Occurrence and Spread of Rice Tungro Spherical Virus in the Philippines

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ABSTRACT

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Rice virus diseases were surveyed in the Philippines from November 1983 to March 1985. Rice tungro was the most prevalent disease. Leaf samples collected in the field were tested for rice viruses by enzyme-linked immunosorbent assay (ELISA). Rice grassy stunt and rice ragged stunt viruses were not observed in the visual survey but were detected by ELISA in some leaf samples collected at Camarines Sur, Iloilo, Laguna, and Antique. In most locations, many plants showing tungrolike leaf yellowing contained rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). In some locations, however, many leaf samples with similar symptoms contained neither virus. Many plants without the symptoms in the same fields contained RTSV alone. RTSV also occurred in the fields where tungrolike symptoms were not observed. A high proportion of vector leafhoppers collected in the fields transmitted RTSV alone. These results indicate that aside from tungro (a disease complex associated with RTBV and RTSV), RTSV also occurs and spreads as an independent disease in the Philippines.

Additional key words: epidemiology, *Nephotettix virescens, N. nigropictus, Recilia dorsalis,* rice tungro virus, rice waika virus

Rice tungro, a destructive virus disease, is one of the most important rice diseases in many South and Southeast Asian countries including the Philippines. Rice tungro spherical virus (RTSV) was believed to be the causal virus of tungro and was once called rice tungro virus (4,5). Now, RTSV is known as a latent virus and acts as a "helper" for the transmission of rice tungro bacilliform virus (RTBV) by leafhopper vectors (6,9). RTBV causes the tungro symptoms and RTSV enhances the symptoms. RTBV and RTSV are transmitted by several species of leafhoppers in a semipersistent manner (7). Tungro is apparently a disease complex associated with RTSV and RTBV.

Because of the complexity of the disease, tungro epidemiology is not well understood. Recently, Cabauatan and Hibino (1) found that RTSV was spreading as an independent virus in experimental fields in the International Rice Research Institute, Philippines, and suggested that this phenomenon might have key importance for understanding tungro epidemiology. Here, we report the results of our survey on rice virus diseases in the Philippines indicating the wide occurrence of RTSV. Preliminary results have been reported (1,12).

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MATERIALS AND METHODS

Surveys and sampling. Rice virus diseases were surveyed in wetland fields in several rice-producing provinces of the Philippines from November 1983 to March 1985. Disease incidence was estimated on the basis of visual observation, and leaf samples were also collected for serological identification of the diseases on early to late tillering stages. To survey symptomless plants, hills with or without tungrolike leaf yellowing symptoms were randomly selected in each field and one leaf sample about 10 cm long was collected from the second or third youngest leaf of each hill. Collections were also made from fields totally infected with tungro and from fields without tungrolike symptoms. The leaf samples were tested separately for the viruses by the enzyme-linked immunosorbent assay (ELISA).

Transmission test. From January to March 1985, vector leafhopper species *Nephotettix virescens* Distant, *N. nigropictus* Stål, and *Recilia dorsalis* Motschulsky were collected with sweep nets. Immediately after collection, adults and nymphs of the leafhoppers were individually confined in test tubes with one 9- to 12-day-old seedling of the rice cultivar TN1 for an overnight inoculation access. After the leafhoppers were removed or killed, seedlings were transplanted in pots and grown in a greenhouse for 14–21 days, then tested by ELISA.

ELISA. Leaf samples were homogenized separately with phosphate-buffered saline (pH 7.4) containing 0.05% Tween 20 in a Combined Leaf and Bud Press (Erich Pollahne, FRG). Extract was directly tested by ELISA following the procedure described by Clark and Adams (2). Immunoglobulin (IgG) was purified from antisera to RTBV, RTSV (14), rice grassy stunt virus (RGSV) (11), and rice ragged stunt virus (RRSV) (8). An Immulon II plate (Dynatech) was coated with IgG at 1, 4, 1, and 1 μg/ml, and IgG-alkaline phosphatase conjugate was diluted 1,000, 500, 1,000, and 1,000 times for RTBV, RTSV, RGSV, and RRSV, respectively. Reactions were evaluated at 405 nm with a Minireader MR590 (Dynatech). ELISA detected RTBV, RTSV, RGSV, and RRSV in virus-infected leaf extracts diluted up to 1/1,024, 1/512, 1/4,096, and 1/1,024, respectively (8,11,12). Presence of these virus antigens in the extracts did not affect the efficiency of the assay to detect RTBV or RTSV (N. B. Bajet and H. Hibino, *unpublished*).

RESULTS

Incidence of rice virus diseases. The most common and widely planted rice cultivars in the fields visited were IR36 and IR42. In 1983–1984, rice tungro was prevalent in many sites regardless of the crop stages (except in the seedbed), especially on IR36 and IR42 (Table 1). In Isabela and Nueva Ecija provinces, infected IR36 and IR42 at early to late tillering were often cut down or used for cattle grazing. In 1985, tungro incidence was lower. Rice cultivars relatively free of tungro were IR50, IR52, IR54, IR58, and IR60.

No plants showing symptoms typical of RGSV or RRSV were observed in the areas surveyed. However, a few leaf samples collected from Laguna, Camarines Sur, ILoilo, and Antique contained RRSV or RGSV antigens (Table 1).

Incidence of RTBV and RTSV. Because RTBV/RTSV incidence was high while RGSV and RRSV incidence was very low or zero in the early survey, subsequent ELISA was done for RTBV and RTSV only. About 53% of leaves showing tungrolike symptoms contained both RTBV and RTSV, whereas about 26% of the nonsymptomatic leaves contained RTSV alone (Table 2). Some leaf samples collected at Pila and Pagsanjan, Laguna, where no tungro symptoms were observed, contained RTSV. On the other hand, leaf samples collected in some fields showing tungrolike leaf yellowing did not react to either RTBV or RTSV.

Infectivity of vector insects collected in the fields. *Nephotettix* spp. were more common than *R. dorsalis* in all fields visited. The three leafhopper species collected transmitted both RTBV and RTSV together or RTBV or RTSV alone (Table 3). However, *N. nigropictus* collected in Bicol transmitted RTSV alone. Of the 972 total leafhoppers tested, 1.74% transmitted both RTBV and RTSV, 2.26% transmitted RTBV alone, and 5.45% transmitted RTSV alone.

DISCUSSION

The survey from 1983 to 1985 showed that rice tungro was the most prevalent virus disease of rice in the Philippines. The disease was severe on IR36 and IR42, indicating their susceptibility to tungro. These became popular cultivars because of their high and stable yield, but unless more tungro-resistant cultivars are grown, the disease may continue to be an important constraint to rice production.

RTBV and RTSV were predominantly associated with rice plants with tungrolike leaf yellowing, whereas many rice plants without symptoms were infected with RTSV alone. In greenhouse tests, rice seedlings are rarely infected with RTSV alone when inoculated by the vector leafhoppers maintained on plants with both RTBV and RTSV (7,10). However, RTSV can be readily transmitted by the leafhoppers that have fed on plants with RTSV alone (7,10). The high incidence of RTSV-infected plants in farmers' fields indicates that RTSV was spreading as an independent disease. These surveys found RTSV even in rice fields without tungrolike symptoms, and the transmission tests showed common presence of RTSV-infective leafhoppers in the fields. These results indicate wide occurrence and spread of RTSV in the Philippines.

These results also confirm and extend the results obtained previously with rice samples from experimental farms (1). The common presence of RTSV in the Philippines suggests that RTSV may have some important role in tungro epidemics.

Symptoms of tungrolike leaf yellowing on samples that did not react in ELISA were probably due to disorders other than tungro. Because tungro is difficult to diagnose by symptomatology, it has often been confused with physiological disorders and damages caused by other pests. It was very unlikely that ELISA was not able to detect RTBV and RTSV in plants with tungro. The assay could detect the viral antigens in seedlings as early as 6–8 days after inoculation access (N. B. Bajet and H. Hibino, *unpublished*).

It is known that *N. virescens* is the most efficient vector of the tungro viruses (7,10,13). In transmission tests conducted in Indonesia and Japan (7,10), *N. nigropictus* transmitted RTBV or RTSV at very low efficiency while *R. dorsalis* failed to transmit the viruses. In these experiments, *N. nigropictus* and *R. dorsalis* collected in the fields transmitted RTBV and/or RTSV. The efficiency of RTBV and RTSV transmission may be different depending on the leafhopper colony used.

RTSV, which was epidemic in 1971–1974 in Kyushu, Japan, was called rice waika virus (3). Rice waika virus caused mild stunting and occasional leaf yellowing on some susceptible Japonica rice cultivars (3). In the Philippines, Indica cultivars are commonly planted, and all Indica rice cultivars tested so far do not show clear symptoms when infected with RTSV alone. Evaluation of

Table 1. Occurrence of rice virus diseases in the Philippines

Region	Province	Sites (no.)	Cultivars	Average disease incidence (%)[a]		
				Tungro	Grassy stunt	Ragged stunt
I	La Union	7	Unknown	1	0	0
	Pangasinan	10	Unknown	18	0	0
II	Cagayan	10	IR36, C-1000 Malagkit sungsong	20	0	0
	Isabela	16	IR36, IR42, IR58 C-1000, R10, unknown	54	0	0
III	Tarlac	5	IR36, unknown	40	0	0
	Pampanga	1	Unknown	15	0	0
	Bulacan	1	Unknown	0	0	0
	Nueva Ecita	15	IR36, IR42, IR2307 R5, unknown	27	0	0
	Zambales	1	IR36	80	0	0
IV	Laguna	16	IR36, IR42, IR48, IR52, IR56, IR58, IR60, Sinandomeng	5	+[b]	+[b]
	Palawan	25	IR36, IR46, IR52, IR56, C4-63G, C-1000, Malagkit sungsong, Pinili, Isang dakot, unknown	6	0	0
V	Albay	5	IR42, IR54	10	0	0
	Camarines Norte	3	IR36, IR42, IR50, IR58	11	0	0
	Camarines Sur	27	IR36, IR42, IR50, IR58, C-1000	23	+[b]	0
VI	Antique	12	IR36, IR42, IR60	51	0	+[b]
	Iloilo	27	IR36, IR42, UPL-Ri 3, BE3	38	+[b]	0
VII	Cebu	1	Unknown	5	0	0
VIII	Leyte	3	IR42	0.1	0	0

[a] Visual observation.

[b] In other tests, some leaf samples tested in ELISA were found to be infected with RGSV or RRSV.

Table 2. Incidence of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) in rice plants with and without tungrolike leaf yellowing collected in several provinces in the Philippines

		With symptoms[a]				Without symptoms[a]			
		Plants	Plants infected (no.)			Plants	Plants infected (no.)		
Location	Cultivar	tested	RTBV + RTSV	RTBV	RTSV	tested	RTBV + RTSV	RTBV	RTSV
Cagayan									
Baculog	IR36	10	3	0	1	10	0	1	0
Dugayong	C-1000	10	0	0	2	10	0	0	3
Nagulisan	IR36	14	11	0	1	14	6	1	3
Isabela									
Sn Fernando	Ri-10	10	9	0	1	10	0	0	1
Soyong	C-1000	10	2	0	0	10	0	0	2
Nueva Ecija									
Guimba	?	20	18	0	1	20	0	0	8
Galvan	IR42	20	20	0	0	20	1	0	10
Banitan	IR42	20	14	0	2	20	1	0	13
M. Marikit	IR42	10	10	0	0	10	1	0	4
Muñoz	?	10	9	0	1	35	13	0	8
Tarlac									
Camiling (Burot)	IR36	20	16	0	4	20	1	0	7
Capas (Dolores)	IR36	10	10	0	0	10	0	0	10
Zambales	IR36	10	10	0	0	10	5	0	3
Laguna									
Los Baños	IR36	45	42	1	0	45	2	0	14
Los Baños	IR42	45	40	0	0	45	5	1	18
Pila	?	0	...	...	...	10	0	0	3
	IR58	10	0	0	0	0	...	...	...
Mabitac	IR56	20	0	0	0	0	...	...	...
Pagsanjan	?	0	...	...	...	20	0	0	9
Cabuyao	IR42	20	0	0	0	0	...	...	...
Camarines Norte									
Talisay	?	30	0	0	0	30	0	0	0
Sn Fernando	?	25	0	0	0	25	0	0	0
Camarines Sur									
(Pili)	IR42	20	0	0	0	20	0	0	0
Albay									
Ligao	?	30	10	1	1	0	...	...	...
Polangui	IR50	0	...	...	...	10	0	0	0
Cagsawa	IR42	0	...	...	...	50	0	0	0
Total		419	224	2	14	454	35	3	116

[a] Leaf samples were randomly collected and tested in ELISA for the presence of RTBV and RTSV.

Table 3. Infectivity of leafhoppers collected in rice fields in Laguna and Bicol provinces (January to March 1985)

		Insects tested	Insects transmitted (no.)[a]		
Location	Insect species	(no.)	RTBV + RTSV	RTBV	RTSV
Laguna	*Nephotettix virescens*	278	8	15	20
	N. nigropictus	186	2	3	7
	Recilia dorsalis	61	2	1	8
Bicol	*N. virescens*	170	3	3	8
	N. nigropictus	271	0	0	10
	R. dorsalis	6	2	0	0
Total		972	17	22	53

[a] Individual leafhoppers were given an overnight inoculation access to rice seedlings, and inoculated seedlings were tested in ELISA for the presence of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV).

cultivars for their reactions to RTSV as well as to tungro is important to prevent possible development of RTSV epidemics similar to the rice waika epidemic in Japan (3).

In these surveys, ELISA was applied to diagnose rice virus diseases. Rice virus disease incidence has been scored previously on the basis of symptomatology. Visual scoring of virus disease incidence is difficult and often unreliable, especially for tungro. These experiments indicated the usefulness of serodiagnosis in tungro monitoring.

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