Resistance to Rice Tungro-Associated Viruses in Rice Under Experimental and Natural Conditions

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Accepted for publication 19 November 1986 (submitted for electronic processing).

ABSTRACT

Hibino, H., Tiongco, E. R., Cabunagan, R. C., and Flores, Z. M. 1987. Resistance to rice tungro-associated viruses in rice under experimental and natural conditions. Phytopathology 77:871-875.

Tungro is a composite disease resulting from infection with both rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). Both viruses are transmitted efficiently by the leafhopper *Nephotettix virescens*. Rice cultivars developed by the International Rice Research Institute with resistance to leafhoppers were evaluated under field and greenhouse conditions. In the field, moderately resistant cultivars had low tungro infection rates under low virus inoculum and leafhopper levels but high infection rates under high levels. Resistant cultivars had low infection rates regardless of inoculum or leafhopper levels. Resistance in cultivars was due to both antibiosis and nonpreference to adult leafhoppers. In mass or test-tube inoculations with leafhoppers that had fed on plants infected with RTBV and RTSV, resistant cultivars showed increasing rates of infection with increasing numbers of leafhoppers. When latex serological tests were used to index these inoculated plants, resistant cultivars had increasing RTBV infection rates, whereas moderately resistant cultivars had increasing RTBV and RTSV infection rates. Susceptible cultivars had high RTBV and RTSV infection rates irrespective of leafhopper number. When resistant cultivars were inoculated with RTSV alone, fairly high infection rates occurred. Low field infection rates in the resistant cultivars can be explained by their resistance to the leafhopper.

Additional key words: Rice green leafhopper, rice tungro disease, rice tungro virus.

Tungro (14), the most important virus disease of rice (*Oryza sativa* L.) in south and southeast Asia, is transmitted only in a semipersistent or transitory manner by leafhoppers (16). The most efficient vector species is the rice green leafhopper *Nephotettix virescens* (Distant).

Polyhedral virus particles isolated from plants with the tungro disease were once called rice tungro virus (7). Later, tungro was recognized as a composite disease caused by a small bacilliform virus and a polyhedral virus (1,11,18,19,23). The two particle types were separated and characterized after purification, and the names, rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV), were adopted (10,19). RTBV and RTSV are not serologically related (19).

Generally, rice plants infected with both RTBV and RTSV exhibit severe yellowing and stunting, those infected with RTBV exhibit mild yellowing and stunting, and those infected with RTSV exhibit almost no symptoms (10,11). RTBV depends on RTSV for transmission by the green leafhopper (10,11). Leafhoppers fed on plants infected with both viruses can transmit the viruses to rice plants together or individually, but the transmission of RTSV alone occurred at low rate. However, leafhoppers fed on plants infected with RTSV alone transmit RTSV at high rate (10–12).

Rice cultivars and lines have been evaluated for resistance to the tungro disease, based on symptoms, after mass inoculation in the greenhouse (15) and under field conditions. However, resistant cultivars so far screened may only be resistant to the vector leafhopper. Leafhopper-resistant cultivars generally show a high level of resistance to tungro disease in the field and sometimes in the greenhouse (15,21,22). It is not known whether the tungro-resistant cultivars developed at the International Rice Research Institute (IRRI) are, in fact, resistant to the tungro viruses or are resistant merely to the vector leafhopper. Inoculation of plants by force-feeding with viruliferous leafhoppers, followed by assay for viruses in inoculated plants was used to provide an answer.

We report reactions of some leafhopper-resistant cultivars to infection with tungro viruses under field and experimental conditions. Preliminary reports have been published (4,13,24).

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MATERIALS AND METHODS

Plants, insects, and viruses. High-yielding cultivars IR36, IR42, IR50, IR52, IR54, and IR56 were used in greenhouse and field experiments. These cultivars have several possible sources of resistance to the green leafhopper in their parentage. The resistance of IR36 and IR42 derive mainly from cultivar Ptb 18, and the others derive resistance mainly from cultivar Gam Pai 30-12-15. Cultivars Taichung Native 1 (TN1) and IR22 were used as susceptible checks in greenhouse and field trials, respectively. The reaction of the test cultivars to the green leafhopper vector based on damage rating and to tungro disease based on visual assessment in the greenhouse and field are summarized in Table 1.

A virus-free *N. virescens* colony that had been reared for several years on TN1 plants was used. Newly emerged adult *N. virescens* were selected for all experiments.

The tungro isolate used was originally collected at Laguna, Philippines, and maintained in TN1 by successive transfers with

TABLE 1. Reactions of cultivars to the green leafhopper *Nephotettix virescens* and to tungro disease in the greenhouse and field

Cultivar	Reaction to *N. virescens*[a]	Reaction to tungro	
		Greenhouse[b]	Field[c]
IR36	MR	S	S-MR
IR42	MR	S	S-MR
IR50	R	MR	R
IR52[d]	R	MR	R
IR54	R	MR	R
IR56	R	S	R
IR22 (S-ck)	S	S	S
TN1 (S-ck)	S	S	S

[a] Based on damage ratings in the greenhouse at the International Rice Research Institute (IRRI) (8). R: resistant, MR: moderately resistant, and S: susceptible.
[b] Based on visual assessment after the mass inoculation method (15) at IRRI (Hibino, *unpublished*).
[c] Based on visual assessment at IRRI (Hibino, *unpublished*).
[d] Sister line of IR54 substituted for IR54 in the field trials.

viruliferous leafhoppers. RTSV was isolated from an inoculated plant and maintained by the same method. TN1 plants, 40–60 days old, infected with both RTBV and RTSV or RTSV alone were used as virus sources. Adult leafhoppers fed on source plants for 4 days were used for inoculation.

Latex test. Antisera against RTBV and RTSV had titers of 1/1280 and 1/640, respectively, in the precipitin ring interface test (19). Immunoglobulin was precipitated with half-saturated ammonium sulfate, pH 6.5. It was dialyzed and suspended in phosphate-buffered saline of original volume. Latex particles (Difco-Bacto-Latex 0.81) were sensitized with immunoglobulin either to RTBV or RTSV, following the procedure of Omura et al (17). One leaf sample about 10 cm long was cut from the second youngest leaf of each test plant 1 mo after inoculation. Leaf samples were homogenized separately in 1 ml of 0.05 M, pH 7.2, Tris-Cl buffer using a combined leaf and bud press (Erich Pollahne, Wennigsen, West Germany). Approximately 50 μl of sap and of sensitized latex suspension were placed in a small test tube, and the tube was shaken at 160 oscillations per minute for at least 30 min. The presence of the viruses was determined by the detection of latex particle clumps under a light microscope at 100× magnification. With this procedure, RTBV and RTSV were detected in extracts of infected TN1 leaves when diluted up to 1/160 and 1/80, respectively. When the latex suspension was mixed with leaf extracts without virus antigen or with the heterologous virus, no clumping of latex particles was observed, even at a 1/2 dilution.

Field evaluation for resistance to tungro. Field trials were conducted in the 1984 wet season at IRRI, Laguna, and at Guimba, Nueva Ecija, Philippines, where incidences of tungro disease were observed to be low and high, respectively. Three-week-old IR22, IR36, IR42, IR50, IR52, and IR56 seedlings were transplanted on a 20- × 20-cm spacing, one seedling per hill, in 5- × 5-m plots in a randomized complete block design and subjected to natural infection. Both trials were replicated four times. Sixty days after transplanting, the percentage of infection was assessed based on symptoms, and leaf samples randomly collected from 120-160 hills per plot were indexed by the latex test.

Reaction of leafhoppers to cultivars. Leafhopper longevity, mortality, and preference were determined on the cultivars IR36, IR42, IR50, IR54, IR56, and TN1. Adult leafhopper longevity was determined by confining a single leafhopper on a 7- to 10-day-old seedling in a test tube until the leafhopper died. The experiment used 120 seedlings of each cultivar in each of four replications. The average adult longevity was calculated by dividing the total daily count of surviving insects from initiation until all were dead by the number of insects tested. Leafhopper mortality was determined by confining 20 leafhoppers with a 45-day-old plant in a cage for 4 days before counting the number of surviving leafhoppers. Each test included all cultivars, and the experiment had five replications. Leafhopper preference for each cultivar was determined by releasing 100 leafhoppers into a cage that contained two pots of each cultivar planted with 10 seedlings per pot. Leafhoppers that settled on each cultivar were counted 24 hr after insect release in each of the five replications.

Mass inoculation. Leafhoppers that had fed on source plants infected with RTBV and RTSV were used for inoculation. Seven-day-old seedlings of IR36, IR42, IR50, IR54, IR56, and TN1 were inoculated in the greenhouse by the mass inoculation procedure (15). Twenty-nine seedlings of each cultivar were planted in clay pots. Two pots of each cultivar were randomly arranged in a cage and exposed to an average one, three, or five leafhoppers per seedling for 2.5 hr (three replications). Two weeks after inoculation, plants were scored for presence or absence of symptoms of tungro disease.

Reaction to infection by RTBV and RTSV. Leafhoppers that had fed on plants infected with both RTBV and RTSV were used. Seven-day-old test seedlings were exposed individually to one, three, or five leafhoppers in test tubes for 1 day. Inoculated seedlings were transplanted in pots and grown in a greenhouse. Infection was determined based on symptoms. Forty seedlings per number of leafhoppers per cultivar were inoculated in each of two replications. In another experiment, 1-mo-old potted plants were confined separately with 1, 5, 10, 20, or 30 leafhoppers in mylar cages for 1 day. Sixteen plants per number of leafhoppers per cultivar were inoculated in each of two replications. All inoculated seedlings were indexed for the viruses by the latex test.

Leafhoppers fed on RTSV-infected plants for 4 days were used to transmit RTSV in cultivar reaction tests. About 40 7-day-old seedlings of each cultivar were exposed individually to one leafhopper in test tubes for 1 day. All inoculated seedlings were indexed by the latex test.

Virus recovery. Virus identity in selected plants from test-tube inoculations was verified by leafhopper transmission to 7-day-old TN1 seedlings. Leafhoppers were given a 4-day acquisition access and a 1-day inoculation access period. All inoculated plants were indexed by the latex test.

RESULTS

Field evaluation for resistance to tungro. Based on symptoms, the incidence of tungro disease in the cultivars varied with the prevalent levels of disease (Table 2). At IRRI, Laguna, where low virus inoculum and leafhopper levels prevailed, IR36 and IR42 had relatively low infection rates, whereas in Nueva Ecija, these cultivars had infection rates similar to that of the susceptible IR22. IR50, IR52, and IR56 had very low infection rates at both locations.

Latex tests indicated that most IR22, IR36, and IR42 plants were infected with both RTBV and RTSV in Nueva Ecija, whereas more plants were infected with RTBV or RTSV alone at IRRI (Table 2). Most plants of IR50 and IR52 were not infected with either virus at IRRI, but IR50, IR52, and IR56 had high infection rates with RTSV alone at Nueva Ecija. These results confirmed a previous report (2) about the occurrence and spread of RTSV as an independent virus in the Philippines.

Reactions of leafhoppers to test cultivars. The longevity of adult *N. virescens* on seedlings of IR50, IR54, and IR56 was shorter than on the other cultivars (Table 3). Leafhopper mortality was higher on 45-day-old IR50, IR54, and IR56 plants than on other cultivars.

TABLE 2. Tungro disease incidence based on symptoms and rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) incidence based on the latex test in rice cultivars 60 days after transplanting in fields at IRRI, Laguna, and in Nueva Ecija, Philippines

Cultivar	IRRI					Nueva Ecija				
	Tungro incidence (%)	Latex test score (%)				Tungro incidence (%)	Latex test score (%)			
		RTBV + RTSV	RTBV	RTSV	Healthy		RTBV + RTSV	RTBV	RTSV	Healthy
IR22	42.7 a[x]	52.5	22.5	16.9	8.1	88.2 a	90.8	0.8	8.4	0
IR36	5.7 c	6.3	8.8	26.4	58.5	87.2 a	89.2	2.5	4.1	4.2
IR42	15.4 b	31.6	12.7	33.5	22.2	87.8 a	89.0	0	9.3	1.7
IR50	0.1 d	0	0.6	0	99.4	0.8 b	0	1.6	6.7	91.7
IR52	0 d	0	0	0	100.0	1.0 b	6.7	5.0	15.8	72.5
IR56	...[y]	...	...	...	...	1.9 b	5.0	1.6	46.7	46.7

[x] Means tested by Duncan's multiple range test at 1% level.
[y] Not tested.

In the leafhopper preference test, fewer leafhoppers were observed on IR36, IR50, IR54, and IR56 than on IR42 and TN1 (Table 3). Resistance to leafhoppers in IR50, IR54, and IR56 was due to both antibiosis and nonpreference to adult leafhoppers.

Greenhouse evaluation based on symptoms. By the mass inoculation method, the number of infected seedlings increased as the number of leafhoppers per seedling was increased from one to five (Fig. 1). With the reaction scale developed for the mass inoculation method when an average of five to six leafhoppers per seedling was used (0–30% infection, resistant; 31–60%, intermediate, and 61–100%, susceptible) (15), the reaction of IR36 and IR42 shifted from resistant to susceptible as number of leafhoppers per seedling was increased (Fig. 1). The reactions of IR50, IR54, and IR56 shifted from resistant to intermediate.

By the test-tube inoculation method, a similar trend in cultivar reactions occurred except that the level of disease incidence was higher (Fig. 1). IR36, IR42, and TN1 gave susceptible reactions even with one leafhopper per seedling. The reactions of IR50, IR54, and IR56 shifted from intermediate to susceptible as the number of leafhoppers increased from one to five.

Plants of each cultivar showing typical tungro symptoms were selected from those inoculated with one leafhopper per seedling in test tubes and indexed for virus infection by the latex test. All TN1 plants sampled contained both RTBV and RTSV, whereas all IR50 and IR54 plants contained only RTBV (Table 4). All plants were also tested for virus recovery using leafhoppers. All test plants that contained both RTBV and RTSV served as virus sources regardless of cultivar. The percentage of leafhoppers that transmitted RTBV and/or RTSV from source plants to TN1 seedlings varied from 63 to 84 among the cultivars. Regardless of cultivar, none of the RTBV-infected plants served as virus sources.

Reaction to infection of RTBV and RTSV. When plants were exposed separately in cages to leafhoppers that had fed on plants infected with RTBV and RTSV, infection with RTBV and RTSV together increased in IR36 and IR42 as the number of leafhoppers per plant increased from one to 30 (Fig. 2). However, IR50 and IR54 showed an increase in RTBV infection rates as leafhopper numbers increased, with some double infections with higher vector numbers. IR56 showed no definite pattern of increase in infection to any of the viruses as the number of leafhoppers increased. Infection with RTSV alone was not found on any cultivar except IR56. TN1 had the highest rate of double infections irrespective of the number of insects used.

Reaction to RTSV infection. When seedlings of the test cultivars were inoculated with RTSV in test tubes, the percentage of RTSV-infection varied among cultivars. IR50 and IR54 had lower infection rates of 16 and 21%, whereas IR36, IR42, IR56, and TN1 had higher rates of infection of 58, 39, 82, and 83%, respectively. These results indicated that the leafhopper-resistant cultivars could be infected with RTSV if exposed to leafhoppers fed on RTSV-infected plants.

TABLE 3. Longevity, mortality, and preference of adult green leafhopper on rice cultivars

Cultivar	Longevity[w] (days)	Mortality[x] (%)	Preference[y] (No./10 seedlings)
IR36	6.5 b[z]	59.3 b	8.6 c
IR42	6.2 b	32.5 bc	15.2 b
IR50	4.0 c	90.0 a	3.8 c
IR54	3.9 c	78.7 a	2.2 c
IR56	4.7 c	69.9 a	6.2 c
TN1	8.5 a	2.7 c	31.4 a

[w] Longevity in days on 7- to 10-day-old seedlings in test tubes.
[x] Determined 4 days after insect confinement with 45-day-old plants in a cage.
[y] Number of leafhoppers settled on each cultivar. Counted 24 hr after insect introduction.
[z] Means tested by Duncan's multiple range test at the 5% level.

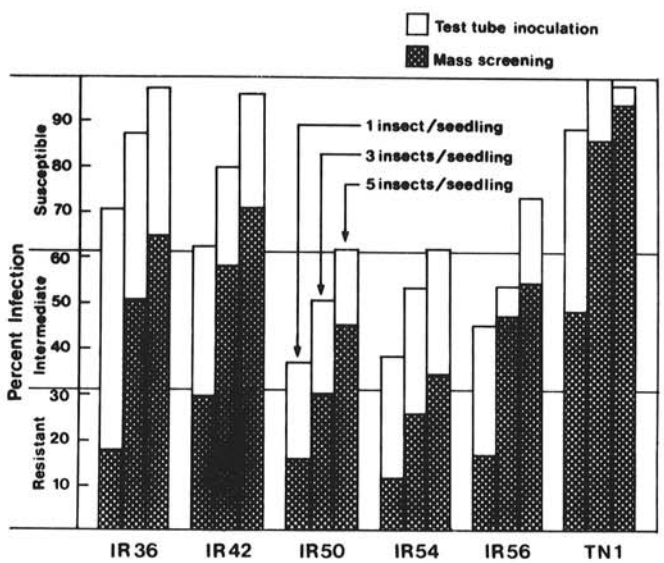

Fig. 1. Visual scores of tungro-diseased plants of rice cultivars inoculated in test tubes or by the mass inoculation method (15) with one, three, or five *Nephotettix virescens* per seedling. The insects used had fed on source plants with rice tungro bacilliform and rice tungro spherical viruses.

DISCUSSION

Pairs of "dependent and helper viruses" so far known are aphid-borne (20) except for RTBV and RTSV. No studies have been done on cultivar reaction to the dependent and helper virus complexes because of the complexity of four components, namely two viruses, the vector, and the host. Recently, serological detection of RTBV and RTSV in rice leaves became available (19) and has been applied in preliminary studies on resistance to the tungro disease (5,6). In these experiments, the latex test was successfully applied to evaluate tungro-resistant cultivars, although its efficiency for RTBV and RTSV detection was not high.

In these experiments, rice cultivars reacted differently to the viruses depending on the resistance of the cultivars to the leafhopper. When inoculated in mass or in test tubes by the leafhoppers that had fed on plants infected with both RTBV and RTSV, leafhopper-resistant cultivars were primarily infected only with RTBV. When inoculated in test tubes by leafhoppers that had fed on an RTSV source, however, the resistant cultivars, notably IR56, did not show a high level of resistance to RTSV infection. Similarly, fairly high rates of RTSV infection in IR56 occurred in the field. These results indicate that the leafhopper-resistant cultivars were not resistant specifically to RTSV infection. Results were similar with other leafhopper-resistant cultivars (5,6). The mechanism of selected RTBV infection in leafhopper-resistant cultivars remains to be clarified.

On the other hand, plants infected with RTBV alone did not serve as a virus source, which agrees with previous reports (10–12).

TABLE 4. Incidence of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) detected by the latex test in rice cultivars artificially infected with tungro

Cultivars	Plants tested[a] (No.)	Positive reactions (%) to: RTBV + RTSV	RTBV	RTSV
IR36	15	27	73	0
IR42	14	57	43	0
IR50	13	0	100	0
IR54	15	0	100	0
IR56	12	42	58	0
TN1	13	100	0	0

[a] Plants were selected from those showing symptoms of tungro disease after inoculation in test tubes by one *Nephotettix virescens* per seedling.

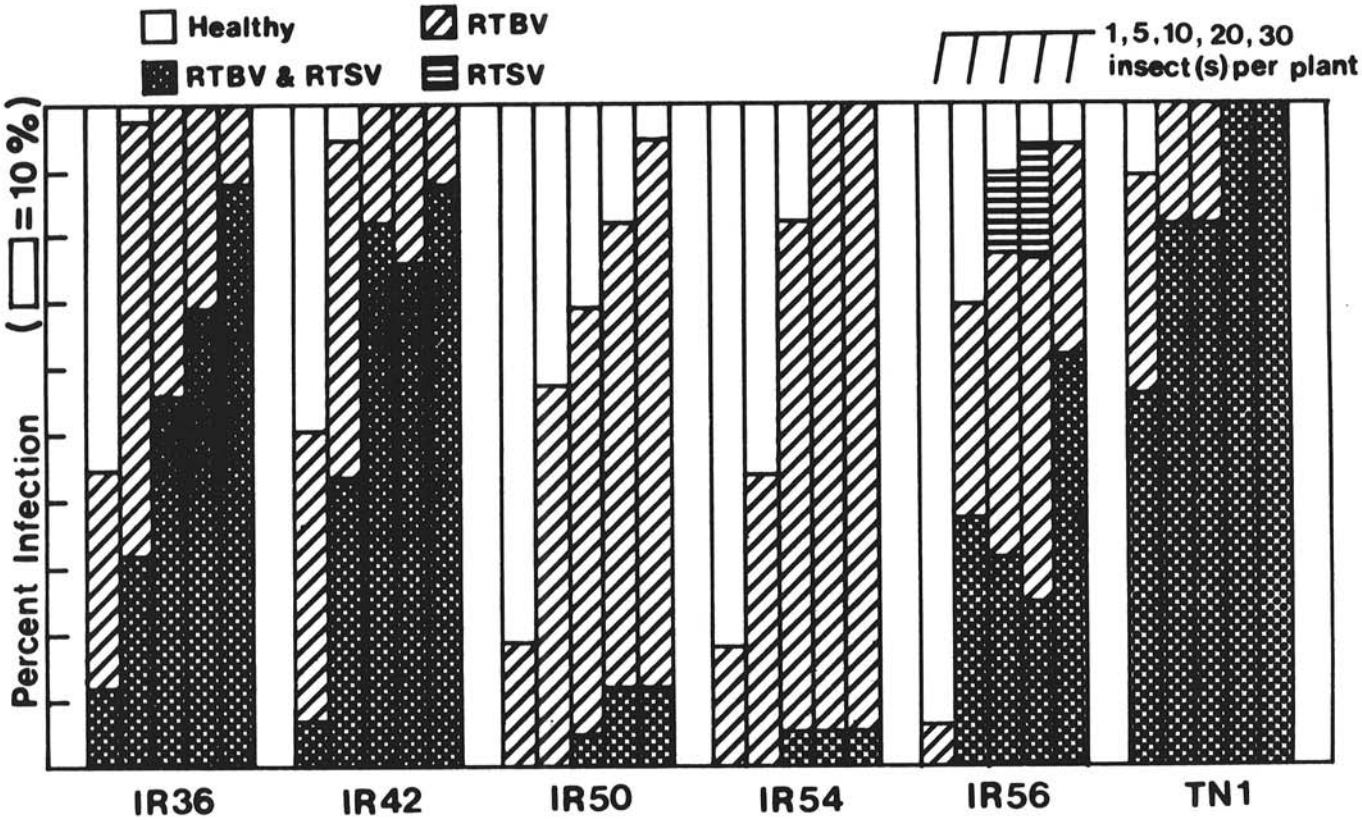

Fig. 2. Percentage of infection of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) detected by the latex test in rice cultivars exposed to 1–30 *Nephotettix virescens* that fed on source plants with RTBV and RTSV.

If RTSV incidence is not very high in the field, the chance that leafhopper-resistant cultivars would be doubly infected with RTBV and RTSV would be very low. The tendency of leafhopper-resistant cultivars to be infected with RTBV would cause slow development of tungro disease in the resistant cultivars because of limited available sources with both RTBV and RTSV.

IR50 and the other recently released IR cultivars tested in these experiments showed a high level of antibiosis and nonpreference to the leafhopper. In fields, these leafhopper-resistant cultivars showed very low levels of tungro-disease. In confined conditions, as in mass inoculation in cages, however, the cultivars had higher infection rates and the percentage of virus-infected plants increased with increasing numbers of leafhoppers per plant. The percentage of infection was even higher in test-tube inoculation, where the effect of leafhopper preference for cultivars was eliminated. These results indicate that the resistance to tungro infection of these IR cultivars can be overcome if force-feeding is used and number of insects is increased. Low infection rates of these leafhopper-resistant IR cultivars in the field can be attributed to their resistance to the leafhoppers. A high level of resistance to the leafhopper alone may be adequate to protect rice crops from the tungro disease. On the other hand, it is known that leafhopper-resistant cultivars that show resistance to tungro infection may be susceptible to tungro when inoculated with leafhopper colonies selected on these resistant cultivars (3,9). Recently, IR50 and IR54 showed high rates of tungro disease in the southern Philippines and they were also susceptible to leafhopper colonies collected from this region (Hibino, *unpublished*).

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