II. Virus diseases of rice

RICE TUNGRO VIRUS

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Abstract

Rice tungro disease was found to be associated with a virus complex composed of two different viruses and these viruses were described as rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV).

Vector species of the disease, transmission efficiencies of the disease by vectors and complex nature of the relationships between the vector and the two viruses are introduced. The vector species present in rice fields in various areas of the tropical and subtropical regions in relation to rice tungro epidemics were studied. It was found that the population density of *N. virescens*, an efficient vector species of rice tungro disease, was much higher than that of *N. nigropictus* in the rice tungro-epidemic regions such as central Thailand, central and east Java, and Lombok Is., whereas *N. nigropictus* was predominant in the hills of Nepal without tungro epidemics.

RTSV and RTBV were separately purified and antisera specific to each virus were prepared. The viruses in infected plants were detected by the latex flocculation test using the antisera.

1. Introduction

Rice tungro disease was described for the first time by Anon in 1964 and Rivera and Ou in 1965. The disease is distributed throughout the South and Southeast Asian countries and Japan. The disease has been reported under different names such as yellow orange leaf disease in Thailand, penyakit habang in Indonesia, penyakit merah in Malaysia, waika disease in Japan and tungro disease in the Philippines and India.

This disease is one of the major rice virus diseases and an important potential threat to rice production in this area due to its occasional severe outbreaks.

Previously, the causal agent of the disease was thought to be rice tungro virus, a spherical virus about 30nm in diameter.

However, it became clear recently that tungro disease is associated with a virus complex composed of two different viruses and these viruses were described as rice tungro spherical virus and rice tungro bacilliform virus (Saito *et al.*, 1981).

2. Host range and symptomatology

Host range of rice tungro disease is restricted mainly to the *Oryza* spp.

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The symptoms of the disease in the rice plant are characterized by slight-to-severe stunting of the plants, yellow-orange to orange-red discoloration of the leaf blades usually starting from the leaf tip and gradually moving downwards. Very small rusty-colored necrotic spots may be found in the discolored areas of the older leaves. Infected plants, especially those of a susceptible cultivar produce few tillers.

The symptoms of the disease were severe in double infection with RTBV and RTSV, moderately severe in single infection with RTBV, while clear symptoms were detected in single infection with RTSV.

Taichung (Native) 1 seedlings show typical symptoms of tungro disease and are suitable sources of virus for purification.

3. Transmission of tungro virus agents by leafhopper vectors

Six leafhopper species are known as vectors of tungro disease, namely *Nephotettix virescens* (Rivera and Ou, 1965), *N. cincticeps* (Hibino, 1983), *N. nigropictus* (Rivera and Ling, 1968), *N. parvus* (IRRI, 1972), *N. malayanus* (IRRI, 1973) and *Recilia dorsalis* (Rivera *et al.,* 1969). *N. parvus* and *N. malayanus* have little biological relationship with rice and *N. cincticeps* is not distributed in South and Southeast Asia where tungro is predominant. Of the three leafhopper species, vectors of tungro, *N. virescens* is the most efficient vector. *N. nigropictus* and *R. dorsalis* are less efficient and their transmission efficiency of tungro variable depending on the colonies and locations tested. Percentage of active transmitters was 50—90 for *N. virescens,* 0—27 for *N. nigropictus* and 0—8 for *R. dorsalis* (Sogawa, 1976).

Recent tungro transmission studies based on the presence of RTBV and RTSV in inoculated plants indicated the complex nature of the relationships between the green leafhopper and the two tungro associated viruses (Hibino *et al.,* 1978, 1979; Hibino, 1983ab). *N. virescens* exposed to both RTBV and RTSV predominantly transmit both viruses together, while some of them may transmit RTBV alone and a few may transmit RTSV alone. The leafhopper can transmit RTSV from plants infected with RTSV alone but cannot transmit RTBV from plants infected with RTBV alone.

N. cincticeps also transmit both viruses together or one virus alone from plants infected with RTBV and RTSV, though the transmission efficiency is lower than that of *N. virescens*. In the case of *N. nigropictus,* transmission ability of RTBV and RTSV

Table 1. Transmission efficiencies of RTBV, RTSV and RWV from plants infected with RTBV, and/or RTSV or RWV by vector leafhoppers in Japan (Hibino, Plant Dis. 67, 774-777. 1983).

Vector	Efficiency of transmission from source plants with				
	RTBV + RTSV	RTBV +RWV	RTBV	RTSV	RWV
Nephotettix virescens	+++	+++	-	+++	+++
N. cincticeps	++	++	-	++	++
N. nigropictus	+	+	-	++	++
R. dorsalis	-	-	-	-	-

Transmission efficiency at two leafhoppers per seedling: +++ = high efficiency (>70%), ++ = intermediate efficiency (10-40%), + = low efficiency (1-5%), - = no transmission.

depended on the colony. Of the plants infected with RTBV and RTSV, *N. nigropictus* transmitted RTSV alone in one case in Indonesia (Hibino *et al.*, 1979), and transmitted either RTBV or RTSV in Japan (Hibino, 1983a), while it transmitted RTBV and/or RTSV in the Philippines, though its transmission efficiency was low (Cabauatan and Hibino, unpublished data). However, the three *N. nigropictus* colonies transmitted the virus efficiently from plants infected with RTSV alone. *R. dorsalis* has been tested in Indonesia and Japan (Hibino *et al.*, 1979; Hibino, 1983a). None of the leafhoppers transmitted either RTBV or RTSV. Rice waika virus (RWV) (Furuta, 1977), a virus identical or very close to RTSV occurring in Kyushu, Japan, is transmitted similarly as RTSV by the *Nephotettix* spp. but not by *R. dorsalis* (Hirao and Inoue, 1978).

RTBV is dependent on RTSV for its transmission by *N. virescens* (Hibino *et al.*, 1978; Hibino 1983b). RTBV was transmitted by leafhoppers which had been given acquisition access to RTSV at the same time or before. Both RTBV and RTSV are transmitted in a semipersistent manner by the leafhopper and their retention periods in the leafhopper are 4 and 3 days, respectively. However, the leafhopper exposed to

Table 2. Transmission manner of RTBV, RTSV and hypothetical "helper factor" by *Nephotettix virescens*.

Virus	Min. acquisition access time	Min. inoculation access time	Retention period	Infectivity loss after moulting
RTBV	10 min	30 min	4 days	yes
RTSV	10 min	30 min	3 days	yes
Helper factor	10 min	-	7 days	yes

RTSV retained the ability to assist RTBV in the transmission for 7 days. This fact indicates that the retention period of the hypothetical "helper factor" is 7 days (Hibino, 1983b; Cabauatan and Hibino, unpublished data). The "helper factor" was not retained by the leafhopper after moulting.

Transmission of RTBV and RTSV by *N. virescens* is also affected by the age of the virus source plants and test seedlings. When source plants are old, transmission efficiency is low and the leafhoppers tend to transmit RTBV alone. In this case, when the resistant varieties were inoculated by using leafhoppers exposed to both RTBV and RTSV, the percentage of infection was low and the infected seedlings mostly contained RTBV alone (Hibino *et al.*, 1983; Daquioag *et al.*, 1984). In the case of the susceptible varieties, the percentage of infection was high and the infected seedlings mostly contained both RTBV and RTSV.

4. Species composition and population density of vector species of rice tungro viruses in paddy field

It is well known that the efficiency of rice tungro viruses (RTV) transmission varies among the vector species; *Nephotettix virescens, N. nigropictus* and *Recilia dorsalis*. The experimental evidences obtained in various tropical countries in Asia indicate that *N. virescens* is the most efficient vector species; the proportion of transmitting individuals ranged from 35 to 83% in the Philippines (Rivera and Ou, 1965) and from 32 to 79% in

Table 3 Species composition and population density of *Nephotettix* spp. in paddy field.

Country	Region	Time	No. of site	No. of leafhoppers obtained per 20 strokes of sweeping[a]						Ratio[b]
				N. virescens			*N. nigropictus*			
				Min.	Max.	Averg.	Min.	Max.	Averg.	
Thailand	North	Aug. 1979	5	0	12	3.6	0	18	3.7	1.0
	North-east	ditto	12	0	23	4.9	0	2	0.1	49.0
	Central	ditto	10	7	37	22.1	0	11	2.4	15.4
	South	ditto	4	0	4	0.8	0	0	0	
	Northeast	Oct. 1980	5	2	11	5.2	0	0	0	
	Central	ditto	16	3	75	11.7	0	8	0.9	13.0
Indonesia	East Java	Feb.—March 1982	6	2	25	12.6	0	6	1.8	7.0
	Central Java	ditto	17	0	28	6.5	0	1	0.1	65.0
	West Java	ditto	9	0	17	5.3	3	12	7.3	0.7
	Lombok Is.	ditto	4	10	37	18.5	0	1	0.5	37.0
	Bali Is.	ditto	7	1	10	5.7	0	0	0	
Nepal	Terai	Sept. 1980	11	0	4	0.9	0	1	0	
	Hill	ditto	5	0	0	0	7	34	21.6	

a) Sweeping survey of 40—50 strokes was standardized in each collection site.
b) Adults and nymphs are included.
c) Number of *N. virescens*/number of *N. nigropictus*.

Thailand (Hino *et al.,* 1974). And, both *N. nigropictus* and *R. dorsalis* are inefficient vectors. These experimental results indicate that predominance and prevalence of *N. virescens* in a certain area could relate significantly to RTV outbreak if diseased rice plants are present as source of inoculum. The present chapter describes the population of vector species in rice fields in various areas in the tropical and subtropical regions in relation to RTV epidemics.

Leafhopper survey was carried out in the rainy season in Thailand in 1979 and 1980, in Indonesia in 1982 and in Nepal in 1980, the former two countries being RTV-epidemic regions unlike the latter. In all cases, 30 — 40 strokes of sweep using an insect-sweeping net were standardized in each field surveyed including paddy fields and nursery beds. The collected specimens were examined taxonomically under a microscope.

The results are summarized in Table 3. A total of 52 sites in Thailand, 43 sites in Indonesia and 16 sites in Nepal were selected for the survey, respectively. Except those in the hill area of Nepal where no RTV incidence has been reported yet, *N. virescens* and *N. nigropictus* were frequently collected and *R. dorsalis* were observed in small numbers or were absent. According to Inoue (personal communication), *R. dorsalis* is almost indigenous to gramineous grass; a significant number of *R. dorsalis* usually infest fields soon after rice transplanting but they usually do not colonize for a whole rice growing period.

In most of the survey sites, the number of *N. virescens* exceeded that of *N. nigropictus* except in the northern region of Thailand and western region of Java where the population of both species was almost identical. A significantly higher population was observed in some survey sites in the central plain of Thailand, east and central Java, and Lombok Is., where RTV had prevailed previously.

Species composition in paddy field in the genus *Nephotettix* was studied previously by Hokyo *et al.,* (1977) in Malaysia and Indonesia in the 1976 rainy season and they showed that the population of *N. nigropictus* exceeded that of *N. virescens.* Consequently, the present results of *N. virescens* predominance over *N. nigropictus* indicate that there has been an alteration of the pest status in the genus *Nephotettix* during the past 5 years. It seems that the most important factor relating to this phenomenon is the subsequent rice cropping from the rainy to the dry season which became a site for *N. virescens;* the species can feed only on rice plants. On the other hand, the condition of rice does not affect directly the population trend of *N. nigropictus* which has a wide host plant range including the genera of *Echinochloa, Leersia, Oryza*, etc.

5. Relation with cells and tissues

The characteristics of the ultrastructural alterations in the cells infected with RTSV are the presence of viroplasm and crystalline aggregates of virus particles, etc. RTBV particles 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. Cross-section of RTBV showed a core 9 nm in diameter, surrounded by a high-density zone of 4.5 nm and a medium-density outer zone of 3.5 nm. Both viruses were localized in the phloem cells and induced necrosis of these

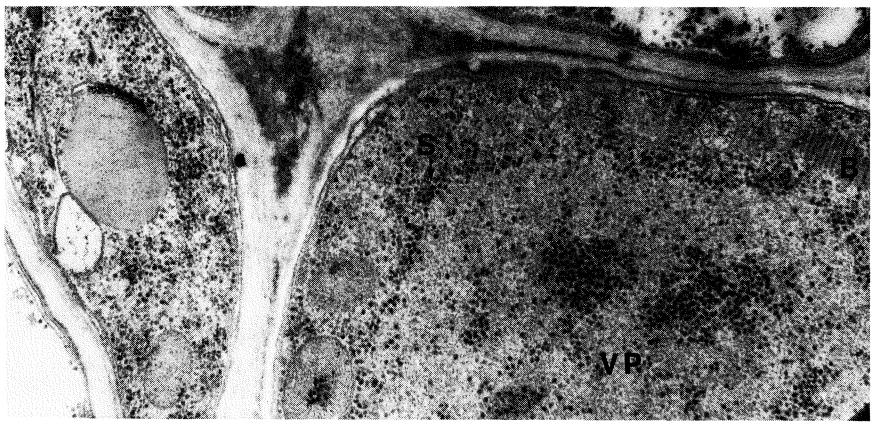

Fig. 1. Ultrathin section of phloem cells of rice leaves infected with rice tungro disease.
B: rice tungro bacilliform virus
S: rice tungro spherical virus
VP: viroplasma

cells. It is possible that the virus in the phloem cells inhibits the translocation of certain food materials and induces tungro symptoms.

6. Purification

As described previously, RTSV was transmitted by the vector *Nephotettix virescens,* but RTBV was transmitted concomitantly only when RTSV was acquired previously or simultaneously, i.e., the propagation of RTBV using plants solely infected with RTBV as inoculum was impossible. Because large quantities of doubly-infected plants were available, such plants were used for virus purification. Infected plants were harvested about 40 days after inoculation and were stored at -80°C. Clarification and partial purification were carried out according to the method applied for rice waika virus (RWV) (Usugi and Saito, 1975). Leaf extracts in 0.01 M ethylenediaminetetraacetic acid (EDTA), pH 6.0 were heated at 40°C for 1 hr. Virus particles were precipitated with 7% polyethylene glycol 6,000 plus 0.2 M sodium chloride (NaCl) containing 1% Triton X-100. The resuspended virus particles were treated with 20% carbon tetrachloride, and were subjected to differential centrifugation (96,000 × *g* for 60 min at 4°C in a Hitachi RP-40 rotor, then 10 min at 3,000 × *g*). Supernatant was layered on 10–40% linear sucrose density gradients (prepared in 0.01 M borate buffer pH 9.0 (BB)) and centrifuged at 60,000 × *g* for 3 hr at 4°C in a Hitachi RPS-25 rotor. Tube contents were scanned at A_{254} and fractionated through an ISCO model UA5 ultraviolet analyzer. Typical density-gradient scanning pattern presented in Fig. 2A showed a peak (arrow with "a") containing RTSV, and a shoulder (arrow with "b") on the descending slope containing RTBV. Peak and shoulder fractions were separately collected, virus particles were pelletted by centrifugation, and were subjected to two additional density-gradient centrifugations (Fig. 2B). After three sucrose density-gradient cycles, RTSV were further purified by equilibrium centrifugation in CsCl. When 3.273 g CsCl was dissolved in 4 ml (45% W/V) of the peak material in 0.01 M phosphate buffer (PB), at pH 7.0 and the suspension was centrifuged at 114,5000 × *g*

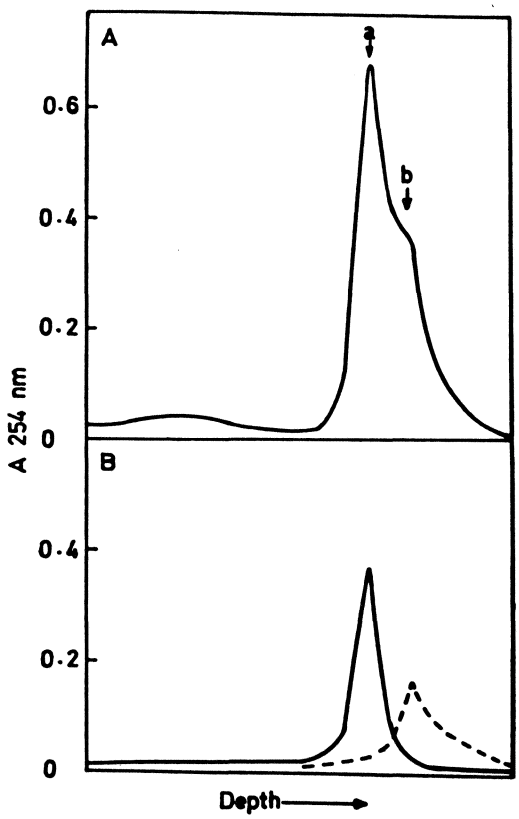

Fig. 2. Sucrose density-gradient profiles (A) of partially purified preparation from rice plants infected with both RTSV and RTBV, (B) following repeated sucrose density gradient centrifugations of peak "a" (solid line) and shoulder "b" (broken line). (Data from Omura *et al.*, 1983).

for 40 hr at 4°C in a Hitachi RPS-40 rotor, a single band was formed at about two-thirds of the distance below the meniscus. The peak material was recovered, and CsCl was removed by two cycles of differential centrifugation. Final pellets were resuspended in 0.01 M EDTA, pH 6.0 and such preparations contained isometric particles about 30 nm in diameter (Fig. 3). Preparations were negatively stained with neutralized phosphotungstic acid (PA) and were examined under a Hitachi H-500 electron microscope. The sedimentation coefficient of the virus determined by analytical centrifugation was 173 S. Equilibrium centrifugation in CsCl was not appropriate for the purification of RTBV, because the particles were degraded during the two cycles of differential centrifugation applied to remove CsCl after the equilibrium centrifugation. After three cycles of density-gradient centrifugation, RTBV was further purified using antiserum to RWV whose titer was 1:640 in the precipitin ring interface test. Partially purified RTBV fraction was mixed with equal volume of the serum which was diluted to 1/100 with 0.01 M PB, pH 7.0 containing 0.85% NaCl. The mixture was incubated for 1 hr at 37°C, followed by overnight

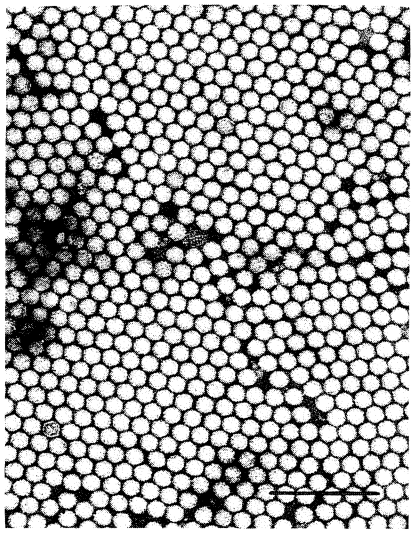

Fig. 3. Purified preparation of RTSV. Bar represents 200 nm.

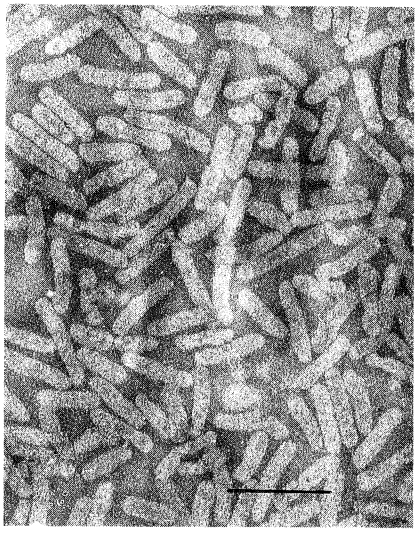

Fig. 4. Purified preparation of RTBV. Bar represents 200 nm.

(Data in Figs. 3, 4 cited from Omura *et al.*, 1983)

incubation at 4°C. Contaminating RTSV particles were clumped and easily sedimented by low speed centrifugation. After two additional serum treatments, final supernatant was layered on sucrose density-gradient, centrifuged, and fractionated as mentioned above. Virus was concentrated by high speed centrifugation and the pellets were resuspended in 0.01 M EDTA, pH 6.0. Purified RTBV were 30–35 nm in width with varying length (Fig. 4).

Ratios of absorbance (260/280) for purified RTSV and RTBV were 1.75 and 1.11, respectively. Buoyant densities of the particles were estimated by equilibrium centrifugation in CsCl. For RTBV, 2.154 g CsCl was dissolved in 4 ml (35%, W/V) of the purified preparation in 0.01 M PB, pH 7.0 and centrifuged at 114,500 × *g* for 40 hr at 4°C in a Hitachi RPS-40 rotor. A single band was formed at about one-third of the distance below the meniscus. The density of the fraction was determined from the refractive index using the method of Brakke (1967). Buoyant densities of RTSV and RTBV were 1.551 g/cm^3 and 1.312 g/cm^3, respectively. The fact that RTBV formed a single band indicated that the composition of RTBV was even, though the virus contained particles of various sizes.

Rabbits were immunized separately against RTSV and RTBV by an intramuscular injection with purified preparations emulsified with an equal volume of Freund's complete adjuvant as well as by repeated intravenous injections. In the precipitin ring interface test, antiserum titers were 1/320 and 1/2048 against RTSV and RTBV, respectively. For double gel diffusion test, 0.8% agar in 0.01 M PB, pH 7.6 containing 0.85% NaCl, 0.001 M EDTA and 0.05% sodium azide was used. No heterologous reactions occurred between these antisera and the two kinds of particles. RTSV and rice waika virus were serologically identical both in agar gel-diffusion test and complement fixation test.

7. Serological detection

Latex flocculation (LF) method (Bercks and Querfurth, 1971) was employed to detect RTBV and RTSV. Antisera obtained above were used in this experiment. The fraction which precipitates when the serum was 50% saturated with ammonium sulphate was used. Phosphate buffer (0.01 M), pH 7.0 containing 0.01 M $MgCl_2$ 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 two fold 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 on a shaker at 150 oscillations per minute at 30°C. For controls, 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). All tests were duplicated.

The most distinct flocculation occurred at globulin dilutions of 1 : 150 and 1 : 120 for RTBV and RTSV, respectively, when the protein concentration in the globulin suspension was calculated to be 1%. Intensity of flocculation did not increase when shaking was extended beyond 50 min for both viruses. As shown in Table 4, virus antigens were detected by LF. Maximum titers of the antigens were obtained 30 days after inoculation and little change in titer occurred during the period with both viruses tested.

8. Rice tungro disease in Nepal

Rice plants showing symptoms of yellow leaf discoloration, plant stunting, and delayed flowering were observed at Hardinath Agricultural Farm, Janakpur, Nepal. The disease occurred in circular field patches several meters in diameter.

Electron microscopic observations of dipped preparations revealed the presence of polyhedral particles about 30 nm in diameter and bacilliform particles about 30–35 nm in diameter and 100–300 nm long. These findings suggest that rice tungro disease in the Janakpur area is caused by double infection with both rice tungro spherical virus and rice tungro bacilliform virus, as has been reported in the Philippines, Thailand, Malaysia, and Indonesia.

Table 4. Detection of virus antigens by latex flocculation test in rice plants at various times after inoculation[a]

	Days after inoculation					
Virus	20	30	40	50	60	70
RTBV	20[b]	80	40	40	40	40
RTSV	40	80	80	80	80	80

a) No flocculation was observed in any of the healthy controls.
b) Reciprocal of the highest dilution with positive reaction. (Data cited from Omura *et al.*, 1984).

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Literature cited

1. Bercks, R. and Querfurth, G. (1971). The use of the latex test for the detection of distant serological relationships among plant viruses. J. Gen. Virol. 12, 25-32.
2. Brakke M. K. (1967). Density-gradient centrifugation. Meds. in Virol. 2, 93-136.
3. Daquioag, R.D., Tiongco, E.R. and Hibino, H. (1984). Reaction of several rice varieties to rice tungro virus (RTV) complex. Int. Rice Res. Newsl. 9(2): 5-6.
4. Furuta, T. (1977). Rice waika: A new virus disease found in Kyushu, Japan. Rev. Plant Prot. Res. 10:70-82.
5. Hibino, H. (1983a). Transmission of two rice tungro-associated viruses and rice waika virus from doubly or singly-infected source plants by leafhopper vectors. Plant Dis. 67:774-777.
6. Hibino, H. (1983b). Relations of rice tungro bacilliform and rice tungro spherical viruses with their vector *Nephotettix virescens*. Ann. Phytopath. Soc. Japan 49:545-553.
7. Hibino, H., Roechan, M., and Sudarisman, S. (1978). Association of two types of virus particles with penyakit habang (tungro disease) of rice in Indonesia. Phytopathology 68:1412-1416.
8. Hibino, H., Saleh, N., Roechan, M. (1979). Transmission of two kinds of rice tungro-associated viruses by insect vectors. Phytopathology 69:1266-1268.
9. Hibino, H., Tiongco, E., Cabunagan, R. (1983). Rice tungro virus complex in tungro-resistant IR varieties. Int. Rice Res. Newsl. (4):12-13.
10. Hirao, J. and Inoue, H. (1981). Transmissionof rice waika virus by green rice leafhoppers, *Nephotettix* spp. (Hemiptera: Cicadellidae). Bull. Kyushu Intl. Agric. Exp. Sta. 21:509-552.
11. Hokyo, N., Otake, A. and Okada, T. (1977). Species composition of *Nephotettix* (Hemiptera: Cicadellidae) in west Malaysia and Indonesia. Appl. Ent. Zool. 12: 83 - 85.
12. Int. Rice Res. Inst. (1972). Annual Report for 1971, 112-113. Los Baños, Philippines. 238 p.
13. Int. Rice Res. Inst. (1973). Annual Report for 1972, 132-133. Los Baños, Philippines. 264 p.
14. Ling, K.C. (1966). Nonpersistence of the tungro virus of rice in its leafhopper vector, *Nephotettix impicticeps*. Phytopathology 56:1252-1256.
15. Omura, T., Hibino, H., Usugi, T., Inoue, H., Morinaka, T., Ong, C. A., Putta, M., Tsuchizaki, T., and Saito, Y. (1984). Detection of rice viruses in plants and individual insect vectors by latex flocculation test. Plant Disease. 68: 374-378.
16. Omura, T., Saito, Y., Usugi, T. and Hibino, H. (1983). Purification and serology of rice tungro spherical and rice tungro bacilliform viruses. Ann. Phytopath. Soc. Japan 49, 73-76.
17. Rivera, C.T. and Ou, S.H. (1965). Leafhopper transmission of "tungro" disease of rice. Plant Dis. Reptr. 49:127-131.
18. Rivera, C.T. and Ling, K.C. (1968). Transmission of rice tungro virus by a new

vector *Nephotettix apicalis*. Philippine Phytopathol. 4:16 (Abst.).
19. Rivera, C.T., Ling, K.C., Ou, S.H. and Aguiero, V.M. (1969). Transmission of two strains of rice tungro virus by *Recilia dorsalis*. Philippine Phytopathol. 5:17 (Abstr.).
20. Saito, Y., Roechan, M., Tantera, D.M., and Iwaki, M. (1975). Small bacilliform particles associated with penyakit kabang (tungro-like) disease of rice in Indonesia. Phytopathology 65: 793–796.
21. Saito, Y., Iwaki, M., Usugi, T. (1976). Association of two types of particles with tungro group diseases of rice. Ann. Phytopath. Soc. Japan 42:375.
22. Saito, Y. (1977). Interrelationship among waika disease, tungro and other similar diseases of rice in Asia. Trop. Agric. Res. Ser. No. 10, 129–135.
23. Saito, Y., Hibino, H., Omura, T., and Usugi, T. (1981). Transmission of rice tungro bacilliform virus and rice tungro spherical virus by leaf hopper vectors. 5th International Congress Virology, 213 (Abst.).
24. Sogawa, K. (1976). Rice tungro virus and its vectors in tropical Asia. Rev. Plant Prot. Res. 9:21–46.
25. Usugi, T. and Saito, Y. (1975). Purification and serology of rice waika virus. Ann. Phytopath. Soc. Japan. 41, 283 (Abstr.)