Transmission of Two Kinds of Rice Tungro-Associated Viruses by Insect Vectors

Hiroyuki Hibino, Nasir Saleh, and M. Roechan

Plant pathologist, Indonesia-Japan Joint Food Crop Research Program, Central Research Institute for Agriculture (CRIA), Jalan Merdeka 99, Bogor, Indonesia (on leave from Institute for Plant Virus Research, Yatabe, Tsukuba, Ibaraki, Japan), and assistant plant pathologists, Pests and Diseases Division, CRIA, Bogor, respectively.
We wish to thank Y. Iwata, D. M. Tantera, and Y. Saito for review and criticism of this manuscript.
Accepted for publication 18 May 1979.

ABSTRACT

HIBINO, H., N. SALEH, and M. ROECHAN. 1979. Transmission of two kinds of rice tungro-associated viruses by insect vectors. Phytopathology 69:1266-1268.

Two kinds of tungro-associated viruses, bacilliform (B) and isometric (I), were transmitted together or separately by *Nephotettix virescens* from rice plants infected with both B and I particles. Both particles were acquired in a 30-min acquisition access period, transmitted in a 10-min inoculation access period, and retained by the insects for 3 days. I particles were transmitted from plants infected with I particles alone, but B particles were not transmitted from plants infected with B particles alone. *N. nigropictus* transmitted I particles from rice plants infected with I particles alone and, occasionally, I particles but not B particles from doubly infected plants.

Additional key words: rice waika virus.

Tungro is one of the most important rice diseases in tropical Asian countries. The disease has been thought to be caused by an isometric "rice tungro virus" (2) transmitted by leafhoppers, efficiently by *Nephotettix virescens* and inefficiently by *N. nigropictus* and *Recilia dorsalis* (2,6). Small bacilliform particles also have been found in thin sections of tungro-diseased rice cells (1,5,11–13). In our previous reports, dip preparations from tungro-diseased rice plants examined with an electron microscope (4,5) contained bacilliform (B) or isometric (I) particles or both. I particles were transmitted alone by *N. virescens* but B particles were dependent on I particles for transmission (4,5). Symptoms were caused mainly by B particles and were intensified by I particles.

Rice tungro virus-vector interactions have been investigated in several countries (3,6,7,9,10,16). In those investigations, infection was assumed on the basis of symptoms on inoculated plants that might have contained B and/or I particles. Because tungro disease is associated with two kinds of virus particles, we reexamined the interactions between the tungro-associated particles and the insect vectors. B and I particles were transmitted together or separately by insects, and infection was judged by virus particles in inoculated rice leaves.

MATERIALS AND METHODS

A tungro isolate collected from South Sulawesi in 1976 was maintained on rice seedlings by successive transfers using *N. virescens*. I particles isolated from a tungro-diseased rice plant (4) were maintained similarly. B particles were isolated from the tungro isolate in each experiment; plants containing only B particles (4) were selected.

Colonies of *N. virescens, N. nigropictus,* and *R. dorsalis* were reared on rice seedlings in screened cages. The insects did not transmit any known viruses or mycoplasmalike organisms in tests with rice seedlings. Male adults generally were used to transmit viruses. Acquisition and inoculation accesses were conducted in an air-conditioned (26–28 C) room. Unless otherwise specified, insects given an acquisition access period of 1 day on a diseased plant were allowed an inoculation access period of 8 hr on each seedling (cultivar Taichung Native 1) at the first or second leaf stage in a test tube. Two insects were allowed for transmission from plants infected with both B and I particles, and five insects were allowed for transmission from plants infected with either B or I particles. Inoculated seedlings were grown in a greenhouse. About 3 wk after inoculation, seedlings were examined for virus particles by the dip method (4); a Hitachi HS-9 electron microscope was used.

00031-949X/79/000226$03.00/0

RESULTS

Transmission of B and I particles by *N. virescens*. B and I particles were transmitted in various periods of acquisition and inoculation accesses from rice plants infected with both particles. Both particles were acquired in a 30-min, but not in a 10-min, acquisition access period and were transmitted in a 10-min inoculation access period (Table 1). Transmission of both particles was more efficient with longer acquisition and inoculation access periods.

Effect of insect number on transmission is shown in Table 2. Both B and I particles were transmitted by 25% of single insects and B particles alone by 45%. B particles were transmitted by 70% of insects and I particles by 25%. The percentage of seedlings infected with both B and I particles increased as the number of insects per seedling increased.

Retention periods of B and I particles by *N. virescens*. Insects given acquisition access to both B and I particles were transferred daily to fresh seedlings for inoculation access. Infectivity of insects decreased markedly at the second inoculation access (Table 3). Retention periods of B and I particles were 3 and 2 days, respectively.

In another experiment, 78 insects given acquisition access to both B and I particles were allowed an inoculation access period of 8 hr on seedlings and then held overnight on a plant infected with I particles only. A second inoculation access period of 8 hr was followed by an overnight acquisition access period to I particles. These acquisition and inoculation access periods were repeated five times. Similar serial transmissions were repeated with 66 insects. Tungro symptoms appeared on seedlings inoculated at the first to third access periods but not on those inoculated at the fourth or later periods, suggesting that the insects retained B particles for 3 days, even when they acquired I particles daily.

Transmission of I particles by *N. virescens*. I particles were transmitted from rice plants infected with I particles alone. Particles were transmitted by 60% of single insects, and nearly 100% of the seedlings were infected when more than four insects were allowed inoculation access. Insects were given acquisition access periods of 10 min to 1 day and an inoculation access period of 8 hr. In another experiment, viruliferous insects were given

TABLE 1. Effect of acquisition and inoculation access periods on transmission of bacilliform (B) and isometric (I) particles from rice plants infected with both particles by *Nephotettix virescens*

Acquisition access period	Inoculation access period[a]	Number of seedlings with particles			
		B + I	B	I	None
10 min	8 hr	0	0	0	20
30 min	8 hr	3	2	1	14
1 hr	8 hr	6	1	0	13
8 hr	8 hr	14	2	1	3
1 day	8 hr	12	7	0	1
1 day	10 min	6	5	1	8
1 day	30 min	5	4	1	10
1 day	1 hr	11	4	0	5
1 day	8 hr	13	3	0	4

[a]Two insects per seedling.

inoculation access periods of 10 min to 8 hr. I particles were acquired in a 1-hr acquisition access period and were transmitted in a 10-min inoculation access period. Retention period of the particles by 275 insects was determined in two separate experiments; five insects were transferred daily to each seedling for inoculation access. The insects retained I particles for 4 days. The insects did not transmit B particles from plants infected with B particles alone.

Transmission of B and I particles by *N. nigropictus*. Efficiency of *N. nigropictus* and *N. virescens* in transmitting B and I particles from a plant infected with both particles was compared. *N. nigropictus* and *N. virescens* inoculated 63 and 65 seedlings, respectively. *N. nigropictus* transmitted I particles but not B particles to one seedling. *N. virescens* transmitted both particles to 29 seedlings, B particles only to 13, and I particles only to one.

Tests for transmission of I particles by *N. nigropictus* and *R. dorsalis*. Seedlings were inoculated with I particles separately by *N. nigropictus* and *R. dorsalis*. *N. nigropictus* infected 13 of 21 seedlings, but *R. dorsalis* did not infect any of 22 seedlings.

DISCUSSION

Rice tungro virus is transmitted by *N. virescens* in a semipersistent manner; the insect retains the agent for 3–5 days, and no definite latent period has been shown. Rice waika virus is related serologically to the I particles (5,11,12) and is transmitted similarly by *Nephotettix* spp. (8,14,15). In our experiments, both B and I particles were transmitted in a semipersistent manner from doubly infected plants by *N. virescens*. I particles were transmitted from plants infected with I particles alone but B particles were not transmitted alone, as reported previously (4,5). The results of our experiments did not indicate that the manner of B particle transmission differed from that of I particle transmission. The insects retained B particles for 3 days, even when they were given daily acquisition access to I particles.

B particles from doubly infected plants were transmitted by 70% of *N. virescens* and I particles, by 25%; I particles from plants infected with I particles alone were transmitted by 60% of the insects. *N. nigropictus* was more efficient in transmitting I particles from a plant infected with I particles alone than from a doubly infected plant, suggesting interaction between B and I particles in the insect vectors. In a previous report, transmission of I particles by *N. virescens* was inhibited when both B and I particles were acquired by the insect (4). These results and the fact that B particles require assistance from I particles for transmission (4,5) indicate complex interactions between the two kinds of particles in insect transmission.

I particles are also transmitted by *N. cincticeps* (H. Hibino et al, *unpublished*) as efficiently as by *N. virescens* and *N. nigropictus*, whereas tungro disease is transmitted efficiently by *N. virescens* and less efficiently by *N. cincticeps*. Therefore, attention must be given to the spread of I particles in tungro-free areas.

TABLE 2. Effect of insect number on transmission of bacilliform (B) and isometric (I) particles from rice plants infected with both particles by *Nephotettix virescens*[a].

Number of insects	Number of seedlings with particles			
	B + I	B	I	None
1	5	9	0	6
2	10	8	0	2
3	12	7	0	1
4	13	4	0	3
5	13	7	0	0

[a]Insects were given an acquisition access period of 1 day and an inoculation access period of 8 hr.

TABLE 3. Results of serial daily transmission of bacilliform (B) and isometric (I) particles by *Nephotettix virescens* after termination of an acquisition access period of 1 day on rice plants infected with both particles[a]

Transfers	Number of seedlings with particles			
	B + I	B	I	None
1	18	10	0	2
2	1	5	0	22
3	0	1	0	27
4	0	0	0	28
5	0	0	0	26
6	0	0	0	22

[a]Two insects per seedling in each transfer.

LITERATURE CITED

1. FAVALI, M. A., S. PELLEGRINI, and M. BASSI. 1975. Ultrastructural alterations induced by rice tungro virus in rice leaves. Virology 66:502-507.
2. GALVEZ, G. E. 1971. Rice tungro virus. C.M.I./A.A.B. Descriptions of Plant Viruses. No. 67. 3 pp.
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. HIBINO, H., M. ROECHAN, and S. SUDARISMAN. 1978. Association of two types of virus particles with penyakit habang (tungro disease) of rice in Indonesia. Phytopathology 68:1412-1416.
5. HIBINO, H., N. SALEH, H. JUMANTO, S. SUDARISMAN, and M. ROECHAN. 1978. Two kinds of virus particles associated with tungro disease of rice (Abstr.) Ann. Phytopathol. Soc. Jpn. 44:394. (in Japanese).
6. HINO, T., L. WATANAKUL, 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. Tech. Bull. TARC No. 7. Tropical Agricultural Research Center, Tokyo. 67 pp.
7. JOHN, V. T. 1968. Identification and characterization of tungro, a virus disease of rice in India. Plant Dis. Rep. 52:871-875.
8. KIMURA, T., I. MAEJIMA, and Y. NISHI. 1975. Transmission of rice waika virus by *Nephotettix virescens*. (Abstr.) Ann. Phytopathol. Soc. Jpn. 41:115. (in Japanese)
9. LING, K. C. 1966. Nonpersistence of the tungro virus of rice in its leafhopper vector *Nephotettix impicticeps*. Phytopathology 56:1252-1256.
10. RIVERA, C. T., S. H. OU, and D. M. TANTERA. 1978. Tungro disease of rice in Indonesia. Plant Dis. Rep. 52:122-124.
11. SAITO, Y. 1977. Interrelationship among waika disease, tungro and other similar diseases of rice in Asia. Pages 129-135 in: Tropical Agricultural Research Series No. 10. Tropical Agricultural Research Center, Tsukuba, Ibaraki, Japan. 7 pp.
12. SAITO, Y., M. IWAKI, and T. USUGI. 1976. Association of two types of particles with tungro-group disease of rice. (Abstr.) Ann. Phytopathol. Soc. Jpn. 42:375. (in Japanese)
13. 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.
14. SATOMI, T., J. HIRAO, and T. KIMURA. 1975. Transmission of the rice waika virus by a new leafhopper vector, *Nephotettix nigropictus* (Stal) (Homoptera: Cicadelloidea). Proc. Assoc. Plant Protec. Kyushu

21:60-63.
15. SHINKAI, A. 1975. Rice waika disease: Transmission by insect vectors and host range. (Abstr.) Ann. Phytopathol. Soc. Jpn. 41:87. (in Japanese)
16. SINGH, K. G. 1969. Virus vector relationship in penyakit merah of rice. Ann. Phytopathol. Soc. Jpn. 35:322-324.