Thailand

Varietal differences in the resistance to rice ragged stunt virus (RRSV), which is one of the most important rice diseases in Thailand, were examined in the field and by seedling inoculation. The present experiments were conducted at the Department of Agriculture, Thailand, from August, 1979 to January, 1981.

Observation in the field: Varietal differences in resistance to RRSV were observed in the field of Chainat Rice Experiment Station located in the northern part of the Central Plain of Thailand. Percentage infection on five RD varieties was scored at the tillering and heading stages.

Seedling inoculation test: Ten breeding lines from the Rice Division, Department of Agriculture, Thailand and 54 varieties (15 indicas, 34 japonicas and 5 upland rice varieties) from the National Chugoku Agricultural Experiment Station, Japan were tested. Colonies of the vector brown planthopper used had been maintained at the Division of Plant Pathology and Microbiology, Department of Agriculture, Thailand. The insects fed on RRSV-infected rice plants for 1 day were allowed to feed on healthy seedlings for 10 days and used for inoculation access. Rice seedlings from the Rice Division at the two-leaf stage were exposed to the viruliferous insects for 1 day at the rate of 3 insects per seedling in test tubes. For the rice varieties from Japan, 40 seedlings of each variety in a tray were confined with 400 viruliferous insects in a screened cage for 1 day. Inoculated seedlings were transplanted in seedling boxes, kept in a screened house, and scored based on symptoms 30 days after inoculation. The inoculation test was replicated 3 times. Reactions to rice tungro virus inoculation

(yellow orange leaf virus in Thailand) were also tested by inoculating seedlings using viruliferous *Nephotettix virescens* for inoculation.

RD varieties: Reactions of RD varieties to RRSV in the field are shown in Table 5. Among the 5 varieties tested, RD9 showed the lowest percentage infection. RD7 showed the highest percentage of infection at both the tillering and heading stages.

Table 5. Varietal resistance to rice ragged stunt disease of RD varieties in the field

Variety	Tillering stage		Heading stage	
	Number of plants infected/observed	Percentage infection	Number of plants infected/observed	Percentage infection
RD 1	70/200	35	49/200	25
RD 5	29/100	29	19/200	10
RD 7	101/200	51	86/200	43
RD 9	13/100	13	13/200	7
RD 11	73/200	37	62/200	31

Date of observations
Tillering stage: August 4, 1980
Heading stage: September 4, 1980

Breeding lines from the Rice Division: The results are shown in Table 6. BKN6321-46-3, SPT58-9-330, SPR7419-862-5 and SPRLR76002-168-1-1 showed resistance to RRSV, while they showed a susceptible reaction to rice tungro virus infection.

Indica and japonica varieties: The result are shown in Table 7. Chinsurah Boro II from India, To-to originating from China and japonica lowland rice Zensho 26 and Fujihikari showed a lower percentage of infection. No varieties tested showed a high resistance.

Hibino *et al.* (12) observed the difference of symptoms on 12 Indonesian rice varieties infected with RRSV, but all the varieties observed were susceptible to RRSV. According to Ling *et al.* (22), Ptb21 from India was resistant in the Philippines, but Ghosh and John (8) reported that Ptb21 was susceptible whereas Ptb18 and IET6288 were resistant in India. So far, few reports have been published on the varietal resistance to RRSV.

Thai variety RD9 has been known to be resistant to the brown planthopper (35). It is considered that the low infection percentage of RD9 is due to the resistance to the brown planthopper. RD7 showed the highest infection percentage in these tests. Since 1975, RD7 had been a prevailing variety in the Central Plain of Thailand because of its high yield, good cooking quality and disease resistance (34). However severe incidence of RRSV was observed on RD7 in some regions in the Central Plain in 1980. The present results confirm the susceptibility of RD7 to RRSV.

Breeding lines of SPR7419-86-2 and SPRLR76002-168-1-1 were found to be resistant to RRSV in the field of Suphanburi Rice Experiment Station in 1980 and were released by the Department of Agriculture in 1981 as new RRSV resistant varieties, RD21 and RD23, respectively (36). Cultivation of the new varieties has rapidly spread in Thailand and RRSV has been successfully controlled since 1982. Resistance of RD21 and 23 to RRSV was confirmed in the test tube inoculation in these experiments.

Table 6. Resistance to rice ragged stunt and rice tungro (yellow orange leaf) diseases of breeding lines from Rice Division and Suphanburi Rice Experiment Station, Thailand

Breeding lines tested	Ragged stunt		Tungro	
	No. of plants infected/ observed	Percentage infection	No. of plants infected/ observed	Percentage infection
Experiment 1				
KTH'65-G_2U-31 (Irrad KTH 17)	9/47	19	4/48	8
BKN6003-242 (KTH17/LY34)	8/44	18	39/48	81
BKN58-4-315 (GR201/PN16)	9/49	18	35/45	78
BKN6321-46-3 (Lon Krok/NMS-4)	2/35	6	32/40	80
SPT'58-9330 (LY2B-72)	3/43	8	19/35	54
SPT6217-297 (Pin Gae Bow 27/JL11)	8/37	17	40/49	82
BKN6113-79 (Khao Tak Oo/KTH) (RD27)	12/45	27	39/42	93
SRRC'70-11-32 (Leuang Hawn)	5/45	11	38/42	91
TN1 (Control)	22/47	47	47/50	94
Experiment 2				
SPR7419-862-5 (KDML105/NMS-4//IR26) (RD21)	2/20	10	19/20	95
SPRLR76002-168-1-1 (RD7/IR26//RD1) (RD23)	2/20	10	19/20	95
RD9 (Control)	2/20	10	19/20	95
RD7 (Control)	17/20	85	17/20	85
TN1 (Control)	12/20	60	19/20	95

Abbreviations
KTH: Khao Tha Haeng, LY: Leuang Yai, GR: Gow Ruang, PN: Pung Nahk, NMS: Nahng Mon S, KDML: Khao Dawk Mali

The indica and japonica varieties or differential varieties tested are the varieties having been used as resistant test varieties for rice blast, bacterial leaf blight, and stripe virus. Chinsurah Boro II, Zensho 26, To-to and Fujihikari showed low infection percentages by seedling inoculation. Correlation of varietal resistance in seedling inoculation and field tests needs to be determined.

10. Discussion

It has been suggested that RRSV is a member of the Fijivirus subgroup of Reoviridae, due to the similarity in morphology, vector interactions and relations to plant and insect cells (10, 12, 23, 39). However, Boccardo and Milne (1) reported that RRSV had double-stranded RNA with 8 segments and not 10 as in the case of the Fijiviruses (27). In uranyl acetate stain, RRSV generally appeared as spherical particles with spikes which forms a truncated cone, and not as double shelled

Table 7. Varietal resistance to rice ragged stunt diseases of indica and japonica rice varieties

Percentage of infection	Variety
0 — 10	none
11 — 20	indica: Chinsurah Boro II, japonica: (lowland rice) To-to, Zensho 26, Fujihikari.
21 — 30	indica: Koentoelan, Java 14, Chiem Chank, Chokoto, Zenith, japonica: (lowland rice) Jikkoku, St. No. 1, Asominori, Asahi, Wase Aikoku 3, Chugoku 45, Toride 1, Fukunishiki, Norin 8, Norin 20, Norin 22, (upland rice) Rikuto Norin 11, Rikuto Norin 12
31 — 40	indica: Russia 33, Russia 41, Loktjan, Usen, Tongil, Te-tep, Dharial, Tadukan, japonica: (lowland rice) Kinmaze, Mineyutaka, Shin 2, 70X-46, Chusei Shinsenbon, Kogyoku, Yamabiko, Mokoto, Nipponbare, Homarenishiki, Aichi Asahi, Ginga, Pi 4, (upland rice) Kagoshima Hakaburi, Kuroka Mochi.
41 — 50	indica: Rantai Emas 2, Taichung (N) 1, japonica: (lowland rice) Senbon Asahi, Yashiromochi, Kanto 51, Ishikari Shiroke, Himekei 16, Tsuyuake, Reiho (upland rice) Rikuto Norin 24.
51 —	none

particles as in the case of the fijiviruses (10, 12, 18, 19, 25, 30, 38). Fijivirus subviral particles have spikes of parallel-sided form (27). Based on these evidences, RRSV was separated from the Fijivirus subgroup (25).

Recently, Omura *et al.* (30) clearly indicated that RRSV had 10 segments of double-stranded RNA as in the case of the Fijiviruses. This finding was confirmed by Kawano *et al.* (19). Omura *et al.* (30) proposed that RRSV can be tentatively included in the Fijivirus subgroup, though distribution of the molecular weight is distinct from that of the other members (18, 30). In contrast, Kawano *et al.* (18) suggested that RRSV should be assigned to a new plant reovirus subgroup, because RRSV has a different morphology and smaller total molecular weight.

Considering the reports of Hibino and his co-workers (10, 12) indicating the possible presence of double shelled particles in RRSV infected plant cells, further information is needed to establish a new subgroup for RRSV. Meanwhile, RRSV can be tentatively included in the Fijivirus subgroup, until new criteria for the Fijivirus taxonomy are developed.

RRSV was detected for the first time in 1976—1977 in Indonesia and the Philippines and soon afterwards in China, Japan, Malaysia, Sri Lanka, Taiwan and Thailand. It remains to be determined whether RRSV occurred inadvertently in these countries or whether RRSV had reached these countries from elsewhere. Ling et al. (23) suspected that the disease had long been present at a low level in rice fields in Indonesia and the Philippines.

It is known that BPH migrated from south to central and north China (3) and also probably from south China to Japan and Korea across the ocean (20). RRSV might have spread to China and Japan through the migration of virus-carrying BPH. In South and Southeast Asia, heavy BPH infestation had occurred since the early 1970's in many countries. If RRSV had long been present in those countries, RRSV incidence

might be very high in the BPH-infested regions. It is unlikely that RRSV would have been present although undetected in the countries where the BPH population was high.

This discrepancy may suggest possible long distance migration of BPH in the tropics. Since RRSV distributes widely in rice growing areas in South and Southeast Asia, the origin and spread of RRSV can be only assumed through speculation. RRSV seems to have a wide host range in gramineae as in the case of the fijiviruses (10, 17, Salamat and Hibino, unpublished data). It is possible that RRSV was a virus of weed(s) transmitted presumably by *N. bakeri* or other unidentified vector planthoppers. RRSV might have been picked up by chance by *N. lugens* from the weed hosts and transferred to rice the BPH population was high. RRSV could spread from the original rice fields to other areas via migrating BPH. Wide distribution of another BPH-borne grassy stunt virus in Asia may support this assumption.

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