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Genetic Analysis and Preliminary Mapping of Two Recessive Resistance Genes to Brown Planthopper, *Nilaparvata lugens* Stål in Rice

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Abstract: An F_2 population derived from the cross of WB01, an introgression line resistant to brown planthopper (BPH) originated from *Oryza rufipogon* Griff. and a susceptible indica variety 9311, was developed for genetic analysis and gene mapping. The population with 303 $F_{2:3}$ families was genotyped by 141 simple sequence repeat (SSR) markers and used for gene mapping. Two softwares, Mapmaker/Exp 3.0 and Windows QTL Cartographer V2.0 were applied to detect QTLs. Totally, two QTLs resistant to BPH, named temporarily as *bph22*(t) and *bph23*(t), were identified to locate on chromosomes 4 and 8, individually had LOD values of 2.92 and 3.15, and explained 11.3% and 14 .9% of the phenotypic variation, respectively.

Key words: brownplanthopper; *Oryza rufipogon*; resistance gene; simple sequence repeat marker; gene mapping

Brown planthopper (BPH, *Nilaparvata lugens* Stål) is one of the most prevalent insects damaging the plants of *Oryza* species (Chen et al, 2006). In recent years, BPH damage has increased dramatically in China. Based on the data from the National Bureau of Statistics (2007), there were 25.765 million hm^2 of rice damaged by BPH in 2006, with the yield loss of 1.88 million tons, of which, 0.2 million hm^2 of rice plants were damaged badly, especially in the provinces of Zhejiang, Jiangsu and Anhui, China. At all times, chemical spray was considered as a major way to control the pest. Actually, wide and continuous spray of pesticide has induced genotype variation of BPH to resist pesticide, and caused environmental pollution as well (Jiang et al, 2005; Wang and Wang, 2006). Therefore, the most effective and economic way to control BPH is the use of resistant varieties in rice production (Wu et al, 2005).

Since the 1970's, many countries have been exploring the possibilities of controlling BPH by various resistance genes. Nowadays, 24 BPH resistance genes (14 dominant genes and 10 recessive genes) have been identified and affirmed. Of them, at least 19 major resistance genes have been located on chromosomes (Liu et al, 2007; Huang and Gong, 2009). Besides, some important BPH resistance QTLs have been appraised (Huang et al, 1997; Alam and Cohen, 1998). It is noticed that there are 11 BPH resistance genes originated from wild rice species, e.g. *Bph10* (Ishii et al, 1994) and *Bph18*(t) (Jena et al, 2006) originated from *O. australiensis*, *Bph12*(t) (Yang et al, 2002) from *O. latifolia*, *Bph13* (Liu et al, 2001) from *O. eichingeri*, *bph12* (Hirabayashi and Ogawa, 1999), *Bph14* (Renganayaki et al, 2002), *Bph15* (Huang et al, 1997), *Bph16* (Yang et al, 2002) and *Bph17* (Sharama et al, 2003) from *O. officinalis*, and *Bph20*(t) and *Bph21*(t) (Rahman et al, 2009) from *O. minuta.* Therefore, it is considered that wild rice can be a valuable gene-pool with rich resistance genes to pest, which will greatly enlarge genetic background in rice improvement (Yan et al, 1997; Chen et al, 1998). Significant benefit could be created in developing rice varieties with high yielding and excellent resistance to pest, reducing spray of chemical pesticide and protecting environment if resistance genes derived from wild rice can be fully utilized (Cai, 1984; Ling et al, 1989; Chen and Li, 1993).

The present study was conducted for genetic analysis and location of rice BPH resistance genes, using the $F_{2:3}$ families from a cross of WB01 and 9311. The tested materials were identified for BPH resistance by the method of the Modified Seedling Screening Test (MSST) (Wu et al, 1984). Based on the evaluation of BPH resistance of $F_{2:3}$ families, the genotype of each F_2 plant could be inferred from the phenotype of corresponding $F_{2:3}$ lines. Combining with the molecular marker-linked gene mapping built by F_2 population, the genetic analysis and gene mapping for BPH resistance were processed. The aim of the study is to search SSR molecular markers linked gene(s) resistant to BPH and to evaluate the gene effect in an attempt to provide basic information for molecular marker-assistant breeding and gene clone in rice.

MATERIALS AND METHODS

Rice materials

The tested rice materials were WB01, an introgression line induced from wild rice *Oryza rufipogon* Griff. (resistant to

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BPH) crossed with a restorer line Minghui 63 (susceptible to BPH) (Minghui 63/*O. ruffipogon*//Minghui 63), and 9311, an indica rice variety (susceptible to BPH). The F_1 seeds (WB01/9311) and self-pollinated F_2 seeds were obtained in 2006 and 2007, respectively. Then, 303 F_2 plants selected randomly were planted and self-pollinated in Hainan Province, China in the spring of 2008 to produce $F_{2:3}$ families.

Identification for BPH resistance

The identification for BPH resistance was carried out for WB01, 9311 and their F_1 and $F_{2:3}$ plants by the MSST method (Wu et al, 1984) in the summer of 2008. The BPH insect used for identification was originated from the rice fields of Hangzhou City, Zhejiang Province, China with major BPH biotype-1 and biotype-2 (Liu et al, 2001). All seeds were sown separately to provide homogeneous growth of the tested seedlings. The tested seedlings were planted in controlled nurseries, and 10 varieties plus 4 check varieties (Mudgo, IR26 and ASD7 as resistant checks and TN1 as a susceptible one) in each block across a completely random design with two replications. Each line/variety had 15 seedlings in one row. At the 2-leaf stage, 8–10 young BPH larvae were inoculated on the leaves of each seedling. When the seedling death rate of susceptible TN1 reached 70%, the number of dead seedlings of each line (variety) was accounted daily until all the TN1 seedlings were dead (Chen et al, 2005). The BPH resistance was evaluated according to the standard issued by the China National Rice Research Institute (Liu et al, 2002).

SSR analysis

Total genomic DNA was extracted from fresh young seedling leaves, following the method of DNA mini-scale DNA extraction protocol as described by Zheng et al (1995). The PCR was performed in a 10 μL reaction mixture containing 1.0 μL of 10×PCR buffer, 1.0 μL of 2 mmol/L dNTPs, 1.0 μL of 25 mmol/L $MgCl_2$, 0.6 μL of each of the forward and reverse primers (10 μmol/L), 0.1 μL of 5 U/μL *Taq* polymerase, and 20 ng of template DNA, with a PTC-100 programmable thermal controller (MJ Research, Inc.) in the sequence of first denaturation at 94 ºC for 5 min, followed by 30 cycles of 94 ºC for 45 s, 50 ºC, 55 ºC, 61 ºC or 67 ºC for 45 s according to different primers and 1 min extension at 72 ºC. A final extension at 72 ºC for 8 min terminated the reaction. PCR products were separated on 6% non-denaturing polyacrylamide gels at constant voltage and the marker bands were revealed using silver staining (Panaud et al, 1996).

Construction of mixed DNA pool

Following the BSA method put forward by Michelmore et al (1991), 12 plants were selected from the F_2 population representing the best resistant and the most susceptible ones to BPH according to the evaluation on $F_{2:3}$ families to BPH response. DNA was extracted separately and mixed by equal amount for setting up BPH resistant and susceptible DNA pools, respectively, which were used for identifying molecular markers linked to the target gene(s).

Construction of linked gene mapping and QTL analysis

First, the polymorphism of parents was tested by 586 pairs of SSR primers covering all 12 rice chromosomes. Then, the genotypes of F_2 individual plants were identified by the polymorphic primers. Band data were recorded using the method of Mapmarker register (Lincoln et al, 1992).

Through Mapmaker/Exp 3.0 (Lander et al, 1987) and Windows QTL Cartographer 2.0, resistant QTL identification, gene mapping and genetic variation analysis were conducted for F_2 population. Taking 2.0 as an LOD critical value, if the LOD in marked region is higher than 2.0, the locus responding the highest LOD value could be considered as a QTL resistant to BPH. The limited gene-linked mapping was determined up by the Mapdraw Ver 2.2 software (Liu and Meng, 2003).

RESULTS

BPH resistance identification and genetic analysis

By the method of MSST, two parents, F_1 plants and $F_{2:3}$ families were evaluated for BPH resistance. The result indicated that the parent WB01 showed resistance to BPH (resistance scale was 3), the parent 9311 was susceptible (resistance scale was 9), and all F_1 plants were susceptible. The $F_{2:3}$ families segregated greatly, showing a continuous distribution (resistance scales ranged from 2.6 to 9.0) (Fig. 1). The χ^2 test showed that the segregation ratio of resistant plants (21) and susceptible plants (282) in the $F_{2:3}$ families fitted into 1:15, indicating that the BPH resistance was controlled by two pairs of recessive genes.

Screen for SSR markers linked to genes resistant to BPH

SSR markers linked to the genes resistant to BPH were

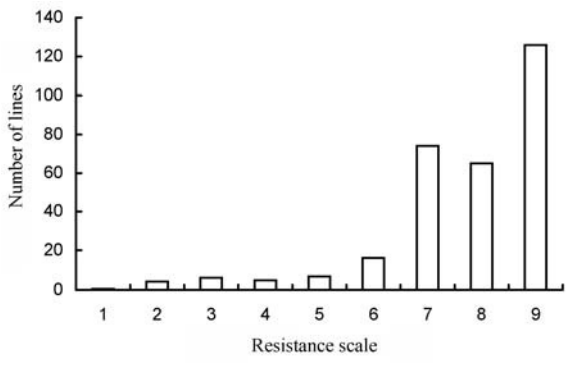

Fig. 1. Distribution of brown planthopper resistance scales of the 303 families of $F_{2:3}$ generation.

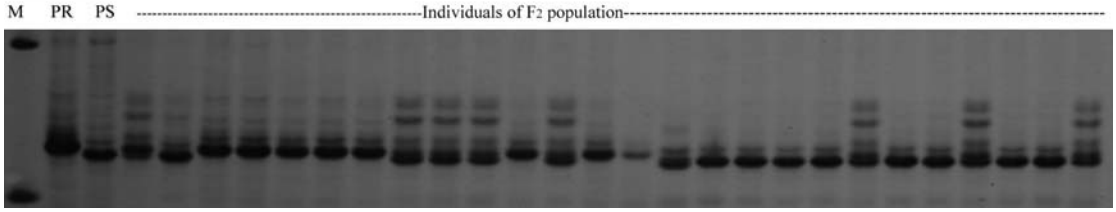

Fig. 2. Electrophoresis of PCR products of RM261 on chromosome 4 for parents and partial individuals of F_2 population.
M, Molecular weight marker; PR, Resistance parent (WB01); PS, Susceptible parent (9311).

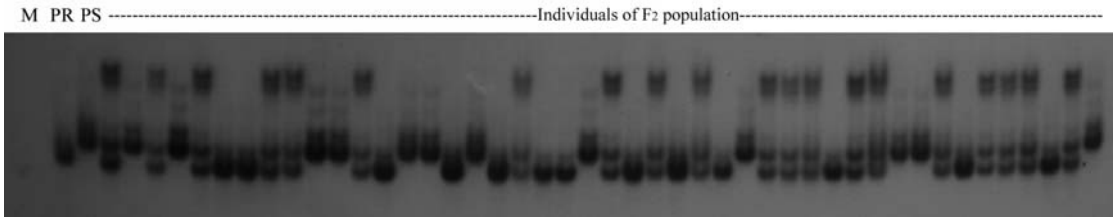

Fig. 3. Electrophoresis of PCR products of RM2655 on chromosome 8 for parents and partial individuals of F_2 population.
M, Molecular weight marker; PR, Resistance parent (WB01); PS, Susceptible parent (9311).

Table 1. Chromosomal locations of QTLs for brown planthopper resistance.

QTL	Marker interval	Chromosome	LOD value	Variance explained (%)	Additive effect
bph22(t)	RM8212–RM261	4	2.92	11.3	-1.02
bph23(t)	RM2655–RM3572	8	3.15	14.9	-1.46

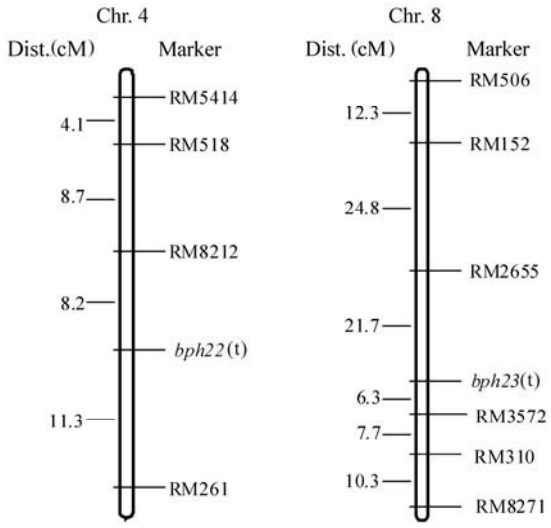

Fig. 4. Locations of two BPH-resistance QTLs *bph22*(t) and *bph23*(t) on rice chromosomes 4 and 8.

screened by the method of bulked segregant analysis. A total of 586 pairs of SSR primers distributed on all 12 rice chromosomes were selected for polymorphism evaluation of the parents WB01 and 9311. It was found that 141 pairs of the primers showed polymorphism between parents, occupying 24% of all the tested primers. Then, the 141 SSR primers were used for screening of resistant and susceptible gene pools, among which three pairs of the primers showing polymorphism between resistant and susceptible gene pools, e.g., RM261 and RM8212 on chromosome 4, and RM2655 on chromosome 8. The PCR amplification was conducted in F_2 population using these three typical primers plus some SSR primers with polymophorism. The electrophoresis of PCR products of RM261 and RM2655 on chromosomes 4 and 8 in the parents and parts of F_2 population is shown in Fig. 2 and Fig. 3, respectively.

The construction analysis by Windows QTL Cartographer 2.0 discovered a QTL locus resistant to BPH on chromosome 4 and another one on chromosome 8, which were located at the interval of RM8212–RM261 and RM2655–RM3572 with LOD values of 2.92 and 3.15, explaining 11.3% and 14.9% of the phenotypic variation, respectively (Table 1). The presumed view of the QTLs additive effect indicated that the gene effect increasing resistance to BPH originated from the parent WB01, thereafter, they were named temporarily as *bph22*(t) and *bph23*(t) (Fig. 4).

DISCUSSION

Wild rice, the ancestor of modern rice cultivars, is the natural gene pool of resistance genes to pest, which has accumulated rich genetic variation due to the experience of a long history of various environments and natural selection. It is generally considered that it would be 50 times more likely to find resistance gene(s) to pest from wild rice compared with cultivated rice varieties, and the resistance gene(s) from wild

rice could be a unique source for the control of pests (Heinrichs et al, 1985; Vaughan, 1994). Because of the scarcity of resistance gene(s) in cultivated rice, the exploration of resistance gene(s) from wild rice is particularly important. Therefore, the exploration, identification and genetic analysis of elite germplasm resistant to BPH in wild rice could be the basis of rice molecular breeding.

The present study indicated the BPH resistance in the introgression line WB01 being controlled by two pairs of genes, e.g. *bph22*(t) on chromosome 4 and *bph23*(t) on chromosome 8. On chromosome 4, there are only two reported recessive genes resistant to BPH, *bph12* (Hirabayashi and Ogawa, 1999) and *bph18*(t) (Li et al, 2006). As compared with *bph12* and *bph18*(t), we found that *bph22*(t) was physically distant from these two genes and they were not in the same region on chromosome 4. Wu et al (2005) reported a BPH resistance QTL on chromosome 8 using a doubled haploid population at the seedling stage, which was located between RM152 and RM310. We compared the genetic distance between *bph23*(t) and this QTL, and found that they were located at the same region (between RM152 and RM310) on chromosome 8. Is *bph23*(t) the same as this QTL? Therefore, it needs further validation.

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