NOTES

Mapping of two new brown planthopper resistance genes from wild rice

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Abstract **A brown planthopper (BPH) resistance line, B5, derived its resistance genes from the wild rice *Oryza officinalis* Wall exwatt, was hybridized with Taichung Native 1, a cultivar highly susceptible to BPH. A mapping population composed of randomly selected 167 F_2 individuals was used for determining the BPH resistance genes by the restriction fragment length polymorphism analysis (RFLP). Bulked segregant analysis was conducted to identify RFLP makers linked to the BPH resistance genes in B5. The results indicated that the markers linked to BPH resistance are located at two genomic regions on the long arm of chromosome 3 and the short arm of chromosome 4, respectively. The existence of the two loci was further assessed by the quantitative trait locus (QTL) analysis. We located the two loci at a 3.2 cM interval between G1318 and R1925 on chromosome 3 and a 1.2 cM interval between C820 and S11182 on chromosome 4. Comparison with the BPH genes that have been reported indicated that the BPH resistance genes in B5 are novel. These two genes may be useful BPH resistance resource for rice breeding. Furthermore, the mapping of the two genes is useful for cloning the BPH resistance genes.**

Keywords: ***Oryza sativa*** **L., brown planthopper resistance gene, molecular marker, gene localization.**

Brown planthopper, *Nilaparvata lugens* Stål (BPH), is one of the most serious pests of rice in Asia. In China the BPH causes the damage of rice on 4 million ha, accounting for half of the total rice area. Applying the resistant varieties is an economical and effective way to control the BPH. Large efforts have been made to identify BPH resistance genes from various sources for developing resistant varieties. According to their reactions to the four BPH biotypes, at least 10 resistant genes have been characterized and reported[1—5]. The dominant gene *Bph1* resists biotypes 1 and 3; and the recessive gene *bph2* closely linked with *Bph1* shows resistance to biotypes 1 and 2. *Bph3* and *Bph4* also link closely and resist all four biotypes. *Bph5*, *Bph6* and *Bph7* only resist biotype 4. The other three genes, *Bph8*, *Bph9* and *Bph10*(*t*), resist biotypes 1, 2 and 3.

Obviously, localizing the BPH resistance genes is the base of using them efficiently. Ikeda and Kaneda mapped *Bph1* and *bph2* on chromosome 4[6], *Bph3* and *bph4* on chromosome 3[7] using trisomic analysis. The development of molecular markers facilitated the construction of genetic linkage maps, which make mapping easier, faster and more accurate than before. Recently some resistance genes have been mapped using the molecular marker analysis. The *Bph1* and *bph2* were remapped on chromosome 12[8—10], and *Bph(10)* was also mapped on chromosome12[5].

Oryza officinalis Wall exwatt, one of the three wild rice species found in China, shows a high resistance to BPH. By introgressing the BPH resistance genes of *Oryza officinalis* into a cultivar, Shu et al.[11] produced BPH-resistant lines. In the research, we used the bulked segregant analysis (BSA)[12] to identify RFLP markers linked to the BPH resistance genes in B5, and then tried to localize the genes at the genetics map. The results indicated that B5 has two new BPH resistance genes.

1 Materials and methods

(i) Plant materials and BPH insects. The genetic material was a random F_2 population consisting of 167 F_2 individuals chosen from a cross between B5, a BPH-resistant line derived from wide-hybridization program involving *O. officinalis*, and Taichung Native 1(TN1), a cultivar highly susceptible to BPH, which is often used as a standard susceptible control at evaluation of BPH resistance. Each F_2 plant was self-pollinated, and 144 F_3 families were obtained.

The BPH insects used for the research were the mixtures of biotype1 and biotype2, which were fed at the Genetics Institute of Wuhan University.

(ii) Evaluation of BPH resistance. In the summer of 1998, we used the Tiller Seedbox Screening Technique (TSST)[13] with some modification to evaluate the BPH resistance of 167 F_2 individuals. First, the seeds of B5, TN1 and each F_2 plant were separately sown in the fields. When the seedlings had 3 — 4 tillers, we separated one tiller from each F_2 plant and two parents, and replanted them in plastic pods. We tried to choose those tillers in similar growth condition. In each plastic pod 8 tillers were planted, 6 of them from the F_2 individuals and the other two from the B5 and TN1, planted along a cycle. About a week later, when the replanted tillers were alive and grew well, the tillers were infested with 4th instar nymphs of the BPH at the density of 15 insects per tiller, 120 insects per pot. 24 h after the infestation, we recorded the proportion of BPH distribution among the F_2 individuals and the parents. Then we let the insects feed, mate, lay eggs and hatch freely. Until TN1, the susceptible parent, died, we evaluated the severity scores of each F_2 plant. In the summer of 1999, we conducted the repeated experiments to evaluate the BPH resistance of each F_3 families twice using the Standard Seedbox Screening Technique (SSST)[14].

(iii) RFLP analysis. Total DNAs of parents, B5 and TN1, 167 F_2 individuals were extracted, digested by the restriction enzymes and electrophoresed. The digested DNA fragments were transferred to nylon membrane and hybridized according to the method described by Zhang et al.[15]. Five restriction enzymes, including *Eco*RⅠ, *Eco*RⅤ, *Hin*dⅢ, *Bam*HⅠ, and *Dra*Ⅰ, were used for the survey of parental polymorphism.

Bulked segregant analysis was used to screen the RFLP markers linked to the BPH resistance in B5. According to the results of the F_2 individuals' BPH resistance evaluation, 19 F_2 individuals extremely resistant to BPH were selected and equal amount of DNA from these plants were mixed to form an extremely resistant bulk. Similarly the DNA from 17 extremely susceptible F_2 individuals were mixed to form an extremely susceptible bulk. The two bulks and the parents were screened for polymorphism with RFLP markers, which were selected from all 12 chromosomes at regular intervals based on the published rice genetic maps of RGP and Cornell University Group[16, 17]. RFLP markers were kindly provided by the Japanese Rice Genome Research Project and the Cornell University Group.

(iv) Data processing and analysis. The RFLP data of F_2 individuals were transferred to the symbols and the raw data file was formed. An RFLP linkage map was constructed by using the Mapmaker/Exp3.0 at a LOD score of 3.0[18]. QTL analysis was processed to localize the BPH resistance genes at the RFLP linkage map by using the Mapmaker/QTL 1.0 at a LOD threshold of 3.0[19].

2 Results

(ⅰ) BPH resistance evaluation. The BPH resistance of each F_2 plants could be evaluated by two groups of data: one is the proportion of BPH distribution among the F_2 individuals and the parents 24 h after infestation; the other is the severity scores evaluated at the time TN1 was completely killed by the BPH. The results indicated that the BPH distribution among the F_2 individuals and the parents had an obvious tendency. BPHs were mainly distributed at TN1, the susceptible parent, whose average proportion of BPH distribution was 30.4%. The average proportion of BPH distribution at B5, the resistant parent, was only 5.5%. The BPH distribution among F_2 individuals was unbalanced, ranging from 0.5% — 45.3%. When TN1 was completely killed by the BPH, the severity scores of the 167 F_2 individuals showed a continuous distribution, and the peak value of the distribution is at the severity score of 3 (fig. 1). Such distribution indicated that there are major dominant genes underlying the BPH resistance in B5.

The BPH resistance evaluation of F_3 families produced the similar results (fig. 1). The average severity scores of them ranged from 2.17 to 9.00. And the average severity scores of B5 and TN1 were 2.23 and 8.71 respectively. Most of the 144 F_3 families' average severity scores matched with those of the F_2 individuals. The correlation coefficient between the two group data was 0.485. Especially those F_2 individuals that were selected as the extremely resistant bulk or susceptible bulk also exhibited extreme resistance or susceptibility to the BPH at the evaluation of F_3 families, indicating that the extremely resistant and susceptible bulks we had chosen were reliable.

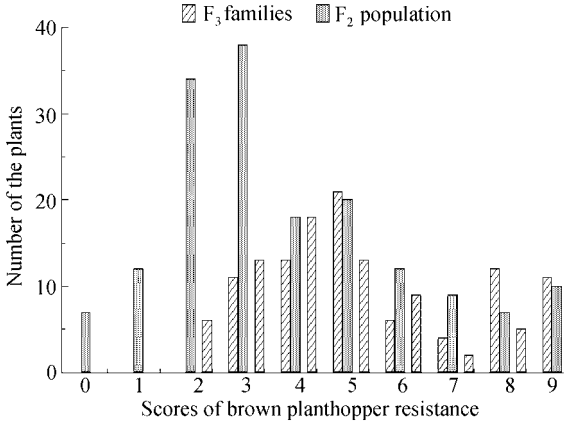

Fig. 1. Distribution of BPH resistance scores in F_2 population and F_3 families from cross TN1/B5.

(ⅱ) Screening the RFLP makers linked to BPH resistance. Bulked segregant analysis was used to screen the RFLP markers linked to the BPH resistance. Totally more than 300 DNA probes distributed among all 12 chromosomes of the rice genome were used for the RFLP analysis. Only the markers on chromosomes 3 and 4 showed co-segregation with two bulks (fig. 2(a)). These markers were from the narrow regions on the long arm of chromosome 3 and the short arm of chromosome 4, respectively. The results indicated the existence of two BPH resistance genes in B5, which were located on the chromosomes 3 and 4.

(iii) Constructing the partial region RFLP linkage map of chromosomes 3 and 4. We selected more markers to survey for polymorphism from the two regions of chromosomes 3 and 4, which contained the positive markers. Those markers that were polymorphic between the parents were used to assay the 167 TN1/B5 F_2 individuals (fig. 2(b)). Based on the RFLP data of F_2 individuals, the partial region RFLP linkage maps were constructed by using Mapmaker/Exp3.0 (fig. 3). On chromosome 3, 8 markers were mapped and covered 62.3 cM, with an average distance of 7.2 cM between adjacent markers. On chromosome 4, 11 markers were mapped and covered 77.5 cM, with an average distance of 7.75 cM between adjacent markers. The marker order in the map is coincide with those published before[17].

(iv) QTL analysis of the BPH resistance genes.

According to the data of the severity scores of each F_2 plant, two QTLs for BPH resistance were detected by using QTL analysis with Mapmaker/QTL1.1(table 1). One was located at a 1.2 cM interval between C820 and S11182 on the short arm of chromosome 4, with a LOD score of 11.76. This QTL can explain the 27.7% phenotypic variance of BPH resistance in the population, and showed dominant effect. The other was located at a 3.2 cM interval between G1318 and R1925 on the end of the long arm of chromosome 3, with a LOD score of 3.37. This QTL contributed to the 9.2% phenotypic variance of BPH resistance in the population.

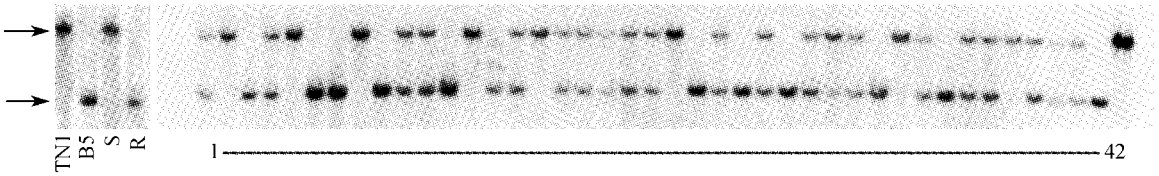

Fig. 2. Southern blotting of probe R288 on chromosome 4 to parents, bulks and portion of F_2 population. (a) Parents TN1, B5, the susceptible bulk (S) and the resistant bulk (R); (b) F_2 plants (lanes 1—42).

Table 1 Quantitative trait loci identified for BPH resistance in B5

Locus	Interval	Chrom.	LOD	Var (%)	Additive	Dominance
C820-S11182	1.2	4	11.76	27.7	−1.602	−0.9072
R1925-G1318	3.2	3	3.37	9.2	−0.64	−1.1296

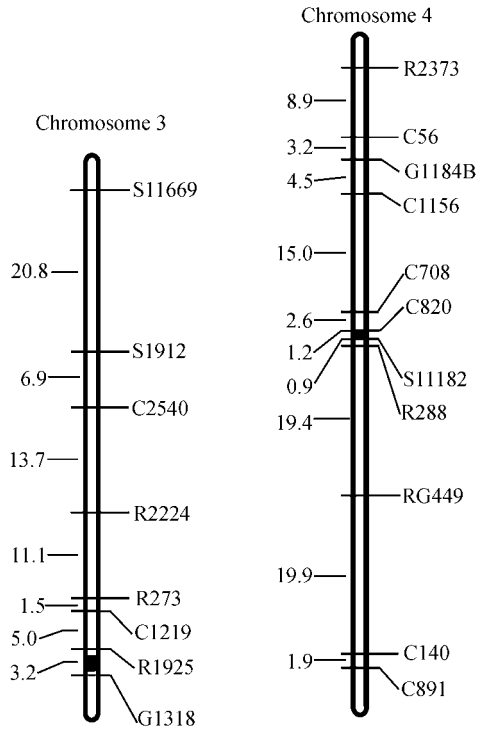

Fig. 3. The locations (solid bars) of two BPH resistance genes on chromosomes 3 and 4. Marker names are listed on the right hand side of the chromosome with the distances (in cM) indicated on the left.

We used another group data, the proportion of the BPH distribution among the F_2 individuals, for QTL analysis. Two main effect loci were scanned at the same chromosomal positions, which just had some differences in the LOD scores. One locus located at the interval between C820 and S11182 explained the 20.7% phenotypic variance of BPH resistance and had a LOD score of 8.38. The other one located at the interval between G1318 and R1925 explained the 9.1% phenotypic variance of BPH resistance and had a LOD score of 3.33.

3 Discussion

Genus *Oryza* has 20 wild species, which contain abundant disease and insect resistance resources for rice genetic improvement. Li et al.[20] reported that most of *O. officinalis* collected from Guangxi of China, were highly resistant to BPH. The resistance character of B5, the resistance parent used in our research, was introgressed from *O. officinalis*[11].

In the research, we constructed an F_2 random population derived from the cross between the introgression line B5 and TN1. By using co-segregant analysis between BPH resistance and RFLP markers and QTL analysis, we found two main effect BPH resistance loci in B5 and located them on the long arm of chromosome 3 and the short arm of chromosome 4 respectively. A number of BPH resistance genes have been reported previously. Three of them, *Bph1*, *bph2* and *Bph*(*10*) are located on chromosome 12; the other two, *Bph3* and *bph4*, are located on chromosome 10. Moreover, *Bph5*, *Bph6* and *bph7* are not resistant to BPH biotypes 1, 2 and 3. Until now, most of the BPH resistance genes that have been tagged with molecular markers are located on chromosome 12. The two BPH resistance genes identified in the present study are distinct from all the previously reported BPH resistance genes. Thus the two BPH resistance genes, derived from *O. officinalis*, are two new BPH resistance

genes.

The near isogenic lines analysis[21] and bulked segregant analysis[12] are often used for the linkage analysis between target genes and molecular markers. But it is time-consuming and laborious to obtain the near isogenic lines. To resolve this problem, Michelmore[12] conducted the bulked segregant analysis by using F_2 segregant population. In our research, this method was used to rapidly identify the RFLP markers linked to the BPH resistance in B5. The using of the bulked segregant analysis required to construct the extremely resistant and susceptible bulks first, and the reliability of the experiment's results is depended on the accuracy of the BPH resistance evaluation. We conducted the BPH resistance evaluation of F_2 individuals by using the modified TSST. We not only evaluated the severity scores of F_2 individuals, but also recorded the proportion of BPH distribution among the F_2 individuals. The results indicated that the two groups of data were positively relative, namely, the F_2 individuals with higher severity score had a higher proportion of BPH distribution. On the basis of the data, we respectively selected 19 and 17 most reliable highly resistant and susceptible F_2 individuals to form the extreme bulks. In order to prove the accuracy of the BPH resistance evaluation of F_2 individuals, we conducted the BPH resistance evaluation of F_3 families twice by using the SSST. The results were in accord with the BPH resistance evaluation of F_2 individuals. The repeated BPH resistance evaluation presented above guaranteed the accuracy of the BPH resistance evaluation, and assured the reliability of the BPH resistance gene loci in B5. Our lab even obtained similar results by using the Minghui63/B5 F_2 population to localize the BPH resistance genes in B5. Our research provided the new useful BPH resistant resources for rice breeding and made a good beginning for finally cloning the BPH resistance genes in B5.

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