# 8. INTERRELATIONSHIP AMONG WAIKA DISEASE, TUNGRO AND OTHER SIMILAR DISEASES OF RICE IN ASIA

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## Introduction

Rice tungro disease is one of the important virus diseases of rice plant in South and Southeast Asian countries. The disease is characterized by a slight-to-severe stunting of the infected plant. The leaf blades exhibit yellow-orange to orange-red discoloration usualy starting from the leaf tip and gradually advancing downwards. Infected plants produce few tillers and the root growth is very poor.

Similar diseases are present under different names in Asia such as yellow orange leaf in Thailand (Lamey *et al.*, 1967), penyakit merah in Malaysia (Singh, 1969), penyakit habang in Indonesia (Saito *et al.*, 1975) and leaf yellowing in India (John, 1968). These diseases are thought to fall into a group mainly on the basis of their symptomatology, varietal resistance, and manners in transmission by vectors.

Spherical particles, 30–33 nm in diameter, with a sedimentation coefficient of 175 S were purified from diseased rice plants infected with severe strain of rice tungro virus (Gálvez, 1968).

A new virus disease of rice plants, waika disease, has occurred since 1971 in Japan. The causal agent which was transmitted by *Nephotettix cincticeps,* and *N. virescens,* seemed to be non-persistent in the vectors (Yokoyama and Sakai, 1975, Nishi *et al.*, 1975). Spherical particles, 30 nm in diameter, were found in the diseased plants (Nishi *et al.*, 1975, Doi *et al.*, 1975).

The characteristic symptoms on diseased rice plant are stunting of the plants and discoloration of the leaves. However, the symptoms, especialy degrees of discoloration of leaves, are milder than those of tungro disease.

Recently small bacilliform particles of about 25x140 nm were found in the phloem cells of rice plant infected with penyakit habang disease in Indonesia (Saito *et al.*, 1975).

It is important to clarify the interrelationship among waika disease, tungro and other similar diseases of rice in Asia.

## Spherical particles associated with the diseases

Spherical particles, ranging from 30 to 33 nm in diameter, were seen in purified preparations of rice tungro virus (Gálvez, 1968) and yellow orange leaf virus (Saito, 1971). The same particles were observed in ultrathin sections in leaf cells of rice plant infected with rice tungro virus in East Pakistan (Gálvez *et al.*, 1971) and in phloem cells of rice plant infected with yellow orange leaf virus (Saito, 1971).

More recently, spherical particles were found in phloem parenchyma and companion cells infected with severe strain of rice tungro virus. The most relevant alternations found in these cells were viroplasms and tubular inclusions (Favali *et al.*, 1975).

In the case of waika disease, in thin section experiments, spherical particles together with viroplasms were observed in phloem cells (Doi *et al.*, 1975).

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In ultrathin section experiments of the preparations collected from various countries of Southeast Asian countries, spherical particles were observed in the phloem cells of rice plant infected with tungro (S, M, and T strains), yellow orange leaf, penyakit merah, penyakit habang, and waika diseases (Saito *et al.*, 1976).

The characteristics of ultrastructural alternations in these cells which are thought to be induced by the spherical particles are as follows;

1. Localization of spherical particles in phloem cells
2. Presence of viroplasms
3. Crystalline aggregates of virus particles
4. Necrosis of the phloem cells
5. Accumulation of starch in chloroplasts of mesophyll parenchyma cells

## Small bacilliform particles associated with the diseases

In an examination of the sections of penyakit habang-infected rice leaves, a few phloem companion cells within the vascular bundles showed necrosis and they contained small bacilliform particles of about 25x140 nm 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 membrances.

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. These particle morphology, especialy the cross-section views, are thought to be characteristic for virus particle.

The small bacilliform particles associated with penyakit habang disease do not seem

**Table 1. Small bacilliform particles found in plants**

| Name of disease | Size of particle | Vector | Source |
|---|---|---|---|
| Cacao swollen shoot | 121–130×28 nm | Mealybug | Brunt et al., 1964 |
| Internal brown spot of yam | 130×29 nm | | Harrison & Roberts, 1973 |
| Citrus leprosis | 100–120×40 nm | Mite | Kitajima et al., 1972 |
| Leaf spot of *Phalaenopsis lueddemanniana* | 110×30 nm | | Lesemann & Begtrup, 1971 |
| Orchid fleck | 100–140×32–35 nm | | Chang et al., 1976 |

**Table 2. Kinds of particles observed in tungro and similar diseases in thin-section experiment**

| Name of disease | Country | Particle | |
|---|---|---|---|
| | | Spherical | Small bacilliform |
| Tungro (S) | Philippines | + | + |
| (T) | | + | + |
| (M) | | + | |
| Yellow orange Leaf | Thailand | + | + |
| Penyakit merah | Malaysia | + | + |
| Penyakit habang | Indonesia | + | + |
| Waika | Japan | + | |

to belong to the Rhabdo virus group because they are not associated with cellular membranes, have neither envelope nor striations, and are smaller in size. The small bacilliform particles are similar in morphology to cacao swollen shoot virus (Brunt, 1 970), small bacilliform particles of raspberry mosaic (Jones *et al.*, 1974), citrus leprosis-associated particles (Kitajima *et al.*, 1972), Phalaenopsis virus (Lesemann & Begtrup, 1971), bacilliform particles found in internal brown spot of yam (Harrison & Roberts, 1973), and orchid fleck virus (Chang *et al.*, 1976).

However, some differences will be found between these viruses (or particles) and the Indonesian penyakit habang virus-like particles, in particle size, type of transmission, and relations to cells and tissues.

The small bacilliform particles were also observed in thin section experiments of diseased plants infected with tungro (S and T strains) in the Philippines, yellow orange leaf in Thailand, and penyakit merah in Malaysia. However, diseased rice plants infected with waika in Japan, some isolates of yellow orange leaf in Thailand, and M strain of tungro in the Philippines did not contain the small bacilliform particles.

The results of the experiment in which the presence of the two kinds of particles, spherical and small bacilliform was investigated, among tungro and similar diseases in Asia by thin section methods, are summarized in Table 2.

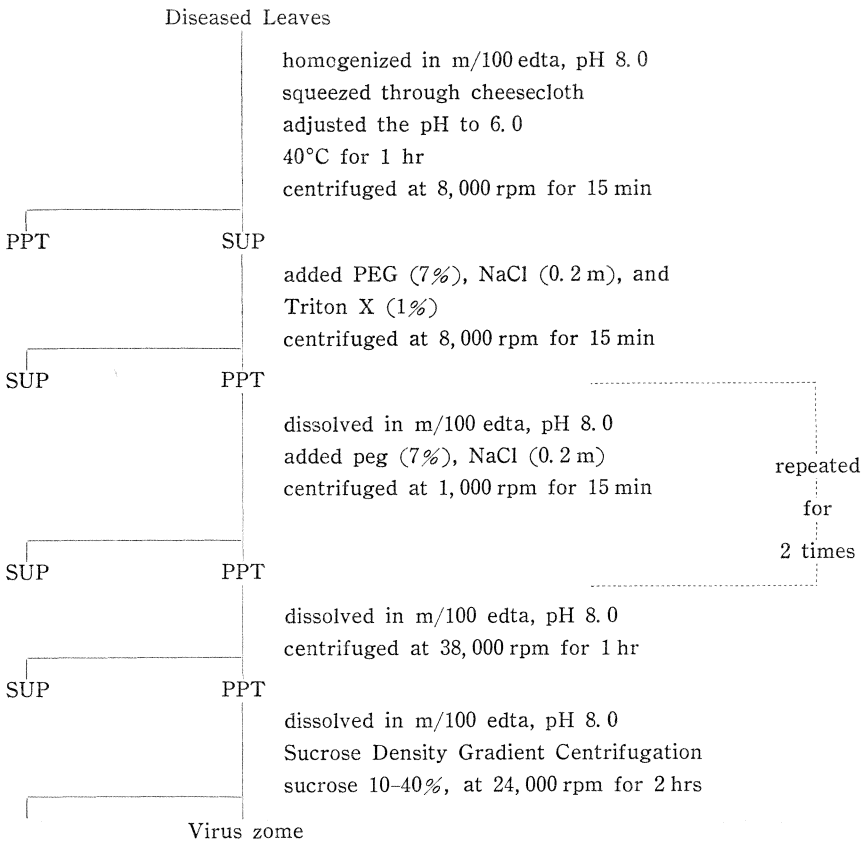


**Fig. 1. Purification procedure for rice waika virus and penyakit habang virus**

## Purifications and serology of rice waika virus and two kinds of particles of penyakit habang

Rice waika virus was purified by heating leaf extracts in 0.01 m EDTA, pH 6.0, at 40°C for 1 hour followed by PEG precipitation and differential centrifugation and by rate zonal centrifugation in sucrose density gradient (Fig. 1). The particles were spherical, 30 nm in diameter, with a sedimentation coefficient of 173 S.

Two kinds of particles, spherical and small bacilliform, were purified from penyakit habang-infected rice plants after same purification procedure for rice waika virus. The spherical particles from penyakit habang had same morphology and sedimentation coefficient as waika virus. The shape, size, and sedimentation coefficient of both particles coincide with those of rice tungro virus reported by Gálvez.

Antisera were prepared by immunizing rabbits with purified preparations of rice waika virus or spherical particles of penyakit habang. Both antisera reacted against both viruses in complement fixation tests and showed the same titer of sera in homologus and heterologus combinations. In double gel-diffusion test, both of the antisera reacted with both of the viruses and showed fused single precipitin line. Purified preparation of penyakit habang, containing both spherical and small bacilliform particles, was mixed with waika virus-antiserum. Then it was examined by electron microscopy. Aggregates of spherical particle-antibody complex were observed and the small bacilliform particles remained unchanged.

PTA negatively stained bacilliform particles are 31 nm in width and 100–160 nm in length, with both ends rounded. There were several peaks in particle-length distribution. The particles about 120 nm in length were sedimented at the same position as the spherical particles, and longer particles were sedimented at lower position after rate zonal centrifugation in sucrose density gradient. The substructure of small bacilliform particles was similar to that of alfalfa mosaic virus or cacao swollen shoot virus, and different from that of rod-shaped or filamentous viruses such as TMV.

## Conclusion

The small bacilliform particles and spherical particles found in tungro and similar diseases 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.

Both of the particles invade phloem cells and were transmitted by the same species of leafhopper, *N. virescens*. It is possible that double infection often takes place. So far as this ultrathin section experiment is concerned, most of the tungro and similar disease in Asia were caused by double infection of the spherical particles and the small bacilliform particles with some exceptions.

The symptoms of rice transitory yellowing disease in Taiwan (Shikata, 1972) are similar to those tungro disease. However, the two diseases differ in the size of virus particles, and in the vector relationships.

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## Discussion

**M. D. Mishra,** India: 1. I think we are forgetting about transitory yellowing reported from Taiwan.

2. In one of your electron micrograph, is there some degeneration of virus particles?

3. What is your opinion regarding strains reported from India?

**Answer:** 1. The symptoms of rice transitory yellowing are similar to those of tungro disease. However, the vector relations and the morphology of the viruses differ. Transitory yellowing virus is persistent in its insect vectors, rice green leafhopers, and has bullet-shaped particles belonging Rhabdo virus group.

2. In the case that the diseased leaves which were kept in glutaraldehyde fixactive for a long time, the spherical particles were degenerated somewhat and it became difficult to distinguish them from ribosomes.

3. I have not examined yet on the strains reported from India. I would like to examine them under your cooperation.

**E. W. Kitajima,** Brazil: You mentioned that treating purified preparation of pneyakit habang with sera against waika, only spherical particles are precipitated. What would happen if you inoculate rice plants (via leafhopper) with these bacilliform particles?

**Answer:** We have a plan to make such experiment, but not yet.

**T. Soelaeman,** Indonesia: Is there any information about the relationship between the spherical and the bacilliform particles, for instance in function of the forming of protein coats and so on, like those in the case of Alfalfa mosaic virus?

**Answer:** At present, there is no information. I think there may be no relationship between the spherical and the bacilliform particles.

**T. Iida,** Japan: Is the 'bacilliform virus' transmitted in a similar manner as the 'spherical virus' ?

**Answer:** We have no exact experimental evidence on the non-persistent mode of the bacilliform particle. However, the penyakit habang diseased plants after 1-day acquisition feeding followed by 1-day inoculation feeding of *N. virescenes,* contained the bacilliform particles together with the spherical particles. On the basis of this that tungro is caused by a multi-particled virus like in Alfalfa mosaic virus.

**D. A. Benigno,** Philippines: Your report is very enlightening. However, your results seem to indicate that probably 2 viruses are really present at the same time in the same plant. Perhaps it is about time to give another name of the disease caused by the basilliform virus to that of tungro caused by spherical virus. Or could it be that tungro caused by a multi-particled virus like in Alfalfa mosaic virus?

**Answer:** From the results of our electron microscopic studies, it is thought that the tungro and similar diseases which are present under different names in Asia, are caused by double infection of the spherical and the bacilliform particles, not by single infection of the spherical particle as previously been thought. Some exceptions are in the case of diseases showing milder symptoms, such as waika disease and the disease caused by M strain of tungro in which single infection of the spherical particle is taking place. I do not think that the spherical and the bacilliform particles belong to a multicomponent virus, because too many differences in properties exist between the spherical and the bacilliform particles.

**E. Shimura,** Japan: I have an interest in varietal reactions. Do you find any typical symptoms in Japonica varieties in an early stage by an artificial inoculation of virus recovered from tungro or yellow orange leaf diseased plants?

**Answer:** Japonica varieties, such as Reiho, Tukushibare, Nihonbare, exhibited typical symptoms of tungro in their seedling stages after artificial inoculation of penyakit habang virus.

**D. P. Shivanathan,** Sri Lanka: What is your evidence to show that the bacilliform particle is infectious?

**Answer:** Inoculation tests with only bacilliform particles have not yet been done, because I have no diseased plant singly infected with the bacilliform particles. I suppose that the bacilliform particles may be infectious, because the phloem cells containing the bacilliform particles only showed necrosis, and the diseased plants containing both particles showed much severer symptoms than those of single infection with spherical particles.

**Y. Nagai,** Japan: Are there any instances to find small bacilliform particles only, not with spherical ones in waika and other similar virus diseases?

**Answer:** I have not yet found the diseased plants infected only with bacilliform particles.

**T. Soelaeman,** Indonesia: Could it be possible that the bacilliform particles act

like the satellite virus of Tobacco necrosis virus, since you did not find the disease with only bacilliform particles?

**Answer:** That is one possibility. Another possibility is that the two viruses are different ones, and cause double infection. I think that there is a possibility to find out diseased plants only with bacilliform particles around tungro infected paddy fields. They will be exhibiting milder symptoms than those of tungro disease.