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Mapping and Marker-assisted Selection of a Brown Planthopper Resistance Gene *bph2* in Rice (*Oryza sativa* L.)

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Abstract: *Nilaparvata lugens* Stål (brown planthopper, BPH), is one of the major insect pests of rice (*Oryza sativa* L.) in the temperate rice-growing region. In this study, ASD7 harboring a BPH resistance gene *bph2* was crossed to a susceptible cultivar C418, a *japonica* restorer line. BPH resistance was evaluated using 134 $F_{2:3}$ lines derived from the cross between "ASD7" and "C418". SSR assay and linkage analysis were carried out to detect *bph2*. As a result, the resistant gene *bph2* in ASD7 was successfully mapped between RM7102 and RM463 on the long arm of chromosome 12, with distances of 7.6 cM and 7.2 cM, respectively. Meanwhile, both phenotypic selection and marker-assisted selection (MAS) were conducted in the BC_1F_1 and BC_2F_1 populations. Selection efficiencies of RM7102 and RM463 were determined to be 89.9% and 91.2%, respectively. It would be very beneficial for BPH resistance improvement by using MAS of this gene.

Key words: *Oryza sativa* L.; *Nilaparvata Lugens* Stål; insect resistance; SSR; marker-assisted selection

Brown planthopper (BPH), *Nilaparvata lugens* Stål, is one of the most serious insect pests of rice (*Oryza sativa* L.) throughout the Asian rice-growing countries. The BPH has been causing serious yield reduction by directly sucking plant sap and also by transferring various diseases such as rice grassy stunt [1] and rugged stunt [2]. Pesticides are the common method of controlling an insect pest like BPH, but this method is costly and harms the environment. Resistant cultivars are the most economical and environmentally sound strategy for pest management. Resistant varieties are being developed by introgressing resistance genes and pyramiding resistance genes from different origins to increase the durability of resistance. Molecular markers closely linked to these genes could be used to follow the incorporation of these genes into new varieties.

An *indica* rice cultivar ASD7 was found to harbor *bph2* [3]; this study on the resistance segregation clearly demonstrated the recessive nature of *bph2*. Later, a line IR 1154–243 derived from a cross involving two susceptible cultivars, IR8 and Zenith, was also found to possess *bph2* [4]. Another study on segregation analysis of a recessive resistance gene *bph*2 in Tsukushibare/PL4 showed that it behaved as a major dominant gene [5]. Norin-PL4 has become an authentic *bph2*-introgression line derived from an IRRI *indica* line "IR1154-243" [6]. AFLP analysis of *bph2* has been mapped on the long arm of chromosome 12 within a 1.0 cM region delimited by map KAM3 and KAM5 [7].

Marker-assisted selection (MAS) should be ap-

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plied to BPH resistance breeding, since BPH must be maintained in the conventional way of evaluating BPH resistance, which is money and time consuming. Among the molecular markers, RFLP analysis needs DNA extraction with high quality, filter making and expensive ECL kit (or radioactive probe). Especially, 10 g of leaves need to be harvested for DNA extraction, so the selection cannot be conducted at early growth stage. Since the number of alleles per locus for RFLPs is limited, it is often difficult to find polymorphisms within a subspecies, and genotypes for breeding materials which can be applied to RFLP are limited. However, simple sequence repeats (SSR) markers can effectively solve these problems and new SSR markers have been isolated either from DNA libraries or else from sequences in data banks. SSRs have been commonly used as genetic markers based on sequence-specific PCR, which can be employed to conduct MAS.

In this study, we conducted a molecular-based genetic analysis of *bph2* in ASD7 to find SSR markers closely linked to *bph2*. The BC_1F_1 and BC_2F_1 populations derived from a cross between "ASD7" and "C418" were further constructed in order to map the major resistant gene on rice chromosome for further gene cloning and to benefit MAS in rice breeding.

1 Materials and Methods

1.1 Plant materials

1.1.1 Construction of population for primary mapping

To investigate the basis of BPH resistance in rice, an SSR linkage map was constructed with an F_2 population of 134 plants and F_1 plants from a cross between ASD7 and C418. The *japonica* restorer line C418 was a BPH susceptible cultivar. Each F_2 plant was self-crossed to obtain $F_{2:3}$ line.

1.1.2 Construction of populations for MAS

The BC_1F_1 population was constructed, which consisted of 89 plants derived from a cross between "ASD7" and "C418". These plants were selected with two SSR markers flanking *bph2* (RM7102 and RM463), and was further backcrossed to C418 to obtain the BC_2F_1. Each BC_1F_1 and BC_2F_1 plant was selfed to obtain their family lines, which were used for investigation of reaction to BPH feeding . Rathu Heenati with *Bph3* [8] and Taichung Native 1 with no resistance gene were used as resistant and susceptible controls, respectively.

1.2 Evaluation of BPH resistance

The BPH population used for infestation included biotypes 1 and 2, which was first collected from rice fields at Hangzhou, China, and has been maintained on TN1 in greenhouse for ten generations. In this experiment, insects were maintained on TN1 under natural condition in greenhouse of Nanjing Agricultural University, Nanjing, China.

The seedling bulk test with minor modifications was conducted to phenotype the reaction to BPH feeding. To ensure all seedlings at the same growth stage for insect infestation, seeds were first germinated in petri dishes. Then about 25 seeds from each F_2 individual were sown in a 10 cm-diameter plastic pot with a hole in the button. The distance between seedlings was about 2.5 cm. Generally, 28 pots including one pot of each parents and control varieties were placed in a 68 cm × 42 cm × 16 cm plastic seed-box. About 2 cm deep water was kept in the seed-box until resistance evaluation was finished. A total of four pots of ASD7, three pots of C418 and three pots of Taichung Native 1 were randomly put among the F_3 lines as controls. Seven days after sowing, seedlings were thinned to 20 plants per pot. At the third-leaf stage, the seedlings were infested with 2nd to 3rd-instarnymphs of BPH at ten insects per seeding. When all of the seedlings of Taichung Native 1 died, the plants of the $F_{2:3}$ lines were examined and each seedling was given a score of 0, 1, 3, 5, 7 or 9 according to the criteria in Table 1, which were based on Athwal *et al*.[3], IRRI [9] and Huang *et al* [10]. The genotype of each F_2 plant was then determined by assaying the phenotype of corresponding $F_{2:3}$ lines which was inferred based on the weighted average of the seedlings tested.

Table 1 The criteria for brown planthopper resistance-scoring used in this study

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

R: resistant; MR: moderately resistant; S: susceptible.

1.3 DNA preparation and SSR analysis

DNA samples were extracted from young leaves of each entry using the method described by Dellaporta *et al* [11]. The original sources and motifs of all the SSR markers used in this study could be found in the gramene database (http://www.gramene.org/) and McCouch *et al*.[12] (or http://www.dna_res.kazusa. or .jp/9/6/05/spl_table1/table1.pdf). PCR reaction was done as described by Chen *et al* [13]. The PCR productions were separated in 8% non-denaturing PAGE gel, and observed by silver staining method based on Sanguinetti *et al* [14]. The bands were then scored on a light box with fluorescent lamps.

1.4 Linkage analysis and SSR marker-assisted selection

Co-segregation of SSR markers with BPH resistance was analyzed by using MAPMAKER/EXP3.0 [15] software to determine the linkage relationships between them. The Kosambi function was transformed into Centimorgan (cM). The selection efficiency of MAS was calculated on BC_1 and BC_2 populations derived from ASD7/C418. Recombination frequency was calculated and transformed to genetic distance with Kosambi mapping function.

2 Results

2.1 BPH resistance evaluation

In bulk seedlings test, the resistance scores of ASD7 and C418 were 1.1 and 8.7, respectively, showing that ASD7 was resistant to BPH while C418 was susceptible. The F_1 plants with resistance score of 1.8 were resistant to BPH, indicating that the resistance was governed by a dominant gene (Table 2).

Table 2 The scores of BPH resistance in parental and F_1 at seedling stage

Varieties	Number of seedlings tested	Resistance score (0–9)
ASD7	40	1.1
C418	40	8.7
F_1	37	1.8
Rathu Heenati	40	0.1
TN1	40	9.0

Note: Rathu Heenati and TN1 were used as resistant and susceptible controls for BPH biotypes 1 and 2, respectively.

The BPH resistance scores of the 134 $F_{2:3}$ lines showed a continuous distribution, ranging from a low of 0.1 to a high of 9.00, with three apparent peaks around 1, 5 and 8 in the distribution curve (Fig.1). Genotype of each F_2 plant was inferred from the corresponding $F_{2:3}$ line. All the 134 $F_{2:3}$ lines were classified into three categories on the resistance scores as resistance, segregating or susceptibility. The corresponding F_2 plant was genotyped as *RR* (homozygous resistance), *Rr* (segregating heterozygous) and *rr* (homozygous susceptibility), accordingly. The segregation of F_2 population showed a good fit to the expected ratio of 1:2:1 (χ^2= 2.95, $\chi^2_{0.05}$ = 5.99) (Table 3).

Table 3 Segregation of BPH resistance in F_2 population derived from the cross ASD7/C418

F_2 genotype	Number of F_2 individuals	Phenotype of corresponding $F_{2:3}$ line
RR	35	RS＜2
Rr	74	2≤RS＜7
rr	25	RS≥7

RR: homozygous resistance; *Rr*: segregating heterozygous; *rr*: homozygous susceptibility. Number in this column was the range of resistance score. RS: resistance score.

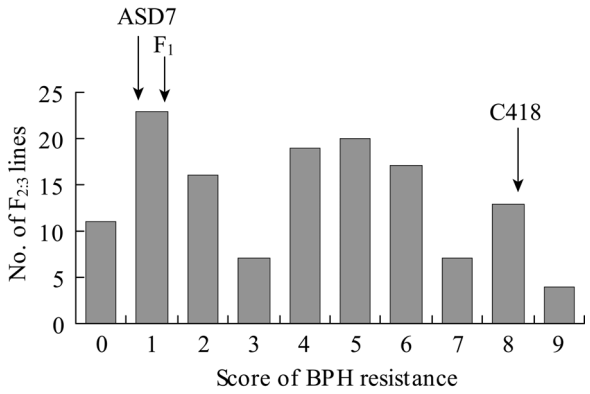

Fig. 1 Distribution of BPH resistance scores of the 134 $F_{2:3}$ lines

The scores of ASD7, F_1 and C418 were 1.1, 1.3 and 8.7, respectively.

2.2 Linkage analysis

According to the previous report on the chromosome position of *bph2* [5], a total of 43 SSR primer pairs on chromosome 12 were first used to survey polymorphisms between ASD7 and C418, of which 12 (28%) SSR primer pairs showed polymorphic. These SSR markers showing polymorphism were further used to analyze segregation in the mapping population and a linkage map was constructed using SSR markers on the long arm of chromosome 12. Finally, the resistant gene *bph2* in ASD7 was located between RM463 and RM7102 with a distance of 7.2 cM and 7.6 cM, respectively (Fig.2).

2.3 SSR marker-assisted selection

Analysis using RM463 and RM7102 as the markers was performed on the BC_1F_1 and BC_2F_1 populations derived from ASD7/C418. Based on the phenotype data of BPH resistance, the selection accuracy of the two markers was calculated. We investigated the selection

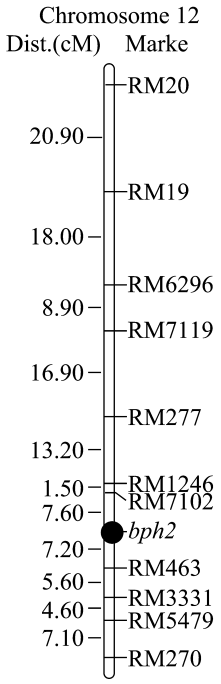

Fig. 2 Molecular mapping *bph2* on the long arm of chromosome 12

efficiency based on RM463 and RM7102 according to the results from the above genetic analysis and from phenotype analysis by BPH resistance (Table 4). The results showed that both RM463 and RM7102 had high selection accuracies of 91.2% and 89.9%, respectively, in the BC_2 generation derived from ASD7/C418, while in the BC_1 generation RM463 and RM7102 had the same selection accuracy of 95.1%. Thus RM463 and RM7102 could be applied to the MAS of the trait of BPH resistance in the progeny of *indica*/*japonica* varieties.

3 Discussion

There has been great progress in the development of MAS in recent years. But relatively few varieties or lines have been reported to be successfully developed

Table 4 Efficiency of RM463 and RM7102 assisted selection

Generation	Marker	A-Bb	A-bb	AaBb	aabb	Total	Recombination value	cM
BC_1	RM463	46	2	0	41	89	2.5%	2.5
	RM7102	46	2	0	41	89	2.5%	2.5
BC_2	RM463	71	5	2	76	154	4.5%	4.6
	RM7102	70	5	3	76	154	5.2%	5.2

A(a): indicating phenotype of plants analyzed; "A-": denoting resistance plants; "aa": susceptible plants; B(b): indicating genotype of SSR locus; "Bb": denoting one allele from ASD7, another from susceptible parent C418; "bb": two alleles from C418.

by this method. One of the problems lies in the segregation of markers and genes in the population and the final deviated selection. In this study, we conducted a molecular-based genetic analysis of *bph2* and found the SSR markers closely linked to *bph2*, which was then verified by the efficiency of MAS. The result indicated that both RM463 and RM7102 had high selection accuracies of 91.2% and 89.9%, respectively, in the BC_2 generation derived from ASD7/C418, while in the BC_1 generation RM463 and RM7102 had the same selection accuracy of 95.1%. Both the two markers showed high selection accuracies, and selection based on single marker could satisfactorily meet the needs of breeding. Thus RM463 and RM7102 could be applied to the MAS of the trait of BPH resistance in the process of breeding.

Another problem concerns the genetic nature of the resistance gene *bph2*, and its dominance or recessiveness. A recessive BPH resistance gene *bph2* was first identified in an *indica* rice cultivar ASD7 [3]. A line IR 1154-243 was found to possess *bph2* which represented as a major recessive resistance gene [4]. Norin-PL4 was an authentic *bph2*-introgression line derived from an IRRI *indica* line "IR1154-243" [6].The study of resistance segregation in a large number of F_2 and F_3 progenies from a cross between "Tsukushibare" and "PL4" clearly showed the dominant behavior of *bph2*[5]. In this study, we constructed an F_2 segregating population derived from the cross between "ASD7" and "C418" (a Japonica susceptible cultivar); the segregation analysis showed that it behaved as a major dominant gene. It is known that recessive resistance genes may behave as dominant genes under different genetic backgrounds and with different pathotypes (races) or biotypes. The Catalogue of Gene Symbols for wheat, for example, recommends the use of capital letters to designate all resistance genes irrespective of dominance or recessiveness [16]. A possible explanation might be that the dominance/recessiveness of *bph2* depends on the interaction between BPH biotypes with different virulence properties and different genetic backgrounds of host rice plants.

Previous studies reported that *bph2* is either allelic or closely linked to *Bph1*, because no recombinants were obtained in a fairly large number of progenies of crosses between *bph2* and *Bph1* carrier lines [3, 17, 18]. Trisomic analysis suggested that *bph2* and *Bph1* were linked to ebisu-dwarf gene, *d2*, on chromosome 4 with a recombination value of 39.4% [19]. RFLP assay and linkage analysis indicated that the resistant gene *Bph1* in Mudgo was located on chromosome 12 with a closest marker G148 being at 1.7 cM [20]. Resistance gene *bph2* in PL4 was mapped at 3.5 cM from the closest RFLP marker, G2140, on chromosome 12 [5]. The map position of *bph2* was 30 cM apart from that of *Bph1* previously determined on Nipponbare/Kasalath map. We reported here that *bph2* in ASD7 was mapped between two SSR markers RM463 and RM7102 on the long arm of chromosome 12 with a distance of 7.2 cM and 7.6 cM, respectively (Fig.2). From the newly published genetic linkage map that integrated RFLP and STS markers with SSR markers, the SSR marker RM1246 was located between G2140 and S2545 and only 0.6 cM to G2140. The other SSR marker RM463 was 1.0 cM to S2545 on the integrated map. We thus confirmed that *bph2* in ASD7 and PL4 were located in the same region of chromosome 12.

The threat of BPH to rice has resulted in a constant search for resistance genes and an accelerated effort to develop resistant varieties [21, 22]. However, the difficulty of resistance breeding was greatly exacerbated by the ability of BPH to develop new biotypes to "breakdown" resistant varieties and become virulent on novel plant genotype rapidly. So far, 17 major effective BPH resistance genes have been identified in *indica* cultivars and four wild relatives, *Oryza australiensis*, *O. eichingeri*, *O. latifolia* and *O. officinalis*. Of these genes, *Bph1*, *bph2*, *Bph*3, *bph4*, *Bph9*, *Bph10*, *Bph11*, *Bph12*, *Bph13*, *Bph14*, *Bph15*, *Bph16* and *Bph17* have been assigned to rice chromosomes [20,5,23-32]. Analysis of quantitative trait loci (QTL) contributed to BPH resistance in IR64, Kasalath, DV85, Teqing and wild rice *Oryza officinalis* has also been carried out [33-36,10]. Advanced lines with a good genetic background and resistance gene combinations have great potential breeding value. The time- and money-saving SSR markers flanking *bph2* described here may serve as useful tools for introducing and pyramiding *bph2* gene to cultivars.

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水稻抗褐飞虱基因 *bph2* 的 SSR 定位和标记辅助选择

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摘 要：利用综合性状较好对褐飞虱敏感的粳稻恢复系 C418 为父本，以含有 *bph2* 基因的抗褐飞虱品种 ASD7 为母本构建了包含 134 个 $F_{2:3}$ 家系的群体，利用苗期鉴定法对 $F_{2:3}$ 家系进行抗性鉴定；用 SSR 标记技术，将 *bph2* 基因定位在第 12 染色体长臂上，标记 RM7102 和 RM463 之间，其遗传距离分别为 7.6 cM 和 7.2 cM。在进行表型选择的同时，利用与 *bph2* 基因连锁的 SSR 标记 RM7102 和 RM463 对 BC_1F_1 和 BC_2F_1 进行了标记辅助选择，选择效率分别为 89.9%和 91.2%，为培育高抗褐飞虱水稻品种奠定了基础。

关键词：水稻；褐飞虱；抗虫性；SSR；分子标记辅助选择

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