Identification of quantitative trait loci associated with resistance to brown planthopper in the *indica* rice cultivar Col.5 Thailand

LIHONG SUN[1], YUQIANG LIU[1], LING JIANG[1], CHANGCHAO SU[1], CHUNMING WANG[1], HUQU ZHAI[2] and JIANMIN WAN[1,2]

[1]*National Key Laboratory for Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, P.R. China*
[2]*Chinese Academy of Agricultural Sciences, Beijing 100081, P.R. China*

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The brown planthopper (BPH) is one of the most serious insect pests of rice throughout Asia. In this study, we constructed a linkage map to determine the locus for BPH resistance gene, using an F_2 population from a cross between a resistant *indica* cultivar, 'Col.5 Thailand', and a susceptible cultivar '02428'. Insect resistance was evaluated using 147 F_3 families and the genotype of each F_2 plant was inferred from the phenotype of corresponding F_3 families. Two QTLs was detected on chromosome 2 (explains 29.4% phenotypic variation) and 6 (46.2% variation explained) associated with resistance to BPH in the mapping population. Comparison of the chromosomal locations and reactions to BPH biotypes indicated that the gene on chromosome 6 is different from at least 18 of the 19 previously identified BPH resistance genes. These two genes have large effects on BPH resistance and may be a useful BPH resistance resource for rice breeding programs.

Jianmin Wan, National Key Laboratory for Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, P.R. China. E-mail: wanjm@njau.edu.cn

The brown planthopper (abbreviated as BPH), *Nilaparvata lugens* Stål, is one of the most serious insect pests in Asia where rice is widely planted. It has been causing serious yield reduction by directly sucking plant sap and transferring various diseases such as rice grassy stunt (Rivera et al. 1966) and rugged stunt (Ling et al. 1978). The usual means for controlling the BPH pest by spraying poisonous chemicals is costly in terms of labor, money and environment. In addition, resurgence, a phenomenon of pest population increase after application of insecticides (Heinrich et al. 1982), has also been reported. The application of resistant cultivars has generally been considered to be the most economic and environmentally sound strategy for pest management.

The 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, 19 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, 16 — *Bph1*, *bph2*, *Bph3*, *bph4*, *Bph6*, *Bph9*, *Bph10*, *Bph11*, *Bph12*, *Bph13(t)*, *Bph13*, *Bph14*, *Bph15*, *Bph17*, *Bph18* and *Bph19* — have been assigned to rice chromosomes (Ikeda 1985; Ishii et al. 1994; Hirabayashi and Ogawa 1995, 1999; Takita 1996; Murata et al. 1998, 2001; Kawaguchi et al. 2001; Liu et al. 2001; Jena et al. 2002, 2005; Renganayaki et al. 2002; Yang et al. 2004; Sun et al. 2005; Chen et al. 2006). 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 (Alam and Cohen 1998; Huang et al. 2001; Su et al. 2002, 2005; Xu et al. 2002).

Akihama collected two unknown varieties from Thailand in 1969, and named Col.5 Thailand (hereafter Col.5 T) and Col.11 Thailand (hereafter Col.11 T). Ikeda (1985) has proved that the recessive genes found in Col.5 T, Col.11 T and Chin saba are non-allelic to the gene *bph4*, and the newly identified resistance genes show different biotype reactions from *Bph1* and *bph2*. Nemoto et al. (1989) reported that resistance of Col.5 T, Col.11 T and Chin saba was governed by a recessive gene that was designated *bph8* and the recessive gene *bph8* showed resistance to biotypes 1, 2 and 3. However, molecular mapping of resistance genes in Col.5 T had not been reported.

In the study reported in this paper, we constructed a linkage map with an F_2 population and conducted a molecular-based genetic analysis of BPH resistance gene in Col.5 T. The objectives were: (1) to determine the amounts and modes of the genetic effects of the resistance genes, (2) to find SSR (simple sequence repeats) markers closely linked to the resistance genes, in order to improve marker assisted selection (MAS)

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in rice breeding and to map the major resistant gene on rice chromosome for further gene cloning.

MATERIAL AND METHODS

Plant material and insects

The genetic materials were an F_2 population consisted of 147 plants and F_1 plants from a cross between Col.5 T and 02428. The Japonica 02428 was a BPH susceptible cultivar with wide compatibility. Each F_2 plant was selfed to obtain F_3 family, which were used to test reaction to BPH feeding. ASD7 with *bph2* (Athwal et al. 1971) and Taichung Native 1 with no resistance gene were used as resistant and susceptible controls, respectively.

The BPH population used for infestation was a mixture of biotype 1 and 2, which was firstly collected from rice fields at Hangzhou, China. According to the report of Hu (1990), the BPH populations in China consisted of BPH biotypes 1 and 2, with biotype 1 occurring at predominantly high frequencies. In this experiment, these insects were maintained on Taichung Native 1 under natural condition in the greenhouse of Nanjing Agricultural University since 2002.

Evaluation of BPH resistance

The seedling bulk test with modification was conducted to phenotype the reaction to BPH feeding. To ensure all seedlings were at the same growth stage for insect infestation, seeds were first germinated in petri dishes. Then about 25 F_3 seeds harvested from each F_2 individual were sown in 10 cm-diameter a plastic pot with a hole in the button. The distance between seedlings was 2.5 cm. Generally, twenty-eight pots including one pot of each parents and control varieties were placed in a $68 \times 42 \times 16$ cm plastic seed-box. About 2-cm deep water was kept in the seed-box until resistance evaluation finished. A total of four pots of Col.5 T, three pots of 02428 and three pots of Taichung Native 1 were randomly put among the F_3 families as controls. Seven days after sowing, seedlings were thinned to 20 plants per pot. At the third-leaf stage, the seedlings were infested with ten BPH (at 2nd to 3rd-instar nymphs) per seeding. When the seedlings of Taichung Native 1 died, the plants of the F_3 families were examined and each seedling was given a score of 0, 1, 3, 5, 7 or 9 according to the criteria shown in Table 1, which were based on Athwal et al. (1971), Irri (1988) and Huang et al. (2001). The resistance level of each F_2 plant was then inferred based on the weighted average of the seedlings in the corresponding F_3 families.

DNA preparation and SSR analysis

Total DNA from the susceptible parent 02428, resistant parent Col.5 T and each F_2 plant were extracted from young leaves using the method described by Dellaporta et al. (1983). The extracted DNA samples were then dissolved in TE buffer (10 mM Tris base, 0.1 mM 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^{-1} with autoclaved double distilled water (dd H_2O) for further analysis.

SSR analysis was performed following the procedure of Chen et al. (1997) 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. (2002) (or http://www.dna_res.kazusa.or.jp/9/6/05/spl_table1/table1.pdf). Amplification reactions were carried out in 10 μl containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 50 μM each of dNTPs, 0.2 μM of each primers, 0.5 U Taq polymerase (TaKaRa, Dalian) and 20 ng of DNA template. Reactions were performed using a PTC-200 thermal cycler (MJ Research Inc.) programmed as 94°C for 4 min, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C 1.5 min at 72°C with a final extension of 7 min at 72°C. Amplification productions were separated in 8% non-denaturing PAGE, and observed by silver staining method based on Sanguinetti et al. (1994). Then the bands were scored on a light box with fluorescent lamps.

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

Mapping QTL for BPH resistance

Linkage groups and the order of markers were determined using MAPMAKER/EXP 3.0 (LANDER et al. 1987). The Kosambi mapping function was used to transform the recombination frequency to genetic distances (cM). Interval QTL mapping was carried out using MAPMAKER/QTL 1.1 (LANDER et al. 1987) with a LOD threshold of 3.0 and a probability level of 0.01 for declaring the presence of putative QTLs.

RESULTS

BPH resistance evaluation

In bulk seedlings test, the resistance score of Col.5 T and 02428 were 1.4 and 8.2, respectively, which indicated that Col.5 T was resistant to BPH while 02428 was susceptible. The F_1 plants with resistance score of 5.8 were middle resistance to BPH insects (Table 2).

The resistance scores of the 147 F_3 families infested with a BPH population mixed with biotype 1 and 2 showed a continuous distribution, ranging from a low of 1. 00 to a high of 9.00, with three apparent peaks around 1, 5 and 8 in the distribution curve (Fig. 1). Such a distribution indicated the involvement of major genes controlling the segregation of BPH resistance in this population.

Rice linkage map construction

A total of 551 SSR primer pairs on all chromosomes were surveyed for polymorphisms between Col.5 T and 02428, of which 185 (33.6%) markers showed polymorphism. With the F_2 population containing 147 individuals, a framework of linkage map with 131 SSR markers was constructed to locate the gene controlling the BPH resistance. The map covered 1476.4 cM on all 12 chromosomes with an average interval of 11.27 cM. The two maps covered 116.4 cM of chromosome 2 and 162.5 cM of chromosome 6, respectively (Fig. 2). The orientations of the 120 markers out of 131 determined were consistent with those of the maps published by TEMNYKH et al. (2000) and MCCOUCH et al. (2002).

Table 2. *The scores of BPH resistance in parents, F_1 and control varieties.*

Variety [a]	Number of seedlings tested	Resistance score (0–9)
Col.5 Thailand	40	1.4
02428	40	8.2
F_1	32	5.8
ASD7	40	1.1
Taichung Native 1	40	9.0

[a] ASD7 and Taichung Native 1 were used as resistance and susceptible controls respectively

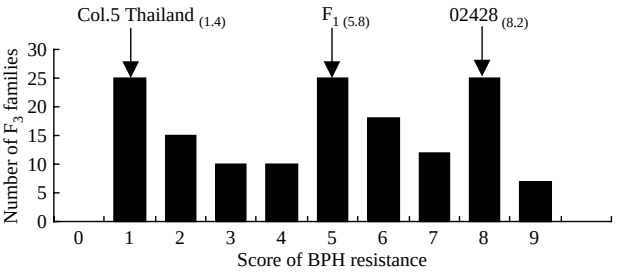

Fig. 1. Distribution of BPH resistance scores of the 147 F_3 families. The scores of Col.5 T, 02428 and F_1 were 1.4, 8.2 and 5.8, respectively.

Identification for the BPH resistance gene

QTL analysis using Mapmaker/QTL 1.1 detected two QTLs for BPH resistance (Table 3, Fig. 2). The first, designated *Qbph2*, detected with a LOD score of 3.40, was located between RM6843 and RM 3355 on the chromosome 2. This QTL explained 29.4% of the

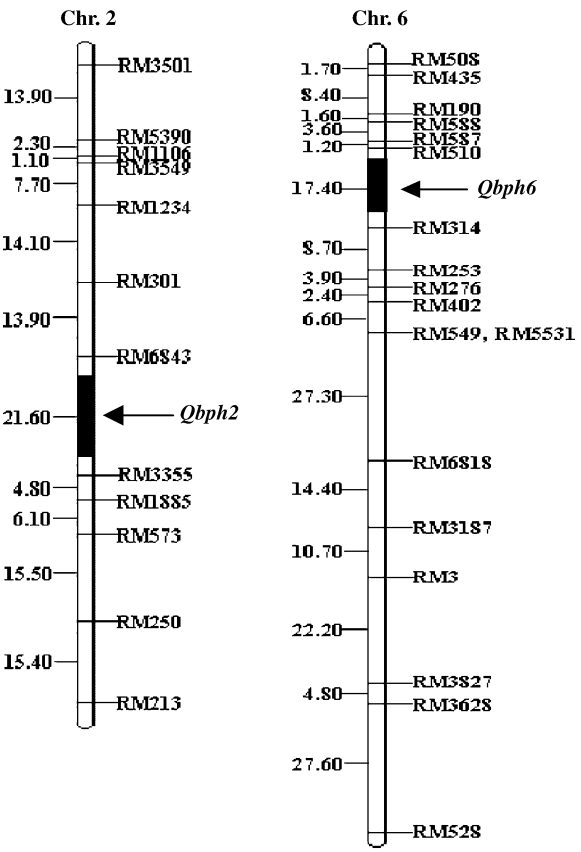

Fig. 2. Linkage map showing chromosomal locations of QTLs detected for resistance to BPH in Col.5 T/02428 F_2 population. Marker names are listed on the right hand side of the chromosome with the distances (in cM) indicated on the left. The solid bars indicates the locations of the two loci for BPH resistance, designated as *Qbph2* and *Qbph6*.

Table 3. *QTLs identified for BPH resistance using the F_2 population of Col.5 T/02428*

QTL	Interval	Chromo-some	LOD	Variance explained (%)	Additive	Dominance
Qbph2	RM6843–RM3355	2	3.40	29.4	−2.1446	−0.8649
Qbph6	RM510–RM314	6	18.301	46.2	−2.547	−0.181

phenotypic variance of BPH resistance in this population. The second QTL, *Qbph6*, was detected with a high LOD score of 18.30 between RM510 and RM 314 on the chromosome 6. This QTL accounted for 46.2% of the phenotypic variance of BPH resistance in this population. The two QTLs jointly explained 75.6% of the phenotypic variance of BPH resistance in this population.

DISCUSSION

The brown planthopper (BPH) is one of the most serious insect pests of rice. In China, BPH caused only occasional damage in the southern rice-growing areas before the 1960s, whereas widespread outbreaks occurred frequently in the 1990s in the rice-producing areas of southern and central China. BPH, as a sucking insect, cause several plant damages as lower plant vigor and height with a reduced number of fertile tiller and consequently lower filled grains. A heavy BPH infestation may be resulted in the crop complete drying and dead, in other word "hopperburn". The insect is also a vector of ragged stunt and grassy stunt viruses that seriously decreases rice production in the south Asia.

In this study, we found the identification of two loci for BPH resistance in *indica* rice Col.5 T using a F_2 mapping population derived from Col.5 T and 02428. Molecular marker-based-QTL analysis resolved these two QTLs to the long arm of chromosome 2 and the short arm of chromosome 6, respectively. The two QTLs jointly explained 75.6% of the phenotypic variance of BPH resistance in this population.

The QTL on chromosome 2 detected in this study, *Qbph2*, was resolved with a LOD score of 3.40 and explained 29.4% of the phenotypic variance of BPH resistance in this population derived from Col.5 T and 02428. Comparison of our results with those reported by Liu et al. (2001), revealed that *Qbph2* and *Bph13 (t)* in wild rice *Oryza eichingeri* were mapped on the long arm of chromosome 2. Whether *Qbph2* being allelic or closely linking to *Bph13 (t)* must be proved in the future.

It should be noted that one major QTL, *Qbph6*, was detected with a high LOD score of 18.30 and PVE (phenotypic variation explained) of 46.2%, which located at the region between two SSR markers RM510 and RM314 on the short arm of chromosome 6. The genetics of BPH resistance is well studied and 19 monogenically controlled resistance genes have been reported until now. Five of these genes, *Bph1*, *bph2*, *Bph9*, *Bph10* and *Bph18*, are located on chromosome 12; *Bph3*, *Bph12*, *Bph15* and *Bph17* on chromosome 4; *Bph11*, *Bph13*, *Bph14* and *Bph19* on chromosome 3; *Bph6* on chromosome 11; *bph4* on chromosome 6; *Bph13(t)* on chromosome 2. In addition, two of the BPH resistance genes, *bph5* and *Bph7*, were demonstrated not to confer resistance to BPH biotypes 1, 2 or 3. Ikeda (1985) proved that the resistance of Col.5 T was controlled by a new recessive gene different from *bph2* and *bph4*. Thus, the major QTL is distinct from at least eighteen of the nineteen previously characterized BPH resistance genes. According to our results, we deduced two possibilities to explain the BPH resistance genes in Col.5 T. First, the gene on chromosome 6 is allelic of a BPH resistance gene *bph8* in Col.5 T. Second, *bph8* in Col.5 T did not represent a single recessive gene but was associated with multiple loci affecting the resistance to the BPH.

BPH populations can quickly overcome single resistance gene under natural conditions. New resistance genes are always needed for rice improvement and breeding against BPH. Therefore, the resistance genes identified in this study could be a new resource of BPH resistance.

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