Molecular mapping and genetic analysis of a rice brown planthopper (*Nilaparvata lugens* Stål) resistance gene

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The brown planthopper (BPH), *Nilaparvata lugens* Stål, is a serious insect pest of rice (*Oryza sativa* L.). We have determined the chromosomal location of a BPH resistance gene in rice using SSR and RFLP techniques. A rice line 'B14', derived from the wild rice *Oryza latifolia*, showed high resistance to BPH. For tagging the resistance gene in 'B14', an F_2 population and a recombinant inbred (RI) population from a cross between Taichung Native 1 and 'B14' were developed and evaluated for BPH resistance. The results showed that a single dominant gene controlled the resistance of 'B14' to BPH. Bulked segregant SSR analysis was employed for identification of DNA markers linked to the resistance gene. From the survey of 302 SSR primer pairs, three SSR (RM335, RM261, RM185) markers linked to the resistance gene were identified. The closest SSR marker RM261 was linked to the resistance gene at a distance of 1.8 cM. Regions surrounding the resistance gene and the SSR markers were examined with additional RFLP markers on chromosome 4 to define the location of the resistance gene. Linkage of RFLP markers C820, R288, C946 with the resistance gene further confirmed its location on the short arm of chromosome 4. Closely linked DNA markers will facilitate selection for resistant lines in breeding programs and provide the basis for map-based cloning of this resistance gene.

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The brown planthopper (BPH), *Nilaparvata lugens* Stål (Homoptera: Delphacidae), is a destructive and widespread insect pest throughout the rice areas in Asia. The BPH feeds mainly on the stems, and sucks assimilates from the phloem of rice plants. Feeding by a large number of BPH may result in drying of the leaves and wilting of the tillers, a condition called hopperburn. Developing resistant cultivars is generally considered the most effective and economical means for BPH control. Accordingly, breeding for resistant cultivars has become a priority in rice improvement. At least 12 resistance genes have been identified and several have been located on the chromosomes via trisomic analysis or linkage to molecular markers (ATHWAL et al. 1971; KHUSH and BRAR 1991; ISHII et al. 1994; HIRABAYASHI and OGAWA 1995; HUANG et al. 2001).

Brown planthoppers in China belong in general to biotypes 1 and 2. They cause heavy losses in rice production in southern and central China. Extensive work is in progress to identify BPH resistance genes from various sources. *Oryza latifolia* has been proven to have a good germplasm for BPH resistance (WU et al. 1986). The cultivated rice, *O. sativa*, has been crossed with *O. latifolia* in a large scale and fertile progenies were recovered through somatic cell culture (SHU et al. 1994). The BPH resistant lines were selected from progenies of this cross (YANG et al. 1999). One of the progeny lines, 'B14', showed high resistance to BPH biotypes 1 and 2 collected in China. Now 'B14' is used as a resistance donor for improving BPH resistance in rice breeding programs.

The chromosomal location of the BPH resistance gene provides the foundation of manipulating the gene. In order to identify DNA markers linked to the BPH resistance gene in variety 'B14', we have employed bulked segregant analysis with SSR (simple sequence repeat) technique. SSR makers are widely distributed in the rice genome (MCCOUCH et al. 1997) and can be easily and economically analyzed by polymerase chain reaction. SSR can detect high allelic variation and provide a new tool for gene mapping and marker-assisted selection (MAS) in rice.

Here we characterized the inheritance pattern of the BPH resistance gene in 'B14' and determined the chromosomal location of this resistance gene using SSR and RFLP analysis.

MATERIALS AND METHODS

Plant materials

An F_2 population consisting of 184 plants and a recombination inbred (RI) population consisting of

209 lines were derived from a cross between Taichung Native 1 and 'B14'. Taichung Native 1 is an *indica* cultivar that is susceptible to all biotypes of brown planthoppers. Both populations were screened for resistance to BPH. The RI population was also used to map the BPH resistance gene.

BPH resistance scoring

For evaluating the BPH resistance of each RI lines, the standard seedling box screening technique was followed with some modifications (IRRI 1988). About 15 seeds of each RI line were sown in a row of 20 cm in length in a plastic box. Two lines of 'B14' and two lines of Taichung Native 1 were randomly planted among the RI lines as controls. At the three-leaf stage, the seedlings were infested with ten BPH per seedling. When all of the seedlings of Taichung Native 1 died, each seedling of the RI lines was examined and given a score of 0, 1, 3, 5, 7 or 9 according to the criterion given in Table 1. The resistance level of each RI line was inferred based on the average value of the seedlings. Accordingly, we employed the modified tiller seedbox screening technique (FU et al. 1994) to evaluate the BPH resistance of each F_2 individual. The resistance scoring experiment was repeated twice.

Plant DNA extraction

Preparation of genomic DNA from the parents and RI population followed the CTAB method as described by MURRAY and THOMPSON (1980). For each sample, the DNA concentration was determined using a fluorometer (DyNA Quant 200, Hoefer company).

SSR amplification and assay

Three hundred and two SSR primer pairs were used to amplify simple sequence length polymorphism (SSLP) of genomic DNA from the two parents. The PCR was performed in a total volume of 12.5 μl containing 20 ng of genomic DNA, 0.2 μM of each primer, 200 μM each of dATP, dTTP, dGTP, dCTP, 0.5 unit of Taq DNA polymerase, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$ and 0.01 % gelatin. Samples were amplified at 94°C for 5 min, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, 2 min at 72°C, and 72°C for 5 min at final extension in a Perkin Elmer Thermal Cycler. After amplification, 12.5 μl stop solution was added to the PCR products, and they were denaturing at 94°C for 4 min. Three microliters of each reaction were run on a 6 % polyacrylamide denaturing gel containing 7 M urea. A silver staining procedure was used as described by PANAUD et al. (1996).

Table 1. *Criterion for brown planthopper resistance scores*

Resistance score	Plant state (investigated when most of the Taichung Native 1 plants died)
0	None of the leaves shrank and the plant was healthy
1	One leaf was yellowing
3	One to two leaves were yellowing or one leaf shrank
5	One to two leaves shrank or one leaf shriveled
7	Three to four leaves shrank or two to four leaves shriveled, the plant was still alive
9	The plant died

RFLP analysis

Total genomic DNA was digested with five restriction enzymes: *Bam*HI, *Hind*III, *Eco*RI, *Eco*RV and *Dra*I. Electrophoresis, hybridization and detection followed the procedures described by LIU et al. (1997). The RFLP probes were kindly provided by the Japanese Rice Genome Research Project and by the Cornell University group.

Identification chromosomal regions containing BPH resistance gene

The detection for markers that were linked to the resistance gene followed essentially the procedures described by ZHANG et al. (1994). Two bulks were made by pooling equal amounts of DNA from 20 highly resistance plants and equal amounts of DNA from 20 highly susceptible plants. The two bulks and the two parents were screened for polymorphism with SSR markers. The polymorphic SSR markers were used to construct linkage map.

Linkage analysis

After the polymorphic SSR markers were mapped on the chromosome, a number of RFLP markers from this region were selected for a parental polymorphism survey. Subsequently, the whole RI population was surveyed for RFLP markers showing polymorphism between parents. The phenotype, SSR and RFLP data were combined for linkage analysis using the Mapmaker/Exp 3.0 at LOD 3.0 (LINCOLN et al. 1992) and a partial linkage map of the chromosome region surrounding the BPH resistance gene was constructed using the KOSAMBI (1944) mapping function. The genetic contribution to the phenotypic resistance by the chromosome region was analyzed using QTLMapper 1.0 at a LOD threshold 3.0 (WANG et al. 1999).

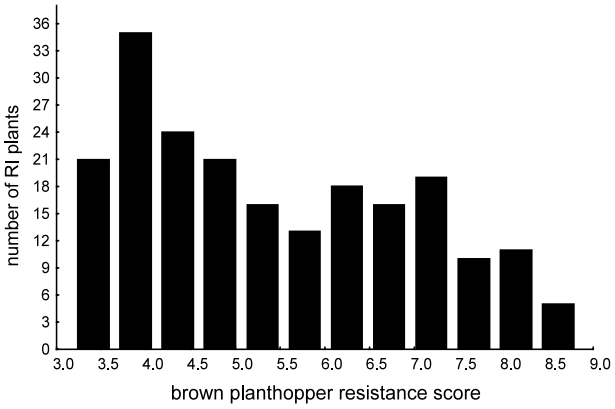

Fig. 1. Distribution of BPH resistance scores of 209 RI lines. The damage severity scores of the two parents, Taichung Native 1 and 'B14', were 8.96 and 3.12, respectively.

RESULTS

Inheritance pattern of BPH resistance gene in 'B14'

The damage severity scores of the two parents, Taichung Native 1 and 'B14', were 8.96 and 3.12, respectively. The damage severity scores of the 209 recombinant inbred lines infested with BPH ranged from 3.0 to 9.0 showing a continuous distribution with two peaks. An apparent valley was around 5.5 in the distribution curve (Fig. 1). A plant was classified as resistant if the average severity score was < 5.5, and as susceptible if the average severity score was between 5.5 and 9.0. Segregation of resistant and susceptible plants fits a 1:1 (113:96) ratio in the RI population ($x^2 = 1.38$, $p > 0.20$). Analysis of the resistance scoring data obtained from the F_2 population revealed that resistant and susceptible plants fits a 3:1 (133:51) ratio ($x^2 = 0.90$, $p > 0.3$). These indicated that a single dominant gene controlled the resistance of 'B14' to BPH.

Identification of SSR markers linked to the BPH resistance gene

To identify the positive SSR markers linked to the BPH resistance gene, a total of 302 SSR primer pairs distributed along 12 chromosomes of rice were used for the parental polymorphism survey. Among the 302 SSR primer pairs, 112 primer pairs showed polymorphism between 'B14' and Taichung Native 1, and the polymorphic frequency was 37.1 %. Bulked segregant analysis using the 112 polymorphic SSR markers revealed that three markers (RM335, RM261, RM185) on chromosome 4 were polymorphic between both the bulks as well as the parents. Linkage analysis with Mapmaker/EXP 3.0 revealed that SSR markers RM335, RM261 and RM185 were linked to the BPH resistance gene. The map distance between the BPH resistance gene and the closest SSR marker RM261 was 1.8 cM.

Confirming the chromosomal location of the BPH resistance gene

To further confirm the chromosome location of the BPH resistance gene and to identify more-closely linked markers for marker-assisted selection purposes, 10 RFLP markers from the region surrounding the BPH resistance gene on chromosome 4 were selected for a parental polymorphism survey. Only three RFLP markers (C820, R288, C946) showed polymorphism between the parents. With analysis of the whole RI population, the three polymorphic RFLP markers (C820, R288, C946) showed linkage to the BPH resistance gene. All the evidence described above led to the conclusion that the BPH resistance gene was located on the short arm of chromosome 4. QTLMapper 1.0 was used to analyze genetic contribution to the BPH resistance by the chromosome region. This locus accounted for 70.6 % of phenotypic variance of BPH resistance in the RI population. According to the denomination rule of plant gene, we designated this BPH resistance gene as *Bph 12(t)*.

DISCUSSION

We have identified a BPH resistance gene carried by 'B14' derived from *O. latifolia* and determined its chromosomal location by SSR and RFLP analysis (Fig. 2). We found a single dominant BPH resistance gene in 'B14' and mapped it on the short arm of chromosome 4. This locus accounted for 70.6 % of phenotypic variance of BPH resistance.

A number of BPH resistance genes have been reported in the literature (ATHWAL et al. 1971; KHUSH and BRAR 1991; ISHII et al. 1994; HIRABAYASHI and OGAWA 1995; HUANG et al. 2001). *Bph1*, *bph2* were assigned to chromosome 12. *Bph3* and *bph4* were located on chromosome 10. ALAM and COHEN (1998) reported two QTLs for BPH resistance that were located on short arm of chromosome 3 and the long arm of chromosome 4, respectively. More recently, HUANG et al. (2001) also reported two BPH resistance QTLs on chromosome 3 and chromosome 4, which were derived from *O. officinalis*. However, the two QTLs reported by Huang et al. were located on the long arm of chromosome 3 and short arm of chromosome 4, respectively. We mapped the BPH resistance gene of rice line 'B14' on the short arm of chromosome 4. This location is very close to one locus reported by HUANG et al. (2001). It is very interesting that BPH

resistance genes derived from different wild rice species were mapped on the same region of chromosome 4. Disease resistance genes of plants often cluster in the same chromosome region (HULBERT and BENNETZEN 1991; GROG et al. 1993; MAHADEVAPPA et al. 1993; SUDAPAK et al. 1993; ELLIS et al. 1995). It remains to be seen whether the distribution of insect pests resistance genes is similar to that of disease resistance genes. We are now working to ascertain whether these BPH resistance genes are allelic genes or different genes clustered. The system existing between BPH and resistance gene seems to be similar to the gene-for-gene system in disease resistance. BPH populations can quickly overcome single resistance gene under natural conditions. New resistance genes are always needed for rice improvement and breeding against BPH. Therefore, the resistance genes identified in this study could be a new resource of BPH resistance.

Building up multiple resistance genes of different origin is obviously an advantageous strategy for increasing the overall resistance. Wild species of rice are important resources of disease and insect resistance in crop genetic improvement. Several wild species, including *O. latifolia*, *O. minuta*, *O. nivara*, *O. officinalis* and *O. punctata*, possess resistance to various biotypes of BPH (WU et al. 1986). Through the wide-hybridization breeding program, BPH resistance genes from several wild species were transferred to the cultivated rice and resistant lines have been produced (ISHII et al. 1994; SHU et al. 1994). Experiments with BPH showed that these introgressed lines were highly resistant (YANG et al. 1999). 'B14' derived from a cross using *O. latifolia* as a pollinator, was one of the lines. 'B14' can be a useful BPH resistance donor for improving BPH resistance in rice breeding programs. The transfer of BPH resistance genes from wild species to different variety backgrounds can be greatly facilitated by using marker-assisted selection.

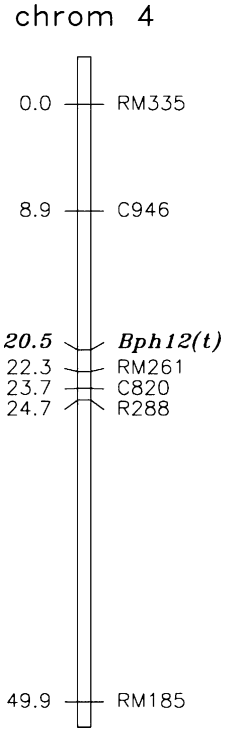

Fig. 2. The locations of the BPH resistance gene identified by SSR and RFLP. Marker names are listed on the right of chromosome and their positions (in cM) are listed on the left.

Molecular markers tightly linked to target gene may be useful in breeding (PARAN and MILCHELMORE 1993). SSR markers are abundant and widely distributed in the rice genome (WANG et al. 1994; PANAUD et al. 1996; MCCOUCH et al. 1997) and often detect more allelic variation than RFLP or RAPD markers (PANAUD et al. 1996). SSR markers have the advantages of both the rapidity and simplicity of RAPD, and the stability of RFLP and can become the markers of first choice for genetic mapping in rice. Our results indicate that the SSR marker RM261 will facilitate selection for resistant genotypes in breeding programs.

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