Resistances in Rice to Tungro-Associated Viruses

H. HIBINO, R. D. DAQUIOAG, E. M. MESINA, and V. M. AGUIERO, International Rice Research Institute, P.O. Box 933, Manila, Philippines

ABSTRACT

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Tungro is a composite disease associated with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). Over a period of 23 yr, 40,000 rice germ plasm accessions were evaluated for resistance to tungro by greenhouse mass inoculation using the vector green leafhopper (GLH), *Nephotettix virescens*. From those, 119 cultivars that showed low levels of infection were selected. Each seedling of the selected cultivars was exposed in a test tube to five GLH that had fed on plants infected with either both RTBV and RTSV or RTSV alone. Inoculated seedlings were indexed by enzyme-linked immunosorbent assay (ELISA). Seedlings inoculated with both viruses combined were also scored for symptom severity on a scale of 1–9. Nine cultivars had low levels of overall infection with RTBV and RTSV, 40 cultivars had low or no infection with RTSV, and 16 cultivars were tolerant to RTBV and developed very mild symptoms even when infected with both viruses. Some cultivars had more than two types of resistance in combination. Some of these cultivars with resistances or tolerance to the viruses also had resistance to GLH. The resistances to virus infection or tolerance to RTBV can be used in the breeding program for tungro resistance. The method adopted would be useful in evaluating breeding lines to tungro and in the analysis of inheritance for tungro resistances.

Tungro is the most important virus disease of rice in South and Southeast Asia. It also occurs in southern China (24). Tungro is a composite disease associated with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV; 12,20,23). Both RTBV and RTSV are transmitted in a semipersistent manner by the green leafhopper (GLH), *Nephotettix virescens* (Distant), and some other leafhopper species (9,10,13). GLH that feed on source plants infected with RTBV and RTSV transmit the viruses either together or separately on rice plants. Plants infected with both RTBV and RTSV generally show severe stunting and yellowing, those infected with RTBV alone show mild stunting and yellowing, and those infected with RTSV alone show very mild stunting but no leaf symptoms (12). RTSV can be transmitted independently by GLH, while RTBV transmission by GLH is dependent on the presence of RTSV (10,12,13). RTSV also spreads widely as an independent disease (2). RTSV was once epidemic in 1972 and 1973 in Kyushu, Japan, and called rice waika virus (23).

In South and Southeast Asia, cultivar resistance to tungro is an important breeding objective for rice improvement (4,16). High-yielding cultivars that had little tungro have been bred and grown widely in several South and Southeast Asian countries. Some of these cultivars have succumbed to tungro after a few years of intensive cultivations in Indonesia (19), Philippines (6,14), and Thailand (15).

These cultivars have resistances to GLH but not to the virus agents (6,8,14, 22). Finding cultivars resistant to the viruses rather than GLH is desired in cultivar improvement. Because of the lack of appropriate methodology, cultivars that showed low tungro incidences in the greenhouse or field screenings (1,4,16) remained to be clarified for their resistances to the viruses. In the recent trials on high-yielding cultivars, serological indexing demonstrated characteristic reactions to infection with RTBV and RTSV of cultivars resistant to GLH (14) or RTSV (11). The precise indexing for viruses and symptom severity after artificial inoculation with tungro would clarify resistances of cultivars selected in the screenings.

We selected 119 rice cultivars from 40,000 rice germ plasm accessions that have been evaluated at the International Rice Research Institute (IRRI) for resistance to tungro in the greenhouse mass inoculation method (18) and characterized some of their resistances through serological and symptom severity indexing using artificial inoculation with an RTBV-RTSV mixture or RTSV alone.

MATERIALS AND METHODS

Virus and insect. The isolate that causes tungro at Laguna, Philippines, has been maintained on rice cultivar Taichung Native 1 (TN1) by successive transfer with GLH. By enzyme-linked immunosorbent assay (ELISA; 3), plants infected with both RTBV and RTSV were selected and used as an inoculum source. RTSV was isolated from an inoculated plant and maintained by the same method. RTSV source plants were also selected by ELISA. The GLH colony collected at Laguna has been reared on TN1 plants in a greenhouse. Newly emerged adults were allowed a 3–4 day acquisition access period on 45- to 60-day-old virus source plants. Immediately after the acquisition feeding, these viruliferous adults were used for inoculation.

Plants. During 1963–1986 at IRRI, 40,000 rice germ plasm accessions in the International Rice Germplasm Center, International Rice Research Institute, Laguna, Philippines, were tested in the mass inoculation method (18). For the mass inoculation, 16 pots with 29 seedlings each at the two- or three-leaf stage were confined in a cage with 2,000–3,000 GLH adults that had fed on tungro source plants. Since 1963, the mass inoculation method in the greenhouse (18) was modified from time to time for efficient large-scale inoculation with no substantial difference in results. The inoculation access period was initially for 24 hr. It was decreased to 8 hr from 1967 to 1974 and to 2.5 hr thereafter. During 1967–1973, viruliferous GLH were used for inoculation of one set of seedlings and then allowed to feed overnight on tungro source plants. After the reacquisition feeding, GLH were used again for inoculation. During 1974–1987, two sets in a day were inoculated and GLH were allowed an overnight reacquisition feeding. Three or four weeks after the inoculations, seedlings were scored for the percentage of infection based on symptoms. Each accession was tested at least two times.

For test tube inoculation, 1-wk-old seedlings of each of 119 accessions were separately confined for 6 hr in test tubes with five virus-exposed GLH adults. Inoculated seedlings were transplanted in pots and grown in a greenhouse. Three to four weeks after inoculation, seedlings were scored for symptom severity (7) and indexed by ELISA (3). Scoring was based on a scale of 1–9 where 1 = no symptoms, 3 = 1–10% height reduction with no leaf discoloration, 5 = 11–30%

Present address of first author: National Agriculture Research Center, Kannondai, Tsukuba, 305 Japan.

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height reduction with no distinct discoloration, 7 = 31–50% height reduction and/or yellow to orange discoloration, and 9 = >50% height reduction and yellow to orange discoloration. The average score was designated as the severity index. Susceptible cultivar Taichung Native 1 (TN1) served as a control.

Resistance to GLH. At IRRI, the seedling balk damage rating test (21) was used to ascertain scores for the accessions' resistance to GLH. Cultivars with scores of 1 or 3 are resistant to GLH and those with scores of 7 or 9 are susceptible.

ELISA. The antisera to RTBV and RTSV had titers of 1/2,560 and 1/640 by the ring interface precipitin tests, respectively (5). A portion approximately 10 cm long was collected from the second or third youngest leaf of each plant. The leaf was extracted with 1 ml of 0.1 M phosphate buffer (pH 7.0) containing 0.15 M NaCl, 0.05% Tween 20, and 1% NaN_3 in a leaf and bud press (Ehrich Pollahne, Wennigsen, FRG). Extracts were directly tested in ELISA following basically the method described by Bajet et al (3). An Immulon II microtiter plate (Dynatech Corp. Chantilly, VA) was coated by immunoglobulin at 0.5 μg/ml for RTBV and 1 μg/ml for RTSV, and an immunoglobulin-alkaline phosphate conjugate was diluted 1,000 times for RTBV and 500 times for RTSV. One well per sample was used for the detection of RTBV or RTSV. On each plate, four wells with extracts of healthy TN1 leaves were added, along with four wells with extracts of TN1 leaves infected with both RTBV and RTSV, and two wells with the extraction buffer as the controls. Presence or absence of the viruses in extracts was determined by measuring absorbance at 405 nm in a Microelisa Minireader (Dynatech Corp., Chantilly, VA). Absorbances over twice the mean of four healthy control readings were considered positive.

RESULTS

Mass inoculation. With the mass inoculation method, the resistance level of 40,000 germ plasm accessions was indicated by the percentage of infection. Out of 15,677 entries that were tested in the recent 6 yr, 4% had infection with tungro lower than 30%. From 40,000 accessions, a total of 119 cultivars that had good agronomic traits and average infections of less than 50% were selected for further evaluation with test tube inoculation.

Test tube inoculation. When susceptible control cultivar TN1 was inoculated with a mixture of RTBV and RTSV, seedlings generally had total infections with both viruses. TN1 seedlings infected with both viruses had a severity score of 9, those infected with RTBV alone had a score of 7 or 9, and those infected with RTSV alone had a score of 1 or 3. TN1 seedlings exposed to five nonviruliferious GLH often gave a severity score of 3 because of damages caused by feeding and transplanting. The symptom severity index ranged from 3 to 9 among accessions tested.

After combined inoculation with RTBV and RTSV, ELISA indexing revealed that nine accessions had overall infections of less than 30% with RTBV and RTSV either together or separately (Table 1). They also had a low level of or no infections when inoculated with RTSV alone. ARC 10343, Utri Merah accession No. 16680, and ARC 5905 had GLH resistance scores of 7 or 9 indicating their susceptibility to GLH, whereas Bale Betor, Chingair, Dol Kochu 2, and Nedpasha had resistance scores of 1 indicating their resistance to GLH. Low levels of infection with both RTBV and RTSV in at least the GLH-susceptible cultivars are likely caused by their resistances to infection with both viruses. Of the nine accessions, six were from Bangladesh, two were from India, and one was from Indonesia.

After inoculation with a mixture of RTBV and RTSV, a total of 31 accessions had low levels of infections with RTSV (less than 7%) but high levels with RTBV (Table 2). They also had low levels of or no infections with RTSV when inoculated with RTSV alone. Including nine accessions that had low overall infections with RTBV and RTSV (Table 1), 40 accessions appeared to have resistance to RTSV infection. Of the 40 accessions, 14 had a severity score of 4 or lower. Many of these accessions were susceptible to GLH. Of the 40 accessions, 15 were from India, 15 were from Bangladesh, four were from Indonesia, four were from Pakistan, one was from Iran, and one was from the Philippines.

A total of 19 accessions had a severity score of 4 or lower. Gam pai 30-12-15, Seratus Hari T36, Tjempo Kijik, Balimau Putih, and Bhoro Nepa had low average scores although they had high overall infections with RTBV and/or RTSV (Table 3), indicating their tolerance to RTBV. Of the accessions that showed resistance to RTBV and RTSV infections (Table 1), ARC 10437, Utri Merah accession No. 16680, and ARC 5905 had low severity scores. The low scores of ARC 10437 and ARC 5905 were likely caused by their low overall infection rates. Utri Merah plants infected with RTBV alone showed very mild symptoms indicating their tolerance to RTBV. Of accessions that showed resistance to RTSV infection (Table 2), PI 184675-2, Habiganj DW8, ARC 10312, Utri Merah (accession No. 16682), Utri Rajapan, ARC 10963, ARC 10980, ARC 11554, ARC 12596, and Schuli 2 appeared to have low severity scores attributable to their tolerance to RTBV. Of the 16 accessions that had low severity scores attributable to their tolerance to RTBV, seven were from Indonesia, four were from India, three were from Bangladesh, one was from Iran, and one was from Thailand.

Table 1. Nine rice accessions that showed low overall infections with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) when inoculated at the seedling stage with a mixture of RTBV and RTSV or with RTSV alone by the vector *Nephotettix virescens* (GLH), and their reactions to GLH[a]

			Inoculation with RTBV + RTSV					Inoculation with RTSV	
					Plants infected (%)				
Cultivar	Accession	GLH score[b]	Plants tested (no.)	Average severity score[c]	RTBV+RTSV	RTBV	RTSV	Plants tested (no.)	Plants infected (%)
ARC 10343	12437	9	38	3	0	3	0	30	0
Utri Merah	16680	7	57	3	0	9	0	62	0
ARC 5905	19675	9	39	4	0	0	0	38	0
Bale Betor	26295	1	36	5	8	19	0	63	10
Chingair	26322	1	37	5	5	22	0	39	0
Dol Kochu 2	26334	1	39	6	15	13	0	36	6
Khoia Motor	26379	5	40	5	0	15	0	36	11
Nedpasha	26515	1	40	6	5	5	8	57	5
Gachia	26615	5	39	5	0	23	0	40	0

[a]Seedlings were separately exposed to five GLH that had fed on plants infected with RTBV + RTSV or RTSV alone.
[b]By the seedling bulk damage rating rest at IRRI (21): 1–3 = resistant, 4–6 = moderately resistant, and 7–9 = susceptible.
[c]Scoring was based on a scale of 1–9, where 1 = no symptoms, 3 = 1–10% height reduction with no leaf discoloration, 5 = 11–30% height reduction with no distinct discoloration, 7 = 31–50% height reduction and/or yellow to orange discoloration, and 9 = >50% height reduction and yellow to orange discoloration.

Of 119 accessions, 29 had a score of 1 or 3 for GLH resistance. Some of these GLH-resistant accessions also had resistances or tolerance to the viruses (Tables 1–3). There was no indication relating GLH resistance to the other resistances. Of the 29 accessions, 12 were from Bangladesh, nine were from India, two were from Sri Lanka, two were from Thailand, and one each was from Burma, China, Pakistan, and Philippines.

DISCUSSION

Resistance to tungro disease has been an important breeding objective for rice improvement in many Asian countries (1,4,16,18). Many cultivars bred as tungro-resistant had resistance to GLH (8,14,22) and did not last long (6,14,15, 19). Consequent development of cultivars with resistance only to GLH could be attributed to the screening methods adapted in the breeding programs. The screening of rice germ plasms and breeding materials has been done generally in the fields where GLH-resistant cultivars had very low tungro incidences (11,14). Lack of appropriate diagnosis also made the analysis of resistances to tungro difficult. Differentiation of resistances to the virus agents and GLH has been of a great concern to develop screening methods for stable resistances. Because of complex interactions between

Table 2. Thirty-one rice accessions that showed low levels of infection with rice tungro spherical virus (RTSV) when inoculated at the seedling stage with a mixture of rice tungro bacilliform virus (RTBV) and RTSV or with RTSV alone by the vector *Nephotettix virescens* (GLH), and their reactions to GLH[a]

			Inoculation with RTBV + RTSV					Inoculation with RTSV	
					Plants infected (%)				
Cultivar	Accession	GLH score[b]	Plants tested (no.)	Average severity score[c]	RTBV+RTSV	RTBV	RTSV	Plants tested (no.)	Plants infected (%)
Adday Sel	177	9	37	5	0	62	0	34	0
Binicol	4021	1	40	6	0	73	0	32	0
Pankhari 203	5999	5	34	7	3	91	0	30	3
Mushkan 41	6828	9	34	7	0	88	0	36	0
PI 184675-2	7366	5	32	3	0	78	0	31	0
G 378	11062	9	38	7	0	97	0	39	0
Habiganj DW8	11751	7	39	4	0	72	0	66	2
ARC 6064	12203	5	34	5	0	94	0	36	0
ARC 6080	12207	5	34	5	0	59	0	35	0
ARC 7007	12310	5	37	5	0	41	0	31	0
ARC 10312	12428	7	39	3	0	67	0	33	3
Utri Merah	16682	7	35	4	3	54	0	61	3
Utri Rajapan	16684	7	31	4	0	97	0	67	0
ARC 10963	19680	9	31	3	0	97	0	36	0
ARC 7140	20533	9	38	5	0	87	0	34	0
ARC 10980	21164	9	37	4	3	92	0	53	0
ARC 11554	21473	3	38	3	0	55	0	59	0
ARC 12596	22176	5	37	3	0	89	0	40	0
Boron	26317	7	34	5	0	97	0	33	0
Shuli 2	26527	9	37	4	5	95	0	37	0
Surjamukhi	26803	3	34	6	0	56	0	38	7
Muktahar	27572	9	37	7	0	73	0	40	0
Naria Bochi	27573	9	37	4	0	35	0	37	3
Basmati	27782	9	40	7	3	68	0	38	0
Basmati 375A	27827	5	36	5	0	92	0	40	0
Basmati 376	27828	5	39	5	0	92	0	39	0
Gasmal 110-2	29327	7	31	6	3	90	0	38	0
Gasmal 735	29349	9	31	7	3	97	0	37	0
Cempo Obang	35588	9	39	5	3	72	3	38	0
Kashiabinni	37488	3	60	5	7	62	0	37	0
Ovarkondoh	49996	9	40	7	3	83	0	40	0

[a]Seedlings were separately exposed to five GLH that had fed on plants infected with RTBV + RTSV or RTSV alone.
[b]By the seedling bulk damage rating test at IRRI (21): 1–3 = resistant, 4–6 = moderately resistant, and 7–9 = susceptible.
[c]Scoring was based on a scale of 1–9, where 1 = no symptoms, 3 = 1–10% height reduction with no leaf discoloration, 5 = 11–30% height reduction with no distinct discoloration, 7 = 31–50% height reduction and/or yellow to orange discoloration, and 9 = >50% height reduction and yellow to orange discoloration.

Table 3. Six rice accessions that showed low symptom severity scores but relatively high infection with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) either together or separately when inoculated at seedling stage with a mixture of RTBV and RTSV by the vector *Nephotettix virescens* (GLH), and their reactions to inoculation with RTSV alone and to GLH[a]

			Inoculation with RTBV + RTSV					Inoculation with RTSV	
					Plants infected (%)				
Cultivar	Accession	GLH score[b]	Plants tested (no.)	Average severity score[c]	RTBV+RTSV	RTBV	RTSV	Plants tested (no.)	Plants infected (%)
Gam Pai 30-12-15	831	1	33	4	6	42	0	64	6
Seratus Hari T36	5346	9	35	4	31	66	0	33	6
Tjempo Kijik	16602	7	32	3	63	28	3	64	28
Balimau Putih	17204	5	53	2	2	21	19	35	40
Bhoro Nepa	26311	1	36	4	17	72	3	63	5

[a]Seedlings were separately exposed to five GLH that had fed on plants infected with RTBV + RTSV or RTSV alone.
[b]By the seedling bulk damage rating test at IRRI (21): 1–3 = resistant, 4–6 = moderately resistant, and 7–9 = susceptible.
[c]Scoring was based on a scale of 1–9, where 1 = no symptoms, 3 = 1–10% height reduction with no leaf discoloration, 5 = 11–30% height reduction with no distinct discoloration, 7 = 31–50% height reduction and/or yellow to orange discoloration, and 9 = >50% height reduction and yellow to orange discoloration.

the virus agents and the vectors (6,9,10, 12) and hosts (11,14), the methods used to differentiate virus resistance and GLH resistance (8,17,22) were not conclusive until serological indexing availed precise analysis of the interactions (6,11,14). So far, such analysis has been adapted on high-yielding cultivars but not to rice germ plasms selected in the screening programs. In these experiments, serological indexing and scoring for symptom severity on artificially inoculated seedlings differentiated into four types the resistances in 119 accessions that were selected after the greenhouse mass inoculation. Some cultivars appeared to have more than two types of resistances.

Cultivars resistant to RTSV infection appeared to be abundant in the rice germ plasm. About 30% of the cultivars tested in these experiments showed resistance to RTSV infection. In our previous studies (11), some high-yielding cultivars were found to have resistances to GLH and RTSV infection derived from a cultivar, TKM6. Tungro incidence in these cultivars was low in the Philippines (11) and Indonesia (Cabunagan and Hibino, *unpublished data*). In fields planted for the RTSV-resistant cultivars, development of tungro disease would be slow because plants infected with RTBV alone do not serve as a virus source (10,12–14). There might be many accessions that were resistant to RTSV infection but not selected after the mass inoculation because of their sensitivity to RTBV. Resistance of cultivar Pankhari 203 to tungro virus (17) was found to be attributable to its resistance to RTSV infection (Table 2).

Cultivars tolerant of RTBV are also promising as resistance sources. In our preliminary trials, RTBV-tolerant cultivars had less reduction in grain yield even when infected at the seedling stage with either both RTBV and RTSV or RTBV alone (7). In the field, infection of plants with the viruses occurs after transplanting, and crop loss caused by tungro would be very low in these cultivars even when plants had high levels of infection. These cultivars serve as virus sources equally effective as intolerant ones (Hasanuddin and Hibino, *unpublished data*). This risk seems to have limited importance as the virus sources are present year round in tungro problem areas where rice is grown throughout the year.

Gam Pai 30-12-15, which has been used as a resistance source (14,16), was found to have resistance to GLH and tolerance to RTBV (Table 3). High-yielding cultivars with Gam Pai 30-12-15 as a parent seem to have resistance only to GLH (6,11,14). These cultivars have succumbed to tungro in Indonesia and Philippines because of the development of GLH populations that colonized these cultivars (6,19). The tolerance to RTBV might be dropped during the breeding processes.

Since the mid-1960s, tungro has been one of the most important problems hindering stable rice production in South and Southeast Asia. Tungro has been managed mainly by cultivar resistance and application of insecticide to reduce GLH populations. The insecticide application was not always efficient, and the instability of resistant cultivars has been the major obstruction in the use of cultivar resistance. Stable resistance for tungro has long been anticipated to solve the tungro problem. Some of the cultivars that showed their resistances or tolerance to the viruses in these experiments are promising and can be used as sources of resistance. The scoring method used in these experiments can be applied in the screening of breeding materials and in studying inheritance of resistance.

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