Association of Two Types of Virus Particles with Penyakit Habang (Tungro Disease) of Rice in Indonesia

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ABSTRACT

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Rice plants with the penyakit habang disease were examined by electron microscopy. Two types of virus particles were detected: isometric particles (I) about 30 nm in diameter, and bacilliform particles (B) about 35 nm in diameter and 150-350 nm in length. Plants showing severe symptoms contained both B and I particles, whereas those showing moderate symptoms contained B particles alone. One of the plants showing no clear symptoms contained I particles alone. The I particles were transmitted by *Nephotettix virescens* but the B particles were transmitted by the insects only when the I particles had been acquired previously or at the same time. Reactions of eight rice varieties to infection with the B and I particles indicated that so called tungro symptoms were caused by the B particles and that the I particles intensified the symptoms caused by infection with the B particles.

Additional key words: rice tungro virus, rice waika virus.

Rice diseases which cause stunting and yellowing or orange discoloration have been known in southeast Asia and these diseases have been called locally by different names (8). The disease is called penyakit habang or mentek in Indonesia. The pathogens and symptoms of these diseases and the factors affecting them have been examined in several countries in the last 10 yr (3, 4, 5, 6, 9, 13). The causal agent(s) all were transmitted by *Nephotettix virescens*, and the diseases were similar to each other in symptomatology, transmission mode, and cultivar reactions. Therefore, they are believed to be the same or similar disease and are called tungro disease or the tungro-group of diseases.

Isometric virus particles were reported in rice plants infected with rice tungro virus in the Philippines and a fraction, which contained purified virus, caused symptoms in infectivity assays (1, 2). Similar isometric virus particles were reported in rice plants affected by tungro-group diseases in Pakistan (3) and Thailand (11). On the other hand, Saito et al (12) found bacilliform particles instead of isometric particles in penyakit habang-diseased rice cells in Indonesia. Recently, Saito et al (10) further investigated tungro or tungro-group diseased rice cells in Indonesia, Malaysia, The Philippines, and Thailand by electron microscopy, and found that both isometric and bacilliform virus particles were associated with the tungro disease and tungro-group diseases in plants collected from four countries. They also found both types of particles in the cells infected with the S and T strains of tungro in The Philippines but isometric particles alone with the M strain. These data indicate that tungro disease and tungro-group diseases are caused by the two types of virus particles or by the isometric particles alone. Isolation and characterization of these two types of particles was necessary to clarify their relationship to the tungro disease and that was the objective of this research.

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MATERIALS AND METHODS

Penyakit habang-diseased rice plants showing stunting and yellow-orange discoloration were collected from South Sulawesi and maintained on the rice cultivar Taichung Native 1 [T(N)-1] by successive transfers using *N. virescens*.

Seedlings of the cultivar T(N)-1 at the first- or second-leaf stage were used for inoculation in most experiments. Seedlings of the cultivar Pelita I/1 were also used in one experiment. After acquisition access on diseased plants for 2 day, five adult insects were allowed an inoculation access period of 8 hr on each healthy seedling. The seedlings then were sprayed with insecticide and grown in a greenhouse. The reactions of seven rice cultivars (FK-135, Pankhari 203, Sukanandi, Pelita I/1, IR-5, IR-8, and Reiho) were tested. The cultivar Reiho also is susceptible to rice waika virus (7, 14). Two to 4 wk after inoculation, test plants were examined for the presence of virus particles by quick dip techniques. Leaf samples were prepared in 1 % neutral phosphotungstate and examined with a Hitachi HS-9 electron microscope.

RESULTS

Detection of two types of virus particles in inoculated rice plants.—Insect vectors were given an inoculation access period of 1 day on rice seedlings (three insects per seedling) after 1 day of acquisition access on a penyakit habang-diseased rice plant. One mo after inoculation, leaf samples were collected from the inoculated seedlings and dip preparations were examined. Two kinds of viruslike particles were found in these preparations. One was isometric (I), about 30 nm in diameter, and the other was bacilliform (B), about 35 nm in diameter and 150-350 nm in length (Fig. 1). The I particles were abundant in the preparations, but B particles occurred in low frequency. Six of 33 inoculated plants contained both B and I particles, but seven plants contained B particles alone and one contained I particles alone; the remainder contained no particles. The plants infected with both B and I particles were severely stunted and discolored (Fig. 2). The plants infected with B particles alone were moderately stunted, and their leaves usually were dark green in color but sometimes a transitory yellow-orange color developed. The plant infected with I particles alone developed no clear symptoms and was not stunted or discolored, but was stunted slightly after heading.

Transmission of the two viruses.—The plant infected with I particles alone and one of those infected with B particles alone were used as inoculum sources, and seedlings were inoculated separately, by means of leafhoppers, with B or I particles. One mo after inoculation, I particles were detected in dip preparations from 18 of 21 plants inoculated with I particles, but no viruslike particles were detected in 24 plants inoculated with B particles. None of those inoculated with B particles developed symptoms, but some of those with I particles became stunted slightly at a later growth stage. Transmission of B particles from B-infected plants was attempted several times but none of the test plants developed symptoms. The I particles were maintained on the T(N)-1 seedlings by successive transfers using the insects and none of the infected plants developed symptoms. These results indicate that I particles were isolated from the penyakit habang-diseased plant and the plants which contained B particles alone were free of I particles. Therefore, B-infected plants were selected from penyakit habang-infected plants by means of electron microscopic examination and used as inoculum sources of the B particles in following experiments.

Transmission of B particles in the presence of I particles.—One group of insects was given an acquisition

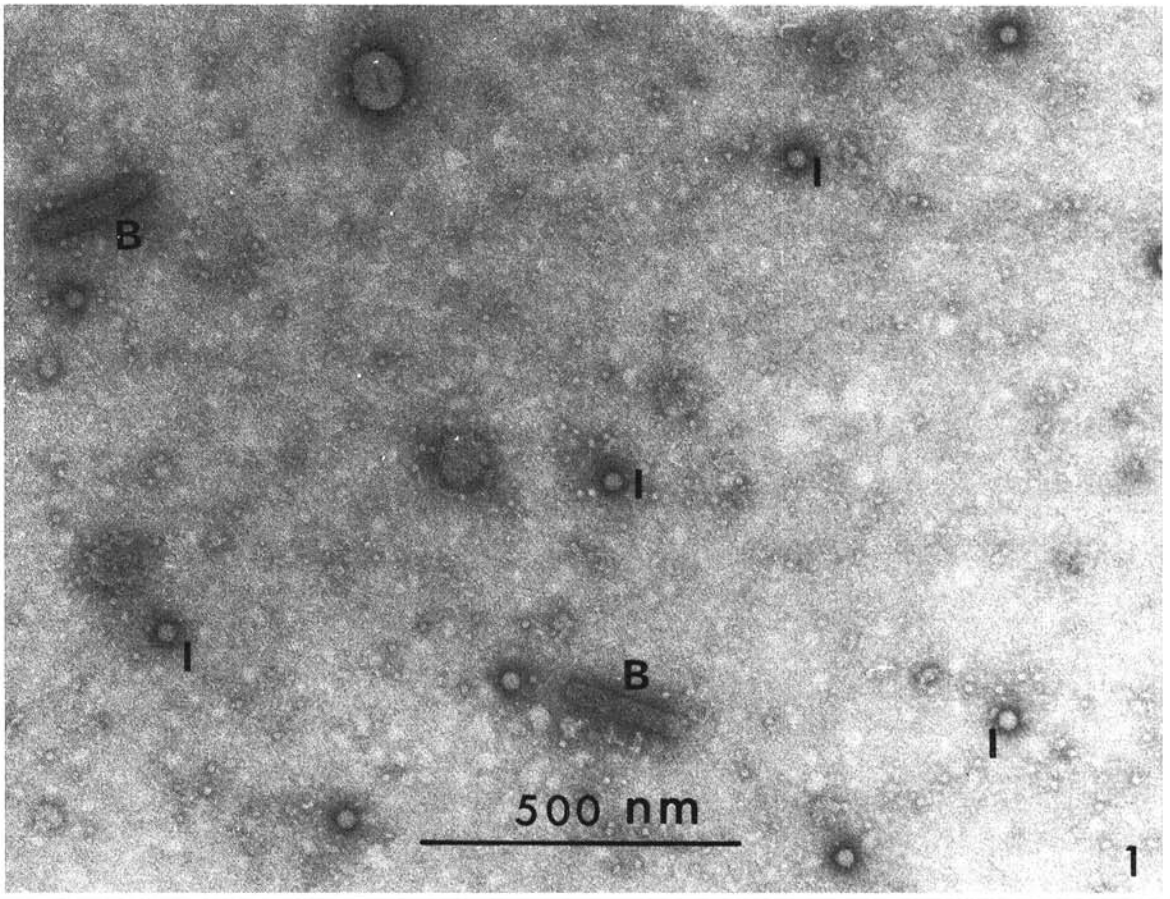

Fig. 1. Electron micrograph of a dip preparation from a rice plant, cultivar Taichung Native 1, with penyakit habang disease, showing isometric (I) and bacilliform (B) virus particles stained negatively with phosphotungstate.

access period of 2 days on I-infected plants, and then half of them were allowed an access period of an additional 8 hr on B-infected plants directly after the first access and another half were allowed also an additional 8 hr on B-infected plants after holding them on healthy seedlings for 1 day. The reciprocal experiments were performed as a second group was given acquisition access periods of 2 days to B particles first and then 8 hr to I particles. As controls, a third group was given an acquisition access period of 2 days on B or I-infected plants and then was held on healthy seedlings for 8 hr. These insects then were allowed an inoculation access period of 8 hr or overnight on healthy seedlings.

The insects transmitted B particles when they acquired I particles first and then B, but failed to transmit B particles when they had access to B particles prior to I (Table 1). Transmission rates of I particles by the insects were reduced when the insects acquired B particles after acquiring I particles. The rate also was reduced in one experiment in which the insects previously had had access to B particles.

Challenge inoculation.—Twenty rice seedlings inoculated either with I or B particles were inoculated again with B or I particles, respectively 7 days after the first inoculation. In a similar experiment, inoculated seedlings were challenged 8 days after the first inoculation. None of these challenged plants developed symptoms or contained B particles, though many of them contained I particles. Seedlings inoculated with B or I particles were examined for the presence of virus particles 7 days after inoculation. Seven of 10 seedlings inoculated with I particles alone contained I particles, but none of the plants inoculated with B particles alone were infected. These results indicate that the B particles failed to infect plants in which the I particles were multiplying.

Reactions of eight rice varieties to the B and I particles.—Seedlings of eight rice varieties were inoculated by means of *N. virescens* given acquisition access on plants infected with I particles alone or with both B and I particles. The seedlings inoculated with a mixture became infected with both B and I particles, or with B particles alone, and no seedlings became infected with I particles alone (Table 2). Rates of infection with both B and I particles, and with B particles alone were different for each cultivar. On the other hand, all cultivars except Pankhari 203 were infected with I particles when they were inoculated with I particles alone (Table 3). Efficiencies of I transmission by the insects from the plants infected with I particles alone were higher than those infected doubly with B and I particles (Table 2, 3). The result indicates that the B particles in the inoculum source may supress transmission of the I particles by the insect.

The symptoms on FK-135 caused by B particles alone were as severe as those caused by double infection of B and I particles, but the symptoms caused on the other cultivars by B particles alone were more moderate than those caused by double infection. The I particles caused no clear symptoms of any of eight cultivars in this experiment, even though seven of them became infected with I particles.

DISCUSSION

The B and I viruslike particles isolated from penyakit habang-diseased plants in these experiments were similar in morphology to the bacilliform and isometric particles

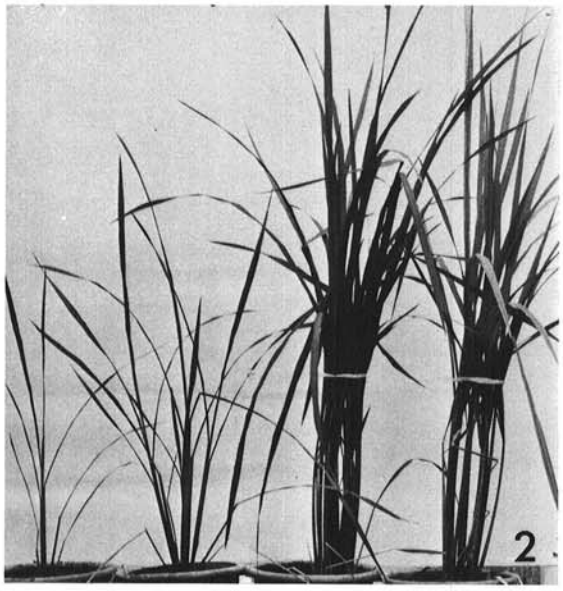

Fig. 2. Symptoms on rice plants, cultivar Taichung Native 1, caused (from left) by double infection with isometric and bacilliform virus particles, and by single infection with bacilliform and isometric particles respectively. A noninfected plant is on the right.

TABLE 1. Transmission of isometric (I) and bacilliform (B) virus particles by *Nephotettix virescens* allowed to acquire these two particles from infected plants separately for 2 days in the first access and for 8 hr in the second access. Insects were given an 8-hr or overnight inoculation access period on seedlings of cultivar Taichung Native 1 or Pelita I/1. Test plants were examined for the presence of virus particles by the use of quick-dip techniques about 1 mo after inoculation

Exp. no.	Acquisition access: 1st	Time after 1st access	2nd	Total plants	Virus transmission (plants with virus particles): B+I	B	I	None
1[a]	I	8 hr[c]	...	13	0	0	12	1
	B	8 hr[c]	...	4	0	0	0	4
	I	0	B	11	5	5	0	1
	I	1 day	B	7	1	5	0	1
	B	0	I	7	0	0	0	7
	B	1 day	I	6	0	0	1	5
2[a]	I	0	B	13	1	9	1	2
	...	...	I	8	0	0	7	1
	B	0	I	8	0	0	7	1
3[b]	I	0	B	12	6	5	0	1
	B	0	I	7	0	0	7	0

[a]Cultivar Taichung Native 1.
[b]Cultivar Pelita I/1.
[c]Insects were held on healthy seedlings for 8 hr after the acquisition access and then were allowed the inoculation access period.

reported in rice plants infected with tungro or tungro-group diseases (1, 2, 3, 10, 11, 12). Present results of the inoculation tests on eight rice cultivars indicate that so called tungro symptoms are caused by the B particles, but not by the I particles. However, the presence of I particles intensifies the symptoms which are caused by the B particles.

It is proposed that the B and I particles interact in some manner during acquisition or inoculation by the vector. The I particles were transmitted singly by the vectors, but the B particles were transmitted only when the I particles were acquired previously or at the same time. On the other hand, some rice plants infected with penyakit habang contained the B particles alone. These results indicate that the B particles can not be transmitted by the vector in the absence of the I particles but can multiply independently in rice plants once transmission occurred. The fact that the plants inoculated with B or I particles and then challenged with I or B particles, respectively, were not infected with the B particles also supports this conclusion. Transmission of the I particles by the vectors often was inhibited when both B and I particles were acquired by the vectors. It is remarkable that only one of 172 plants inoculated with a mixture of B and I particles was infected with I particles alone in the experiments, including the isolation and cultivar-reaction tests, and that many of the other plants were infected with both particles or with B particles alone.

Penyakit habang has been believed to be caused by rice tungro virus because of its symptoms, virus-vector interactions, and cultivar reactions (9). Recent investigation by electron microscopy support this evidence; Saito et al. (10) found that both isometric and bacilliform particles were associated with tungro disease in The Philippines and also with penyakit habang. In these experiments, the association of the two types of virus particles with penyakit habang was confirmed. These facts strongly indicate that tungro and penyakit habang are the same disease.

If tungro and penyakit habang are the same disease, the present findings conflict with the previous report that isometric particles purified from tungro infected plants caused symptoms in infectivity assay (2). The results might have been caused by contamination of bacilliform particles in the purified isometric virus fraction or the isometric particles from tungro-diseased plants in The Philippines may cause symptoms on rice plants. Further investigations are required to clarify this.

Rice waika virus (7, 14) in Japan has the same morphology as the I particles isolated here and induces mild stunting of rice plants. Furthermore, antiserum to rice waika virus reacted with the isometric virus particles purified from penyakit habang-diseased plants (13). In recent experiments, reaction of the antiserum with purified I particles was confirmed (Hibino et al *unpublished*). These results indicate that the I particle of penyakit habang is the same as or related to rice waika virus. Rice waika virus causes slight stunting of plants, but the I particles caused no clear symptoms on eight rice cultivars, although some infected plants developed slight stunting at rate growth stage. It is possible that the disease caused by the I particles may have spread throughout southeast Asia where tungro disease occurs but has not been noticed in fields due to the lack of symptom development.

TABLE 2. Relation between symptoms on rice plants of eight cultivars, inoculated using *Nephotettix virescens* with a mixture of isometric (I) and bacilliform (B) virus particles from penyakit habang-diseased plants, and the occurrence of the two types of particles in the inoculated plants

Cultivar	Inoculated plants (no.)	Virus particles detected[a]	No. of plants	Symptoms[b]
T(N)-1	8	B + I	6	St(S), Dc, Ivc
		B	1	St(M), Dc
		I	0	...
FK 135	30	B + I	9	St(S), Dc, Ivc
		B	17	St(S), Dc, Ivc
		I	0	...
Pankhari 203	15	B + I	0	...
		B	0	...
		I	0	...
Sukanandi	15	B + I	2	St(S), Dc, Ivc
		B	13	St(M), Dc
		I	0	...
Pelita I/1	14	B +I	1	St(S), Dc
		B	10	St(M), Dc
		I	0	...
IR-5	22	B + I	3	St(S), Dc
		B	14	St(M), Dc
		I	0	...
IR-8	22	B + I	3	St(S), Dc
		B	14	St(M), Dc
		I	0	...
Reiho	13	B + I	9	St(S), Y, Dc
		B	2	St(M), Dc
		I	0	...

[a]The plants were examined for the presence of virus particles by the use of quick-dip techniques about 1 mo after inoculation

[b]Symptoms are designated as follows: St = stunting; S = severe; M = moderate; Dc = red or yellow-orange discoloration; Ivc = interveinal chlorisis; and Y = yellowing.

TABLE 3. Transmission of isometric virus particles from rice plants infected with the isometric particles alone to seedlings of eight rice cultivars by *Nephotettix virescens*

Cultivar	Plants inoculated (no.)	Plants infected[a] (no.)
T(N)-1	18	15
FK 135	16	10
Pankhari 203	9	0
Sukanandi	9	4
Pelita I/1	12	8
IR-5	8	5
IR-8	10	9
Reiho	11	11

[a]The seedlings were examined for the presence of virus particles by the use of quick-dip techniques about 1 mo after inoculation.

LITERATURE CITED

1. GALVEZ, G. E. 1967. The purification of virus-like particles from rice tungro virus-infected plants. Virology 33:357-359.
2. GALVEZ, G. E. 1968. Purification and characterization of rice tungro virus by analytical density-gradient centrifugation. Virology 35:418-426.
3. GALVEZ, G. E., E. SHIKATA, and M. S. A. MIAH. 1971. Transmission and electron microscopy of a rice tungro virus strain. Phytopathol. Z. 70:53-61.
4. HINO, T., L. WATHANAKUL, N. NABHEERONG, P. SURIN, U. CHAIMONGKOL, S. DISTHAPORN, M. PUTTA, D. KERDCHOKCHAI, and A. SURIN. 1974. Studies on rice yellow orange leaf virus disease in Thailand. Trop. Agric. Res. Center (Tokyo) Tech. Bull. TARC No. 7. 67 p.
5. JOHN, V. T., 1968. Identification and characterization of tungro, a virus disease of rice in India. Plant Dis. Rep. 52:871-875.
6. LING, K. C. 1966. Nonpersistence of tungro virus of rice in its leafhopper vector, Nephotettix impicticeps. Phytopathology 56:1252-1256.
7. NISHI, Y., T. KIMURA, and I. MAEJIMA. 1975. Causal agent of "waika" disease of rice plants in Japan. Ann. Phytopathol. Soc. Japn. 41:223-227.
8. OU, S. H. 1972. Rice diseases. Commonw. Mycol. Inst., Kew, Surrey, England. 368 p.
9. RIVERA, C. T., S. H. OU, and D. M. TANTERA. 1968. Tungro disease of rice in Indonesia. Plant Disease Rep. 52:122-124.
10. SAITO, Y., M. IWAKI, and T. USUGI. 1976. Association of two types of particles with tungro-group diseases of rice. Ann. Phytopathol. Soc. Japn. 42:375 (Abstr.) (In Japanese).
11. SAITO, Y., N. NABHEERONG, T. HINO, H. HIBINO, S. SRIMARUT, and S. WUNGKOBKIAT. 1971. Purification of rice yellow orange leaf virus and observation on ultrathin sections of the disease. Ann. Phytopathol. Soc. Japn. 37:195 (Abstr.) (In Japanese).
12. SAITO, Y., M. ROECHAN, D. M. TANTERA, and M. IWAKI. 1975. Small bacilliform particles associated with penyakit habang (tungro-like) disease of rice in Indonesia. Phytopathology 65:793-796.
13. SINGH, K. G. 1969. Virus vector relationship in penyakit merah of rice. Ann. Phytopathol. Soc. Japn. 35:322-324.
14. YOKOYAMA, S. and H. SAKAI. 1975. Transmission by green leafhopper, Nephotettix cinticeps Uhler in new dwarf disease of rice plants. Ann. Phytopathol. Soc. Japan 41:219-222.