RICE GRASSY STUNT VIRUS
- Purification and Serology -

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1. Introduction

Rice grassy stunt (RGS) is one of the most important virus diseases of rice in South and Southeast Asia (19). RGS is widely distributed in the rice growing areas in South and Southeast Asia and also in Taiwan and Japan (3, 16, 19). The virus agent is transmitted by the brown planthopper in a persistent manner and has a narrow host range. So far only rice and several species of wild rice are known hosts (19). RGS-infected plants show severe stunting, profuse tillering and narrow, short and yellow colored leaves. (cf. color plate No.6). Rice plants infected at the seedling stage or early tillering stage produce no panicles or a few, small panicles. Plants infected at the later growing stage develop panicles which may bear dark brown and unfilled grains.

RGS has been controlled by varietal resistance. The resistance gene from a wild rice *O. nivara* has been incorporated into many improved varieties (17, 19). Cultivation of the varieties with the resistance gene may be the reason why the grassy stunt incidence has been low in the Philippines since 1974. Recently, a new strain of RGS has occurred in the Philippines (cf. color plate No.7) (2, 8, 10). The new strain can overcome the resistance gene and all the improved varieties with the resistance gene became susceptible to the new strain. Similar RGS strain was also reported in Thailand (5). In Taiwan, three RGS strains causing different symptoms were reported (3). Among the three strains, a severe strain (rice wilted stunt) shows a similarity with the new strain in the Philippines in the symptomatology induced. Many varieties resistant to the brown planthopper have been identified and those varieties also show resistance to RGS in the field.

The RGS has been suspected to be a virus disease but the causal organism had not been identified. The occurrence of 70-nm virus-like particles (13) or of mycoplasma-like bodies (4, 14) in RGS-exposed planthopper or RGS-diseased rice tissues was reported but has not been confirmed. Virus-like particles, 20–25 nm in diameter, were recently reported in RGS-infected rice and RGSV-exposed planthopper tissues (21, 22).

2. Purification and properties of RGS-associated filamentous particles

1) Virus-like particles in leaf extract

Extracts of RGS-infected rice leaves and RGS-exposed planthoppers were injected into the abdomen of second instar nymphs of the planthopper (9, 12). Infectivity was recovered in pellets after high speed centrifugation or polyethylene glycol (PEG) treatment, and the infectivity was retained after freezing and thawing, and clarification with CCl_4 or Triton X-100. These results suggest that the infectious agent shows the characteristics of a virus. Two kinds of virus-like particles-filamentous and isometric - were observed in partially purified preparations (Fig. 1 and 2). Filamentous particles were 6–8 nm in diameter and were numerous in the

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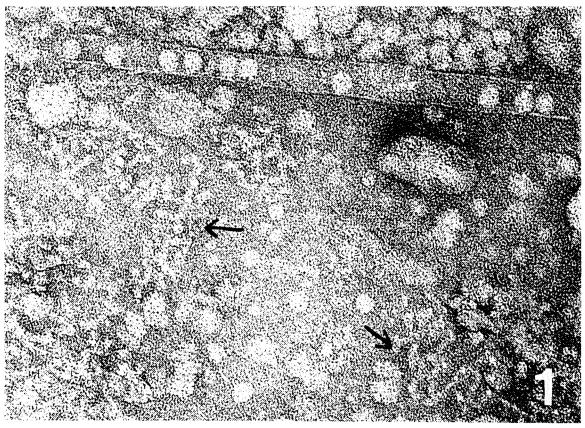

Fig. 1. Isometric particles in tubules and filamentous particles (arrows) in clarified extracts from grassy stunt-infected rice plants. Stained with uranylacetate. ×150,000.

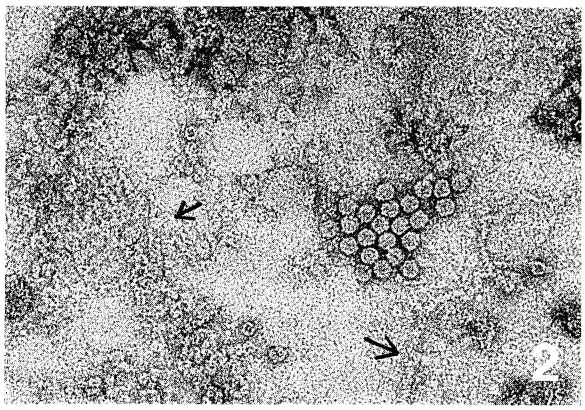

Fig. 2. Filamentous particles (arrows) and a clump of isometric particles in clarified extracts from grassy stunt-infected rice plants. ×150,000.

extracts, while isometric particles were often clustered or aligned in tubules 22–30 nm in diameter.

2) Purification of filamentous virus-like particles

RGS-infected rice plants with roots were ground with 0.1 M borate buffer (pH 8.0) containing 0.01 M Na_2SO_3 with a mincer (9, 12). The extracts were clarified by low speed centrifugation and treatment with Mg-bentonite and CCl_4. The clarified extracts were mixed with PEG and then the filamentous particles were collected in a pellet by low speed centrifugation. The pellet was suspended in buffer and treated once more with PEG under the presence of Triton X-100. The pellet obtained was suspended in buffer, layered onto 5–30% sucrose density gradient and centrifuged for 3 hr at 54,000 g. A peak zone containing filamentous particles was pooled and the particles were sedimented by high speed centrifugation at 130,000 g for 90 min.

Purified fractions thus obtained had a maximum absorbance at 259-260 nm and minimum of 246-247 nm. The 260/280 ratio was 1.28 ± 0.03. Yields of the filamentous particles from 300 g fresh tissues ranged from 1.0 - 2.5 OD_{260} unit.

3) Properties of filamentous particles

Purified filamentous particles consisted of long threads up to 2 μm long and 6-8 nm in diameter as visualized in uranyl acetate stain (Fig. 3) (9, 12). They were often circular (Fig. 4) and the length of the circular filaments ranged from 200 to 2400 nm with a peak at 1,000 to 1,300 (Fig. 5).

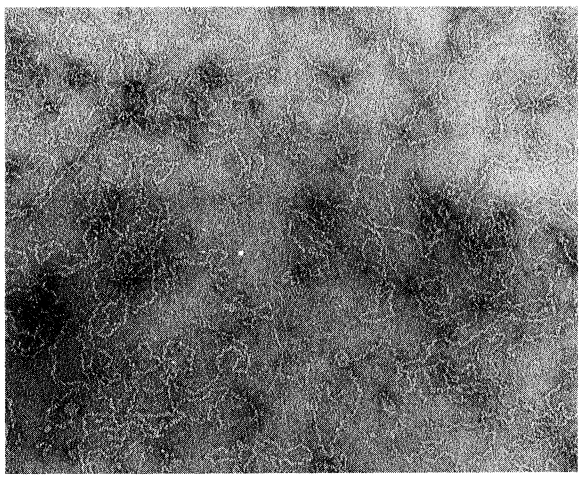

Fig. 3. Purified preparation of filamentous particles from rice grassy stunt-infected rice plants. Stined in uranyl acetate. ×100,000.

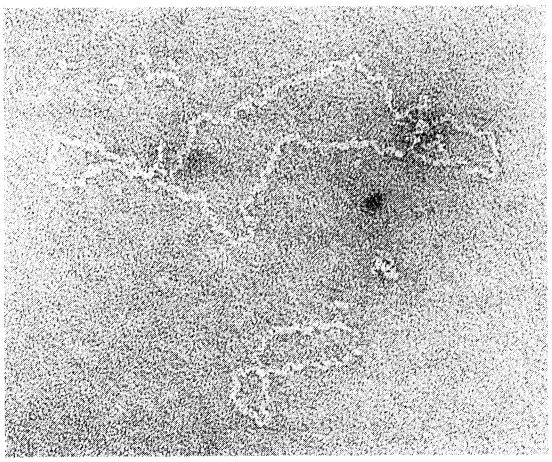

Fig. 4. Circular filamentous particles stined in uranyl acetate. ×150,000.

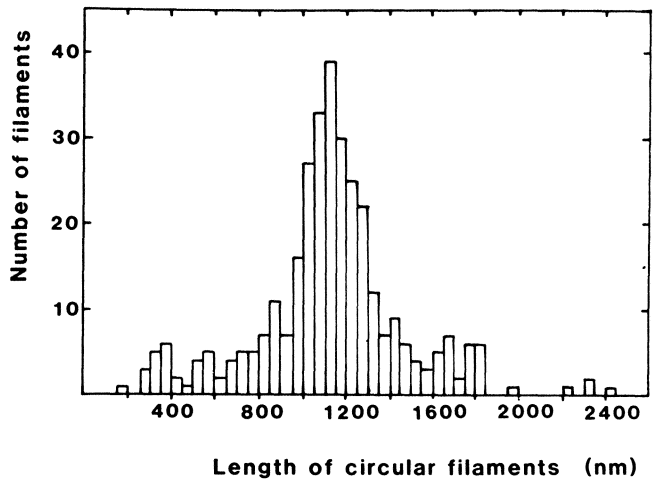

Fig. 5. Length distribution of circular filamentous particels.

Purified filamentous particles gave a positive orcinol reaction, and negative reaction with diphenylamine or cysteine-concentrated H_2SO_4. Proteins released from the purified filamentous particles migrated on the SDS-polyacrylamide gel as a single species with a molecular weight of 31,000 daltons. These results indicate that the filamentous particles consist of ribonucleoprotein.

Infectivity of the filaments was tested by injecting fractions obtained in each step of the purification into the abdomen of planthopper nymphs. Purified fraction was not infective and infectivity was recovered from the fractions before sucrose density gradient centrifugation.

4) Antiserum and serological detection of nucleoprotein

Antiserum to the filamentous nucleoprotein was obtained by injecting the purified filaments into rabbits. The antiserum (RGSV antiserum) had a titer of 1/1280 against the purified filaments (19).

Nucleoprotein was detected from crude sap of RGS-infected leaf and planthoppers diluted up to 1/4096 and 1/1024, respectively, by the latex test (12) using the RGSV antiserum. Extracts of virus-free rice leaves and planthoppers were negative down to the 1/4 dilutions. In ELISA, nucleoprotein was efficiently detected also from crude sap (15). Nucleoprotein was detected in leaf and planthopper extracts diluted up to 1/5120, while extracts of virus-free leaf and planthoppers were negative down to 1/10 dilutions. These results indicate that the necleoprotein is specific to RGSV infection.

3. Relations of filamentous particles to planthopper-borne viruses

1) Serological relationships

Serological relationships between the nucleoprotein and rice stripe virus (RSV) and maize stripe virus (MStpV) were tested by the precipitin ring interface and agar-gel

immunodiffusion tests (11). In the ring test, RSV antiserum reacted to the nucleoprotein but not to the extract of healthy rice leaves (Table 1). The antiserum to the nucleoprotein weakly reacted to RSV but the reaction between the antiserum and RSV was not clear as the serum also weakly reacted to healthy extracts. The MStpV antiserum reacted to RSV but not to the nucleoprotein.

A single band was formed between the RSV antiserum, and RSV and the nucleoprotein in immunodiffusion tests (Fig. 6). A spur was formed on the reaction band between the antiserum and RSV. A band was formed between the antiserum to the nucleoprotein and the nucleoprotein, but not between the antiserum and RSV.

Table 1. Serological reactions of rice grassy stunt-associated nucleoprotein (RGSV), rice stripe virus (RSV) and maize stripe virus (MStpV) in the precipitin ring interface test (Hibino *et al.*, Int. Rice Res. Newsl. 8(1):9-10, 1983).

Antigen	Reaction to antiserum			
	RGSV	RSV	MStpV	Normal
RGSV	1280	80	0	0
RSV	20	1280	160	0
Healthy leaf	10	0	0	0

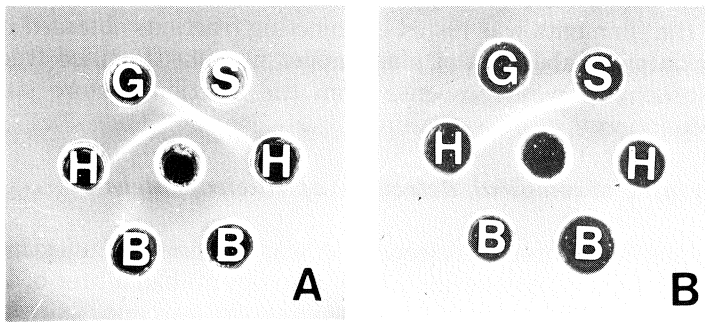

Fig. 6. Serological cross reactivity between rice grassy stunt-associated nucleoprotein (RGSV) and rice stripe virus (RSV).
A. The central well contains antiserum to RSV and the peripheral wells contain RGSV (G), RSV (S), healthy extracts (H) and buffer (B).
B. The central well contains antiserum to RGSV.

2) Similarity in properties

Purified RSV was observed under the electron microscope (11). RSV appeared as a thread-like filament, 11-13 nm in diameter of varied length. RSV filaments had a helical configuration and were often circular. The loosened helix appeared to be a filament 6-8 nm in diameter and the degenerated filaments were shorter and often

branched (Fig. 7). Coiled configuration of RSV has been reported but the circular form was not known (18, 23). The nucleoprotein associated with RGS was a circular filament but had no such helical configuration. In the known planthopper-borne filamentous viruses, MStpV and rice hoja blanca virus (RHBV) are also known to be filamentous in morphology (6, 20). RHBV also had a helical configuration (6). The filamentous planthopper-borne viruses contain RNA and coat proteins with a molecular weight of 32,000 - 34,000 daltons (6, 7, 20).

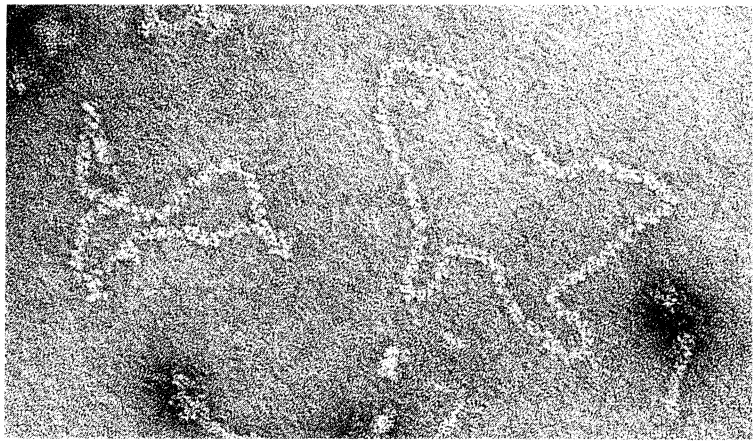

Fig. 7. Circular rice stripe virus particles with a helical configuration. ×150,000.

4. Conclusions

The nucleoprotein purified from RGS-infected rice plants was specific to RGS infection. The nucleoprotein was serologically related to RSV and the RSV was related to MStpV (7). Furthermore, the nucleoprotein showed a similarity to RSV, MStpV and RHBV in its morphology and relationships to vector planthoppers. These facts indicate that the nucleoprotein is the virus agent of RGS. In these experiments, purified nucleoprotein fraction had no infectivity. The nucleoprotein may lose its infectivity during the purification procedures or other factors may be required for the infection of the nucleoprotein. It is also known that infectivity of MStpV and RHBV was lost during the purification process (6, 20). The name rice grassy stunt virus (RGSV) is proposed for the nucleoprotein.

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