RICE DWARF VIRUS

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Abstract

Polyhedral particles about 70 nm in diameter were detected in a dipped preparation under the electron microscope from rice plants with symptoms including stunting of the plants and appearance of white chlorotic specks on foliage in Nepal. Similar particles were also observed in ultra-thin sections of infected leaves. In agar gel diffusion test using antiserum to rice dwarf virus, a precipitin line was formed with sap from infected tissues. However, no such a line was observed with the sap from healthy tissues. These results together with the symptomatology confirm that the disease was caused by rice dwarf virus.

Nephotettix nigropictus the only known vector of rice dwarf virus in Nepal, occurred prevalently on weeds and paddy fields in the late growing season of rice in the hill area of Nepal such as Kathmandu.

Latex agglutination method was applied to detect virus antigen of rice dwarf disease in both plants and insect vectors.

1. Introduction

The characteristic symptoms of rice dwarf disease are stunting of the plant and appearance of white chlorotic specks on foliage (Iida, *et al.*, 1972). The virus is polyhedral, about 70 nm in diameter and is transmitted by leafhoppers in a persistent manner. Occurrence of the disease has been restricted to Japan, Korea and China. Recently, a similar disease transmitted persistently by *Nephotettix nigropictus,* was reported to occur in Kathmandu Valley, Nepal (John, *et al.*, 1979). The present work was carried out to study the disease by electron microscopy and serology, and to survey insect vectors of the disease in Nepal. The studies on the application of serological methods for the detection of virus antigen in infected plants as well as in viruliferous individual insects are also included.

2. Identification of the virus in Nepal

1) Electron microscopy

Rice plants with the disease were collected in Kathmandu Valley, Nepal in 1978 and the disease was maintained by successive inoculations to young seedlings of rice (cv. Taichung Native 1) using *Nephotettix nigropictus* in a greenhouse. Plants with white chlorotic specks on the foliage at the tillering stage were used for the examination. Rice plants infected with rice dwarf virus (RDV) maintained in the Institute for Plant Virus Research, Japan were used for comparison. Leaf samples were dipped in 2% neutral phosphotungstic acid and were examined under a Hitachi H-500 electron

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microscope. For thin sectioning, leaf samples were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) for 2 hr. After being washed with cold buffer, they were post-fixed with 2% osmium tetroxide in the same buffer for 3 hr. After dehydration in an acetone series, they were embedded in Epon 812. Thin sections were cut transversely from samples using a diamond knife mounted on a Sorvall MT2-B ultramicrotome. The sections were stained with uranyl acetate and lead citrate.

Polyhedral particles about 70 nm in diameter were abundantly observed in dipped preparations of leaves from the diseased plants. The size and structure of the particles were similar to those observed in RDV in Japan. Electron micrographs of sections of affected leaves revealed the presence of polyhedral particles scattered in the cytoplasm, in the cells of chlorotic tissues (Fig. 1). These particles were uniform in size and shape approximately 70 nm in diameter with central electron dense cores. No such particles were observed in the nuclei, mitochondria and chloroplasts of the cells. Such particles were not found in tissues of healthy rice leaves. The size, shape and distribution of the particles in the cells were similar to those found in tissues affected with RDV in Japan.

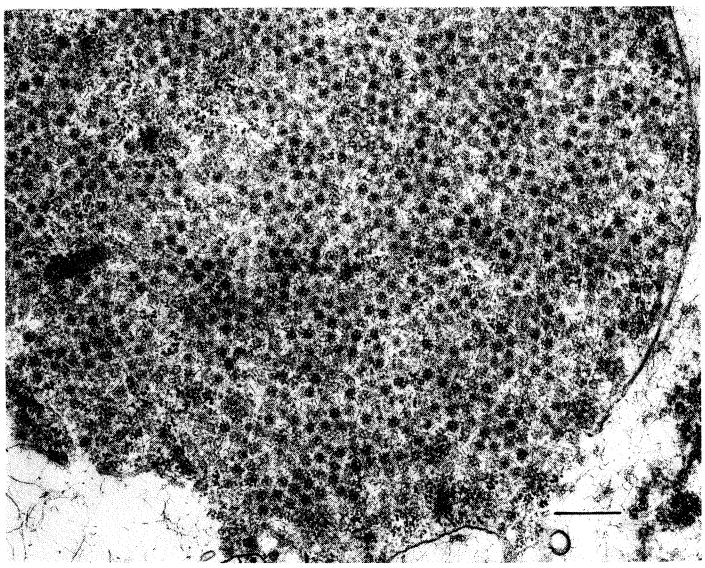

Fig. 1. Electron micrograph of an ultra-thin section of rice plant infected with rice dwarf virus. Bar represents 300 nm.

2) Serological reaction

Serological tests were performed by the double gel diffusion method. The gel consisted of 0.8% agar in 0.1 M phosphate buffer pH 7.6, in the presence of 0.85% sodium chloride, 0.001 M ethylenediaminetetraacetic acid and 0.05% sodium azide. The diameter of the wells was 8mm. The distance from the edge of the well for antigen and that for the antiserum was 5 mm. Infected leaf tissues were crushed in 10-fold amount of 0.1 M phosphate buffer, pH 7.0 containing 0.85% sodium chloride (PBS), and were passed through four-folded gauze. Healthy leaf tissues were processed

in the same manner. Antiserum against RDV (titer 1 : 2,000 in precipitin ring interface test) was used at a dilution of 1 : 100 in PBS. The agar gel plates were incubated for 2 days at room temperature.

Precipitin line was observed with sap from infected tissues, however, no such a line was observed with the sap from healthy tissues.

3. Occurrence of the vector of rice dwarf virus in Nepal

In Nepal, *Nephotettix nigropictus* is the only known vector of RDV. During the course of the present survey, we could also detect the species and no *N. cincticeps,* an efficient vector species in Japan, was observed though the temperature conditions of the hill area of Nepal resemble those in Japan. As for the vector species population in the country, it was shown that the population of *N. nigropictus* was much higher in the hill area such as Kathmandu, whereas in the Terai plain, a low altitude area, the population was low. In the hill area, however, a high population density was recognized on weeds in grasslands and ditches whereas the populations in the paddy fields were low; 43 - 217 individuals including adults and nymphs on weeds and 7 - 34 individuals on rice (20 strokes of net sweeps).

According to Pradhan (1980), *N. nigropictus* in Kathmandu prevails from April to November and its peak occurrence appears in August or September (Fig. 2). Based on the symptoms of the disease on affected rice plants, though the incidence of the rate of diseased hills was less than 1%, virus infection appeared to occur at the early tillering stage of rice.

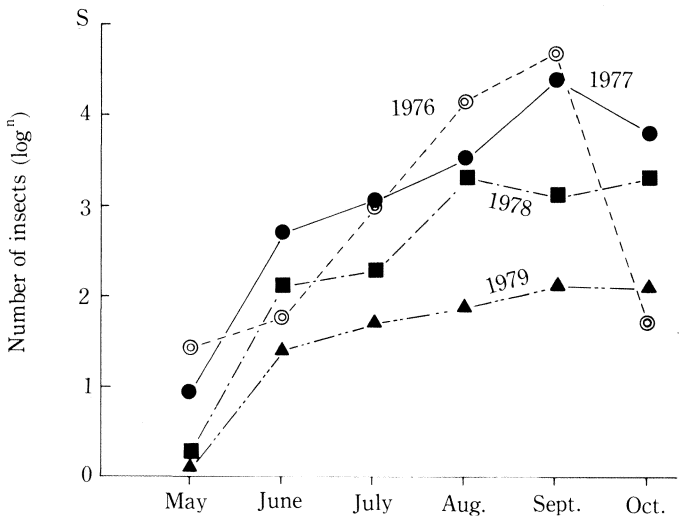

Fig. 2. Seasonal population fluctuation of *Nephotettix nigropictus* in light trap in Khumal Tar, Lalitpur, Nepal (Pradhan, 1980).

4. Serological detection

1) Detection from plants

Latex flocculation test (LF) was employed to detect RDV antigen in infected rice plants and viruliferous insects. The procedure of Bercks and Querfurth (1971) was employed for latex sensitization with serum. The fraction which precipitated when the serum was 50% saturated with ammonium sulphate was used. Phosphate buffer (0.01M), pH 7.0 containing 0.01M $MgCl_2$ and 0.1% Tween 20 was used to homogenize and to dilute virus-infected samples. The homogenate was centrifuged at 3,000 g for 10 min. Serial twofold dilutions of the supernatant were made with the buffer and 0.1 ml of each dilution was placed in a small test tube (11 × 75 mm). Two drops (ca. 0.1 ml) of sensitized latex suspension were then added to each tube and the tubes were shaken for 20 min on a shaker (150 oscillations per minute) at 30°C. For the control, sensitized latex was mixed with (i) buffer, (ii) sap from healthy plants, and (iii) sap containing viruses unrelated to antiserum used to sensitize the latex. The latex particles formed aggregates in a positive reaction, or remained as a milky suspension in a negative one. Doubtful readings were checked under a microscope (× 200).

As shown in Table 1, RDV antigen was detected by LF. Maximum titers of the antigens were obtained 30-60 days after inoculation and little change in titer occurred during this period.

Table 1. Detection of RDV antigen in rice plants at various times after inoculation by LF test[a] (adapted from Omura *et al.*, 1984).

Exp. no	Days after inoculation					
	20	30	40	50	60	70
I	1,280[b]	5,120	5,120	5,120	5,120	2,560
II		5,120	5,120			

a) No flocculation was observed in healthy controls tested.
b) Reciprocal of the highest dilution with positive reaction.

2) Detection from insects

Individual insects were crushed in 0.4 ml buffer and used either directly or after centrifugation at 8,000 g for 10 min. Crude insect macerates (0.1 ml) or supernatants after centrifugation were used for the detection of viruses after twofold serial dilutions. Ten insects with a positive reaction were used for each experiment. For the negative reactions, 30 insects were used.

As shown in Table 2, virus antigen was detected from individual insect vectors and the titers did not vary significantly after the 15th day of acquisition access.

Table 2. Detection of RDV antigen in viruliferous *N. nigropictus* at various times after acquisition access by LF test[a] (adapted from Omura *et al.*, (1984)

	Days after acquisition access started				
Sex	5	10	15	25	35
Female			32	64	32
Nymph	0[b]	8[c]			
Male			16	32	16

a) No flocculation was observed in healthy controls tested.
b) Negative reaction.
c) Reciprocal of the highest dilutions of crushed insects as mentioned in the text.

5. Discussion

The size and structure of the particles and particle-plant cell relationships were quite similar to those of RDV in Japan. Furthermore, the sap of the infected tissues reacted with the antiserum against RDV. These results, together with the symptomatology and transmission tests (John *et al.*, 1979), led to the conclusion that the disease occurring in Nepal is rice dwarf disease.

There is no clue to understand the origin of rice dwarf disease in Nepal, though it is very interesting from the etiological point of view. Virus perpetuation may be possible in rice stubbles after harvest which remain alive to overwinter in the hill area in Nepal. Furthermore, the virus is considered to overwinter in *N. nigropictus,* an efficient vector of the virus in Nepal, because the virus can be transmitted from viruliferous females to their progenies congenitally (Nasu, 1963). These facts made us assume that the virus can easily overwinter and last long once the disease invades the area. The disease may become important due to the high transmission ability of *N. nigropictus* (Nasu, 1963), which is predominant in the area.

Only 20 min were required to detect the virus antigen of RDV. Working time was less than one min for one sample with this method. From these results, LF proved to be an excellent and convenient serodiagnostic method to detect virus antigen of RDV in both plant and insect vectors.

Acknowledgements

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