Rice Gall Dwarf, A New Virus Disease

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ABSTRACT

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A previously undescribed virus disease was found on rice plants in central Thailand in 1979 and named rice gall dwarf. Symptoms consisted of gall formation along leaf blades and sheaths, dark green discoloration, twisted leaf tips, and reduced number of tillers. Some plants died in the greenhouse in later stages of infection. The causal agent was transmitted by the green rice leafhopper *Nephotettix nigropictus* after an incubation of about 2 wk. Polyhedral particles about 65 nm in diameter observed in the cytoplasm of phloem cells were always associated with the disease. No serologic relationship was found between the virus and rice dwarf virus.

In August 1979, dark green, stunted rice plants with small galls on the leaf blades and sheaths were found in paddy fields at Uthaithani, central Thailand. Tips of some leaves of diseased plants were slightly twisted. None of the known virus diseases of rice produces these symptoms. Preliminary experiments showed that the green rice leafhopper *Nephotettix nigropictus* transmitted the causal agent of the disease and that polyhedral particles about 65 nm in diameter were always associated with the disease. Some of the diseased plants maintained at the Rice Pathology Branch of the Division of Plant Pathology and Microbiology in Bangkok were brought to the Institute for Plant Virus Research, Ibaraki, Japan, for further studies.

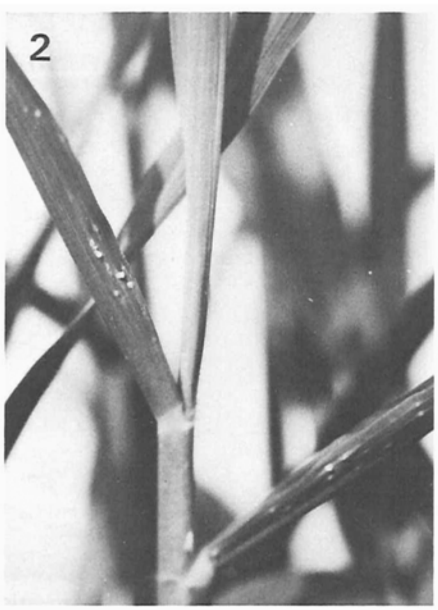
This paper describes the symptomatology of rice gall dwarf, the transmission of the disease agent by the insect vector, and the morphological and serologic characteristics of the viruslike particles associated with the disease.

MATERIALS AND METHODS

Vector transmission. Early-instar nymphs of *N. nigropictus*, collected at Kagoshima, Japan, and maintained at Kyushu National Agricultural Experiment Station, were used for transmission tests. After a 1-day acquisition access on diseased source plants, the insects were serially transferred twice a week to healthy rice seedlings (cv. Taichung Native 1) for an inoculation access in a controlled-environment greenhouse (23–27 C). Inoculated seedlings were grown in the same greenhouse.

Electron microscopy. Electron microscopic grids prepared by the leaf dip method with 2% neutral phosphotungstic acid as a negative stain were examined under a Hitachi H-300 electron microscope in Thailand and a Hitachi H-500 electron microscope in Japan. For thin sections, leaf samples were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) for 2 hr. They were washed in cold buffer and then fixed with 2% osmium tetroxide in the same buffer for 3 hr, dehydrated in an acetone series, and embedded in Epon 812. Thin sections were cut transversely from samples by using a diamond knife mounted on a Sorvall MT2-B ultramicrotome. The sections were stained with uranyl acetate and lead citrate.

Serum-specific electron microscopy. The clumping technique of immunoelectron microscopy (5) was used to study serologic relationships between the rice gall dwarf agent and rice dwarf virus. Antiserum against rice dwarf virus (titer 1:2,000 in the precipitin ring test) was used at dilutions of 1:20, 1:200, and



Figs. 1 and 2. (1) Healthy rice plant (right) and plant with rice gall dwarf disease (left). **(2)** Galls on plant with rice gall dwarf disease.

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1:2,000 in 0.1 M phosphate buffer (pH 7.0). The virus-serum mixture was stained with 2% neutral phosphotungstic acid and examined under an electron microscope.

RESULTS

Symptomatology. About 1 mo after inoculation of rice seedlings at the one- to three-leaf stage, marked dwarfing (Fig. 1), small galls along the leaf blades and sheaths (Fig. 2), and dark green discoloration and twisting of some leaf tips appeared. Some plants died in the greenhouse in the later stages of infection. Symptoms were milder when plants were inoculated at the 8- to 10-leaf stage. Dwarfing and discoloration were the characteristic symptoms of diseased plants in the field.

Galls or vein swellings appeared on the undersurfaces of leaf blades (Fig. 2) as well as the outer sides of leaf sheaths. Initially light green and rather translucent, galls later became white. Galls were more abundant in plants infected early, and some leaves had more than 10 galls. Galls varied from 0.4 to 8 mm in length, with most less than 2 mm long, and from 0.4 to 0.5 mm in width.

Vector transmission. Of 128 individual *N. nigropictus* tested, 62 transmitted the rice gall dwarf agent. As shown in Table 1, the leafhoppers transmitted the disease agent in a persistent manner and often retained infectivity for their entire life spans. The minimum latent period in the insect was 11 days.

Electron microscopy. Polyhedral particles about 65 nm in diameter were always observed in dip preparations of leaves and roots of diseased plants (Fig. 3A); they were more abundant in preparations from galls than from other leaf tissues. Spherical particles had visible capsometric structures; empty particles were also observed.

Study of ultrathin sections revealed that similar particles occurred in the cytoplasm of phloem cells of diseased plants (Fig. 3B).

Serology. Grids prepared with extracts from leaves infected with rice dwarf virus and rice dwarf virus antiserum consistently showed large numbers of clumped particles even at 1:2,000 dilution, but only a few dispersed particles were observed on grids prepared with extracts from leaves infected with rice gall dwarf and rice dwarf virus antiserum at the three dilutions.

Table 1. Serial transmission of the causal agent of rice gall dwarf by *Nephotettix nigropictus*[a]

Insect number	Sex	Days after virus acquisition started: 1–4	4–8	8–11	11–15	15–18	18–22	22–25	25–29	29–32
1	F	−	−	−	−	+	+	+	+	*[b]
2	F	−	−	−	−	−	+	*	+	+
3	M	−	−	−	−	−	+	+	+	+
4	M	−	−	−	+	+	+	*	+	+

[a] Test insects at the early instars were placed on diseased source plants for 1-day acquisition feeding. Inoculation test was conducted for 32 days after acquisition access.
[b] * = plant death.

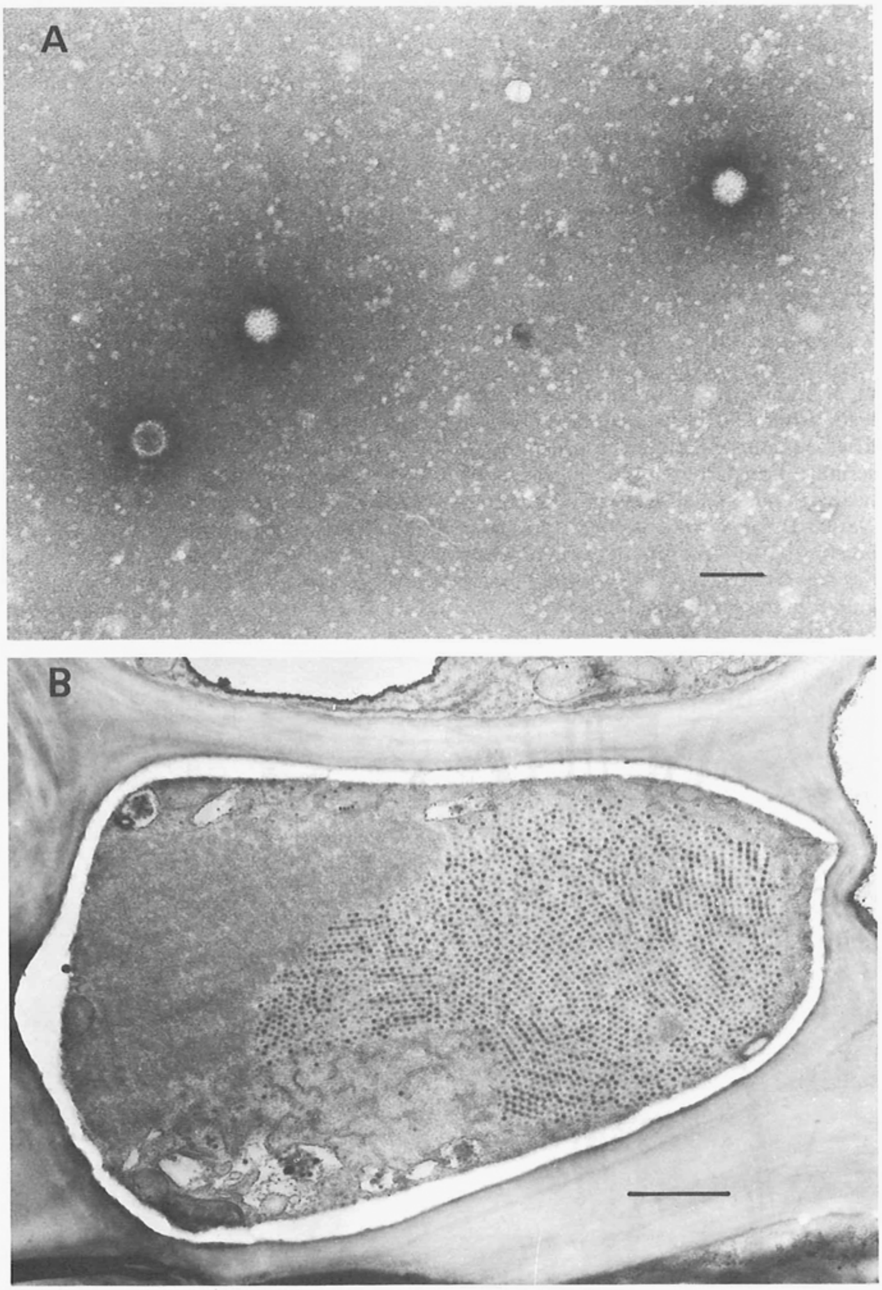

Fig. 3. Electron micrographs of viruslike particles in **(A)** a dip preparation from a plant with rice gall dwarf disease (one particle is empty); scale bar represents 100 nm; and **(B)** a phloem cell of a plant with rice gall dwarf disease; scale bar represents 1,000 nm.

DISCUSSION

Of the known virus and viruslike rice diseases, black-streaked dwarf (7) and ragged stunt (1,4) produce galls or vein swellings. Black-streaked dwarf galls appear on culms, outer sides of leaf sheaths, and undersurfaces of leaf blades; however, the galls are gray or dark brown, grow larger than those of rice gall dwarf, and appear at later growth stages of rice. Symptoms of ragged stunt include elongated vein swellings on the upper portions of leaf sheaths, ragged leaves, and nodal branches, which do not occur in rice gall dwarf. Rice gall dwarf resembles rice dwarf virus in its dark green discoloration, but lacks rice dwarf disease's characteristic symptom of yellowish white specks along the veins of leaf blades (3).

Polyhedral particles about 65 nm in

diameter with capsometric structure similar to that of the plant reovirus group are assumed to cause rice gall dwarf. Of the three plant reoviruses known to cause rice diseases—rice dwarf virus (3), black-streaked dwarf virus (6), and ragged stunt virus (2)—only rice dwarf virus has a visible capsometric structure. Immunoelectron microscopy indicated that the particles associated with rice gall dwarf are not serologically related to rice dwarf virus.

From these results, we conclude that rice gall dwarf is a new virus disease.

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LITERATURE CITED

1. HIBINO, H., M. ROECHAN, S. SUDARISMAN, and D. M. TANTERA. 1977. A virus disease of rice (kerdil hampa) transmitted by brown planthopper, *Nilaparvata lugens* Stal. in Indonesia. Cont. Centr. Res. Inst. Agric. Bogor 35:1-15.
2. HIBINO, H., N. SALEH, and M. ROECHAN. 1979. Reovirus-like particles associated with rice ragged stunt diseased rice and insect vector cells. Ann. Phytopathol. Soc. Jpn. 45:228-239.
3. IIDA, T. T., A. SHINKAI, and I. KIMURA. 1972. Rice dwarf virus. Commonw. Mycol. Inst./Assoc. Appl. Biol. Descriptions of Plant Viruses, No. 102.
4. LING, K. C., E. T. TIONGCO, and V. M. AGUIERO. 1978. Rice ragged stunt, a new virus disease. Plant Dis. Rep. 62:701-705.
5. MILNE, R. G., and E. LUISONI. 1977. Rapid immune electron microscopy of virus preparation. Methods in Virol. 6:265-281.
6. SHIKATA, E. 1977. Rice black-streaked dwarf virus: Its properties, morphology and intracellular localization. Virology 77:826-842.
7. SHINKAI, A. 1966. Studies on insect transmission of rice virus diseases in Japan. Natl. Inst. Agric. Sci. Jpn., Bull. Ser. C, 14:1-112. (In Japanese, English summary)