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RESEARCH PAPER

Analysis of Quantitative Trait Loci for Resistance to Brown Planthopper in Dongxiang Wild Rice (*Oryza rufipogon* Griff.)

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Abstract: The wild species in genus *Oryza* are important resources of genes resistant to brown planthopper (*Nilaparvata lugens*, BPH). The objective of this study was to identify quantitative trait loci (QTLs) in Dongxiang wild rice (*O. rufipogon* Griff.). A BC_1F_5 population and a BC_3F_3 population were developed for QTL mapping from the cross between Dongxiang wild rice and an *indica* rice cultivar, Xieqingzao B (*O. sativa* L. subsp. *indica*) as the recurrent parent. The BC_1F_5 population was infested with BPH collected from paddy fields. Two QTLs were identified, of which *qBph2* was located in the interval of RM29–RG157 on chromosome 2 and *qBph7* in the interval of RM11–RM234 on chromosome 7. The alleles from Dongxiang wild rice on *qBph2* and *qBph7*, which explained 21.8% and 67.1% of the phenotypic variations, respectively, and reduced seedling mortality by 22.2% and 43.7%. These QTLs were validated using the BC_3F_3 population infested by BPH biotypes I, II, and III. This result shows that *qBph2* confers resistance to biotypes I and II, and *qBph7* confers resistance to biotypes I and III. Both QTLs show a great potential for improving BPH resistance in rice cultivars.

Keywords: Dongxiang wild rice; brown planthopper; biotype; quantitative trait loci

Brown planthopper (BPH), *Nilaparvata lugens*, is one of the most destructive pests to rice (*Oryza sativa* L.) in China and other Asian countries. BPH usually damages rice by sucking plant sap, leading to yield loss and even no harvest. In addition, BPH acts as a vector for rice grassy stunt virus and rags stunt virus. In recent years, BPH infests increasingly due to change of cultivation system, replacement and disposal of cultivars, and increase of rice yield [1, 2]. Resistant cultivar is considered as the most economical and efficient solution for BPH control, which depends on identification and utilization of resistance genes.

In the past 5 decades, 21 major genes for resistance to BPH have been identified from both cultivated and wild rice, i.e., 11 from cultivated rice, 5 from *O. officinalis*, 2 from *O. australiensis*, 2 from *O. minuta*, and 1 from *O. eichingeri* [3, 4]. Eighteen of them have been located on 7 chromosomes. Two BPH resistance genes, *Bph14* on chromosome 3 and *Bph18* on chromosome 12, have been cloned with map-based cloning method [5, 6]. Recently, increasing attention has been paid to searching new BPH resistance genes using quantitative trait locus (QTL) analysis, and the detected QTLs distribute on all of the 12 rice chromosomes [7–14].

Dongxiang wild rice (hereafter referred as Dwr) belongs to *O. rufipogon*, which is indigenous to Jiangxi Province of China, the northernmost for all wild rice species to be located up to date. This species has received much attention for carrying cold-tolerance genes [15, 16]. Besides, Dwr has been reported to carry drought-tolerance gene(s) and resistance gene(s) against whitebacked planthopper (*Sogatella furcifera*) [17, 18]. In this study, we mapped the resistance loci in Dwr using the population derived from the cross between Dwr and Xieqingzao B (hereafter referred as XB). The results will provide a basis for utilizing the BPH-resistance of wild rice in rice breeding.

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1 Materials and methods

1.1 Rice materials and populations

Two mapping populations, BC_1F_5 and BC_3F_3, were developed by crossing XB (female parent) and Dwr (male parent) and backcrossing to XB. The BC_3F_3 population was developed from BC_1F_5. The recurrent parent, XB, is an *indica* rice cultivar, also known as the maintainer line of Xieqingzao A, a cytoplasmic male-sterile line in dwarf-abortion type. Dwr, the resistant donor parent, is an accession from the *O. rufipogon* population in Dongxiang, Jiangxi Province, China.

The BC_1F_5 population consisting of 202 lines [19, 20] was used for primary mapping of QTLs, and the BC_3F_3 population with 195 lines was used to validate the effect of QTLs. In an earlier study, *qBph2* and *qBph7* from Dwr were identified. Six BC_1F_5 lines carrying introgression segments harboring *qBph2* and *qBph7* were selected to backcross with XB twice and selfed once. As a result, the BC_3F_2 population was produced. After genotyping with simple sequence repeat (SSR) markers closely linked to *qBph2* (RM29, RM262, RM106, RM6, and RM250) and *qBph7* (RM11 and RM234), 14 BC_3F_2 plants heterozygous in both introgression regions were selected for further selfing to yield the BC_3F_3 population. Further selection with agronomic traits and molecular markers for the target regions fixed 195 individuals (seeds) from 12 BC_3F_3 lines to construct a new BC_3F_3 population.

1.2 Evaluation of BPH resistance

BPH evaluation of the BC_1F_5 population was conducted in summer of 2004 at the China National Rice Research Institute (CNRRI), Hangzhou, Zhejiang Province, China. The BPH population is a mixture of biotypes I, II, and III with proportions of 30.6%, 31.5%, and 12.0%, respectively. The 202 BC_1F_5 lines, together with the recurrent parent, were grown in plastic trays with the dimension of 60 cm × 45 cm × 10 cm. Each line was planted in a single row with 20 cm in length. The spacing between rows was 3 cm. Twenty seeds were sown in a row, and 15 plants were retained at 2-leaf stage by removing weak seedlings. In each tray, 3 rows of Taichung Native 1 (TN1) were randomly planted as the susceptible control. The BPH at the second or third instar nymph stage were released to each tray at a density of 8–10 insects per seedling. Seedling mortality was investigated when TN1 plants exhibited seedling mortality of 90–100%.

For the BC_3F_3 population, BPH resistance was evaluated with biotypes I, II, and III separately in summer of 2007. The BPH insects were collected from the laboratory of CNRRI. All 195 lines of BC_3F_3 were infested with biotype I, while only 152 and 173 BC_3F_3 lines were infested with biotypes II and III, respectively, due to limitation of seeds. The procedure for evaluating BPH resistance in the BC_3F_3 was the same as that in the BC_1F_5 population. The evaluation of BC_3F_3 population had 2 replicates, and the higher mortality value was used in further analysis.

1.3 Marker genotyping

Genomic DNA was extracted from individuals of BC_3F_2 and 14 BC_3F_3 populations following the method described by Zheng et al. [21]. All of the DNA samples were assayed using SSR markers RM29, RM262, RM106, RM6, and RM250 on chromosome 2 and RM11 and RM234 on chromosome 7. The procedure DNA amplification, gel electrophoresis, and silver staining were referred to the method reported by Shi et al. [22].

1.4 Data analysis

We used MAPMAKER (EXP3.0b) [23] to build the linkage map. Marker distances in centiMorgan (cM) were derived from the Kosambi function. The linkage map was constructed with marker data from the BC_1F_5 population, which contained 149 markers spanning 1306.4 cM [19, 20]. Two linkage maps, RM29–RM250 on chromosome 2 and RM11–RM234 on chromosome 7 were constructed using SSR data from BC_3F_3 population.

Windows QTL Cartographer 2.5 [24] was used to detect QTLs and to estimate the effect of a single QTL. Under the composite interval mapping (CIM) option, a QTL was declared when the LOD value was larger than 3.0. In the multiple interval mapping (MIM) model, the positions of putative QTLs were modified under "Optimizing QTL" order in "Refine model" program, and the significance of each QTL was determined under the "Testing for exiting" order. Finally, "Summary" order was run to acquire the joint explanations of phenotypic variance, additive effects, and explanation of phenotypic variance for each QTL.

2 Results

2.1 Mapping of QTL for BPH resistance using BC_1F_5 population

The seedling mortalities in the BC_1F_5 population ranged from 0 to 100.0% with a skewed and continuous variation. The average seedling mortality was 85.6%. The recurrent parent, XB, was highly susceptible to BPH with the seedling mortality of 93.0%. Two QTLs, *qBph2* and *qBph7*, were allocated into the linkage map containing 149 DNA markers [19, 20]. The alleles from Dwr decreased seedling mortality and jointly explained 88.6% of the phenotypic variation (Table 1).

2.2 QTL validation using BC_3F_3 population

In the BC_3F_3 population, the ranges of seedling mortalities were 0–100.0% for biotype I, 63.2–100.0% for biotype II, and 25.0–100.0% for biotype III, which also showed skewed and continuous distributions. According to linear correlation analysis, the seedling mortalities caused by different biotypes were not significantly correlated.