Mapping and Progress toward Map-Based Cloning of Brown Planthopper Biotype-4 Resistance Gene Introgressed from *Oryza officinalis* into Cultivated Rice, *O. sativa*

K. Renganayaki,* Allan K. Fritz, S. Sadasivam, Sujata Pammi, Sandra E. Harrington, Susan R. McCouch, S. Mohan Kumar, and Avutu Sam Reddy

**ABSTRACT**

Brown planthopper (BPH), *Nilaparvata lugens* (Stål.), is a serious insect pest of rice in Asia, causing direct losses and vectoring *Rice grassy stunt virus* (RGSV) and *Rice ragged stunt virus* (RRSV). Recombinant inbred lines (RILs) developed from a cross between ‘IR50’ and ‘IR54745-2-21-12-17-6’ were used to identify random amplified polymorphic DNA (RAPD) markers closely linked to a BPH Biotype-4 resistance gene (*Bph*13 (*t*)) derived from *Oryza officinalis* Wall. Bulked segregant analysis (BSA) using RAPD primers identified 11 polymorphic fragments. Six fragments, *AJ09*_13*a*, *AJ05*_22*b*, *AK10*_33*a*, *AK10*_33*5*, *AK10*_33*d*, and *AJ01*_99*a*, were linked in coupling phase to the *Bph*13 (*t*) locus. The remaining five fragments, *AJ09*_22*b*, *AJ09*_13*b*, *AJ09*_13*c*, *AL05*_68*b*, and *AK10*_22*a*, were linked in repulsion. The most closely linked RAPD marker, *AJ09*_22*b*, was converted to a codominant linked sequence tagged sites (STS) marker. This marker mapped L3 centimorgans (cM) from the resistance gene and was placed on rice chromosome 3 by means of ‘IR64’ × ‘Azucena’ doubled haploid (DH) population. The tightly linked STS marker could be used for marker-assisted selection (MAS). In addition, these markers will be useful for a positional cloning strategy to isolate the resistance gene.

**THE BROWN PLANTHOPPER** is one of the most serious insect pests of rice (*O. sativa L.*) and can cause significant yield losses. In addition to direct damage, BPH also acts as a vector for RGSV and RRSV. Heavy infestations cause complete drying and plant death, a condition known as hopper burn. The primary methods of control are chemical insecticides and host plant resistance as part of an integrated pest management (IPM) strategy. The cost of chemical control is often exorbitant, destroys the natural balance of BPH-predators that help keep the BPH populations in check, and can ultimately cause development of new, insecticide resistant strains. Therefore, the most economical and efficient method to control BPH is through host plant resistance as part of IPM.

Many researchers have investigated the genetics of resistance to brown planthopper. To date, 12 genes have been reported. Studies conducted by Athwal et al. (1971) revealed that a dominant gene, *Bph-1*, governs resistance in ‘Mudgo’, ‘MTU15’, ‘Co22’, and ‘MGL2’, while a single recessive gene, *bph-2*, conveys resistance in ‘ASD7’ and ‘Ptb18’. According to Lakshminarayana and Khush (1977), the Sri Lankan cultivar Rathu Heenati has a dominant gene for resistance, which is non-allelic to, and independent of, *Bph-1* and was designated as *Bph-3*. Another Sri Lankan cultivar, Babawee, has a recessive gene for resistance (Lakshminarayana and Khush, 1977). This gene is independent of *bph-2* and is designated as *bph-4*. Kabir and Khush (1988) identified *bph-5* in ‘ARC10550’, *Bph-6* in ‘Swarinalatha’ and *bph-7* in ‘T12’, by means of BPH biotypes from Bangladesh. Nemamoto et al. (1989) identified a new recessive gene, *bph-8* in ‘Thai Col.5’, ‘Thai. Col.11’, and ‘Chin Saba’, and a new dominant gene, *Bph-9*, in the Sri Lankan local cultivars, Pokkali, Balamavee, and Kaharamana. *Oryza australiensis* Domin, a wild relative of rice, was also demonstrated to possess a dominant resistant gene, *Bph-10* (*t*) that was introgressed into an indica breeding line (Ishi et al., 1994). More recently, two more genes, *bph-11* (*t*) and *bph-12* (*t*), have been reported (Kawaguchi et al., 2001).

Wild species of *Oryza* are potential sources of new genes for resistance to BPH. The evaluation of 11 000 wild rice accessions by means of biotypes-1, 2, and 3 at International Rice Research Institute (IRRI) revealed that 19 accessions belonging to four wild species were resistant or moderately resistant to all three biotypes (Wu et al., 1986). The *N. lugens* population from South India (Tamil Nadu), which differs in virulence from the biotypes of *N. lugens* maintained at IRRI (Velusamy et al., 1984), was used in a study of wild rice species by Velusamy (1988), which revealed that *O. officinalis* and *O. punctata* Kotsch ey Steud. were highly resistant to southern Indian populations of BPH. These two species were also reported to be highly resistant to all three previously described biotypes of BPH, as well as the green rice leafhopper (*Nephotettix cincticeps* Uhler) and the white backed planthopper (*Sogatella furcifera* Horvath).

Populations of BPH were categorized into five biotypes on the basis of their differential reactions to a set of reference cultivars (Chelliah and Bharathi, 1993). The general field population or wild strain in the Philippines that can only damage varieties with no resistance genes are called biotype-1. Biotype-2 was identified in the Philippines, Indonesia, and Vietnam in 1976–1977. The general field population or wild strain in the Philippines, Indonesia, and Vietnam in 1976–1977. It was the dominant biotype after the introduction and wide scale cultivation of cultivars with the *Bph-1* gene (Khush, 1979). Biotype-3 was identified after rearing the

**Abbreviations**: BPH, brown planthopper; bp, base pairs; BSA, bulked segregant analysis; cM, centimorgans; DH, doubled haploid; MAS, marker-assisted selection; QTL, quantitative trait locus; RILs, recombinant inbred lines; RAPD, random amplified polymorphic DNA; STS, sequence tagged sites.

K. Renganayaki, Dep. of Soil and Crop Sciences, Texas A&amp;M Univ., College Station, TX 77843; A.K. Fritz, Dep. of Agronomy, Kansas State Univ., Manhattan, KS 66506; S. Pammi, and A.S. Reddy, Dow Agro Sciences, 9330 Zionville Road, Indianapolis, IN 46268-1054; S.E. Harrington and S.R. McCouch, Dep. of Plant Breeding and Biometry, Cornell Univ., Ithaca, NY 14853-1901; S. Sadasivam and S. Mohan Kumar, Centre for Plant Molecular Biology, Tamil Nadu Agricultural Univ., Coimbatore 641 003, India. Received 8 Aug. 2001. *Corresponding author (renga@tamu.edu).

Published in Crop Sci. 42:2112–2117 (2002).
insect on ASD7, a rice cultivar with the bph-2 resistance gene for 130 generations at IRRI (Pathak and Heinrichs 1982). Biotype-4 BPH populations originated from South Asia and have been referred to as the “South Asian biotype” (Khush, 1984). Systematic study on brown plant-hopper and related species revealed that the *N. lugens* populations from Asia and Australia were separate (Jones et al., 1996).

The advent of DNA marker technology has resulted in the mapping of BPH genes to several linkage groups. To date, at least 12 major BPH resistance genes have been identified and characterized (Kawaguchi et al., 2001). Of these, *Bph1*, *bph2*, and *Bph10* were mapped to chromosome 12, *bph4*, *Bph9*, *bph11* (t), and *bph12* (t) have been mapped to rice chromosomes 6, 12, 3, and 4, respectively (Ishii et al., 1994; Hirabayashi and Ogawa, 1995; Murata et al., 1998; Jeon et al., 1999; Kawaguchi et al., 2001). In addition, 16 major quantitative trait loci (QTLs) associated with Bph resistance have been identified from *indica* and *O. officinalis* derived sources (Alam and Cohen, 1998; Huang et al., 2001; Xu et al., 2002).

In this paper, we report the molecular tagging of a dominant gene introgressed from *O. officinalis* that conveys resistance to BPH biotype-4. This locus is designated as *Bph13* (t).

**MATERIALS AND METHODS**

**Plant Materials and Screening for BPH Resistance**

The cultivar IR50, which is susceptible to BPH Biotype-4 and IR54745-2-12-17-6, a line with *O. officinalis*-derived resistance to BPH biotype-4 were obtained from the International Rice Research Institute (IRRI) and used as parental materials for this study. A random sample of 300 F1 plants was forwarded to F2. Five plants from each F1 line were forwarded to F3, F4, F5, F6, and F7. Fifty-four F1 derived RILs were used for mapping and linkage analysis of RAPD markers. The Modified Seed Box Screening Test (MSST) developed at IRRI (Heinrichs et al., 1985, p. 356) was used to screen parents and segregating generations for resistance to BPH. The BPH biotype-4 population of South Asia (Khush, 1984) maintained at Tamil Nadu Agricultural University was used for the insect bioassay. The seeds were presoaked and sown in rows in 60 to 700 base pairs (bp). Six of the fragments (AJ09b-STS, AJ09260a, AL05, AK10, and AJ01) were mapped was determined by a LOD score of 3.0 and map distances to chromosome 12,

**DNA Extraction and RAPD Analysis**

DNA from the parents and the 54 RILs was extracted following Dellaporta et al. (1983) and the concentration was determined with Hoechst dye 33258 in a TKO mini fluorometer (Hoefer Scientific, San Francisco, CA). The quality of the DNA was checked on an agarose gel (0.8%, w/v). Equal amounts of DNA from 10 resistant (score 1) and 10 susceptible (score 9) individuals were pooled to constitute the resistant and susceptible bulks, respectively. Three hundred random 10-mer primers were obtained from Operon Technologies Inc. (Alameda, CA). The parents and the two bulks were subjected to RAPD analysis, following the conditions of Williams et al. (1990) with minor modifications. Amplification reactions were performed in 15-µL volumes containing 100 μM each of dATP, dGTP, dCTP, and dTTP, 20 ng of primer, 10 ng of genomic DNA, and 0.075 U of Ampli-Taq DNA polymerase (Perkin Elmer, Foster City, CA) in a Perkin Elmer Cetus 9600 thermocycler. The amplification profile was 94°C for 2 min followed by 30 cycles of 94°C for 30 s, 38°C for 1 min, and 72°C for 1 min with a final extension of 10 min at 72°C. Amplified PCR products were denatured and resolved by electrophoresis on a 5% (w/v) polyacrylamide gel and visualized by means of silver staining (Panaud et al., 1995).

Linkage analysis was performed on 54 RILs by MAP-MAKER software (v3.0) (Lander et al., 1987). Marker order was determined by a LOD score of 3.0 and map distances were estimated by the Kosambi function (Kosambi, 1944).

**Conversion of RAPD Fragment to STS**

The *AJ09b* RAPD fragment, tightly linked to the *Bph13* (t), was excised from the silver stained gel and transferred to a 1.5-mL Eppendorf tube containing 25 µL of 1× TE. The gel slice was crushed with a plastic pestle and centrifuged. The supernatant was used for reamplification under the previously described RAPD-PCR conditions. The fresh PCR product was cloned by means of the TA cloning kit (Invitrogen, Carlsbad, CA). The ABI Dye–Terminator sequencing kit (Perkin Elmer) was used for cycle sequencing and products were analyzed on an ABI 377 sequencer (PE Applied Biosystems, Foster City, CA). STS primers were designed from the sequenced RAPD fragment with the Oligo 5.0 program (Molecular Biology Insights, Inc., Cascade, CO). Amplification of genomic DNA (parents and 54 RILs) with the STS primers was performed by means of a two-step program (68°C for 1 min and 94°C for 30 s) for 35 cycles. The amplified products were separated on a 5% polyacrylamide gel and visualized by silver staining.

**Assigning Chromosomal Position to STS Marker**

A DH population consisting of 96 individuals derived from an IR64 × Azucena cross (Tennnykh et al., 2000) and a recombinant inbred (RI) population consisting of 252 individuals derived from a ‘Lemont’ × ‘Teqing’ cross (Li et al., 1995; Tabien et al., 2000) were used for mapping the *AJ09b-STS* marker. Amplification followed the previously described protocols. The resulting PCR products were separated on 4% polyacrylamide gels and visualized via the silver staining process described by Panaud et al. (1995). The marker was placed on the two maps by means of the Kosambi mapping function and Mapmaker software (v3.0) (Lander et al., 1987). In this manner, the *AJ09b-STS* marker, and by association, the *Bph13* (t) locus, was placed on the DH framework SSR map described by Tennnykh et al. (2000) and on the Lemont × Teqing restriction fragment length polymorphism (RFLP) map (Tabien et al., 2000).

**RESULTS**

BSA was employed to identify RAPD markers linked to the *Bph13* (t) locus. Of the 300 primers tested, 19 produced polymorphisms in the parents and bulks. RAPD analyses of parents and the individuals constituting the bulks yielded four polymorphic primers, *AJ09*, *AL05*, *AK10*, and *AJ01* (Fig. 1). These four primers generated 11 polymorphic fragments ranging in size from 100 to 700 base pairs (bp). Six of the fragments (AJ09, AJ05,
linked (1.3 cM) to the resistance gene, was cloned. The *O. officinalis* and mapped 1.3 cM from the *Bph13 (t)* locus. These two markers cosegregated with one another as a QTL for BPH resistance identified by Alam and AJ09b on the other side (Fig. 2). Of the RAPD fragments, some 3 (Kawaguchi et al., 2001). The gene described in AJ01 was a major gene. This clearly indicates the *O. officinalis*-derived gene in our study appeared to be linked to an STS marker. The STS marker produced the same pattern as *AJ09b* and thus mapped 1.3 cM from the *Bph13 (t)* locus. The closely linked *AJ09b*-STS marker cosegregated with RG100 on chromosome 3 when mapped by the 96 DH lines by Temnykh et al. (2000). RG 100 and *AJ09b*-STS were flanked by RZ892 and RG 191 (Fig. 2). Using the 252 RI lines from Lemont × Teqing population, *AJ09b*-STS mapped to chromosome 3 flanked by RG100 and RM7.

**DISCUSSION**

RAPD technology has proven effective for identification of markers closely linked to genes for agronomically important traits. The goal of our study was to find RAPD markers linked to BPH resistance gene, *Bph13 (t)* for biotype-4, which is prevalent in South Asia (Velusamy et al., 1984). In the present study, we identified four RAPD primers that produced 11 fragments closely linked to the *Bph13 (t)* locus from *O. officinalis*. Two markers, *AJ09b* and *AJ09c*, mapped 1.3 cM from the resistance gene. RAPD markers are generally dominant in nature. However, three of the markers isolated here (*AJ09*, *AK10*, and *AL05*) were found to be codominant. Codominant markers are especially useful, as they allow identification of heterozygotes in segregating populations. We used BSA to screen the RAPD primers, which is a powerful technique to find markers linked to a gene of interest in a relatively short time.

RAPD PCR analysis using arbitrary primers can be less repeatable than PCR amplification from more specific primers. Conversion of RAPDs to STS markers improves efficiency, cost effectiveness, and practicality of MAS. To this end, we successfully converted the RAPD marker *AJ09b* to an STS marker. The STS marker produced a single amplification product that yielded the same information as the original RAPD marker, but can be more readily applied in breeding to evaluate germplasm for the presence of the resistance gene.

The tightly linked STS marker from our study was placed on the short arm of rice chromosome 3 by means of populations derived from IR64 × Azucena and LeMont × Teqing crosses. Other genes for BPH resistance have been previously mapped to chromosomes 3, 9, and 12 (Huang et al., 2001; Alam and Cohen, 1998; Ishii et al., 1994; Hirabayashi and Ogawa, 1995; Murata et al., 1998; Jeon et al., 1999) and the long arm of chromosome 3 (Kawaguchi et al., 2001). The gene described in this study is placed in the same region of chromosome 3 as a QTL for BPH resistance identified by Alam and Cohen (1998). The QTL accounted for between 5.6 and 13% of the variation in their population, while the *O. officinalis*-derived gene in our study appeared to be a major gene. This clearly indicates the *O. officinalis*...
'IR64' x 'Azucena'

Chromosome 3, Short arm

Fig. 2. Linkage map of *Bph13 (t)* locus. Left is the standard IR64 × Azucena map (Temnykh et al., 2000) and right is the map constructed for *Bph13 (t)* locus.

derived gene described in this study is different from the previously tagged genes. However, allelism tests with BPH resistance genes that have not been placed on other chromosomes will be necessary to definitively establish the relationship of this gene to previously described BPH genes.
There are other examples of QTLs mapping to the same site as major genes. A major QTL for heading date in rice (Yano et al., 1996) maps to the same location as Se1, a major gene for photoperiod sensitivity in rice (Yokoo et al., 1980). The resistance of rice to bacterial blight pathogen had both qualitative and quantitative components (Li et al. 2001). Similarly the QTL for BPH resistance identified by Alam and Cohen on rice chromosome 3 and the gene identified through the present study may refer to the same locus. In contrast, the QTL identified by Huang et al. (2001) on chromosome 3 mapped to the long arm, while the locus identified through the present study mapped to the short arm indicating that the relationships between the QTL identified by Alam and Cohen (1998) and Huang et al. (2001) and the locus identified here needs further delineation.

The STS marker will provide the opportunity to screen the genotypes efficiently for the Bph13 (t) locus. Traditionally, plant breeders and entomologists used the seed box screening procedures to select plants possessing BPH resistance. This process is both time consuming and cumbersome, taking a minimum of 3 to 4 wk to obtain results. The use of linked markers requires no insect inoculation, and should take 2 to 3 d (with the available quick DNA extraction protocols), thus improving the efficiency of selection for BPH resistance. Linked markers could facilitate incorporation of multiple resistance genes into one genetic background (gene pyramiding), possibly allowing the development of more durable resistance. Sanchez et al. (2000) and Singh et al. (2001) used sequence tagged site markers to develop lines possessing resistance to three bacterial blight resistance genes and pyramiding the three genes into one genetic background in rice.

Cloning the gene would allow it to be transformed into elite cultivars and would open avenues of studying mechanisms of resistance. Identification of bacterial artificial chromosomes (BAC) carrying the most closely linked markers is an important step toward cloning of the gene. We have initiated a chromosome walk by probing BAC filters (Nipponbare) with the polymorphic RAPD fragment (AJ096). Further analysis of positive BACs in conjunction with physical mapping will be required to build a contig spanning the BPH biotype-4 resistance gene region.

ACKNOWLEDGMENTS

Major funding for this project was sponsored by The Rockefeller Foundation, New York as a Postdoctoral fellowship to Dr. K. Renganayaki. The authors greatly appreciate support of The Rockefeller Foundation. The authors acknowledge Dr. G.S. Khush and Dr. D.S. Brar for providing the O. officinalis-derived line used for generating the mapping population. The authors also thank Dr. William Park and Dr. Nicola Ayres for providing the Lemont × Teqing rice mapping population and assigning the chromosomal location of the STS marker.

REFERENCES


