TARC Note

Identification of Rice Dwarf Virus in Nepal

The characteristic symptoms of rice dwarf disease are stunting of the plant and appearance of white chlorotic specks on foliage[2]. 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[1,2]. Recently, similar disease transmitted persistently by *Nephotettix nigropictus* was reported to occur in Kathmandu Valley, Nepal[4]. The present work was carried out to confirm the disease by electron microscopy and serology.

This work was done as a part of the collaborative research project on virus diseases of rice and legumes in the tropics supported by the Tropical Agriculture Research Center, Japan.

Materials and methods

The disease was isolated from a rice plant collected in Kathmandu Valley, Nepal in 1978, and was maintained successively by inoculating it to young seedlings of rice (cv. Taichung Native 1) using *N. nigropictus* in a greenhouse. Plants with white chlorotic specks on foliages at tillering stage were used for the examination.

Electron microscopic methods were essentially the same as previously reported[6]. Rice plants infected with rice dwarf virus (RDV) maintained in the Institute for Plant Virus Research, Japan, were used for comparison.

Serological test was performed by double gel diffusion method. The gel was 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 wells was 8 mm. The distance from the edge of the well for antigen and that for 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.

Results and discussions

By electron microscopy, polyhedral particles about 70 nm in diameter were abundantly observed in dipped preparations of leaves from 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 polyhedral particles scattered in the cytoplasm in cells of chlorotic tissues (Plate 1-A). These particles were uniform in size and shape approximately 70 nm in diameter with central electron dense cores. As shown in Plate 1-B, the particles were frequently arranged in rows and/or large mass of aggregates. No such particles were observed in nuclei, mitochondria and chloroplasts in 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.

As to serology, precipitin line was observed with sap from infected tissues, however, no such a line was observed with the sap from healthy tissues. The size and structure of the particles and particle-plant cell relationship were quite similar to those of RDV in Japan[3]. Furthermore, the sap of the infected tissues reacted with the antiserum against RDV. These results, together with the symptomatology and transmission tests[4] led to the conclusion that the disease occurring in Nepal is rice dwarf disease.

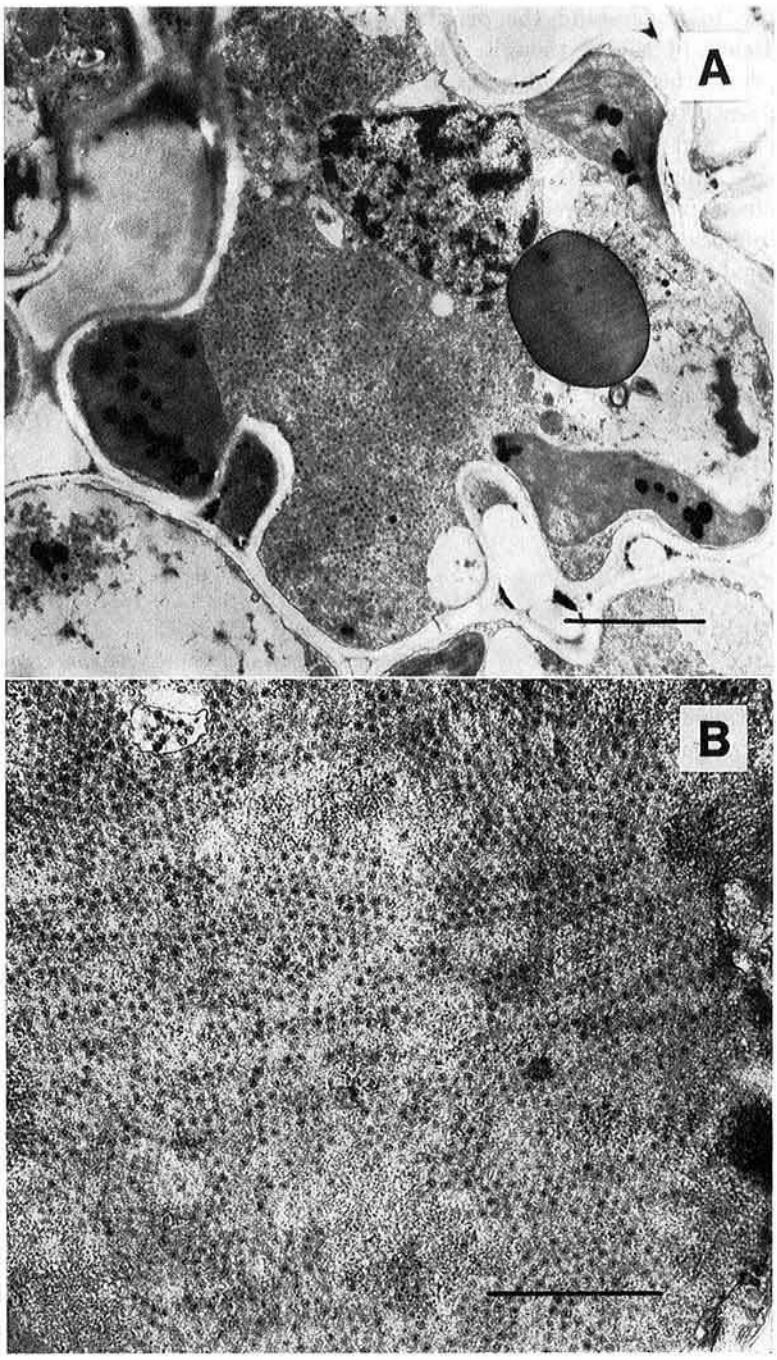

Plate 1. Electron micrographs of virus particles

A: Parenchyma cell of a rice plant infected with rice dwarf disease. Bar represents 2,000 nm.

B: Large mass of agregate. Bar represents 1,000 nm.

There is no key to understand the origin of rice dwarf disease in Nepal, though it is very interesting from the etiological point of view. Rice stubbles after harvest do not die even in dry season and may let the disease overwinter in some hill area in Nepal. Furthermore, the virue is considered to overwinter in *N. nigropictus*, an efficient vector of the virus in Nepal, because the virus can transmit from viruliferous females to their progenies congenitally[5]. These facts made us to assume without difficulty that the virus can easily overwinter and last long once the disease invaded into the area. The disease may become important due to the high transmission ability of *N. nigropictus*[5], being predominant in the area.

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