

Small Bacilliform Particles Associated with Penyakit Habang (Tungro-like) Disease of Rice in Indonesia

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ABSTRACT

Small bacilliform particles of about 25×140 nm were found in the phloem cells of rice plants infected with penyakit habang disease in Indonesia. No spherical particles of 30 nm in diameter, such as rice tungro virus, were detected in these preparations. Necrosis of phloem cells was also observed in the diseased tissues. The bacilliform particles were found not

only in the diseased rice plants collected from paddy fields, but also in the plants inoculated by means of viruliferous leafhoppers, *Nephotettix virescens* Distant. Transmission appeared to be a nonpersistent type.

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Penyakit habang is one of the important virus diseases of rice plants in Indonesia. The disease is characterized by a slight-to-severe stunting of the infected plant. The leaf blades exhibit yellow-orange to orange-red discoloration usually starting from the leaf tip and gradually advancing downward. The younger leaves generally have a mottled appearance. Very small rusty colored necrotic spots may be found in the discolored area of the older leaves.

Infected plants, especially those of a susceptible cultivar produce few tillers. The degree of reduction in tillering appears to depend on the stage of plant growth during which infection took place. The earlier infection took place the more the tillering capacity of the plant was reduced. In the later stages of infection, almost all leaves turn yellow to orange-red. The infected plants were severely stunted, and the root growth was very poor.

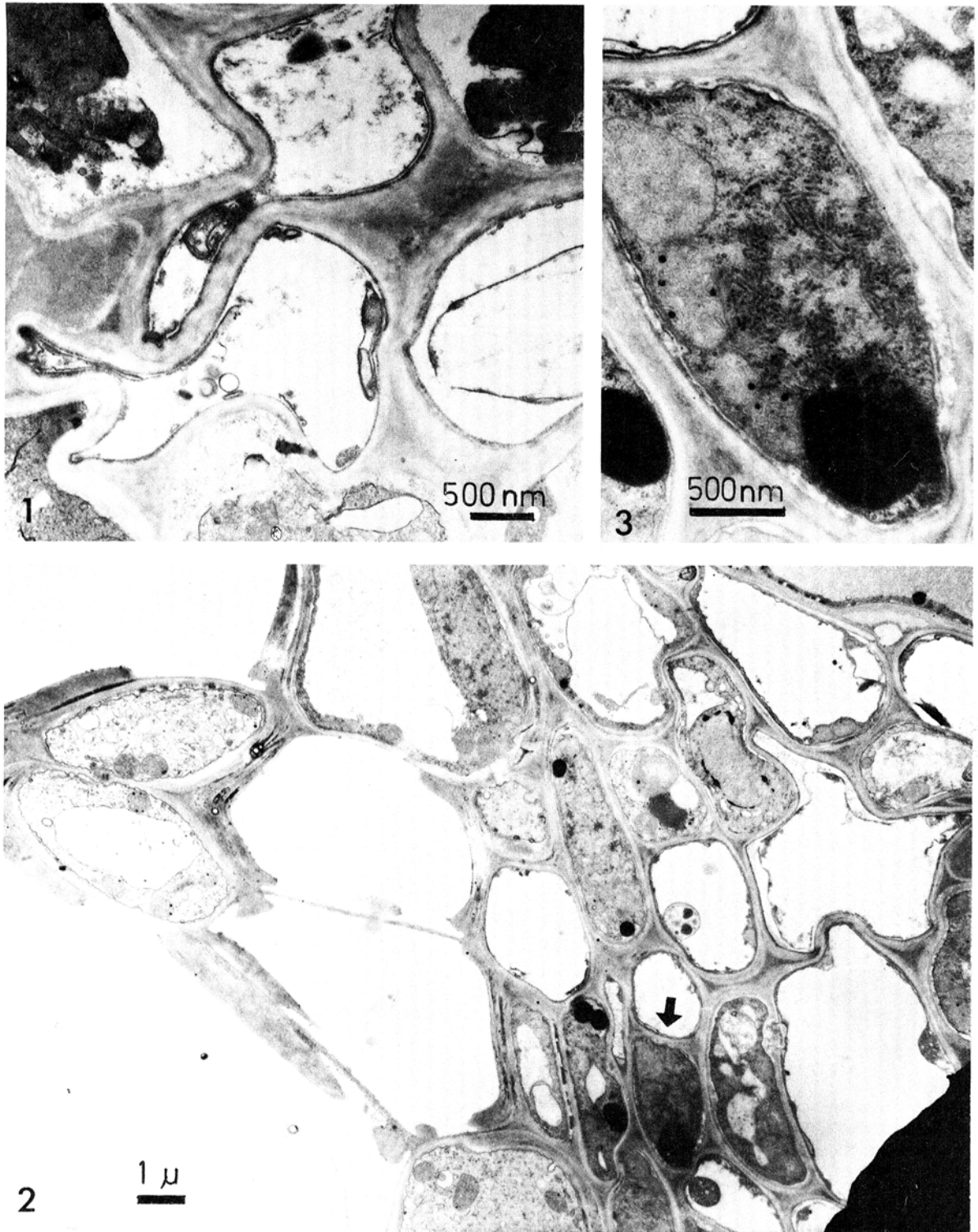
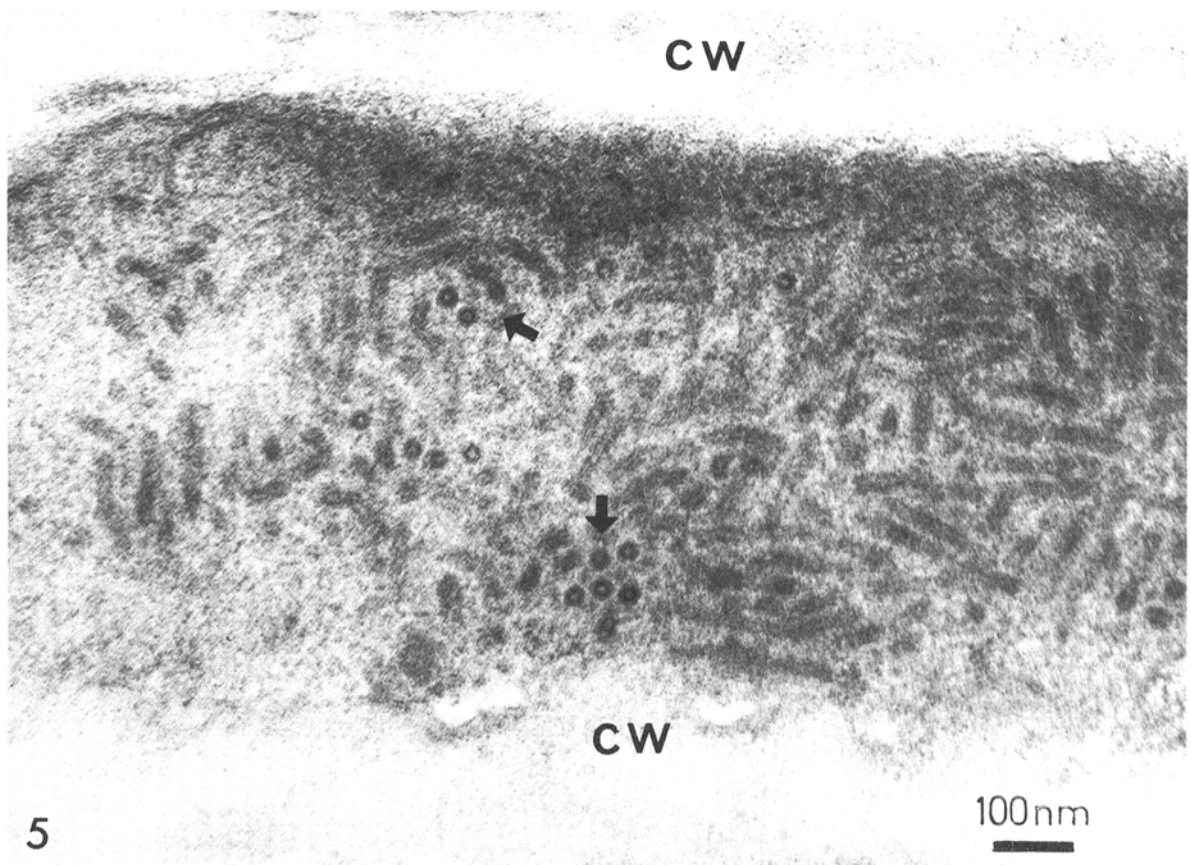
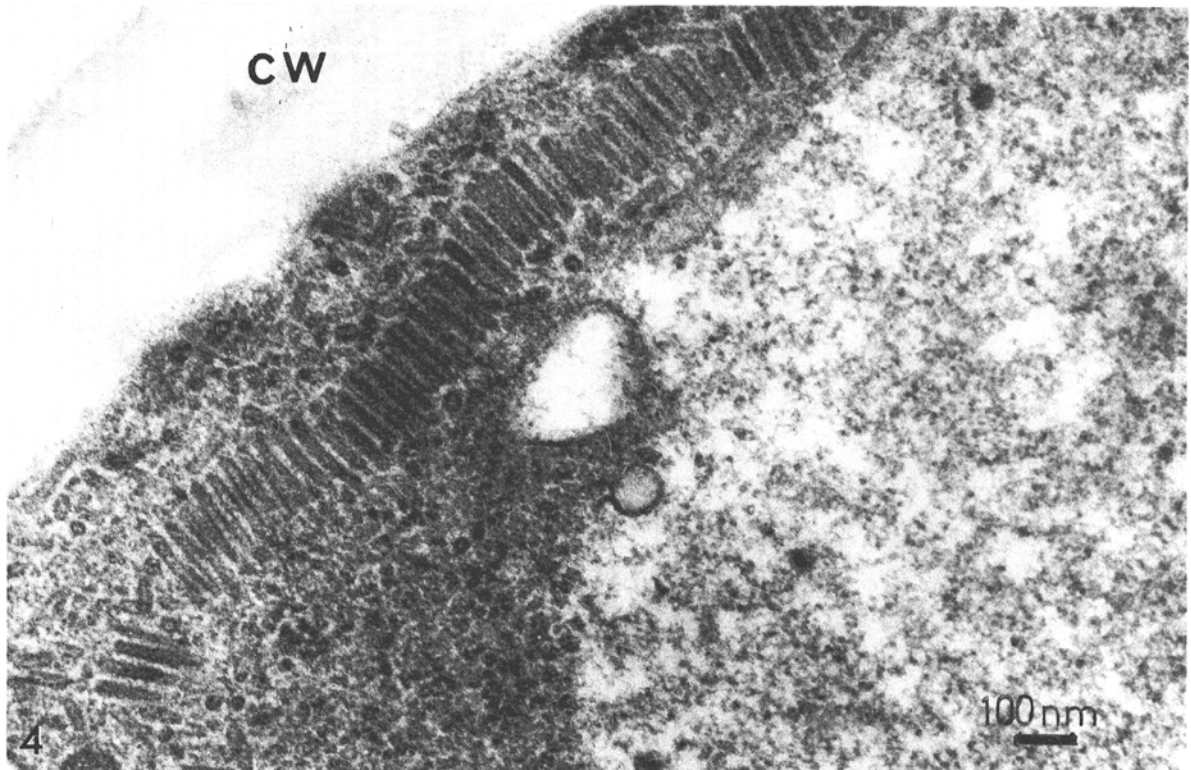


Fig. 1-3. Ultrathin sections of rice leaves infected with penyakit habang disease in Indonesia. **1)** Portion of phloem cells. Two companion cells in the upper part show necrosis. Sieve elements show some deformations ($\times 20,000$). **2)** Portion of a larger vascular bundle. A companion cell (arrow) contains the small bacilliform particles ($\times 6,600$). **3)** Enlarged section of the cell which indicated by arrow in Fig. 2. The small bacilliform particles are randomly dispersed in the cytoplasm ($\times 30,000$).

Fig. 4-5. **4)** A companion cell containing an aggregate in which the particles are arranged side-by-side and formed a single layer ($\times 82,400$). **5)** A sieve element containing the particles. Arrows show the cross-sections of the particle ($\times 103,000$). CW = cell wall.



These characteristics are similar to those of tungro disease in the Philippines, yellow-orange leaf disease in Thailand, and penyakit merah disease in Malaysia.

In this paper, we report the results of an electron microscopic investigation of diseased tissues of the Indonesian penyakit habang disease. Some cellular changes associated with the disease and the association of small bacilliform virus-like particles with the disease are described.

MATERIALS AND METHODS.—The virus isolates were collected from South Sulawesi and South Kalimantan, Indonesia. Young rice plants (*Oryza sativa* L. 'Taichung Native 1') were inoculated by means of viruliferous leafhoppers, *Nephotettix virescens*, after a 1-day acquisition feeding on penyakit habang-infected plants. The insects were allowed to feed 1-6 days before they were removed. After the symptoms developed, small pieces of diseased leaves were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0, and sent to Japan by air. Original sources of diseased rice leaves and healthy controls were also examined. These preparations were postfixed for 4 hours in 2% osmium tetroxide in 0.1 M phosphate buffer, pH 7.0. Fixed materials were dehydrated in acetone and embedded in Epon 812. Ultrathin sections were cut on a Porter-Blum MT-2 ultramicrotome, with a diamond knife. The sections were doubly stained with uranyl acetate and lead citrate. Observations were made with a Hitachi HU-11 B electron microscope at 75 kV.

RESULTS.—In an extensive examination of the sections of penyakit habang-infected rice leaves, a few phloem companion cells within the vascular bundles showed necrosis (Fig. 1), but no spherical particles 30 nm in diameter were found. However, they contained small bacilliform particles of about 25×140 nm (Figs. 2, 3) which were not found in healthy rice plants. These particles were confined to the phloem cells. They were usually randomly dispersed in the cytoplasm of companion cells or in sieve elements, but occurred sometimes as an aggregate in which the particles were arranged side-by-side and formed a single layer without any apparent association with cellular membranes (Fig. 4). Cross-sections of these particles had a core of 9 nm in diameter, surrounded by a high-density zone of 4.5 nm and medium-density outer zone of 3.5 nm (Fig. 5).

DISCUSSION.—Small bacilliform particles were found in all the preparations of diseased rice examined. No other particle, such as distinct spherical particles found in rice infected with yellow-orange leaf virus in Thailand (7) and with a tungro strain in West Pakistan (2) could be found in these Indonesian preparations. Healthy rice plants, including those fed on by nonviruliferous leafhoppers, did not contain these particles. In addition, the particle morphology, the localization in phloem cells, and the insect transmission suggest the viral nature of the penyakit habang disease of rice in Indonesia.

The small bacilliform particles found in penyakit habang-infected rice leaves were restricted to phloem cells and caused some necrosis in these cells. It is possible that the association of the disease with the phloem inhibits the

translocation of elaborated food materials and induces an accumulation of starch in leaf blades which ultimately results in tungro-like disease symptoms such as stunting of plants, discoloration of the leaves, etc. Spherical virus particles were also found in the phloem cells of rice plant infected with yellow orange leaf virus in Thailand (7). It is possible that penyakit habang disease in Indonesia and yellow orange leaf disease in Thailand are caused by different viruses, both of which invade phloem cells and cause similar symptoms in rice plants. The bacilliform and the spherical particles are transmitted by the same species of leafhopper, *N. virescens*. The possibility of double infection should be considered in some cases. The authors believe that future clarification of the causal agent of the tungro group of rice diseases in southeast Asia needs an international cooperative effort.

The symptoms of rice transitory yellowing disease in Taiwan (8) are similar to those of the penyakit habang disease. However, the two diseases differ in the size of virus particles, and in the vector relationships.

The small bacilliform particles described in this paper are similar in morphology to cacao swollen shoot virus (1), small bacilliform particles of raspberry mosaic (veinbanding) (4), citrus leprosis-associated particles (5), Phalaenopsis virus (6), and bacilliform particles found in internal brown spot of yam (3). However, some differences exist between these viruses (or particles) and the Indonesian penyakit habang virus-like particles in particle size, in the type of transmission, and in the relations to cells and tissues.

The small bacilliform particles associated with penyakit habang disease do not seem to belong in the Rhabdo virus group, because they are not associated with cellular membranes, have neither envelope nor striations, and are smaller in size.

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