遗传学报 *Acata Genetica Sinica*, March 2006, 33(3): 262–268 ISSN 0379-4172

SSR Mapping of Brown Planthopper Resistance Gene *Bph*9 in Kaharamana, an *Indica* Rice (*Oryza sativa* L.)

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Abstract: The brown planthopper (BPH) is one of the most serious insects pests of rice, and the host resistance has been recognized as one of the most economic and effective measures for BPH management. In this study, we conducted a molecular-based genetic analysis of *Bph*9 in Kaharamana, a Sri Lanka rice variety resistant to BPH insects of East and Southeast Asia. An F_2 segregating population composed of 180 plants was constructed from the cross between Kaharamana and 02428, and each F_2 plant was self-crossed to obtain $F_{2:3}$ family. The bulked seedling test method was used to evaluate the resistance of $F_{2:3}$ families, and the genotype of each F_2 plant was inferred from the phenotype of corresponding $F_{2:3}$ family. Linkage analysis indicated that the resistant gene *Bph*9 in Kaharamana was located between SSR markers RM463 and RM5341 on chromosome 12 with linkage distances of 6.8 cM and 9.7 cM, respectively. The time- and money-saving SSR markers would be helpful in the application of *Bph*9 in breeding program via marker-assisted selection.

Key words: SSR mapping; *Nilaparvata lugens* Stål; *Bph*9; rice (*Oryza sativa* L.)

The brown planthopper (BPH), *Nilaparvata lugens* Stål, is one of the most serious insect pests of rice (*Oryza sativa* L.) in Asian rice growing area. BPH causes direct damage by sucking plant sap, and transmitting several viral diseases such as rice grassy stunt [1] and rugged stunt [2].

Using of insecticides to control BPH insects is not only costly in terms of labor and money, but also causes environment damage. In addition, resurgence, a phenomenon of pest population increase after application of insecticides [3], has also been reported. To solve the problem, naturally evolved resistance systems would provide a promising and readily acceptable means of control.

Utilization of host resistance has been recognized as one of the most economic and effective measures for BPH management. Many donors of BPH resistance have been identified and genetics of resistance have been investigated. So far, 13 BPH resistance genes have been reported, of which *Bph*1, *Bph*2 and *Bph*10 (t) were mapped on chromosome 12 [4-6]. Four additional resistance genes *Bph*4, *Bph*11(t), *Bph*12 (t) and *Bph*13(t) were assigned to chromosome 6, 3, 4 and 2 [7-9]. Analysis of quantitative trait loci (QTL) contributing to BPH resistance in IR64, Kasalath, Teqing and wild rice *Oryza officinalis* has also been carried out [10-13].

Three Sri Lanka varieties, Kaharamana, Balamawee and Pokkali, were found to be resistant to BPH insects of biotype1, biotype2 and biotype3 but not biotype4 from Bangladesh, and the resistance of these three varieties was controlled by a dominant gene which was allelic to each other but was different from

Received: 2005-04-11; Accepted: 2005-08-02

This work was supported by the National High Technology Research and Development Program of China (863 Program, No. 2003AA222131, 2003AA207020), High Technology Project of Jiangsu Province (No. BG2004303), National Science and Technology Attack Project (No. 2004BA525B02-04).

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*Bph*1 and *Bph*3 [14]. Despite of the versatility of *Bph*9, the use of the gene in breeding program has not yet been reported. Nemoto *et al.*[15] carried out allelism tests between Kaharamana, Balamawee and Pokkali, and proved that a dominant gene, which was designated as *Bph*9, governed the resistance. RFLP and RAPD analysis of the resistant gene has been carried out in Pokkali, and this gene has been mapped between OPRO4 and S2545 on the long arm of chromosome 12 with a map distance of 8.8 cM from OPRO4 and 12.5 cM from S2545 [16]. But the molecular analysis of resistant gene in Kaharamana and Balamawee has not yet been reported.

To facilitate a marker-assisted selection in breeding BPH resistant variety, we carried out SSR tagging of *Bph*9 in Kaharamana. In the study reported here, we constructed an F_2 segregating population and conducted a molecular-based genetic analysis of *Bph*9 in Kaharamana. The objective was to determine the chromosomal location of *Bph*9 in Kaharamana and find SSR markers closely linked to *Bph*9 that might be useful for cloning this gene and for improving BPH resistance in rice breeding programs.

1 Materials and Methods

1.1 Plant materials and insects

The genetic materials were an F_2 population of 180 plants and F_1 plants from a cross between Kaharamana and 02428. The *japonica* 02428 is a BPH susceptible cultivated rice variety with wide compatibility. Each F_2 plant was selfed to obtain $F_{2:3}$ family. Rathu Heenati, carrying *Bph*3 [17] and Taichung Native 1 (TN1) with no resistance genes were used as resistant and susceptible controls, respectively.

The BPH population used for infestation was biotype 1, which was first collected from rice fields at Hangzhou, China, and then maintained on TN1 in the greenhouse for ten generations. In this experiment, insects were maintained on TN1 under natural condition in the greenhouse of Nanjing Agriculture University, Nanjing, China.

1.2 Evaluation of BPH resistance

A modified bulked seedling test was conducted to phenotype the reaction to BPH infestation on parents, F_1 plants and 180 $F_{2:3}$ families. To ensure all seedlings at the same growth stage for all seedlings for insect infestation, seeds were first germinated in petri dishes. After which about 70 germinated seeds of each entry were sown in two plastic pots of 10 cm-diameter with a hole in the button. Generally, 28 pots including one pot of parents and control varieties were placed in a 68 cm × 42 cm × 16 cm plastic seed-box. About 2cm deep water was kept in the seed-box until resistance evaluation was completed.

At the second-leaf stage, 15 d after the seeds were sown; the seedlings were infested with 2nd to 3rd-instar BPH nymphs at a density of six insects per seedling. When all seedlings of TN1 died, the plants of $F_{2:3}$ families were examined and each seedling was given a score of 0, 1, 3, 5, 7 or 9 according to Su *et al.* [11], which were based on IRRI [18] and Athwal *et al* [19]. The genotype of each F_2 plant was then determined by assaying the phenotype of corresponding $F_{2:3}$ families, which was inferred based on the weighted average of the seedlings tested.

1.3 DNA preparation and SSR assay

DNA samples were extracted from young leaves of each entry using the method described by Dellaporta *et al*[20]. The extracted DNA samples were then dissolved in TE buffer (10 mmol/L Tris-base, 0.1 mmol/L EDTA) and tested for quality and quantity using a MBA 2000 UV/VIS Spectrometer (Perkin Elemer Co.). The samples were then diluted into 20 ng/μL with autoclaved double distilled water (dd H_2O) for further analysis.

SSR analysis was performed following the procedure of Chen *et al.* [21] with minor modifications. The original sources and motifs for all the SSR markers used in this study could be found in the gramene database (http://www.gramene.org/) and McCouch *et al.* [22](or http://www.dna_res.kazusa.or.jp/9/6/05/spl_table1/table.pdf). Amplification reactions were carried out in 10 μL containing 10 mmol/L Tris-HCl pH 8.3, 50 mmol/L KCl,