Resistance to Rice Tungro Spherical Virus in Rice

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ABSTRACT

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Twenty-nine improved rice cultivars were evaluated in the field and in the greenhouse for resistance to infection with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). Many cultivars had low field infection rates with RTBV and RTSV combined but had relatively high infection rates with RTBV alone. These cultivars also had similar infections when seedlings were inoculated by leafhoppers (*Nephotettix virescens*) that had fed on plants infected with RTBV and RTSV. When seedlings were inoculated by leafhoppers fed on RTSV-infected plants, only IR20, IR26, IR30, and IR40 had low infection rates. TKM6, which is common in the parentages of these four cultivars, reacted in the same manner as these cultivars in the field and greenhouse. A leafhopper colony selected on IR20 increased in its ability to colonize IR20 and IR26 but did not transmit RTSV to IR20 and IR26. These results suggest that the resistance of the four cultivars to RTSV infection may not be because of resistance to the leafhopper. Possible advantages of these cultivars in tungro management are discussed.

Tungro (26) is the most important viral disease of rice (*Oryza sativa* L.) in South and Southeast Asia. It is a composite disease caused by rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) (22). RTBV causes mild tungro symptoms on rice plants, and RTSV causes no clear symptoms on most rice cultivars and enhances the symptoms caused by RTBV infection (16). RTBV and RTSV are transmitted in a semi-persistent manner by the rice green leafhopper *Nephotettix virescens* (Distant) and other leafhopper species (14,16,17). RTBV depends on RTSV for transmission by the leafhopper (5,15,16). RTSV also spreads as an independent disease in the Philippines (2,4).

Tungro has been managed mainly by planting resistant cultivars and applying insecticides to reduce vector populations. Rice cultivars resistant to tungro have been bred in many countries. The International Rice Research Institute (IRRI) has developed high-yielding cultivars resistant to tungro, and many of them have been planted widely and used as parents in breeding programs in Asian countries.

Recent studies on tungro resistance in IRRI-bred cultivars (indicated as IR) suggested that a low level of tungro disease in some IR cultivars was due to their resistance to the leafhoppers (11,19,25). When leafhopper-resistant cultivars were artificially inoculated by leafhoppers that had fed on plants infected with RTBV and RTSV, they showed relatively high infection rates, primarily with RTBV only (7,18,19). The nature of resistances to tungro in other IR cultivars remains to be characterized.

We evaluated 29 improved rice cultivars for their reactions to infection with the tungro viruses and documented characteristic resistance to RTSV infection in some cultivars. A preliminary report has been published (9).

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MATERIALS AND METHODS

Viruses and insect. A colony of *N. virescens* that had been reared for several years on plants of the rice cultivar Taichung Native 1 (TN1) was used. From time to time, leafhoppers from that colony were tested for their infectivity and were found to be virus-free. The tungro isolate used was originally collected at Laguna, Philippines, and was maintained on TN1 by successive transfers with viruliferous *N. virescens*. Tungro-inoculated TN1 plants were individually tested by latex serology (21), and plants infected with both RTBV and RTSV were selected as a source. RTSV was isolated by selecting a plant infected with RTSV alone and was maintained similarly (19).

Reaction in fields. IR cultivars planted in the rice tungro nursery during the wet season in 1983 and the dry season in 1984 at the IRRI farm were used to assess incidences of RTBV and RTSV. Samples were also collected from IR cultivars during the wet season in 1985 in the tungro nursery. Fifty-two seedlings of each cultivar and of susceptible TN1 were transplanted alternately in two rows, 26 hills per row, with a 20-cm space between plants and a 20-cm space between rows. Tungro-infected TN1 plants were transplanted in four rows running perpendicular to the rows of entries. Cultivars planted in the 4×4 m^2 demonstration plots of IR parents in July 1985 were also used to assess tungro incidence. Leaf samples were collected from the fields and indexed by the latex test or by ELISA (2,3) about 50 days after transplanting.

Reaction in artificial inoculation. Newly emerged adult leafhoppers were allowed a 3- or 4-day acquisition access feeding on a source plant and then individually confined with 7-day-old seedlings in test tubes for 1 day. Inoculated seedlings were transplanted in pots and grown in a greenhouse. One month after inoculation, all inoculated seedlings were indexed by the latex test.

Reaction of leafhoppers to cultivars. Populations of *N. virescens* were reared on mixtures of IR20 and TN1 seedlings at increasing ratios of IR20 seedlings from generation to generation for nine generations and then maintained on IR20 seedlings for nine generations. Adult leafhoppers of each generation were tested for their abilities to transmit virus to IR20, IR26, and IR30 after feeding for 3–4 days on source plants infected with RTBV and RTSV. The ninth generation of the *N. virescens* population selected on IR20 seedlings

was compared with the original colony for its reaction on selected rice cultivars. Nymphal survival was determined by confining five second-instar nymphs on a 7-day-old seedling in a test tube for 4 days prior to counting the number of surviving leafhoppers; 100 nymphs were tested for each cultivar. Population growth was determined by confining one pair of adult leafhoppers with a 1-mo-old plant in a cage for 24 days prior to counting the number of first-generation leafhoppers; eight pairs were tested for each cultivar.

Feeding behavior and virus transmission efficiency were determined by confining individual adult females that had fed on plants infected with both RTBV and RTSV on a 1-wk-old seedling in a specially designed feeding cage (23) for 1 day. Feeding behavior (phloem and xylem feeding) was monitored by the reaction of honeydew spots on a bromocresol green-treated filter paper disk placed at the bottom of each cage. Blue spots (basic honeydew) indicate phloem feeding and orange or brown spots (acidic honeydew) indicate xylem feeding (1). Eighteen leafhoppers were tested for each cultivar. Inoculated seedlings were indexed by the latex test (21).

Table 1. Incidence of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) in tungro nurseries planted during 1983 wet season and 1984 dry season in an International Rice Research Institute experimental field at Laguna, Philippines[a]

	1983 Wet season				1984 Dry season			
		Percent plants infected[b] with:				Percent plants infected[b] with:		
Cultivar	No. plants tested	RTBV + RTSV	RTBV	RTSV	No. plants tested	RTBV + RTSV	RTBV	RTSV
IR5	42	50	5	43	52	96	0	4
IR8	25	96	0	4	46	96	4	0
IR20	45	20	60	7	42	5	95	0
IR22	33	76	0	21	52	87	13	0
IR24	43	0	58	2	51	84	14	2
IR26	36	6	19	0	52	0	92	0
IR28	49	43	14	20	52	6	15	8
IR29	48	21	13	10	52	8	12	12
IR30	49	18	29	8	52	2	50	0
IR32	48	56	10	25	52	75	6	10
IR34	52	74	0	19	52	17	10	17
IR36	40	45	0	40	52	92	2	2
IR38	50	40	28	14	52	62	2	0
IR40	49	12	53	4	52	0	90	0
IR42	45	91	0	9	52	73	19	8
IR43	42	71	10	2	51	92	4	2
IR44	41	69	0	17	49	92	6	0
IR45	36	86	0	14	52	87	4	10
IR46	50	96	2	2	52	67	29	4
IR48	43	88	0	12	50	90	4	4
IR50	49	18	14	22	52	21	15	19
IR52	31	19	10	42	52	0	31	13
IR54	50	18	10	36	48	2	25	10
IR56	44	14	11	11	52	44	6	35
IR58	30	20	0	43	39	3	8	8
IR60	52	13	13	27	52	4	19	2
IR62[c]	52	33	6	46	...[d]	...	...	...
IR64[c]	50	0	6	12	...	...	...	...
IR65[c]	52	6	4	2	...	...	...	...

[a] Plants were indexed by the latex test.
[b] Balance of plants tested were free from both viruses.
[c] Samples were obtained from nursery planted during 1985 wet season.
[d] Not tested.

Table 2. Incidence of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) in four rice cultivars planted during 1985 dry season in an International Rice Research Institute demonstration plot at Laguna, Philippines[a]

		Percent plants infected[b] with:		
Cultivar	No. plants tested	RTBV + RTSV	RTBV	RTSV
Gam Pai 30-12-15	90	0	1	9
Sigadis	90	50	22	7
Peta	90	2	12	7
TKM6	90	2	20	1

[a] Plants were indexed by ELISA.
[b] Balance of plants tested were free from both viruses.

Serological tests. One leaf sample about 10 cm long was cut from the second youngest leaf of a tiller of each plant. Leaf samples were collected from all plants artificially inoculated in test tubes or cages and from all plants of each cultivar planted in the tungro nurseries. Leaf samples were homogenized separately in 1 ml of 0.05 M Tris-HCl buffer, pH 7.2, using a combined leaf and bud press (Erich Pollahne, Wennigsen, Federal Republic of Germany). Approximately 20 μl of sap was mixed with the same volume of latex suspension, which was sensitized with anti-RTBV or anti-RTSV serum (21) in a well of a Microtiter Plate (Dynatech, Chantilly, VA). After the plate had been shaken for about 1 hr, the presence of RTBV or RTSV antigen was determined by the appearance of latex particle clumps under a light microscope at 100× magnification. Extracts of uninoculated and RTBV + RTSV infected TN1 leaves served as the control. One well was used for each sample. When the latex suspension was mixed with healthy leaf extracts or with heterologous virus, no clumping of latex particles was observed.

In the demonstration plots, leaf samples were collected from randomly selected plants and sap was directly tested by ELISA for separate detection of RTBV and RTSV, following the procedure described by Bajet et al (2,3). One well was used for each sample, and presence or absence of the viruses in extracts was determined by measuring absorbance at 405 nm in a Microelisa Minireader (Dynatech). Extracts of uninoculated and RTBV + RTSV infected TN1 leaves served as the control, and samples with absorbance at 405 nm more than three times the absorbance (means from four wells) of the uninoculated control were considered positive.

RESULTS

Reactions to RTBV and RTSV in the fields. Tungro incidence was very high in most cultivars in the rice tungro nurseries planted during the 1983 wet season and 1984 dry season. Percentages of infection with RTBV and RTSV combined or RTBV or RTSV alone differed according to the cultivar (Table 1). RTSV incidence was higher in the 1983 trial than in the 1984 trial. IR20, IR26, IR30, IR40, IR50, IR52, IR54, IR58, and IR60 had relatively low combined RTBV and RTSV infection rates in both trials. IR64 and IR65 had low infection rates with both viruses in the 1985 trial.

In the demonstration plot in July 1985, Gam Pai 30-12-15, Peta, and TKM6 had low combined RTBV AND RTSV infection rates (Table 2).

Reactions to RTBV and RTSV in artificial inoculation. When seedlings were inoculated by the leafhoppers that had fed on plants infected with both RTBV and RTSV, all cultivars tested

were generally infected with RTBV and RTSV combined or with RTBV alone (Table 3). IR20, IR26, IR28, IR29, IR30, IR34, IR40, IR50, IR52, IR54, IR58, IR60, IR64, and IR65 had low infection rates with combined RTBV and RTSV infection and relatively high infection rates with RTBV alone. When those cultivars were inoculated with RTSV alone, IR20, IR26, IR30, and IR40 had infection percentages of 0–4, whereas all other cultivars tested had relatively high infection rates (Table 3).

When seedlings of the IR parents were artificially inoculated by leafhoppers that had fed on plants infected with both RTBV and RTSV, all cultivars tested were infected, mostly with RTBV alone (Table 4). When seedlings were inoculated by leafhoppers that had fed on RTSV-infected plants, only TKM6 was not infected.

Reactions of selected leafhoppers to rice cultivars. The population of *N. virescens* was successfully maintained on IR20 when transferred to IR20 seedlings after having been reared on a mixture of IR20 and TN1 seedlings for nine generations. The ability of leafhoppers to transmit virus did not differ significantly at each generation (*data not shown*). In the transmission tests at each generation, IR20, IR26, and IR30 had no or very low infection rates with RTSV.

The leafhopper population maintained on IR20 for nine generations had higher nymphal survival on IR20 and IR36 and higher population growth on IR20, IR26, and IR36 than the original TN1 colony (Fig. 1A and B). In the transmission test, the IR20 colony transmitted RTBV alone to IR20, IR26, and IR54 (Fig. 1C). During the inoculation feeding on IR20, IR26, and IR36, the IR20 colony excreted slightly more basic honeydew (therefore fed more on phloem) (Fig. 1D). Ratios of leafhopper numbers that fed on both phloem and xylem or on phloem or xylem alone on IR20 and IR26 were similar for the IR20 and TN1 colonies.

DISCUSSION

In these experiments, IR28, IR29, IR34, IR50, IR52, IR54, IR58, IR60, IR64, and IR65, which had Gam Pai 30-12-15 in their parentage, reacted similarly to that parent against infection with RTBV and RTSV in the field and in the artificial inoculation tests. Likewise, IR20, IR26, IR30, and IR40, which had TKM6 in their parentage, reacted similarly to that parent. The Gam Pai 30-12-15 progenies were mostly infected with RTBV alone when inoculated by leafhoppers that had fed on plants infected with both RTBV and RTSV. The predominant RTBV infection was not because of their resistance to RTSV infection, since they were infected with RTSV at relatively high rates when inoculated by leafhoppers that had fed on RTSV-infected plants. On the other hand, the TKM6 progeny showed consistent resistance to RTSV infection by natural and artificial inoculations. The resistances in the Gam Pai 30-12-15 and TKM6 progenies are probably controlled by different major genes.

IR20, IR26, IR30, and IR40 have different levels of resistance to feeding and colonization by the leafhopper *N. virescens* (25). Generally, leafhopper-resistant cultivars are predominantly infected with RTBV alone when inoculated by leafhoppers that had fed on plants infected with both RTBV and RTSV (7–10,18,19,24,27). On the other hand, leafhopper-resistant cultivars may change their reactions to tungro infection when leafhopper populations selected on the resistant cultivars are used for inoculation (6,12,13). To clarify whether or not the resistance of the four cultivars to RTSV was due to resistance to the leafhopper, we compared the ability of the leafhopper

Table 3. Percentage of infection with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV), combined or alone, in rice seedlings separately inoculated in test tubes by confining single *Nephotettix virescens* adults that had fed on plants infected with both RTBV and RTSV or with RTSV alone[a]

	Inoculation with combined RTBV and RTSV				Inoculation with RTSV alone	
		Percent plants infected[b] with:				
Cultivar	No. plants tested	RTBV + RTSV	RTBV	RTSV	No. plants tested	Percent plants infected[b]
IR5	49	29	43	0	56	45
IR8	47	11	72	0	47	32
IR20	28	0	86	0	55	0
IR22	56	46	45	0	56	82
IR24	55	13	49	0	59	20
IR26	55	2	80	0	58	0
IR28	51	6	47	0	55	29
IR29	49	6	49	0	58	21
IR30	45	0	67	0	56	4
IR32	38	32	26	8	57	53
IR34	45	7	36	2	54	31
IR36	33	12	45	3	57	47
IR38	41	32	39	12	54	52
IR40	50	0	66	2	46	0
IR42	51	29	45	0	58	53
IR43	47	11	49	0	58	29
IR44	52	31	31	2	43	56
IR45	53	11	45	2	53	28
IR46	32	53	25	6	32	78
IR48	48	44	21	4	42	57
IR50	56	9	29	5	56	43
IR52	46	4	28	4	51	24
IR54	48	6	29	2	54	33
IR56	58	16	24	3	50	52
IR58	51	4	31	4	49	27
IR60	57	4	37	2	49	29
IR62	55	15	42	5	45	11
IR64	53	0	53	4	51	6
IR65	55	4	45	0	54	9

[a] Plants were indexed by the latex test.
[b] Balance of plants tested were free from both viruses.

Table 4. Percentage of infection with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV), combined or alone, in rice seedlings separately inoculated in test tubes by confining single *Nephotettix virescens* adults that had fed on plants infected with both RTBV and RTSV or with RTSV alone[a]

	Inoculation with combined RTBV and RTSV				Inoculation with RTSV alone	
		Percent plants infected[b] with:				
Cultivar	No. plants tested	RTBV + RTSV	RTBV	RTSV	No. plants tested	Percent plants infected[b]
Gam Pai 30-12-15	56	0	55	0	33	9
Sigadis	56	5	64	0	32	28
Peta	55	15	56	0	33	12
TKM6	50	0	84	0	35	0

[a] Plants were indexed by the latex test.
[b] Balance of plants tested were free from both viruses.

colony selected on IR20 to transmit the viruses to selected rice cultivars with that of the original colony. The IR20 colony had higher nymphal survival and population growth and fed more on phloem, indicating its ability to colonize IR20 and IR26, but could not efficiently transmit RTSV to the cultivars. We consider this to be indirect evidence that resistance of the four IR cultivars to RTSV infection may not be due to their resistance to the leafhopper. IR26 and IR30 showed consistent resistance to RTSV infection in several Philippine sites where reactions of other cultivars, including IR50, IR54, and IR64, to tungro varied (E. R. Tiongco and H. Hibino, *unpublished*). IR26 and IR30 also showed resistance to RTSV infection in South Sulawesi, Indonesia (R. C. Cabunagan and H. Hibino, *unpublished*).

IR20 was widely planted in the Philippines during 1972–1977, IR26 during 1974–1977, and IR30 during 1975–1977. IR40 was occasionally planted during 1977–1979. During those periods, the level of tungro disease on these cultivars was low. In a survey conducted in five central Luzon provinces from 1973 to 1980 (20), average tungro incidence during 1972–1977 was 1.62%; incidences were 0.65% on IR20, 0.36% on IR26, 0.12% on IR30, and 0.01% on IR40 (Hibino, *unpublished*). Although low tungro incidence in the four cultivars could also be due to their resistance to the leafhopper, the resistance to RTSV infection might have an important role in lowering the level of tungro disease on these cultivars. Rapusas and Heinrichs (25) also suggested a possible resistance to tungro infection not due to resistance to *N. virescens* in IR20 and IR30.

In fields planted to IR20, IR26, IR30, or IR40, source plants infected with RTBV and RTSV combined would be few or absent. Virus infection in these fields would occur only when leafhoppers fly from nearby fields affected with tungro and feed on the plants. Development of the disease from sources within these fields would be slow because plants infected with RTBV alone do not serve as a virus source (14,16,18,19), and therefore tungro disease would depend on the tungro inoculum level and leafhopper populations in surrounding fields. In artificial inoculation by confined feeding of vectors and in field screening where test cultivars are planted in rows or in small plots, the four cultivars showed high infection rates because of their susceptibility to RTBV infection. Hence, their possible importance to tungro management has been neglected since 1977. If plot size is sufficiently large, however, these cultivars would have low levels of tungro disease, as observed in farmers' fields from 1972 to 1977. We suggest that more attention should be given to this type of resistance for tungro disease management.

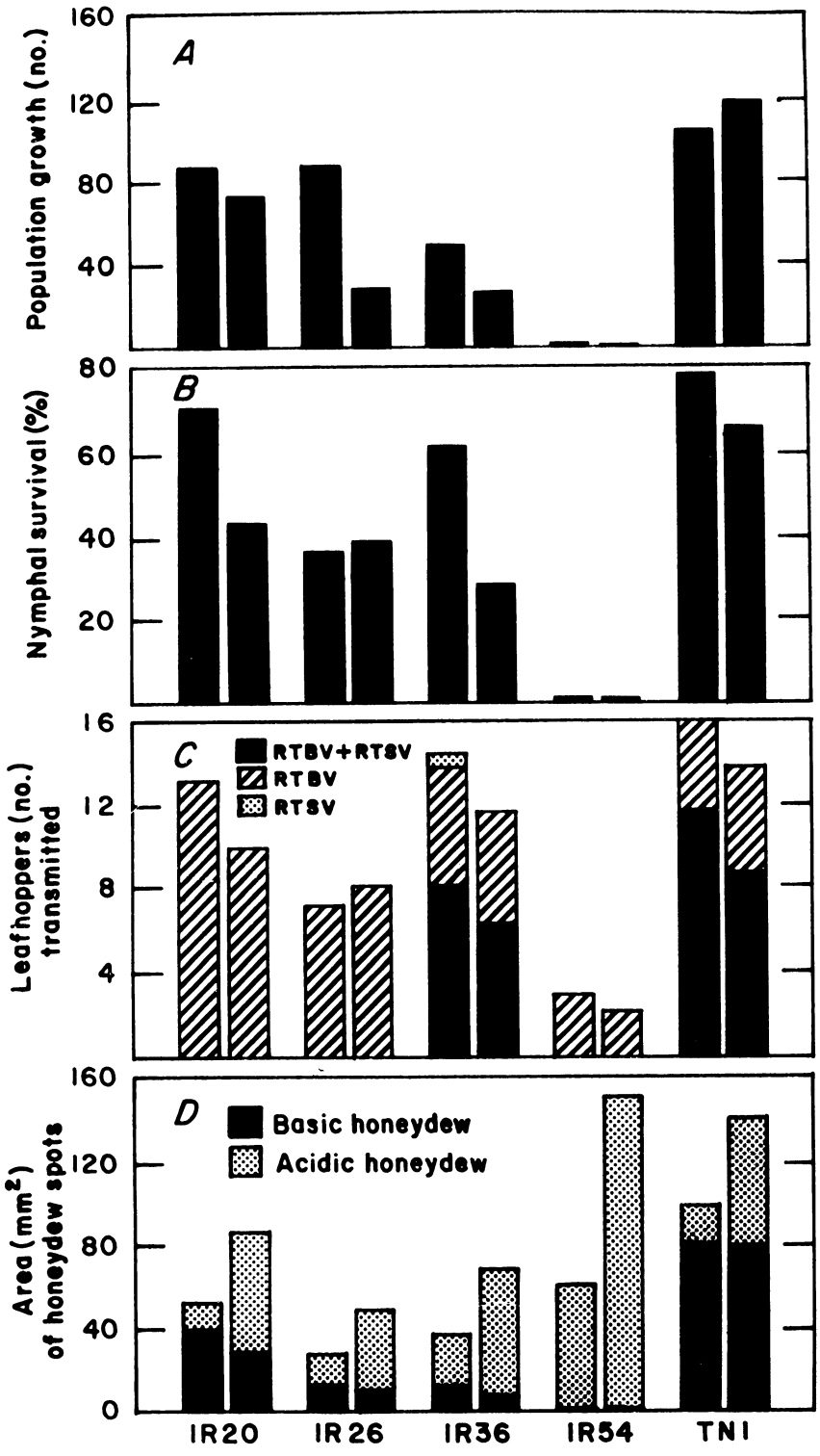

Fig. 1. Comparison of population characteristics, vector ability, and feeding locations on rice cultivars IR20, IR26, IR36, IR54, and TN1 between *Nephotettix virescens* populations selected on IR20 (left bar) and TN1 (right bar). **(A)** Population growth from one adult breeding pair after 24 days. **(B)** Nymphal survival after 4 days. **(C)** Percentage transmission of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) by individual *N. virescens* adult females caged for 1 day on 7-day-old seedlings of the five listed rice cultivars after a 4-day acquisition access period on TN1 plants infected with both RTBV and RTSV. **(D)** Areas (mm^2) of basic and acidic honeydew spots obtained during the day that single *N. virescens* females were confined to the rice seedlings used in Fig. 1C.

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