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Genetic analysis and fine mapping of a rice brown planthopper (*Nilaparvata lugens* Stål) resistance gene *bph19*(t)

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Abstract Genetic analysis and fine mapping of a resistance gene against brown planthopper (BPH) biotype 2 in rice was performed using two F_2 populations derived from two crosses between a resistant *indica* cultivar (cv.), AS20-1, and two susceptible *japonica* cvs., Aichi Asahi and Lijiangxintuanheigu. Insect resistance was evaluated using F_1 plants and the two F_2 populations. The results showed that a single recessive gene, tentatively designated as *bph19*(t), conditioned the resistance in AS20-1. A linkage analysis, mainly employing microsatellite markers, was carried out in the two F_2 populations through bulked segregant analysis and recessive class analysis (RCA), in combination with bioinformatics analysis (BIA). The resistance gene locus *bph19*(t) was finely mapped to a region of about 1.0 cM on the short arm of chromosome 3, flanked by markers RM6308 and RM3134, where one known marker RM1022, and four new markers, b1, b2, b3 and b4, developed in the present study were co-segregating with the locus. To physically map this locus, the *bph19*(t)-linked markers were landed on bacterial artificial chromosome or P1 artificial chromosome clones of the reference cv., Nipponbare, released by the International Rice Genome Sequencing Project. Sequence information of these clones was used to construct a physical map of the *bph19*(t) locus, *in silico*, by BIA. The *bph19*(t) locus was physically defined to an interval of about 60 kb. The detailed genetic and physical maps of the *bph19*(t) locus will facilitate marker-assisted gene pyramiding and cloning.

Keywords Rice brown planthopper (BPH) · Resistance gene · Genetic map · Physical map · Sequence information

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Introduction

The brown planthopper (*Nilaparvata lugens* Stål, BPH), with characteristics of migration, paroxysm and rampancy, is one of the most destructive insect pests in the rice-producing areas, especially for shift to short-stature and heavy-tilling rice cultivars (cvs.) (Dyck and Thomas 1979). The severity of damage and the frequency of outbreak have increased since 1960s, because of the use of greater quantities of nitrogenous fertilizers and insecticides (Pathak 1972; Sogawa 1982, Sogawa et al. 2003; Holt et al. 1996). In China, this insect has caused yield losses of over 500,000 tons each year (Zhu et al. 2004b). Using resistant cvs, which is friendly to the environment than chemical pesticides, has proven to be one of the most efficient ways to control this pest (Pataki 1969; Sogawa 1982).

It is well known that the native *indica* cvs. expresses a much higher level of resistance than that of another subspecies *japonica* cvs. Up to now, about nine major BPH resistance genes were identified in the diversified *indica* cvs. Athwal et al. (1971) identified the first dominant gene *Bph1* and the first recessive gene *bph2* in cvs. Mudgo and Karsamba Red ASD7, respectively, through genetic segregation analysis. Later, Hirabayashi and Ogawa (1995) identified the *Bph1* in cv. IR28 on the long arm of chromosome 12 through restriction fragment-length polymorphism (RFLP) analysis. As for the *bph2* gene, Murata et al. (1998) and Murai et al. (2001) also assigned *bph2* on the long arm of chromosome 12 through RFLP and amplified fragment-length polymorphism analyses.

Lakshminarayana and Khush (1977) identified the second dominant gene *Bph3* and the second recessive gene *bph4* in cvs. Rathu Heenati and Babawee, respectively, through genetic segregation analysis. Later, Huang (2003) mapped the *Bph3* gene to the long arm of chromosome 4 through simple sequence repeat (SSR) and position-specific microsatellite (PSM) analysis. Kawaguchi et al. (2001) mapped the *bph4* gene on the short arm of chromosome 6 through linkage analysis with SSR and RFLP markers. Khush et al. (1985), and Kabir and Khush (1988) reported that three new resistant genes, *bph5*, *Bph6* and *bph7*, were found in rice cvs. ARC10550, Swarnalata and T12, respectively, through genetic segregation analysis. Nemoto et al. (1989) reported that three cvs., Col. 5 Thailand, Col. 11 Thailand and Chin saba, carry the same resistant gene *bph8*(t), and *Bph9*(t) in other three cvs., Balamawee, Kaharamana and Pokkali, through allelism tests. Murata et al. (2001) later identified *Bph9* on the long arm of chromosome 12, through RFLP and random-amplified polymorphic DNA (RAPD) analyses.

The rice wild species have been considered to be the most important resistant resources other than the native *indica* cvs for the BPH. Up to date, about nine BPH resistance genes have been identified in the various wild species. Ishii et al. (1994) first identified a resistance gene, *Bph10*, in an introgression line IR65482-4-136-2-2 derived from the wild species *Oryza australiensis*, on the long arm of chromosome 12, through RFLP analysis. Yang et al. (2002) identified the resistance gene *Bph12*(t) on the short arm of chromosome 4 in cv. B14, which is derived from *O. latifolia*, through SSR and RFLP analysis. Liu et al. (2001) identified a major dominant gene, *Bph13*(t) on the long arm of chromosome 2 in an *O. eichingeri*-derived line, acc105159, through RFLP and SSR analyses. Renganayaki et al. (2002) identified a resistance gene, which was also designated as *Bph13*(t), in IR54745-2-21-12-17-6, a line with *O. officinalis*-derived resistance to BPH biotype 4, on the short arm of chromosome 3, through RAPD analysis. Huang et al. (2001) reported that B5, a highly resistant line that derived its resistant genes from *O. officinalis*, carries two major resistance genes, *Qbp1* and *Qbp2* (later named *Bph14* and *Bph15*, respectively) on the long arm of chromosome 3 and the short arm of chromosome 4, respectively, through linkage analysis and quantitative trait loci (QTL) analysis with RFLP markers. Jena et al. (2005) mapped the resistance gene *Bph18*(t) on the subterminal region of the long arm of chromosome 12 in an *O. australiensis*-derived line, IR65482-7-216-1-2, through SSR and sequence-tagged site (STS) analysis, in combination with statistical analysis. Hirabayashi et al. (1998) also found two recessive genes, *bph11*(t) and *bph12*(t) on the long arm of chromosome 3 and the middle arm of chromosome 4, respectively, in introgression lines of *O. officinalis*, through RFLP analysis.

Consequently, a total of 18 BPH resistance genes have been identified and about half of these resistant genes were derived from the wild species. It is noteworthy that nine resistance genes assigned *Bph1* to *Bph9* were identified by classical genetic approach in the 1970s and 1980s, of which *Bph1*, *bph2*, *Bph3*, *bph4* and *Bph9* were further identified by molecular genetic approach in the 1990s and 2000s. However, the other nine resistance genes assigned *Bp10* to *Bph18* were identified by molecular genetic approach, only, from the 1990s. This is partially due to the fact that allelism test is a tedious task, especially in the case that the donor cvs. of resistance genes cannot be exchanged, freely, and that the number of known resistance genes are strikingly increased. On the other hand, the target gene may be more reliable to differentiate from other BPH gene(s) by comparing the relative map positions determined.

On the other hand, genetic mapping studies of these 18 resistance genes have led to rice and the BPH becoming ideal model systems for the study of interactions between plants and sucking herbivorous insects (Yang et al. 2004). However, the only example of a cloned resistance gene to a pest is the *Mi* gene, and none of the BPH resistance genes have been cloned in crop plants (Vos et al. 1998). Therefore, cloning BPH resistance genes and elucidating its resistance mechanism in rice become very necessary. As we know, map-based cloning is an effective method to get the target genes. However, this way is often tedious and time-consuming, particularly in the process of construction and screening of artificial chromosome library, such as bacterial artificial chromosome (BAC) or P1 artificial chromosome (PAC, Peters et al. 2003; Chen et al. 2005; Liu et al. 2005). Fortunately, the availability and utilization of the sequence information for the rice whole-genome of two subspecies, i.e. the *japonica* reference cv. Nipponbare released by the International Rice Genome Sequencing Project (IRGSP) (http://rgp.dna.affrc.go.jp) and the *indica* reference cv. 93-11 released by Beijing Genomics Institute (BGI) (http://www.genomics.org.cn), have made map-based cloning in rice much more efficient. That is, bioinformatics analysis (BIA) of the reference sequences using various software tools has enabled map-based cloning as a routine work in the laboratories related. Doubtlessly, sequence-based map of the target gene is a crucial step for both marker-assisted gene pyramiding and cloning (Schuler 1998; Pan et al. 2003; Gu et al. 2004; Yang et al. 2004).

In the present study, we analyzed the inheritance pattern of a recessive resistance gene against BPH in an *indica* cv. AS20-1, which is tentatively named *bph19*(t), and finely mapped to a 60-kb interval, on rice chromosome 3, using PCR-based markers including SSR, STS and CAPS (cleaved amplified polymorphic sequence) markers.

Materials and methods

Plant materials

An *indica* rice (*Oryza sativa* L.) cv. AS20-1, which is resistant to the BPH biotype 2, and two susceptible *japonica* cvs., Aichi Asahi and Lijiangxintuanheigu

(LTH), were used as the parental cvs. in this study. Two F_2 populations, F_2-1 and F_2-2, derived from crosses AS20-1/LTH and AS20-1/Aichi Asahi, which consist of 1,330 and 1,403 plants, respectively, were used as a mapping population. For determining the inheritance pattern of the BPH resistance in cv. AS20-1, 13 F_1 plants derived from the cross of AS20-1/LTH were also tested. To confirm the success of infestation and objectively evaluate the progeny plants, the parental cvs. and the *indica* cv. Taichung Native 1 (TN1), which is highly susceptible to all biotypes of the BPH, were used as resistant and susceptible controls.

Insects

The BPH colony used for the research was biotype 2, which was identified by using a set of international testers (Li et al. 1999), and kindly provided by Dr. Huang FK at Guangxi Academy of Agricultural Science, China. The biotype 2 was recognized as a predominant biotype in most of the rice fields in China since 1990s (Wang et al. 1999). The insects were fed on TN1 for about 5 weeks to produce a sufficiently large population, and second-to-third-instar nymphs were selected for infestation. The rest were maintained with alternative plants of TN1 for subsequent infestation.

Evaluation of the BPH resistance

The evaluation of BPH resistance was conducted, using the standard seedbox screening technique (IRRI 1988) with some modifications. At the 3.5- to 4-leaf stage, half a leaf of each seedling was clipped for DNA extraction after removing the weak plants. On the next day, the tray was transferred into an iron sheet case (80×55×8 cm^3), water, 2- to 3-cm deep, being flowed into the tray, for keeping humidity high and ants off the seedlings. Then, the plants were infested with the selected nymphs at a density of six to seven nymphs per seedling as evenly as possible. After infestation, each tray was covered with a nylon-net cage (size: 100 meshes/cm^2; 65×45×50 cm^3), immediately. To objectively evaluate the F_2 plants, all the plants in a tray were rated individually every 2 days from the fourth day of infestation. The scoring system proposed by the International Rice Research Institute (IRRI 1988) with some modifications was used to rate each seedling: 0 = no visible damage; 1 = partial yellowing of first leaf; 3 = first and second leaves partially yellow; 5 = pronounced yellowing or some stunting; 7 = mostly wilting, the plant was still alive; 9 = the plant completely wilted or died. Since the damage of severities of plants is dynamic, the score of each seedling rated on a day when more than 90% of the susceptible parents died, was recognized as its reaction. The mean value of damage severity scores for parental and F_1 plants were used as their reaction. For data analysis, plants with a scale of 0–5 and 7–9 were designated as resistant and susceptible, respectively. Segregation in the two F_2 populations was tested by Chi-square analysis for goodness of fit to the appropriate ratio.

Initial localization of the BPH resistance gene locus

Genomic DNA, from the clipped leaves of F_2 plants as well as the parental plants, was extracted, using the CTAB method (Murray and Thompson 1980).

For SSR analysis, amplification reactions were performed with the Programmable Thermal Controller PTC 100™ (MJ Research Inc., Watertown, MA, USA) or the GeneAmp® PCR System 9700 (PE Applied Biosystems, Foster City, CA, USA). Primers of the SSR markers (Temnykh et al. 2000) were synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). The PCR procedures described by Temnykh et al. (2000) were followed, with the exception that amplified PCR products were separated on 6.0% polyacrylamide denaturing gels and visualized by means of silver staining (Zhu et al. 2004a).

The bulked segregant analysis (BSA, Michelmore et al. 1991) was used to identify the candidate SSR markers linked to the BPH resistance gene. According to the results of the BPH resistance evaluation, two contrasting bulks were prepared, each containing DNA from 10 extremely resistant (score: 0 or 1) or susceptible (score: 9) F_2 plants (both were selected from the F_2-1 population). Based on the results of the segregation analysis, candidate markers were validated by means of the recessive class analysis (RCA, Pan et al. 2003), using 497 extremely resistant individuals of the F_2-1 and F_2-2 populations (Fig. 1, and also see subsequently). The recombination frequency between a marker and the resistance gene was calculated using the following formula: $c = N_r/2N$, in which N is the total number of the resistant individuals tested, N_r is the number of recombination events occurred at the respective locus (Pan et al. 2003; Gu et al. 2004). The recombination frequency was transformed into centimorgans according to the Kosambi function (Kosambi 1944).

Fine mapping of the BPH resistance gene locus

The BPH resistance gene locus was finely mapped by BIA. On the one hand, known SSR markers, covering the BPH resistance gene region, were adopted from the public database released by the International Rice Microsatellite Initiative (IRMI, http://www.gramene.org/microsat). On the other hand, additional PCR-based markers were subsequently developed according to the sequence information of the reference cvs., Nipponbare and 93–11. For SSR marker, the putative simple-sequence repeat motifs were searched and identified using Simple Sequence Repeat Identification Tool (SSRIT, http://www.gramene.org/db/searches/ssrtool). For STS

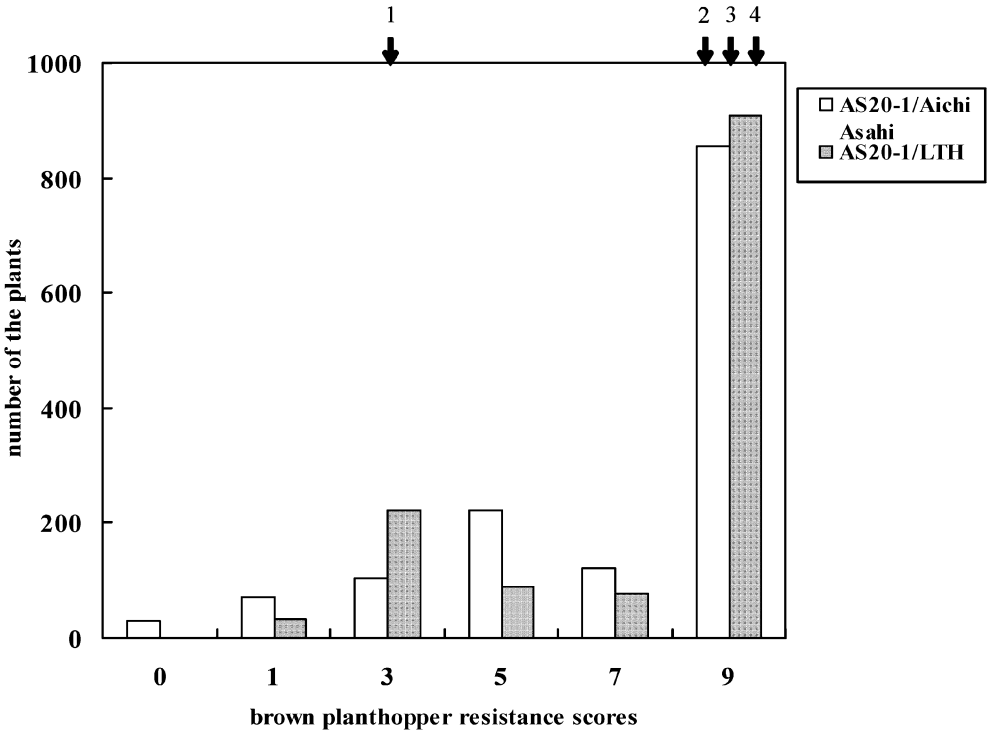

Fig. 1 Distribution of the brown planthopper (BPH) resistance scores in two F_2 populations of AS20-1/Aichi Asahi and AS20-1/Lijiangxintuanheigu (LTH). The *arrows* with number *1*, *2*, *3* and *4* represent the average severities of resistant cultivar (cv.) AS20-1, F_1, and susceptible cvs. Aichi Asahi and LTH, respectively

markers, the putative sequences, which are highly divergent between the two reference genomes, were targeted. All PCR primers were designed using Primer Premier 5.0 (http://www.premierbiosoft.com) and were synthesized by SBS Genetech Co., Ltd. (Beijing, China). The amplification products of the monomorphic STS marker developed were digested with the appropriate restriction enzyme, and converted into CAPS markers, if possible.

Physical mapping of the BPH resistance gene locus

Physical map of the BPH resistance gene locus was constructed, *in silico*, via BIA. Molecular markers linked with the BPH resistance gene locus were landed on the BAC or PAC clones of the reference cv. Nipponbare, released by IRGSP, using sequence homology search tool, BLASTN (http://www.ncbi.nlm.nih.gov/BLAST). Sequences of these clones were downloaded and aligned using sequence olignment tool, Pairwise BLAST (http://www.ncbi.nlm.nih.gov/blast /bl2seq/bl2.html) for constructing the BAC/PAC contigs spanning the BPH resistance gene locus.

Results

Genetic analysis of the BPH resistance and construction of a mapping population

When more than 90% of susceptible parental plants died, the average severity scores of the three parents, AS20-1, Aichi Asahi and LTH, were 3.88, 8.56 and 8.89, respectively. The average severity score of the 13 F_1 plants was 8.43, which resembled to the susceptible parents Aichi Asahi and LTH, indicating that the BPH resistance in AS20-1 was under recessive gene(s) control.

To further confirm the inheritance pattern of the BPH resistance, the two F_2 populations were separately tested with the same biotype of insects. In the F_2-1 population, the damage severity scores of the 1,330 individuals ranged from 0 to 9, and performed a bimodal distribution (Fig. 1). The resistant and susceptible plants well fitted a 1:3 ratio (347R: 983S, $\chi^2 = 0.79$, $P > 0.05$), suggesting that a recessive gene confers the BPH resistance in cv. AS20-1 (Fig. 1). In the F_2-2 population, a total of 1,403 individuals, also produced the similar result (1R: 3S = 371: 1032, $\chi^2 = 1.48$, $P > 0.05$), which further confirmed the BPH resistance in AS20-1 conditioned by a single recessive gene (Fig. 1). Since the resistance gene in cv. AS20-1 showed essentially the identical performance in the two F_2 populations, the mapping population for the resistance gene was constructed by pooling the two F_2 populations.

Identification of SSR makers linked with the BPH resistance gene locus

To efficiently identify the candidate SSR markers linked with the BPH resistance, the BSA method was employed. A total of 142 SSR markers, selected from 12 chromosomes with an interval of ≈15 centimorgans (cM), were tested. Among the selected markers, only three markers, RM231, RM545 and RM7, were polymorphic between

both two parents and bulks. These three markers are positioned in a contiguous region on the short arm of chromosome 3 (Fig. 2) and, therefore, considered as candidate markers for the resistance gene. For confirming the candidate markers, the plants consisting of the resistant and susceptible bulks were tested, individually. The results showed that these three markers were linked with the resistance gene (data not shown). Additional 4 SSR markers, RM517, RM546, RM36 and RM218, around these three markers were selected to survey for polymorphism, of which two markers i.e. RM517 and RM218 were also polymorphic between both two parents and bulks.

The candidate markers identified by BSA were used for linkage analysis in the mapping population consisting of 497 extremely resistant plants by the RCA. The results showed that most of the recombinant plants identified at the RM231, RM545 and RM517 loci were distinguished from those of RM218 and RM7 loci (data not shown). It, therefore, revealed that the resistance gene locus was flanked by these markers on both sides. RM231, RM545 and RM517 were linked to the BPH resistance locus on telomere side with genetic distances of 11.7, 7.1 and 2.0 cM, respectively, while RM218 and RM7 were found to be 3.8 and 6.5 cM, respectively, on the centromere side (Fig. 2a). According to this result, the BPH resistance locus was roughly mapped within a 5.8-cM region flanked by markers RM517 and RM218 (Fig. 2a), and was therefore tentatively designated as *bph19*(t). A total of 58 recombination events, 20 detected at RM517 locus and 38 at RM218 locus, were subsequently used to finely map the *bph19*(t) locus.

Fine mapping of the *bph19*(t) locus

In order to fine map the *bph19*(t) locus, additional SSR markers developed in the region defined by RM517 and RM218 were adopted from IRMI database. Fortunately, 9 out of the 10 SSR markers selected showed

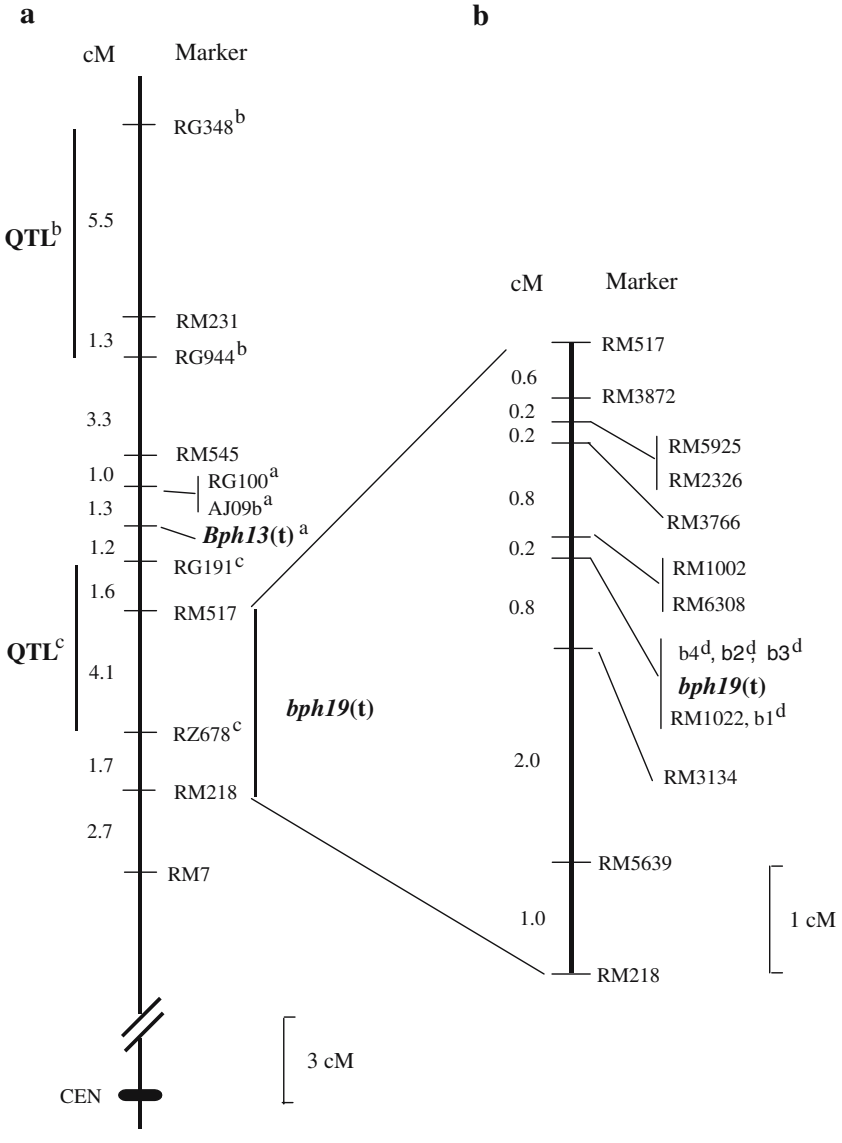

Fig. 2 **a** Genetic map of the *bph19*(t) locus and three other BPH resistance genes/QTLs on the short arm of chromosome 3. The locations of three genes/QTLs other than *bph19*(t) identified in the present research were estimated based on the data from *a*, Renganayaki et al. 2002; b, Xu et al. 2002; and c, Alam and Cohen 1998. CEN: centromere. **b** Finely genetic map of the *bph19*(t) locus. The distance (cM) between markers is indicated to the *left*; markers used in this phase of the study are indicated to the *right*; d: new markers used in the present paper

polymorphism between the resistant and susceptible parents. These nine markers were used to test the 58 recombination events. The results showed that 14, 12, 12, 10, 2 and 2 recombination events occurred at the RM517 locus were detected at RM3872, RM5925, RM2326, RM3766, RM1002 and RM6308 loci, respectively. That is, these marker loci were located on the telomere side with genetic distances of 1.4, 1.2, 1.2, 1.0, 0.2 and 0.2 cM, respectively (Fig. 2b). In contrast, 28, 8 and 0 recombination event(s) occurred at the RM218 locus were detected at RM5639, RM3134 and RM1022 loci, respectively, suggesting that these three markers were located on the centromere side of the resistance gene with genetic distances of 2.8, 0.8 and 0.0 cM, respectively (Fig. 2b). Hence, the *bph19*(t) locus was mapped in a smaller 1.0-cM region, delimited by RM6308 and RM3134, and co-segregated with RM1022. Notably, the orders of two pairs of SSR markers, RM218 and RM7, and RM2326 and RM3872, disagreed with those in the previous maps (McCouch et al. 2001, 2002). It may be because of the inversions taken place in those small chromosome regions in the donor cv. AS20-1.

For map-based cloning of the *bph19*(t) gene conveniently in the future, additional four markers were created in the RM6308-RM3134 interval based on the sequence information from the reference cv. Nipponbare. Of the newly designed markers, two (SSR marker b1 and STS marker b3) were polymorphic between the parents, and another two STS markers b2 and b4 were monomorphic on the parents. The monomorphic STS markers were then converted into CAPS markers, successively (Table 1). Thus, these four polymorphic markers were also tested with the 58 recombination events. The results showed that all the four markers co-segregated with the *bph19*(t) locus (Fig. 2b). According to the previous results, a high-resolution genetic map was constructed in which the *bph19*(t) locus was finally localized to the 1.0-cM interval flanked by markers RM6308 and RM3134, and completely co-segregated with five PCR-based markers, RM1022, b1, b3, b2 and b4 (Fig. 2b).

Physical mapping of the *bph19*(t) locus

According to the primer sequences and genomic sequence information of the reference cv. Nipponbare, the *bph19*(t)-linked SSR, STS and CAPS markers were landed on the 19 Nipponbare BAC clones by the BLASTN analysis. Sequences of the 19 clones were downloaded and aligned through Pairwise BLAST analysis. The results showed that two BAC clones, OSJNBa0049C20 and OJ1081D05, which were anchored by two closely linked markers RM6308 and RM3134, respectively, are overlapped. Thus, a physical map of the *bph19*(t) locus was generated as shown in Fig. 3 (partial data not shown). The physical distance between RM6308 and RM3134 is estimated to be ≈60 kb in length based on the Nipponbare sequence.

Discussion

In the present paper, a powerful strategy, which consists of BSA, RCA and BIA approaches, was employed to construct both genetic and physical maps of the rice BPH resistance gene, *bph19*(t). Firstly, BSA provides a rapid, technically simple means for identifying candidate markers in the target region (Michelmore et al. 1991). Secondly, RCA enables to confirm the candidate markers for mapping the target gene on its chromosome using the homo-recessive progenies (Pan et al. 2003; Zhu et al. 2004a). Third, BIA allows us to adopt and/or develop molecular markers in the target region, and to construct a physical map spanning the target gene locus, *in silico* (Gu et al. 2004; Chen et al. 2005; Liu et al. 2005). As a result, the *bph19*(t) locus was genetically defined to a chromosomal interval of 1.0 cM in length with five co-segregated markers, and physically mapped to a DNA fragment of 60-kb in length.

It is of interest that plant resistance genes, including major (qualitative) and minor (quantitative, QTL) genes, tend to be clustered in the particular regions of chromosomes (Beavis et al. 1991; Wang et al. 1994; Xu et al. 2002; Ramalingam et al. 2003; Yang et al. 2003). Aside from the *bph19*(t) gene, one major gene *Bph13*(t) and two QTLs were mapped on the short arm of rice chromosome 3 (Fig. 2a). The *Bph13*(t) gene was located near the *bph19*(t) gene (Renganayaki et al. 2002), but it is clearly different from the *bph19*(t) gene for the following reasons: (i) *Bph13*(t) is a dominant gene, while *bph19*(t) is a recessive gene based on the evaluation of BPH resistance in our two populations; (ii) According to the report of Renganayaki et al. (2002), *Bph13*(t) resides in the region flanked by RG100 and RG191, while

Table 1 Summary of new markers developed in the present study

Marker name	Marker type	PCR primer (5′-3′)	Motif or enzyme	Annealing temperature (°C)	Expected product size (bp)
b1	SSR	F: gcatcatggcatcctacagcR: gccgacctattagtgttgc	ga	58	177
b2	STS-CAPS	F: acatgtcggcagagcttgagR: ttgcaagtgaactcctagcc	*Hha* I	64	593
b3	STS	F: gcactagccagttccatcgR: cccacacctcaaaggataag	–	60	700
b4	STS-CAPS	F: gttgtgcttgcttcttgccR: cctgcgttgattgaagagc	*Hinf* I	58	1,141

F, forward; R, reverse

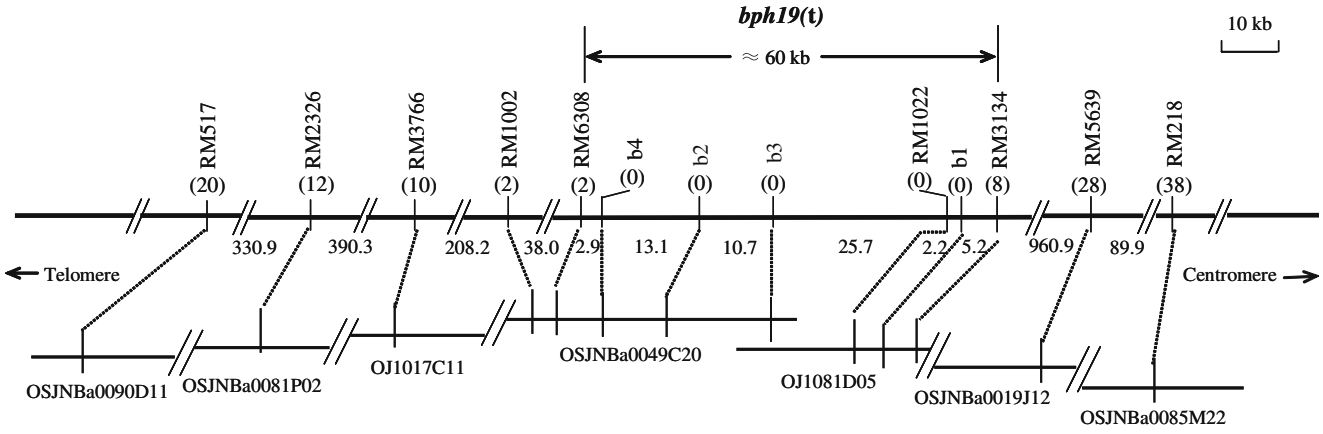

Fig. 3 Physical map of the *bph19*(t) locus. The *numerals in parentheses* indicate the recombination events occurred at the corresponding marker loci, and the *numbers below the long line* are relative physical distances in kilobytes estimated based on the positions of markers linked to the *bph19*(t) locus. The *short horizontal lines* represent bacterial artificial chromosome clones of cv. Nipponbare, which contain the *bph19*(t)-linked markers and were released by International Rice Genome Sequencing Project (IRGSP). The *vertical line* denotes the position of the respective markers

bph19(t) was mapped in the region flanked by RM517 and RM218 in the present study (Fig. 2a). One QTL accounted for only 3.8% of the phenotypic variance was identified to a marker interval, RG944-RG348, which is farther than the *Bph13*(t) locus from the *bph19*(t) locus (Fig. 2a; Xu et al. 2002). Another QTL predominantly associated with a single resistance mechanism, antixenosis, was also identified to a marker interval, RG191-RZ678, which covers the *bph19*(t) location (Fig. 2a; Alam and Cohen 1998). Currently, four hypotheses could explain their genetic relationships: (i) they are different loci but tightly linked to each other; (ii) they are different alleles at the same locus; (iii) they are the same allele but show differential reactions to different biotypes of the BPH; and (iv) they are the same gene but expressed different reactions in the different background (Wang et al. 1994; Monna et al. 2002; Ramalingam et al. 2003). One way to test the hypothesis above is to isolate the single QTL line and then construct its high-resolution map (Paterson et al. 1990; Inukai et al. 1996). Since this QTL has not been named and finely mapped, the resistance gene identified in the present study was reasonably designated as *bph19*(t).

As for disease resistance genes, recessive resistance genes generally do not cluster with other resistant genes in rice (Ronald 1997, 1998; Richter and Ronald 2000). But this may not be the case for BPH resistance genes. The most striking example is that a recessive BPH resistance gene *bph2*, with other three dominant genes, *Bph1*, *Bph9* and *Bph10*, were mapped to a 25-cM interval on the long arm of chromosome 12 (Murai et al. 2001; Murata et al. 2001; Sharma et al. 2002). One more interesting phenomenon was observed by the same research group. That is, the recessive gene *bph2* behaved as a dominant gene (Murata et al. 1998; Murai et al. 2001). A possible explanation might be that recessive resistance genes behave as dominant genes under different genetic backgrounds and with different pathotypes (races) or biotypes (Murai et al. 2001). Another possible reason is that gene conversion or unequal crossing-over events occurred at the *bph2* locus during meiosis, and resulted in the instability of *bph2*, which is similar to the rust resistance gene *Rp1* (Sudupak et al. 1993; Richter and Ronald 2000). A similar case is on the short arm of chromosome 3, where the recessive gene, *bph19*(t), the dominant gene, *Bph13*(t), as well as two QTLs were located (Fig. 2a). It seems that the *bph19*(t) locus is unstable, due to inversions taken place in the two marker pairs, RM218-RM7 and RM2326-RM3872 on the both sides. Although allelism of the BPH resistance genes resided at the clusters, including the *bph19*(t) cluster, are still not well understood, fine mapping and molecular isolation of these BPH genes will shed light on this issue.

On the other hand, only one natural insect resistance gene, *Mi*, has been cloned, so far. It belongs to the nucleotide-binding site-leucine-rich repeat (NBS-LRR) class of resistance genes, and resembles to that of most disease resistance genes (Vos et al. 1998). In our work, putative open reading frames (ORFs) were predicted in the 60-kb interval based on the Nipponbare sequences, using gene annotation systems (http://ricegaas.dna.affrc.go.jp; http://www.softberry.com/berry.phtml). The results show that there are ten ORFs, but none possesses the NBS-LRR structure (data not shown). Therefore, it is probably that the BPH resistance gene *bph19*(t) encodes a novel form of insect resistance. Recent research indicates that plant responses to phloem-feeding insects, such as aphids, have similarities to those by pathogens, because of the similar effects insect stylets and fungal hyphae have on their hosts during insect feeding and pathogen infection (Fidantsef et al. 1999; Walling 2000). The related knowledge may be helpful to clarify the resistance mechanism in rice against BPH, because it also belongs to the phloem-feeding insect. We have undertaken the isolation of *bph19*(t), which will certainly discover the real resistance mechanism in rice against BPH.

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