Molecular Mapping of Resistance to Rice Tungro Spherical Virus and Green Leafhopper

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We thank Carole Morehouse for help in formatting the manuscript. This research was supported by grants from the Rockefeller Foundation to IRRI, to Cornell University, and for the graduate fellowship of L. Sebastian.

Accepted for publication 2 October 1995.

ABSTRACT

Sebastian, L. S., Ikeda, R., Huang, N., Imbe, T., Coffman, W. R., and McCouch, S. R. 1996. Molecular mapping of resistance to rice tungro spherical virus and green leafhopper. Phytopathology 86:25-30.

The green leafhopper (GLH), *Nephotettix virescens* (Distant), vectors rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV) to cause tungro disease in rice (*Oryza sativa* L.). Results of this study demonstrate that a dominant gene(s) conferring resistance to GLH and RTSV is located within 5.5 cM of restriction fragment length polymorphism (RFLP) marker RZ 262 on rice chromosome 4. Segregation analysis was based on a cross between rice cultivars ARC11554 (resistant to both GLH and RTSV) and TN1 (susceptible to GLH, RTSV, and RTBV). Two hundred forty F_2 plants were evaluated for GLH resistance in antibiosis experiments, and 111 additional F_2 plants from the same cross were evaluated for virus resistance by enzyme-linked immunosorbent assay (ELISA). Forced feeding by viruliferous GLH was used to inoculate plants with both RTSV and RTBV. RTSV resistance cosegregated with GLH resistance, and high levels of RTBV in plants resistant to both GLH and RTSV indicated that inoculation was effective. Future studies will clarify whether resistance to GLH and RTSV in ARC11554 is governed by two linked genes or is the result of pleiotropy at a single locus. This is the first report of the map location of an RTSV resistance gene in rice and the first time a GLH resistance gene has been reported on chromosome 4.

Additional keywords: disease resistance, gene tagging, linkage analysis, molecular markers.

Rice tungro disease (RTD) is a composite disease caused by rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) (18,41). Plants infected with both viruses show severe yellowing and stunting; those infected with RTSV alone show only mild stunting, and those with RTBV alone show mild yellowing and stunting (15,18). RTD is the most widespread and important virus disease of rice in Asia (16). In Southeast Asia, the affected area is estimated to be 6.2% of the total area planted to rice (14,16), or about 2 million ha.

It is difficult to screen for resistance to either of the tungro viruses. The etiology of the disease is complex and, until recently, was not well understood. Disease occurrence and spread involve interactions among three different causal agents: the green leafhopper (GLH) vector (*Nephotettix virescens* (Distant)), RTSV, and RTBV (3,15,18,41). GLH is currently the only reliable way to inoculate plants with RTSV and RTBV (7,10,20,21,27,38). Effective transmission of RTBV depends on RTSV, but RTSV can infect plants independently of RTBV (3). Very little is known about the presence of tolerance (high virus titer but few symptoms) to RTD in rice germ plasm (17), making it difficult to correlate results from enzyme-linked immunosorbent assay (ELISA) and visual symptom assessment in a screening program.

Hundreds of rice germ plasm accessions have been screened for resistance to RTD over the past 20 years (13). Screening of rice germ plasm for GLH resistance demonstrated that both antibiosis and antixenosis (nonpreference) were relatively common (14). When plants are resistant to the vector, infection by RTSV and RTBV is low, making it difficult to distinguish between resistance to GLH and resistance to tungro disease caused by the viruses. Thus, field resistance to GLH has often been interpreted as resistance to the viruses. Screening for vector resistance is easier and more direct than screening for virus resistance; however, GLH-resistant plants can be severely attacked by GLH if the "virulence" of the GLH population in the field shifts (8). As a result, many cultivars released as resistant become susceptible to the disease after a few years of extensive cultivation (8,16,42,46).

Serological techniques (i.e., ELISA) have improved the accuracy of disease diagnosis (2,35). This helps in giving a more definite assessment of virus infection in plants and has been very helpful in screening cultivars for their reaction to tungro virus (17). However, the use of serological techniques is an indirect way of assessing whether a cultivar is resistant or not, and confusion can result when plants demonstrate tolerance. The technique is effective in determining whether the viruses have infected a plant but gives only indirect indication of whether the plant carries resistance genes. Because both artificial and field inoculation rely on GLH as the vector, escapes are common. It is also difficult to inoculate large numbers of plants under controlled conditions. A more useful screening method would allow breeders to find out directly whether a plant carries RTD resistance genes at the DNA level, thus avoiding reliance on the many complex reactions involved in phenotype expression.

Tagging and cloning of genes of interest is possible with current molecular techniques. Identifying markers linked to the resistance genes would make selection for the desired gene(s) more reliable and efficient (45). In rice, extensive efforts have resulted in the construction of a molecular map (4,22,32). Many important

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Publication no. P-1996-1205-01R

characters of rice, such as bacterial leaf blight resistance (30,39, 49), blast resistance (6,47,50), photoperiod sensitivity (28), white-backed planthopper resistance (31), root morphology related to drought avoidance (5), and yield components associated with heterosis (48), have been tagged through the use of molecular markers. Tagging of other important genes pertaining to disease resistance, abiotic stress tolerance, yield, and other characters of agronomic importance is now in progress.

The tagging of genes conferring resistance to RTD through linkage to molecular markers is one approach being used to assist in the development of tungro-resistant cultivars. In this study, we undertook to identify restriction fragment length polymorphism (RFLP) and randomly amplified polymorphic DNA (RAPD) markers linked to gene(s) conferring resistance to RTSV and GLH in rice cultivar ARC11554.

MATERIALS AND METHODS

Parental lines. The parents used in this study were ARC11554 (International Rice Germplasm Collection [IRGC] accession #21473) and Taichung Native 1 (TN1) (IRGC accession #105). These cultivars were selected because of their clear resistance and susceptibility to RTD: ARC11554 is characterized by resistance to GLH, a very low rate of infection by RTSV, and an intermediate rate of infection by RTBV; TN1 is highly susceptible to GLH and to both RTSV and RTBV.

F_2 materials. The F_2 materials used in this study were derived from the cross ARC11554 × TN1. Different F_2 subpopulations from the same cross were used in the GLH antibiosis and ELISA studies. It was not possible to test the same F_2 or F_3 plants for both GLH antibiosis (nonviruliferous insect feeding experiment) and ELISA (involving viruliferous insect feeding), because most of the susceptible plants died or failed to grow normally following infestation with GLH in either experiment.

Near-isogenic lines and derived populations. A BC_6F_2 population derived from the cross ARC11554/7*TN1 was developed at the International Rice Research Institute. After each backcross (BC_nF_1), BC_nF_2 lines were subjected to viruliferous GLH feeding and tested with ELISA to determine whether they exhibited RTSV resistance. One near-isogenic line (NIL) segregating for resistance to RTSV in the BC_6F_2 was used in this study. Two hundred BC_6F_2 plants were used for DNA extraction and were advanced to BC_6F_3. One hundred eight of the BC_6F_2 plants yielded sufficient BC_6F_3 seeds for antibiosis and ELISA experiments.

Phenotypic evaluation for resistance to GLH. Three methods of GLH phenotyping were used: mass screening (seedbox test), test tube antibiosis, and small-pot antibiosis. All three involved infesting seedlings with GLH nymphs and observing plant families that survived as well as counting the number of dead GLH nymphs. Details of each procedure are described below.

Mass screening. Seedboxes were divided into two columns; each column had 13 rows planted with 30 seedlings per row. Eleven BC_6F_3 families (one per row) and two controls were planted in each column. Eight days after seeding (DAS), first- or second-instar GLH nymphs were released as evenly as possible into the seedboxes at an approximate density of five nymphs per seedling. The nymphs were allowed to feed selectively on the plants to which they were most attracted. One week after the GLH nymphs were added, the families were scored as "resistant" (R) if none of the seedlings were dead, "heterozygous" (H) if three to 24 seedlings (10 to 80%) were dead, and "susceptible" (S) if more than 80% of the seedlings were dead. This method was used to characterize 90 BC_6F_3 families.

Test tube antibiosis. At 10 DAS, seedlings were transferred to test tubes (one seedling per test tube) containing 1 ml of water, and five first- to second-instar nymphs were added to each tube. Nymphal survival was calculated as a measure of antibiosis. Scoring was done each day for 4 days. The initial count was done 6 h after nymphs were added. Dead GLH nymphs were counted for each tube 1, 2, and 3 days after nymphs were added. The antibiosis score (AS) was computed as follows:

$$AS = (A_1 \times 1) + (A_2 \times 2) + (A_n \times n) \times 100 / (1 + 2 + n),$$

where n is the number of days after adding GLH nymphs and A_n is percentage nymph survival at n. Scores were arcsine-transformed before analysis to normalize the distribution. Sixty-seven BC_6F_3 families were phenotyped by this screening method. Twenty BC_6F_3 seedlings were tested per BC_6F_2 line, and 10 to 15 families plus two controls were tested per batch (240 to 340 tubes).

Small-pot antibiosis. Two hundred forty F_2 plants were planted directly into 9-cm. pots. At 10 DAS, the pots were covered with a Mylar cage and infested with five second-instar nymphs. Three batches of 80 F_2 plants each were screened. Plants were scored as in the test tube antibiosis experiments described above.

RTD resistance screening. Seeds were soaked for 3 days in distilled water and then seeded directly into pots. In the first experiment, 108 BC_6F_3 families were planted at a rate of five seeds

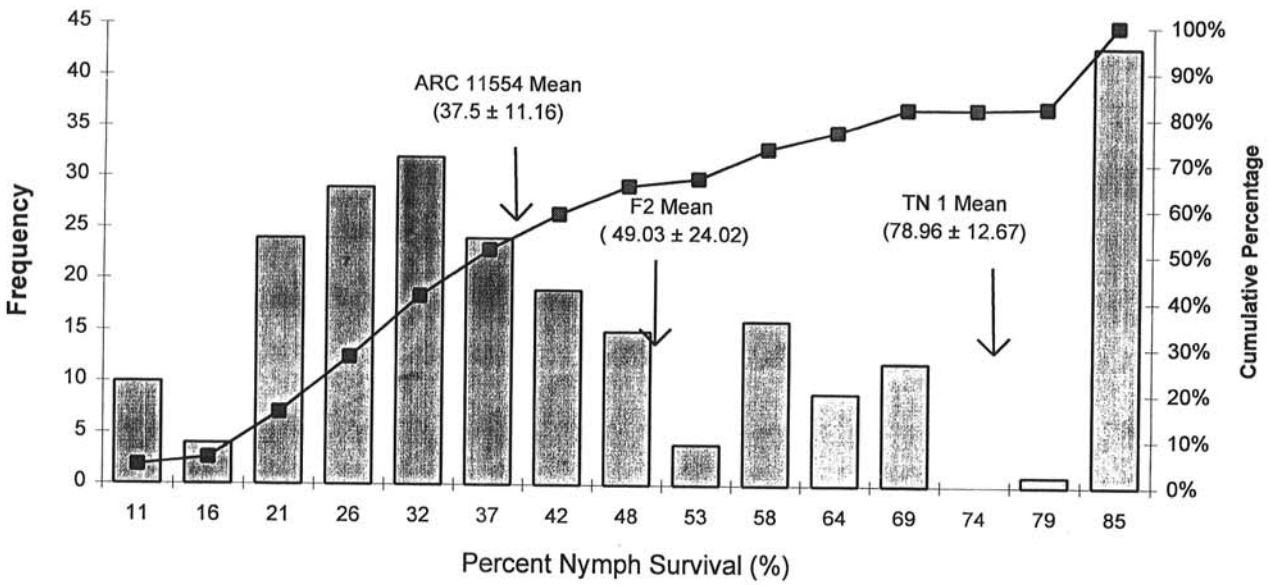

Fig. 1. Frequency distribution of nymph survival among F_2 plants derived from the cross ARC11554 × TN1 in the test tube antibiosis experiment (nymph survival is arcsine-transformed value of percentage nymph survival). Line graph shows cumulative nymph survival. Numbers in parentheses are the means ± standard deviations.

per pot, with six pots (30 plants) per family. At 10 DAS, plants were capped with a Mylar cage and inoculated with 50 viruliferous GLH adults (carrying both RTSV and RTBV) per pot. This rate (10 insects per seedling) is twice the inoculation intensity of the antibiosis experiments described above and was used in order to force insect feeding on all plants to minimize the possibility of escapes. There were 30 families per batch (including two controls), and on average, two batches were evaluated per line. In the second experiment, 111 F_2s were planted at a rate of one seedling per pot. At 10 DAS, plants were capped with a Mylar cage and inoculated with 10 viruliferous GLH adults per seedling. In both experiments, inoculation feeding continued for 4 h before the insects and the cage were removed. This form of virus inoculation was required because there was no reliable way to infect plants with RTSV and RTBV in the absence of GLH.

Three weeks after inoculation, the second and third youngest leaves from each plant were sampled for ELISA. A second sampling was done for the F_2s 4 weeks after inoculation. The procedure for ELISA was described by Bajet et al. (2). ELISA readings greater than the mean of the healthy controls plus four standard deviations were considered positive reactions (i.e., susceptible phenotypes) (1). In this experiment, readings of 0 to 0.11 absorbance units were considered a resistant reaction; readings above 0.11 were considered susceptible. Percentage infection was computed by dividing the number of plants with an ELISA score greater than or equal to 0.11 by the total number of plants sampled and multiplying by 100. Data for percentage infection were arcsine-transformed for statistical analyses.

DNA extraction. DNA samples were extracted by a method modified from Tai and Tanksley (44) and Dellaporta et al. (11). Leaf samples were harvested 30 days after transplanting, dipped in liquid N_2 immediately after harvest, and stored at −70°C. The samples were ground by hand on liquid N_2 with a mortar and pestle and extracted immediately. DNA quality was checked by digestion with *Eco*RI.

Restriction digests, electrophoresis, and southern analysis. Genomic DNA was digested with five restriction enzymes—*Dra*I, *Eco*RV, *Hind*III, *Sca*I, and *Xba*I—and size-fractionated by electrophoresis with 0.9% agarose gels. Southern analysis was performed using Hybond N for nonradioactive labeling and Hybond N+ (Amersham Corp., Chicago, IL) for radioactive labeling. Nonradioactive labeling was done with dig-UTP (36); Lumiphos (Boehringer, Mannheim, Germany) was used as the chemiluminescent substrate. Radioactive labeling was done using ^{32}P by the random hexamer method (12).

Filters hybridized with ^{32}P-labeled probes were washed with 0.5 × SSC at 65°C for 15 to 20 min (up to 10 filters per batch), or longer if more filters were used, and exposed to X-ray films for 1 to 5 days. Filters labeled with nonradioactive probes were washed as described by Panaud et al. (36).

Survey and mapping of markers linked to GLH and RTSV resistance. An NIL survey was conducted with 186 previously mapped RFLP clones (31 CDO, 69 RG, 83 RZ, and 3 xNpb) (4,32,40) to look for markers putatively linked to the gene(s) conferring resistance to RTD. The plants used in the survey included ARC11554 (R donor), TN1 (S recurrent parent), and a BC_6F_2 population derived from the resistant NIL described above. Equal amounts of leaf samples from 200 BC_6F_2 plants were bulked for DNA extraction in order to reconstruct the BC_6F_1 NIL genotype. Based on a comparison of hybridization patterns in the parents and the NIL, markers were classified as monomorphic, negative, or putatively positive in terms of linkage to the resistance character.

The mapping population consisted of 108 BC_6F_2 plants that had been phenotyped for GLH resistance and for RTBV and RTSV using derived F_3 families. Linkage analyses were done with the computer programs Mapmaker/Exp version 3 (23,24) and Mapmaker QTL (23,25).

RAPD analysis. RAPD markers were used to saturate the region containing RTSV and GLH resistance genes. Bulked segregant analysis was used (33); each bulk consisted of DNA from three BC_6F_2 lines known to be homozygous for the ARC11554 or the TN1 allele at the RFLP locus most closely linked to the resistance gene(s). The RAPD protocol described by Martin et al. (29) was modified by adjusting the $MgCl_2$ concentration in the reaction mixture to 1.9 mM. Approximately 10 to 20 ng of genomic DNA was used per amplification reaction for each set of primers (total of 300 Operon primers [Operon Technologies, Alameda, CA]). Reaction products were visualized on 0.9% agarose gels stained with ethidium bromide.

RESULTS

F_2 segregation for GLH resistance. Three batches of F_2 plants (a total of 240 plants derived from ARC11554 × TN1) were scored for antibiosis by the small-pot method. The reaction of this F_2 population suggested that a single dominant gene confers GLH resistance in ARC11554 (Fig. 1). Plants expressing antibiosis formed a curve that was skewed toward resistance, while the susceptible group formed a separate peak. Seventy-four percent of the plants were classified as resistant (nymphal survival scores of 0 to 81% [0 to 65 with arcsine-transformed data]), and 64 plants (26%) were classified as susceptible (greater than 81 to 100% nymphal survival [65 to 90 with arcsine-transformed data]). The χ^2 test confirmed the 3:1 ratio (χ^2 value = 0.27 [not significant]).

F_2 segregation for RTSV and RTBV resistance. The ELISA scores of another ARC11554 × TN1 subpopulation consisting of 111 F_2 plants from the small-pot experiment were used to assess the inheritance of virus resistance. A single dominant gene for RTSV resistance was suggested by a segregation ratio of 3:1 (χ^2 value = 0.42 [not significant]), and two recessive genes for RTBV resistance were indicated by a segregation ratio of 1:15 (χ^2 value = 0.6 [not significant]). ARC11554 had an average percentage infection with RTBV of 27.2% ± 6.8 in this study. As such, the resistance of ARC11554 to RTBV is not complete. This is in contrast to the level of RTSV resistance observed in this cultivar: the percentage infection was almost zero (0.69% ± 1.6). The detectable RTBV infection also served as an internal control in that many plants showing RTBV infection did not show RTSV infection, and therefore, the lack of RTSV in two samplings could not be attributed to escapes.

RTSV and GLH resistance in BC_6F_3 families. Segregation for RTSV resistance in the 108 BC_6F_3 families did not fit a 1:2:1 ratio ($\chi^2 = 14.67$, $P < 0.01$) but was skewed toward the resistant phenotype (Table 1). This was likely the result of elimination of some susceptible lines during generation advance due to TN1-derived susceptible alleles for many diseases in addition to RTD. This skewness was also observed for GLH antibiosis ($X^2 = 9.8$, $P < 0.01$) in the same BC_6F_3 families.

There was no segregation for RTBV resistance in these families: all plants were susceptible. This indicated that the particular

Table 1. Segregation of green leafhopper (GLH) antibiosis (test tube evaluation) and rice tungro spherical virus (RTSV) resistance (enzyme-linked immunosorbent assay) based on BC_6F_3 family analysis from a cross between rice cultivars ARC11554 and TN1[a]

	GLH			
RTSV	Resistant	Segregating	Susceptible	Total
Resistant	24	5	0	29
Segregating	1	28	0	29
Susceptible	0	2	7	9
Total	25	35	7	67

[a] Test for independence of RTSV resistance and GLH antibiosis: $X^2 = 19.41$, $P < 0.01$.

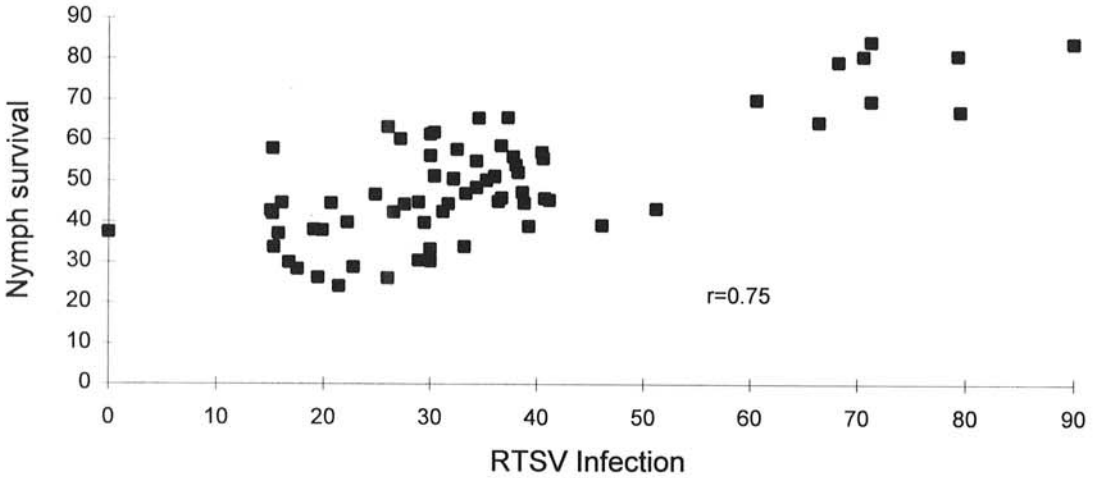

Fig. 2. Distribution of nymph survival (test tube antibiosis) and rice tungro spherical virus (RTSV) infection (enzyme-linked immunosorbent assay) among BC_6F_3 families derived from the cross of rice cultivars BC_5F_1 × TN1 (values are arcsine transformation of percentage nymph survival and percentage infection); *r* value is the computed Pearson product moment correlation coefficient.

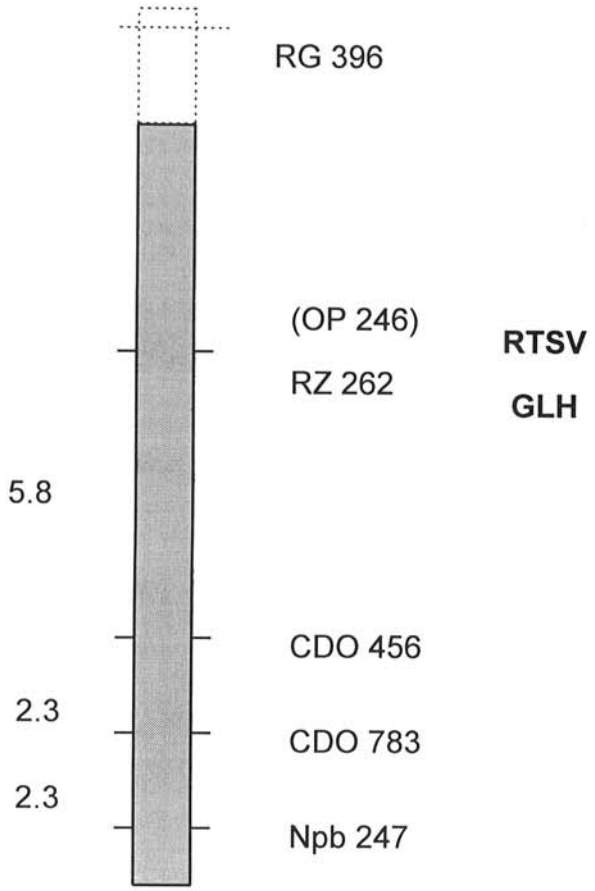

Fig. 3. The most likely arrangement of the green leafhopper (GLH) and rice tungro spherical virus (RTSV) resistance genes relative to molecular markers on rice chromosome 4, derived using Mapmaker/Exp 3.0 at LOD 3.0. OP 246 is a random amplified polymorphic DNA marker that cosegregated with RZ 262.

BC_5F_1 NIL used as a parent in the backcross with TN1 did not carry the RTBV resistance gene(s) from ARC11554.

Cosegregation of GLH and RTSV resistance. Linkage between percentage RTSV and GLH nymph survival was observed when R, H, and S phenotypes were distinguished on the basis of an analysis of 67 BC_6F_3 families and data from test tube antibiosis (Table 1). A test for independence of RTSV and GLH resistance confirmed that these characters do not segregate independently ($\chi^2 = 19.41$, $P < 0.01$). Two-point analysis gave a distance of 6.6 cM (LOD 18.8) between the resistance characters. The Pearson product moment correlation coefficient of GLH resistance with RTSV resistance was also significant ($r = 0.75$) (Fig. 2).

Cosegregation of RFLP markers with RTSV and GLH resistance. Of the 178 previously mapped RFLP markers that were used to survey for polymorphism between ARC11554 and TN1, 108 (60.6%) detected polymorphism for at least one of the five restriction enzymes used. Of the polymorphic markers, 13 were putative positive markers. These markers had bands that were shared by ARC11554 and bulked BC_6F_2 plants but were not present in TN1. When the putative positive markers were used as probes in RFLP analysis, four markers on chromosome 4 (RZ 262, CDO 456, CDO 783, and NPB 247) showed cosegregation with RTSV and GLH resistance. RZ 262 appeared to be the closest to both RTSV resistance and GLH resistance; therefore, this clone was used in refining the conversion of nymph survival and RTSV infection into discrete phenotypic classes.

The approximate positions of the markers and the two traits are shown in Figure 3. The map presented here estimates the most likely positions for RTSV and GLH resistance genes. An RTSV resistance gene maps above RZ 262 (5.5 cM, LOD 30.7), while a GLH resistance gene maps between RZ 262 (3.2 cM, LOD 23.2) and CDO 456 (5.1 cM, LOD 19). However, because of the complex nature of phenotyping and the possibility of errors, the scores may not accurately represent the genotype. Therefore, RTSV and GLH resistance genes are shown in the vicinity of RZ 262.

When quantitative data (percentage infection and percentage nymph survival) were used to map the resistance character based on QTL analysis, a highly significant association between markers in the region of RZ 262 and both RTSV and GLH resistance was detected. RZ 262 was the most significant marker in the region associated with GLH resistance (LOD 19.69) and RTSV resistance (LOD 41.6). The R-squared values, or percentages of the phenotypic variance explained by a gene(s) at this location, are 60 and 76% for RTSV and GLH resistance, respectively. Thus, both analytical approaches support the interpretation that the gene(s) governing resistance to RTD is located toward the end of rice chromosome 4 in ARC11554.

RAPD. One RAPD marker (OP 246) was identified which cosegregated with the ARC11554 (resistant) allele of RZ 262. Forty-five individual lines homozygous for the parental alleles of RZ 262 were then used for segregation analysis. The tentative location of this marker (3.5 cM from RZ 262) is shown in Figure 3.

DISCUSSION

Test tube antibiosis, small-pot antibiosis, and mass screening were evaluated during the course of this study. Each method had certain limitations. The small-pot antibiosis was useful for phenotyping F_2 plants because it provided each rice seedling with optimal conditions during antibiosis feeding and helped to clearly separate resistant and susceptible individuals. However, it was very difficult to count the leafhoppers inside the cage, and seedling maintenance was very tedious, limiting the number of seedlings that could be scored at one time. The scores of the controls (TN1 and ARC11554) also varied greatly among batches.

Mass screening allowed the largest number of individuals to be assayed with the least amount of effort. However, it was also the least reliable technique, because some plants escaped feeding by the insects. Specifically, antixenosis as well as antibiosis could have prevented the insects from infesting the plants.

The small test tube was the best test for the antibiosis reaction. The reaction to antibiosis was clear (dead nymphs), and it was easier to score than the small-pot method. The standard errors for the resistant and susceptible controls for different batches were lower than in the other methods. Hence, we used test tube antibiosis to analyze the antibiosis reaction of several batches of BC_6F_3 families.

The results show that ARC11554 has resistance to GLH (the vector) and to RTSV and a low level of resistance to RTBV. Transmission studies have concluded that cultivars that are resistant to GLH generally have a low level of infection by RTSV (9,16). It has also been shown that in GLH-resistant plants, the insect feeds on the xylem rather than the phloem. Because RTSV resides specifically in the phloem (41,43), whereas RTBV has been found in both the phloem and the xylem (43), plants resistant to GLH frequently show infection with RTBV but not RTSV.

In this study, these observations were taken into account by force-feeding the GLH (10 GLH per plant) on F_2 and BC_6F_3 seedlings for 4 h. Only a short period of inoculation feeding (5 min) is required to transmit the viruses (26). Although the insect avoids prolonged feeding on the phloem in GLH-resistant cultivars, it is known to probe on the phloem for short periods (4 to 92 min) (20). This is enough time for GLH to transmit both viruses and makes the plant vulnerable to increased insect pressure. As the number of GLH per plant increases, the amount of RTSV detected in GLH-resistant cultivars also increases (19,37). Regardless of the number of GLH (1, 5, 10, 20, or 30 viruliferous GLH per plant), ARC11554 supported a low titer of infection by RTSV + RTBV or RTSV alone (19). The percentage infection by RTBV in the RTSV- and GLH-resistant BC_6F_3 lines was consistently high (87.5 to 100%), which indicated effective GLH feeding and virus transmission. The fact that we continued to observe low levels of RTSV in these plants, many of which showed high levels of RTBV (which cannot be transmitted without RTSV), rules out an artifactual relationship between RTSV and GLH resistance.

In the segregating population, the expression of resistance to RTSV is very high, as indicated in the ELISA reading obtained for both heterozygous and homozygous resistant plants. The reaction of the F_2 plants and BC_6F_3 families to GLH feeding was more variable. The different antibiosis screening methods used in this study also gave slightly different results regarding GLH antibiosis, indicating either variability in the insect population or in the pest-plant interactions or variation in the level of expression of antibiosis in the different plants.

Because phenotypic evaluation of GLH, RTSV, and RTBV resistance is so difficult, we used a variety of approaches to ensure that our classification methods were as accurate as possible. We aimed to identify R, H, and S plants with respect to both GLH and RTSV using independent BC_6F_3 families derived from the same BC_6F_2 lines and three interpretations of the data. These interpretations were based on (i) the phenotypic mean of the BC_6F_3 families, (ii) the magnitude of the variance of the BC_6F_3 families, and (iii) molecular marker profiles. Historically, the classification of each F_3 family or individual into resistant and susceptible categories has been achieved by using arbitrary cutoff values for the mean GLH antibiosis score (percentage nymph survival) or RTSV infection. For GLH antibiosis, the variance of each BC_6F_3 family was also considered. A high phenotypic variance in the BC_6F_3 was indicative of heterozygosity in the BC_6F_2 for the locus under consideration. This hypothesis was validated by testing the equality of variances of groups classified as segregating and homozygous. Tests of the variances (34) indicated a significantly higher phenotypic variance associated with heterozygotes for antibiosis ($R = 2.46$, $F_{30,35}$, $P < 0.0056$). Lastly, molecular marker genotype was considered because markers in the region of the putative resistance genes offered a window into the pattern of recombination and suggested which specific phenotypic interpretation was most likely to be valid.

Resistance to GLH and to RTSV appear to be linked traits that are controlled by a dominant gene(s). Although ARC11554 had been known to have resistance to both RTSV and GLH (17), no information was available regarding the linkage relationships between these genes. Table 1 summarizes the number of putative recombinant lines obtained in this study, and these data suggest that there may be two different genes governing these two characters. However, the putative recombinants need to be confirmed because of the inherent difficulties in unequivocally distinguishing among R, H, and S phenotypes. To clarify the relationship between RTSV and GLH resistance, putative recombinant families (F_3) will be genotyped using the linked molecular markers on a per plant basis and phenotyped in the F_4. This will allow us to phenotype only those individuals most likely to be recombinants for the trait of interest. Through this scheme, we aim to identify lines resistant to RTSV alone and to GLH alone, to confirm whether two genes are involved.

RFLP and RAPD markers on a single chromosomal segment at the end of chromosome 4 were associated with GLH and RTSV resistance. This is the first report of the map location of any gene related to RTD resistance in rice germ plasm. Molecular markers should provide a more accurate way to determine the presence of disease and insect resistance genes by facilitating identification at the genotypic level. Currently, it is risky to inoculate and select in early generations, because plants may die as a result of infection caused by either virus. The use of these molecular markers offers great advantages for those interested in studying the genetics and etiology of this complex disease. It will hasten the transfer of the gene(s) to cultivars and eventually its deployment for RTD management.

Our results provide a model for uncovering and distinguishing other genes related to RTD resistance. A repertoire of mapped RTD resistance genes would provide breeders and pathologists with a tool for further evaluating the role of these valuable genes in moderating resistance to RTD. When these markers are converted to a user-friendly form (e.g., polymerase chain reaction [PCR]-based markers), they will provide an invaluable complement to the tedious and highly variable screening method currently used to evaluate RTD resistance. The RAPD marker identified in this study is now being cloned so that its map position can be confirmed and PCR primers can be designed for use in selection.

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