Theor Appl Genet
DOI 10.1007/s00122-009-1125-z

ORIGINAL PAPER

High-resolution mapping of two rice brown planthopper resistance genes, *Bph20(t)* and *Bph21(t)*, originating from *Oryza minuta*

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Received: 28 December 2008 / Accepted: 21 July 2009

Abstract Brown planthopper (BPH) is one of the most destructive insect pests of rice. Wild species of rice are a valuable source of resistance genes for developing resistant cultivars. A molecular marker-based genetic analysis of BPH resistance was conducted using an F_2 population derived from a cross between an introgression line, 'IR71033-121-15', from *Oryza minuta* (Accession number 101141) and a susceptible Korean *japonica* variety, 'Junambyeo'. Resistance to BPH (biotype 1) was evaluated using 190 F_3 families. Two major quantitative trait loci (QTLs) and two significant digenic epistatic interactions between marker intervals were identified for BPH resistance. One QTL was mapped to 193.4-kb region located on the short arm of chromosome 4, and the other QTL was mapped to a 194.0-kb region on the long arm of chromosome 12. The two QTLs additively increased the resistance to BPH. Markers co-segregating with the two resistance QTLs were developed at each locus. Comparing the physical map positions of the two QTLs with previously reported BPH resistance genes, we conclude that these major QTLs are new BPH resistance loci and have designated them as *Bph20(t)* on chromosome 4 and *Bph21(t)* on chromosome 12. This is the first report of BPH resistance genes from the wild species *O. minuta*. These two new genes and markers reported here will be useful to rice breeding programs interested in new sources of BPH resistance.

Communicated by T. Tai.

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Introduction

Brown planthopper (BPH), *Nilaparvata lugens* Stål (Homoptera: Delphacidae), is one of the most destructive insect pests of rice (*Oryza sativa* L.), especially of temperate *japonica* rice cultivars where few resistance genes against BPH have been incorporated (Jena et al. 2006). BPH extracts phloem saps of rice plants using its stylet-type mouthparts, resulting in a severe damage symptom known as 'hopper-burn'. BPH also transmits rice grassy stunt virus and ragged stunt virus as vectors (Rivera et al. 1966; Heinrichs 1979). On the basis of differential reactions, BPH populations in different countries have been categorized into four biotypes (Khush et al. 1985). The original populations in East and Southeast Asia belonged to biotype 1. Biotype 2 originated in Phillippines, Indonesia and Vietnam (Khush 1979) and is the dominant biotype in these countries. Biotype 3 was produced in the laboratory at

Published online: 11 August 2009

the International Rice Research Institute (IRRI) (Pathak and Khush 1979) and in Japan (Ikeda and Vaughan 1991). Biotype 4 is found only in South Asia (Khush 1984). In Southeast Asia, the BPH populations shifted from biotype 1 to biotype 2 in the 1970s (Feuer 1976; Mochida et al. 1977; Stapley et al. 1979), and at present comprise mostly a complex of biotypes 2 and 3 (Medrano and Heinrichs 1985; Sogawa et al. 1987; Huynh and Nhung 1988). On the other hand, Chelliah and Bharathi (1993) categorized BPH populations into five biotypes on the basis of their differential reactions to a set of reference cultivars. Of the biotypes (1, 2, and 3) known to be present in Korea, biotype 1 is the most prevalent (Park and Song 1988). Conventional methods of controlling BPH are highly dependent on spraying poisonous chemicals, which is expensive in terms of labor, money, and the environment. In addition, the occurrence of resurgence, a phenomenon of pest population increase after application of insecticides (Heinrichs et al. 1982), is problematic. Thus introduction of resistant cultivars is beneficial economically and environmentally (Huang et al. 2001).

Incorporating resistance gene(s) from wild species into cultivated species can be an alternative approach to develop BPH resistance in susceptible commercial cultivars. In general, wild species are poor in important agronomic traits such as yield, plant type, grain type, eating quality, seed shattering habit, etc. However, the wild species of *Oryza* are regarded as a treasure trove of novel genes for resistance to disease or insect pests and tolerance against environmental stresses. Since few useful genes from wild germplasm accessions have been explored, there is still great potential for exploring novel genes. Several wild species including *O. minuta, O. latifolia*, *O. nivara, O. officinalis* and *O. punctata* were reported to possess resistance genes to different BPH biotypes (Wu et al. 1986). Nine resistance loci among a total of 19 BPH resistance loci reported so far in rice have been identified from wild species. They are *Bph10* on the long arm of chromosome 12 from *O. australiensis* (Ishii et al. 1994), *Bph12(t)* on the short arm of chromosome 4 from *O. latifolia* (Yang et al. 2002), *Bph13(t)* on the long arm of chromosome 2 from *O. eichingeri* (Liu et al. 2001), another *Bph13(t)* against BPH biotype 4 on the short arm of chromosome 3 from *O. officinalis* (Renganayaki et al. 2002), *Qbp1* and *Qbp2* (later named as *Bph14* and *Bph15*, respectively) on the long arm of chromosome 3 and the short arm of chromosome 4, respectively, from *O. officinalis* (Huang et al. 2001), *Bph18(t)* on the long arm of chromosome 12 from *O. australiensis* (Jena et al. 2006), and *bph11(t)* and *bph12(t)* on the long arm of chromosome 3 and chromosome 4 respectively, from *O. officinalis* (Hirabayashi et al. 1999).

O. minuta ($2n = 48$, BBCC genome, Acc No.101141), an allotetraploid wild species, belonging to the *O. officinalis* complex, is endemic to Philippines and Papua New Guinea. It is known to have useful genes for resistance to bacterial blight (BLB), rice blast, and BPH (Brar and Khush 1997). However, few studies have been conducted to transfer the resistance genes from *O. minuta* to commercial rice cultivars. Amante et al. (1998) evaluated the resistance of advanced progenies introgressed from *O. minuta* for resistance against BLB and rice blast disease. The introgression line IR71033-121-15 derived from the cross of *O. sativa*/*O. minuta* showed resistance to BPH biotypes of Korea (Jena KK, unpublished). However, molecular mapping of these resistance gene(s) has not been reported.

The present study was conducted to identify the BPH resistance genes in IR71033-121-15 and to develop the markers for use in breeding BPH resistance in rice.

Materials and methods

Plant materials

The introgression line, IR71033-121-15, derived from an interspecific cross between IR31917-45-3-2 and a wild species *O. minuta* (Acc. No. 101141) showed strong resistance to the Korean BPH biotype 1. IR71033-121-15 and Junambyeo (a Korean *japonica* cultivar susceptible to BPH) were used as parents to develop an F_2 population of 190 plants for genetic analysis. The 190 F_3 lines harvested from each of the F_2 plants were bioassayed for BPH resistance. IR31917-45-3-2 (progenitor of IR71033-121-15) and two Tongil-type rice cultivars, Taebakbyeo and Andabyeo, were used as check cultivars in the bioassays. IR31917-45-3-2, Taebakbyeo, and Junambyeo are susceptible to Korean BPH whereas IR71033-121-15 and Andabyeo are resistant. Seeds of IR71033-121-15, IR31917-45-3-2, and *O. minuta* were obtained from the Plant Breeding and Genetics and Biotechnology Division of IRRI, Los Baños, Philippines. Seeds of Junambyeo, Taebakbyeo and Andabyeo were obtained from Rice Research Division of National Institute of Crop Science (NICS), Rural Development Administration (RDA), Korea.

Bioassay for BPH resistance

A pure BPH population developed from Korean BPH biotype 1 was used for bioassay of parents and F_2/F_3 population. Bioassays were conducted at the greenhouse facility of the Rice Research Division of NICS, RDA. The bioassay was performed with a modified bulk seedling test following the method of Pathak et al. (1969). Fifteen seedlings from each of 190 F_3 lines were planted in a row per line with three replications. Junambyeo and Taebakbyeo were used as susceptible (S) checks, and IR71033-121-15-2 and Andabyeo were used as resistant (R) checks. Seedlings at

the two and a half leaf stage were infested with second or third-instar nymphs at a density of 10–12 nymphs per seedling. The reaction against the BPH was scored following the guidelines of Standard Evaluation Systems for Rice (IRRI, 1988): 0, no damage; 1, very slight damage; 3, first and second leaves of most plants partially yellowing; 5, pronounced yellowing and stunting or about 10 to 25% of the plants wilting; 7, more than half of the plants wilting or dead and remaining plants severely stunted or dying; 9, all plants dead.

PCR amplification and marker detection

Plant DNA was extracted from the frozen leaves of rice plants using the CTAB method (Murray and Thompson 1980). For PCR amplification of markers, each 20 µL reaction mixture contained 50 ng DNA, 5 *p*mol of each primer, 10× PCR buffer [100 mM Tris (pH 8.3), 500 mM KCl, 15 mM $MgCl_2$, 2 µg gelatin], 250 µM of each dNTP and 0.5 U of *Taq* polymerase. PCR was performed in a PTC-100 Programmable Thermal Controller thermocycler (MJ Research Inc, Waltham, MA). The thermocycler profile was: 5 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 48°C (for STS) or 55°C (for SSR), and 2 min at 72°C, with a final extension of 5 min at 72°C. Amplified PCR products were resolved by electrophoresis in 3% agarose gels with ethidium bromide staining or 8% polyacrylamide denaturing gels with silver-staining for SSR markers (Panaud et al. 1996).

Construction of a framework map, detection of QTLs for BPH resistance and high-resolution mapping

Linkage analysis was conducted using MAPMAKER version 3.0 software (Lander et al. 1987). Map distances were estimated by the Kosambi function (Kosambi 1944). The linkage map in this study was basically the same as previously reported (Rahman et al. 2007). In this study, 46 more F_2 plants were added to construct a framework map. The M-QTL and E-QTLs between random marker intervals were determined using QTLMapper version 2.0 with the mixed linear model approach (Wang et al. 1999). After detecting QTL positions for BPH resistance based on LOD and R^2 values, fine mapping of the resistance loci was performed with SSR and STS markers. Graphical data of the recombinants between markers flanking the target regions were analyzed to clarify QTL positions, and introgressed segments from *O. minuta* were checked at the QTL regions for confirmation of the QTLs. SSR markers located within the two flanking markers of the resistance QTL regions were adopted from the public database released by the International Rice Microsatellite Initiative (IRMI, http://www.gramene.org/microsat), and PCR-based STS markers were developed according to the sequence information which is available at http://www.ncbi.nlm.nih.gov/ (for *indica*) and at http://www.rgp.dna.affrc.go.jp/ (for *japonica*) as described in Chin et al. (2007). For developing new SSR markers, the putative simple sequence repeat motifs were searched and identified using the Simple Sequence Repeat Identification Tool (SSRIT, http://www.gramene.org/db/searches/ssrtool) and for new STS markers, the putative sequences which were highly divergent between the two reference genomes were targeted. All PCR primers were designed using Primer3 version 4.0 (http://frodo.wi.mit.edu/). The markers used for fine mapping of the target regions are listed in Table 1.

Physical map construction

The physical map of the target QTLs was constructed by bioinformatics analysis using bacterial artificial chromosome (BAC) and P1-derived artificial chromosome (PAC) clones of cv. Nipponbare released by the International Rice Genome Sequencing project (IRGSP, http://rgp.dna.affrc.go.jp/IRGSP/index.html). The clones were anchored with the target gene-linked markers and then alignment of sequences was carried out using pairwise BLAST (http://www.ncbi.nlm.nih.gov/blast/bl2seq/b12.html).

Results

Inheritance of BPH resistance in "IR71033-121-15"

The introgression line IR71033-121-15 expressed strong resistance to the Korean BPH biotype 1, while the recurrent parent, Junambyeo, was completely susceptible to BPH (Fig. 1). The F_1 plants from the cross between Junambyeo and IR71033-121-15 showed partial resistance to BPH, indicating that BPH resistance of IR71033-121-15 was controlled by more than one dominant gene and the dominance was not complete. The resistance scores of the 190 F_3 lines infested with BPH ranged from 0.0 to 9.0 and showed a continuous variation with two peaks around 1.5 and 3.5 in the segregation curve (Fig. 1), demonstrating the involvement of major genes or QTLs controlling the segregation of resistance.

Linkage map construction and identification of QTLs for BPH resistance

A linkage map was constructed by genotyping 190 F_2 individuals using 143 markers. Using the QTLMapper version 2.0, two chromosomal loci for the resistance were identified on the short arm of chromosome 4 delimited by MS10 and RM5953 (3.5 cM distance) and the long arm of chromosome

Table 1 Summary of PCR-based markers developed for two specific QTLs

Marker[a]	Forward	Reverse	Expected amplicon size (bp)
C61009	5′-ggccagcaaggtgtagtaag	5′-acaaaccccagcaccctaag	205
MS5	5′-ttgtgggtcctcatctcctc-3′	5′-tgacaacttgtgcaagatcaaa-3′	215
MS10	5′-caatacgagaagcccctcac-3′	5′-ctgaaggaacacgcggtagt-3′	168
B40	5′-caataccggatatcttgactcc	5′-cgaccacgctgcctatattc	102
R288	5′-cctcatcgccagcaaga	5′-atagcagacttagcagcact	2,453
B41	5′-gctggtcttgaccaacgatt	5′-gaagttgccggagtcgag	154
B42	5′-ctgggctgcatacctagctt	5′-agggtgtgttcggtagatgg	117
B43	5′-actccaattggttcctgtgg-3′	5′-tggactaaaagccgatgagc-3′	764
S4019A	5′-ccaccgtttgatcattcatct-3′	5′-aacaaatttgagggcaaaaa-3′	231
S4019B	5′-ttctcggtttcttcgggtta-3′	5′-atctttggcttgctccacac-3′	198
B44	5′-tctcaaaccggctctaccag-3′	5′-ttactggtatggcaggagca-3′	207
S12091A	5′-tggggttaaatgttgcctct	5′-catatgtgggagcagactagca	167
S12091B	5′-ggctttcttcctcacactgc	5′-cgaggacgagatgagacga	204
S12094A	5′-tgcaatgctgtggcaataat	5′-tgcaatgctgtggcaataat	116
B120	5′-ttgaaaactacggggtgagg	5′-tacccgcaggatgagatac	1,496
S12094B	5′-tgcaacatggtaagcgattt-3′	5′-gcaaggtccttttcatggttt-3′	234
B121	5′-cgtcgtacattctgaaatggag-3′	5′-ggacatggagatggtggaga-3′	101
B122	5′-tcgtcaccaaacagcactaca-3′	5′-gtgacgactccccaattgtc-3′	214

[a] C61009, R288 (http://rgp.dna.affrc.go.jp/publicdata/caps/chr4.html); MS5, MS10 (Yang et al. 2004); B- and S- markers were developed in this study

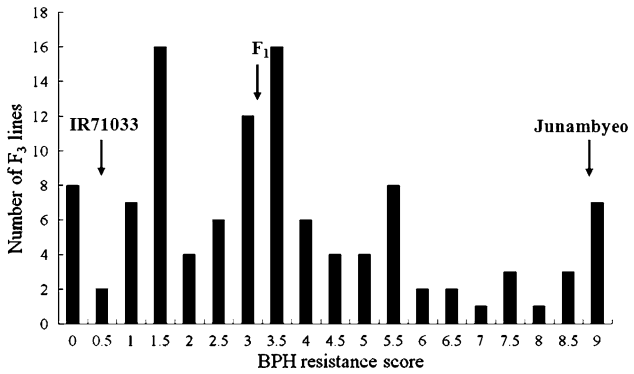


Fig. 1 Frequency distribution of the brown planthopper (BPH) resistance scores among F_3 families from the cross Junambyeo/IR71033-121-15

12, delimited by RM3726 and RM5479 (4.1 cM distance) (Table 2; Figs. 2, 3). At both loci, alleles from the resistant parent IR71033-121-15 significantly increased BPH resistance. These two QTLs were tentatively designated as *QTL-4* on chromosome 4 and *QTL-12* on chromosome 12. The R^2 values of *QTL-4* and *QTL-12* were fairly high representing 26.6% and 14.5% of phenotypic variation, respectively, and the summed R^2 of the two QTLs was 41.1% (Table 2). In addition, a total of two significant epistatic interactions between random marker intervals were identified which included a significant interaction between the two QTLs (Table 2). Total phenotypic variation explained by these QTLs and significant interactions was 47.2%. However, there was no dominance effect in the two major QTLs and other marker loci, explaining the intermediate resistance phenotype of F_1 plants shown in Fig. 1. The allele effect of each major QTL on BPH resistance was greater in *QTL-4* than in *QTL-12*.

Position of QTLs on the physical map

All the anchor markers used for fine mapping of the two resistance loci were landed on the reference sequences of cv. Nipponbare by bioinformatics analysis using a software tool BLASTN. The 1.31 Mb virtual contig map of chromosome 4 composed of 14 overlapping BAC/PAC clones was constructed to locate the physical regions for markers MS10 and RM5953 and thus delimit the *QTL-4* locus. Similarly, the 1.13 Mb virtual contig map of chromosome 12 composed of 13 overlapping BAC/PAC clones was constructed to locate the physical regions for markers RM3726 and RM5479 and delimit the *QTL-12* locus. Additional markers were developed using publicly available sequence databases aided by bioinformatics analysis to narrow down the physical distance between the two flanking markers (Table 1). Finally, two saturated genetic maps and subsequent contig maps were constructed for *QTL-4* on the short arm of chromosome 4 (Fig. 2) and *QTL-12* on the long arm of chromosome 12 (Fig. 3).

Table 2 Major QTLs and significant digenic epistatic interactions identified for BPH resistance in $F_{2,3}$ from a cross between Junambyeo and IR71033-121-15

QTL	Chromosome	Marker interval	LOD	Add.(a)	Dom.(d)	a/d	h^2 (%)
Major QTLs							
QTL4	4	MS10-RM5953	16.69	1.94*[a]	−0.14 ns	−0.07	26.6
QTL12	12	RM3726-RM5479	11.28	1.09*	−0.32 ns	−0.29	14.5
Ch-i[b]	Marker interval	Ch-j[b]	Marker interval	LOD	aaij[c]	adij	daij	ddij	h^2 (%)
Digenic epistatic interactions									
4	MS10-RM5953	12	RM3726-RM5479	17.23	0.56*	−0.14 ns	0.17 ns	−0.33 ns	2.6
8	S80036-S80016	12	S120712-RM3726	9.12	0.71*	−0.36 ns	0.27 ns	−0.21 ns	3.5

h^2 is the percentages of the phenotypic variations explained by the QTL

[a] * indicate $p < 0.05$; ns = non significant

[b] Ch-i and Ch-j represent the chromosome number to which the marker interval belongs

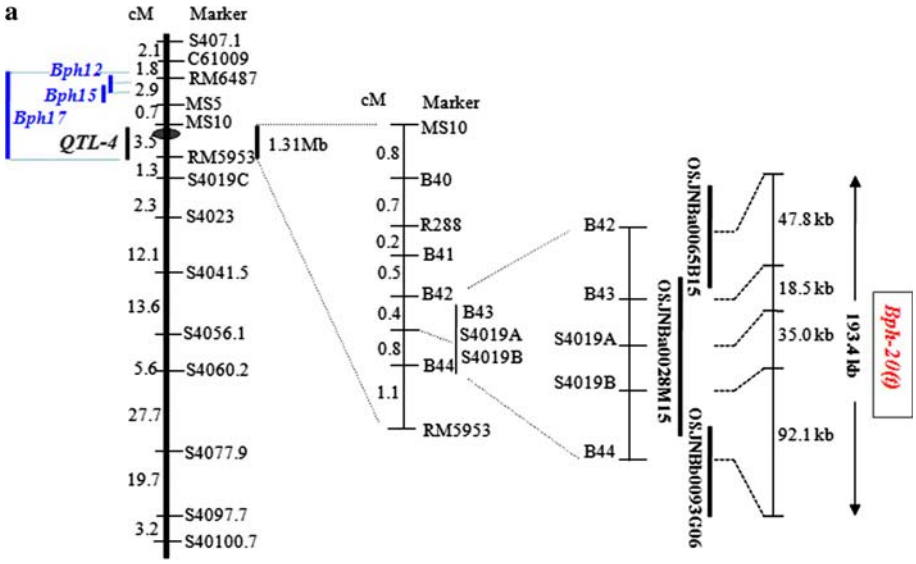
[c] aaij, adij, daij, and ddij are the effects of additive by additive interaction, additive by dominant interaction, dominant by additive interaction, and dominant by dominant interaction between the marker interval i and j

For *QTL-4*, the markers B43, S4019A, and S4019B derived from OSJBa0028M15, flanked by B42 and B44, co-segregated with the BPH resistance phenotypes. Association of marker genotypes with phenotypes (BPH resistance) of F_3 lines that contained the crossover event on the *QTL-4* region was analyzed (Fig. 2b). Based on comparisons of the genotypes at both *QTL-4* and *QTL-12* together with the phenotypes, the lines 73 and 91 delimited the left margin of the *QTL-4* position, and similarly, the lines 76 and 115 delimited the right margin of the *QTL-4* position. From these results, it was concluded that *QTL-4* resides between B42 and B44, which spans 1.2 cM and corresponds to a 193.4-kb region in the reference physical map (http://www.rgp.dna.affrc.go.jp/) (Fig. 2a).

For *QTL-12*, the markers B120, S12094B and B121 derived from OJ1310_CO3, flanked by S12094A and B122, co-segregated with BPH resistance. Using the same procedure as for *QTL-4*, the association of marker genotypes with BPH resistance of F_3 lines containing the crossover event in the *QTL-12* region was analyzed (Fig. 3b). The lines 13 and 28 delimited the left margin of the *QTL-12* position, and the lines 43 and 99 delimited the right margin of the *QTL-12* position. Therefore, it was concluded that *QTL-12* resides between 12094A and B122, which spans 1.0 cM and corresponds to a 194.0 kb region in the reference physical map (Fig. 3a).

Evidence of *O. minuta* chromosome segment integration in two putative QTLs

To confirm that the two chromosomal regions containing resistance QTLs were introgressed from the wild species *O. minuta,* the alleles of markers B43, S4019A, S4019B, and B44 on chromosome 4, and B120, S12094B, B121, and B122 on chromosome 12 were compared between IR71033-121-15 and *O. minuta*. In the *QTL-4* region, the same PCR amplicon (≥200 bp) was produced for markers S4019A and S4019B in both *O. minuta* and IR71033, which was different from the amplicon generated in the recurrent parent IR31917-45-3-2 and Junambyeo (Fig. 4a). However, B43 at the upper position and B44 at the lower position were negative for introgression. Similarly, analysis of markers in the *QTL-12* region revealed that the same PCR amplicons were produced for markers S12094B and B121 in IR71033-121-15 and *O. minuta,* which were different from the amplicons generated in the recurrent parent IR31917-45-3-2 and Junambyeo (Fig. 4b). However, B120 at the upper position and B122 at the lower position were not positive for introgression. These results indicate that the two resistance QTLs correspond to the introgression of *O. minuta* DNA into chromosome 4 (S4019A to S4019B) and 12 (S12094B to B121) of IR31917-45-3-2.



b

Line	Phen.	Chr. 4													Chr. 12
		MS5	MS10	B40	R288	B41	B42	B43	S4019A	S4019B	B44	RM5953	S4019C	S4023	B121
IR71033	0.0	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Junam	9.0	J	J	J	J	J	J	J	J	J	J	J	J	J	J
4	5.3	J	J	J	H	H	H	H	H	H	H	H	H	H	H
10	8.1	J	J	J	J	J	J	J	J	J	J	J	H	H	H
12	7.0	H	H	H	H	H	H	H	H	H	H	I	I	I	J
14	4.2	H	H	H	H	H	H	H	H	H	H	I	I	I	H
54	6.1	H	J	J	J	J	J	J	J	J	J	J	J	J	H
66	1.2	H	H	I	I	I	I	I	I	I	I	I	I	I	H
73	6.0	I	I	I	I	I	I	H	H	H	H	H	H	H	H
76	6.2	H	H	H	H	H	H	H	H	H	J	J	J	J	H
88	3.0	H	H	H	H	H	H	H	H	H	H	H	J	J	I
91	8.1	H	H	H	H	H	H	J	J	J	J	J	J	J	J
115	2.3	I	I	I	I	I	I	I	I	I	H	H	H	H	H
121	3.2	J	J	J	J	H	H	H	H	H	H	H	H	H	I
128	5.4	J	J	H	H	H	H	H	H	H	H	H	H	H	H
134	0.0	H	H	H	H	H	I	I	I	I	I	I	I	I	I
145	0.0	I	I	I	I	I	I	I	I	I	I	I	H	H	I
151	4.9	H	H	H	J	J	J	J	J	J	J	J	J	J	I
167	4.6	I	I	I	I	I	H	H	H	H	H	H	H	H	H

Fig. 2 **a** Genetic and physical map of the *Bph20(t)* locus on the short arm of chromosome 4. Positions of QTLs (*blue vertical bars*) previously reported near the *Bph20(t)* locus were estimated based on the sequence analysis of markers flanking the QTLs using Gramene DB (http://www.gramene.org/): *Bph12* from *O. latifolia* (Yang et al. 2002); *Bph15* from *O. officinalis* (Huang et al. 2001; Yang et al. 2004); *Bph17* from Rathu Heenati (Sun et al. 2005). **b** Phenotype (BPH resistance) and graphical genotype of *selected F_3 lines* showing crossovers near by the *Bph20(t)* locus. *I* IR71033 allele, *J* Junambyeo allele, *H* heterozygous

Discussion

Nineteen major BPH resistance loci have been reported in *indica* cultivars along with four wild species, *O. australiensis*, *O. eichingeri*, *O. latifolia*, and *O. officinalis*. Of these, 17 resistance loci (*Bph1, bph2, Bph3, bph4, Bph6, Bph9, Bph10, bph11, bph12(t), 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; Hirabayashi et al. 1999; Murata et al. 1998, 2001; Kawaguchi et al. 2001; Liu et al. 2001; Jena et al. 2002, 2006; Renganayaki et al. 2002; Sharma et al. 2003, 2004; Yang et al. 2004; Kim and Sohn 2005; Sun et al. 2005; Chen et al. 2006; Jairin et al. 2007). QTL studies involving the BPH-resistant cultivars IR64, Kasalath, DV85, Teqing, Col.5, and B5 introgression line created through the introgression of wild rice *O. officinalis* have also been carried out (Alam and Cohen 1998; Huang et al. 2001; Su et al. 2002, 2005; Xu et al. 2002; Ren et al. 2004; Soundararajan et al. 2004). Candidate genes for BPH resistance have been reported for the *indica* cv. *Samgangbyeo* (Park et al. 2007).

The main goal of this study was to identify new BPH resistance loci originating from the wild species, *O. minuta,* which has the BBCC genomes. Our analysis resulted in the identification of two major BPH resistance QTLs on chromosomes 4 and 12. These QTLs together explained over 40% of the observed phenotypic variance. Studies involving BPH resistance introgressed from other wild species have also resulted in the identification of loci mapping to chromosome 4. Previously, Huang et al. (2001) reported that two QTLs for BPH resistance, introgressed from *O. officinalis* (CC), were located on the short arm of chromosome 4 and long arm of chromosome 3. Later, Yang et al. (2004) identified one BPH resistance locus on chromosome 4 (*Bph15*) using the same introgressed line from *O. officinalis*. Yang et al. (2002) also reported that a BPH-resistant gene, *Bph12(t)* from *O. latifolia* (CCDD), was located on the short arm of chromosome 4. Of

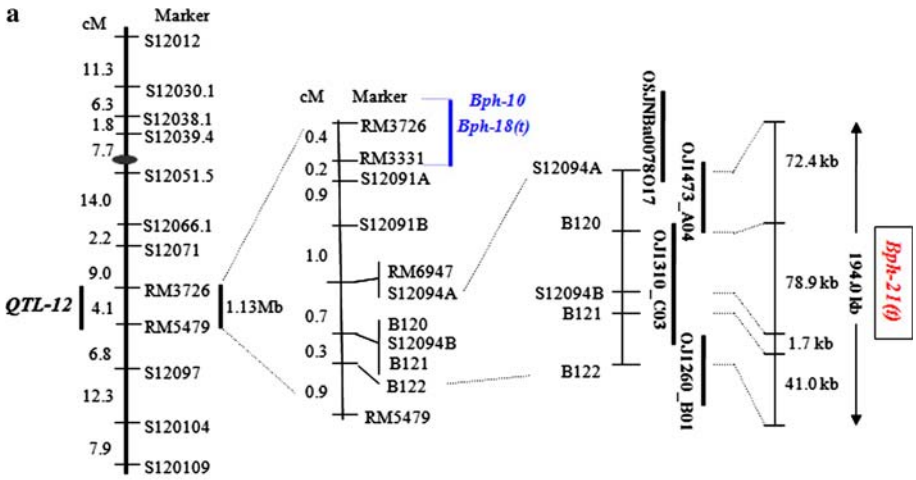


Fig. 3 **a** Genetic and physical map of the *Bph21(t)* locus on the long arm of chromosome 12. Positions of QTLs (*blue vertical bars*) previously reported near the *Bph21(t)* locus were estimated based on the sequence analysis of markers flanking the QTLs using Gramene DB: *Bph10* from *O. australiensis* (Ishii et al. 1994), *Bph18(t)* from *O. australiensis* (Jena et al. 2006). **b** Phenotype (BPH resistance) and graphical genotype of selected F_3 lines showing crossovers near by the *Bph21(t)* locus. *I* IR71033 allele, *J* Junambyeo allele, *H* heterozygous

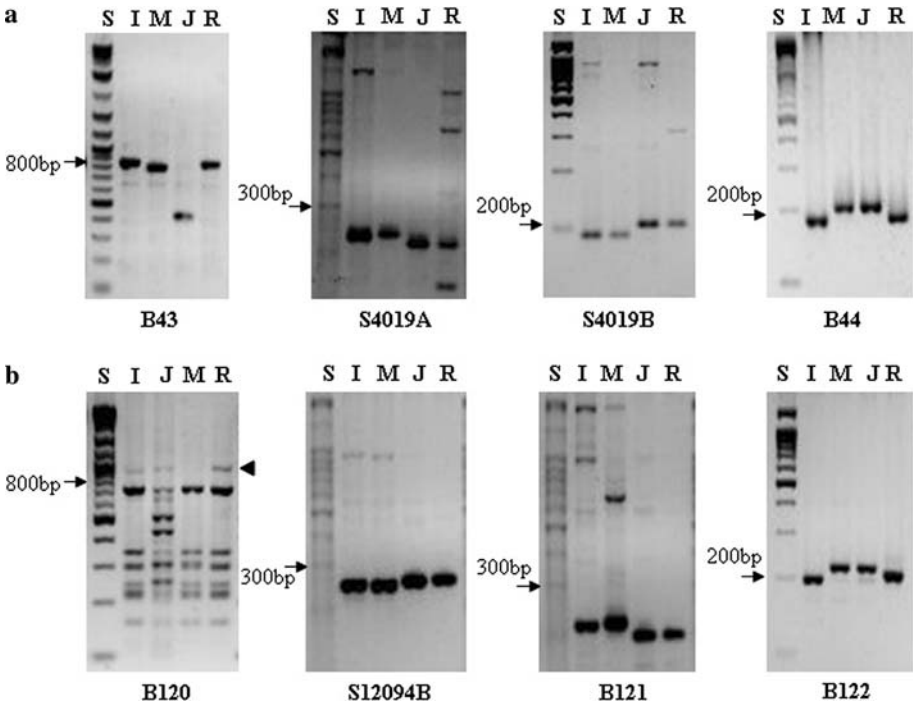


Fig. 4 Introgression test of *O. minuta* segments into IR71033-121-15 **a** at the *QTL4* locus on chromosome 4, and **b** at the *QTL-12* locus on chromosome 12. *S* Size marker, *I* IR71033-121-15, *M O. minuta* (accession # 101141), *J* Junambyeo, *R* IR31917-45-3-2. An *arrow* indicates the B120 marker

the *Bph* genes that have been mapped to chromosome 4, *Bph17* from the Sri Lankan cultivar Rathu Heenati is the closest to *QTL-4* described in our study (Fig. 2a). *Bph17* is reportedly located between two SSR markers RM8213 and RM5953 with map distances of 3.6 cM and 3.2 cM, respectively (Sun et al. 2005). Part of this region covers the *QTL-4* locus; however, the fact that *QTL-4* originates from the wild species *O. minuta* suggests *Bph17* is a different gene. Further studies such as the cloning of these genes will clarify their relationship.

For the long arm of chromosome 12, six resistance loci have been reported: *Bph1* from 'Mudgo', 'MTU15',

'Co22', 'MGL2', 'Samgangbyeo' (Kim and Sohn 2005) and 'Gayabyeo' (Jeon et al. 1999); *bph2* from 'Karsamba Red, ASD7' (Murata et al. 1998); *Bph9* from 'Pokkali' (Murata et al. 2001) and Kaharamana (Su et al. 2006); *Bph10* (Ishii et al. 1994),and *Bph18(t)* (Jena et al. 2006) from *O. australiensis*. Of these loci, *Bph10* and *Bph18(t)* are located nearby *QTL-12*. However, as with *QTL-4*, comparative analysis of marker loci revealed that these resistance genes actually map apart from *QTL-12* (Fig. 3a). Given that the two major QTLs in this study differ in chromosomal location and/or origin (*O. minuta*) from BPH resistance loci reported previously, we have designated these loci as *Bph20(t)* and *Bph21(t)* on chromosome 4 and 12, respectively. This is the first report on BPH resistance loci introduced from a BBCC genome wild species.

It is notable that BPH resistance genes are clustered in the same regions of chromosome 4 and 12 despite their different origins. Similar findings were also observed in the case of BPH resistance loci *Bph13(t)* (Renganayaki et al. 2002) and the recessive *bph19(t)* clustered on the short arm of chromosome 3 (Chen et al. 2006), and *Bph10* (Ishii et al. 1994) with *Bph18(t)* (Jena et al. 2006) on the long arm of chromosome 12.

Besides the two major QTLs, significant epistatic interactions between two random markers were found to play a certain role in the expression of resistance to BPH (Table 2). This suggests that epistatic interactions as well as major QTLs should be taken into consideration for breeding BPH-resistant cultivars through MAS, although this might complicate the MAS process (Qiao et al. 2008). The existence of an additive effect for the two major QTLs reported here indicates that lines with stronger BPH resistance can be developed by incorporating multiple QTLs. We are now developing resistant *japonica* lines by backcrossing the resistant progenies with the recurrent parent Junambyeo using MAS for *Bph20(t)* and *Bph21(t)*.

Of the five biotypes reported (Chelliah and Bharathi 1993), resistance genes have been identified against four of them (Panda and Khush 1995). *Bph1* confers resistance against biotypes 1 and 3, *bph2* is closely linked with *Bph1* and provides resistance against biotypes 1 and 2, *Bph3* and *bph4*, which are closely linked, confer resistance against four biotypes, and *Bph5*, *Bph6*, and *Bph7* provide resistance against only biotype 4. The relationship between other resistance genes and corresponding BPH biotypes has not been comprehensively documented. Considering the potential changes of BPH biotypes, due to the extensive cultivation of high yielding rice cultivars and different environmental factors, new resistance genes need to be identified to ensure the durability of host resistance. Wild species of rice are important genetic resources for breeding insect resistant cultivars. The parental line, IR71033-121-15, used in this study carries two new resistance genes, *Bph20(t)* and *Bph21(t)*, introgressed from *O. minuta*, and is expected to be a valuable source of BPH resistance.

Acknowledgments This research was supported by a grant (code# CG3111) from the Crop Functional Genomics Center of the 21st Century Frontier Research Program funded by the Ministry of Science and Technology, Republic of Korea. We are also thankful to Rural Development Administration, Korea for providing necessary greenhouse facilities for BPH bioassay.

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