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ORIGINAL PAPER

Fine mapping and characterization of *BPH27*, a brown planthopper resistance gene from wild rice (*Oryza rufipogon* Griff.)

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Abstract The brown planthopper (*Nilaparvata lugens* Stål; BPH) is one of the most serious rice pests worldwide. Growing resistant varieties is the most effective way to manage this insect, and wild rice species are a valuable source of resistance genes for developing resistant cultivars. *BPH27* derived from an accession of Guangxi wild rice, *Oryza rufipogon* Griff. (Accession no. 2183, hereafter named GX2183), was primarily mapped to a 17-cM region on the long arm of the chromosome four. In this study, fine mapping of *BPH27* was conducted using two BC_1F_2 populations derived from introgression lines of GX2183. Insect resistance was evaluated in the BC_1F_2 populations with 6,010 individual offsprings, and 346 resistance extremes were obtained and employed for fine mapping of *BPH27*. High-resolution linkage analysis defined the *BPH27* locus to an 86.3-kb region in Nipponbare. Regarding the sequence information of rice cultivars, Nipponbare and 93-11, all predicted open reading frames (ORFs) in the fine-mapping region have been annotated as 11 types of proteins, and three ORFs encode disease-related proteins. Moreover, the average BPH numbers showed significant differences in 96–120 h after release in comparisons between the preliminary near-isogenic lines (pre-NILs, lines harboring resistance genes) and BaiR54. BPH growth and development were inhibited and survival rates were lower in the pre-NIL plants compared with the recurrent parent BaiR54. The pre-NIL exhibited 50.7 % reductions in population growth rates (PGR) compared to BaiR54. The new development in fine mapping of *BPH27* will facilitate the efforts to clone this important resistant gene and to use it in BPH-resistance rice breeding.

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Introduction

The brown planthopper (*Nilaparvata lugens* Stål; BPH) is the most destructive pest to rice production by sucking sap from phloem and transmitting viruses (Cha et al. 2008), such as grassy stunt virus (Rivera et al. 1966) and rugged stunt virus (Ling et al. 1977). Heavy BPH infestation results in extensive damage to the rice plants, known as "hopper burn", i.e., complete drying of the plants (Sogawa 1982). In recent years, infestations of this insect have intensified across Asia, resulting in heavy yield losses (Normile 2008). In rice production practice, BPH is managed mainly by use of chemical pesticides, which are expensive in terms of cost and environment. Moreover, pesticides kill BPH predators, and overuse of pesticides prompts the insect to evolve resistance, which in turn leads

to pest resurgence. Therefore, utilization of BPH-resistance genes to develop resistant varieties is the most economical and environment-friendly strategy to control this insect.

Rice *indica* cultivars probably express a relatively higher level of BPH resistance than that of *japonica* subspecies, and 26 major BPH-resistance genes have been identified in *indica* cultivars and wild rice species (http://www.shigen.nig.ac.jp/rice/oryzabase_submission/gene_nomenclature/). *Bph1*, the first identified dominant resistance gene from an *indica* cultivar Mudgo (Athwal et al. 1971), has been mapped to the long arm of chromosome 12 (Hirabayashi and Ogawa 1995). The first recessive gene *bph2*, identified from the *indica* cultivar ASD7 (Athwal et al. 1971), has been located on the long arm of chromosome 12 (Murata et al. 1998; Murai et al. 2001). *Bph3* and *bph4*, both of which were mapped to the short arm of chromosome 6 (Jairin et al. 2007; Kawaguchi et al. 2001), have been identified in rice cultivars Rathu Heenati and Babawee, respectively (Lakshminarayana and Khush 1977). Three resistance genes, *bph5*, *BPH6* (formerly *Bph6*) and *bph7*, were found in cultivars ARC10550, Swarnalata and T12, respectively (Khush et al. 1985; Kabis and Khush 1988); and *BPH6* has been located on the long arm of chromosome 4 flanked by the markers RM6997 and RM5742 (Qiu et al. 2010). A recessive gene *bph8*(*t*) was found in three cultivars Col. 5 Thailand, Col. 11 Thailand and Chin Saba, and a dominant gene, *Bph9*, in other three cultivars Balamawee, Kaharamana and Pokkali (Nemoto et al. 1989). *Bph9* was located on the long arm of chromosome 12 (Murata et al. 2001). Another dominant gene, *Bph17*, was also found to be located on the short arm of chromosome 4 in cultivar Rathu Heenati (Sun et al. 2005), while another recessive gene, *bph19*(*t*), was identified from cultivar AS20-1 and mapped to the short arm of chromosome 3 (Chen et al. 2006).

Wild rice species is rich in resistant sources to BPH. As of today, at least ten major resistance genes have been identified from the wild rice species. *Bph10* was identified on the long arm of chromosome 12 in *O. australiensis* (Ishii et al. 1994). The *bph11*(*t*) gene was identified on the long arm of chromosome 3 in *O. officinalis* (Hirabayashi et al. 1998). A dominant gene *BPH12* (formerly *Bph12*(*t*)) was detected on the short arm of chromosome 4, and flanked by RM16459 and RM1305 in *O. latifolia* (Yang et al. 2002; Qiu et al. 2012). The recessive resistance gene, *bph16* (formerly *bph12*(*t*)), was mapped to the long arm of chromosome 4 and harbored by two RFLP markers, G271 and R93 (Hirabayashi et al. 1998). Two dominant genes, both named *Bph13*(*t*), were found on the long arm of chromosome 2 of *O. eichingeri* (Liu et al. 2001) and on the short arm of chromosome 3 of *O. officinalis* (Renganayaki et al. 2002). In *O. officinalis*, two more dominant resistance genes, *Bph14* and *Bph15*, were mapped to the long arm of chromosome 3 and the short arm of chromosome 4, respectively (Huang et al. 2001). *Bph18*(*t*) was mapped to the long arm of chromosome 12 in *O. australiensis* (Jena et al. 2006), and two newly identified genes from *O. minuta*, *Bph20*(*t*) and *Bph21*(*t*), were mapped to the short arm of chromosome 4 and to the long arm of chromosome 12, respectively (Rahman et al. 2009).

Plant generally may employ three resistance mechanisms against insects with respect to physiological function. These mechanisms are: antixenosis (non-preference), reduction in colonization or oviposition; antibiosis, reduction in insect survival, growth rate or reproduction after ingestion of host tissue; and tolerance, production of a crop of high quality and yield despite insect infestation (Painter 1951; Alam and Cohen 1998a). Previous studies of IR64 have documented each of these mechanisms with regard to BPH–rice interactions (Cohen et al. 1997; Alam and Cohen 1998a, b). Recent research has identified the antibiotic and tolerant levels to the BPH in several rice lines carrying different BPH-resistance genes (Qiu et al. 2011). In the case of *Bph14*, resistance appears to be due to antibiosis (Du et al. 2009). Furthermore, we also found that the *BPH6* gene confers both antixenotic and antibiotic effects as identified using the *BPH6* near-isogenic line (NILs) plants (Qiu et al. 2010).

In the present research, we have renamed the BPH-resistance loci according to the new gene nomenclature system for rice proposed by McCouch and CGSNL (2008) to avoid confusion among the different loci for BPH resistance. In a previous study, we identified a recessive BPH-resistance gene *bph18*(*t*) derived from a wild rice accession (*O. rufipogon* Griff., accession GX2183), which shows a broad spectrum resistance to BPH biotypes, including biotypes 1 and 2, Bangladesh, Cuu Long (Vietnam) and Pantnagar (India) (Li et al. 2006). However, Jena et al. (2006) reported the same gene nomenclature in a different donor IR65482-7-216-1-2, derived from *O. australiensis*. In addition, the gene symbols from *Bph20*(*t*) to *Bph26* have already been designated. We have therefore revised the gene names by renaming *bph18*(*t*) as *BPH27* and have registered this gene name in the *Oryza* base (Yamazaki et al. 2010: http://www.shigen.nig.ac.jp/rice/oryzabase/). *BPH27* was mapped to a 17-cM region flanked by SSR markers RM273 and RM471 on the long arm of chromosome 4 (Li et al. 2006). In the present study, we aim to: (1) confirm and fine map the *BPH27* resistance gene to facilitate marker-assisted breeding and gene map-based cloning; (2) characterize its resistance mechanisms using preliminary-NILs (pre-NILs).

Materials and methods

Plant materials and mapping populations

Wild rice accession GX2183 of *O. rufipogon* Griff. was collected from Guangxi, China, and maintained by our

research group. GX2183 was first crossed and then backcrossed twice with rice *indica* cv. TeQing to develop BC_2F_2 BPH-resistant lines. These lines were further crossed twice with *japonica* lines BaiMao and BaiR54, respectively, to develop two BC_1F_2 mapping populations, ME488 and ME1458 (Fig. 1). The extremely resistant plants were selected for further backcrossing. The BC_1F_2 populations were bioassayed for BPH resistance, and individuals with resistance extremes (higher or at the same level as GX2183) were used for fine mapping of the resistance gene. Furthermore, the resistant BC_1F_2 plants with BaiR54 genetic background were backcrossed twice with BaiR54 to obtain the BC_3F_1 generation, from which one plant was selected to produce BC_3F_2 progenies. Finally, one BC_3F_2 individual that was homozygous at the target region of the *BPH27* was self-pollinated to generate BC_3F_3 lines. The homozygous BC_3F_3 lines were designated as pre-NIL and used in antibiosis and antixenosis tests (Fig. 1). Furthermore, the BC_3F_2 plants which were homozygous for GX2183 and BaiR54 or heterozygous at the *BPH27* region in BaiR54 genetic background were designated as pre-NILR, pre-NILS and pre-NILH, respectively. During this process, the nearest flanking markers tightly linked to the gene were used to select the positive progenies. For the positive selection of the pre-NIL plants, we also performed background selections using SSRs which were polymorphic between GX2183 and BaiR54 as described by Hospital et al. (1992). GX2183, together with *indica* cv. Ptb33 (highly resistant to BPH biotype 2), was used as resistance control. TeQing, BaiMao, BaiR54 and *indica* cv. Taichung native 1 (TN1), which are highly susceptible to the tested biotype of the BPH, were used as susceptible controls.

BPH insects and bioassay for resistance

A sib line of BPH was developed from a single colony of biotype 2, which was a predominant biotype in most of the rice-growing regions in China (Chen et al. 2006). The insects were fed on the variety Mudgo to produce enough nymphs for infestation. Bioassays were conducted following the method described by Chen et al. (2006) in a greenhouse facility. At third-leaf stage (ca. 13–14 days old), the seedlings in trays (52 × 37 × 6 cm) were infested with second- or third-instar nymphs, 10–12 nymphs per seedling. After infestation, the trays were covered with a fine gauge nylon-net cage (42 × 32 × 45 cm). When all plants of the susceptible parents and TN1 were dead (scored 9.0), the BPH resistance was evaluated with the six-scale standard of scoring system, proposed by the International Rice Research Institute (IRRI 1988): 0 = no damage; 1 = very slight damage; 3 = first and second leaves partially yellowing; 5 = pronounced yellowing and stunting; 7 = mostly wilting, the plant still alive; 9 = the plant completely wilted or died. The lower score indicates higher resistance. When all of the control seedlings had died (scored as 9), the score of each seedling was recorded and recognized as its reaction.

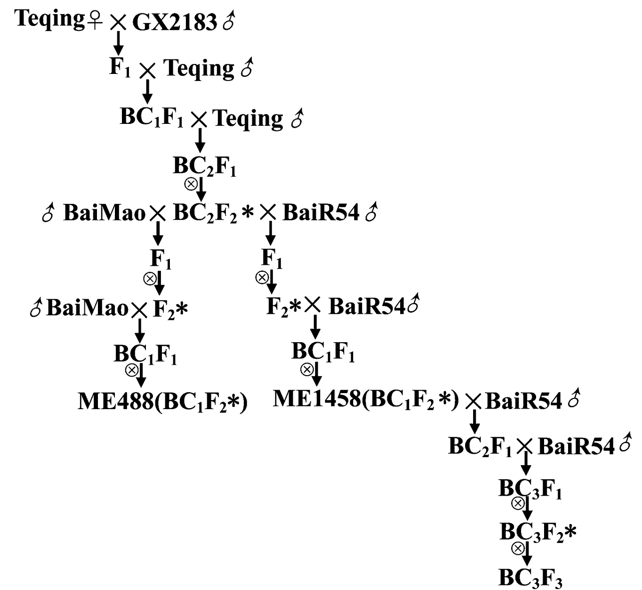


Fig. 1 The pedigree for developing fine-mapping populations and pre-NILs. *Asterisk* indicates the populations bioassayed for BPH resistance and resistant individuals selected for backcross

DNA extraction, gene mapping and gene annotation

Total DNA was extracted from fresh leaves at the seedling stage using the CTAB method (Murray and Thompson 1980). PCR was performed as described by Li et al. (2009). PCR products were separated on 8 % polyacrylamide non-denaturing gels and visualized by silver staining. SSR markers and genomic sequence in the target region of 17 cM were identified from the Gramene database (http://www.gramene.org/markers/index.html). The primers were screened for polymorphism between GX2183 and TeQing, and BaiMao and BaiR54 for efficient detection of the gene locus in GX2183 in gene mapping analysis.

For bulked segregant analysis (BSA), two contrasting DNA bulks were prepared based on the phenotype of the BC_1F_2 individuals from ME488 and ME1458. The bulks consisted of DNA from 20 extremely resistant or susceptible individuals of the BC_1F_2 populations and were screened with SSRs to identify those linked to BPH resistance. Gene mapping was conducted using the method described by Luo et al. (2008). These markers were used for genotyping the selected individuals from BC_1F_2 populations, ME488 and ME1458. Recombinants in the target region were detected, and the linkage relationship between markers and the *BPH27* locus was analyzed for gene

location. By analysis of recombinant events, the *BPH27* locus was fine mapped and landed on the BAC clone.

Gene annotation within specific genomic regions was carried out using Rice Genome Annotation Project (TIGR, http://rice.plantbiology.msu.edu/overview.shtml). The putative open reading frame (ORF) in the target region was predicted by the online software GENESCAN and FGENESH of Softberry (www.softberry.com/berry.phtml) with monocot plants as the model organism.

Host selection behavior

The experiment was conducted as described by Qiu et al. (2010). Two 14-day-old seedlings of pre-NIL and BaiR54 were transplanted in a plastic bucket (15-cm diameter, 14-cm height) with seedlings of the same genotype at opposite ends of roughly perpendicular diagonals. The bucket was then completely covered with fine, light-transmitting mesh and a total of six buckets were used for each pair of genotypes. To observe the host selection of the BPH, 40 s-instar nymphs were placed in each bucket and allowed to choose host plants (35 days old) on which to feed and reproduce over a 120-h period. The BPH insects which settled on each plant were counted at 3, 6, 12, 24, 48, 72, 96 and 120 h after release.

BPH development on rice plants

To measure BPH survival and growth on the pre-NIL and BaiR54 plants, seedlings (14 days old) were transplanted to individual 0.4-L plastic cups under natural conditions. One week before treatment with BPHs, the plants were cultured in a greenhouse at a constant temperature (26–28 °C). To examine the BPH survival rate on plants (35 days old), each cup/plant was infested with 15 s-instar nymphs, and the surviving insects in each cup/plant were recorded every day for 9 days.

The BPH growth was measured after 4 days on the pre-NIL and BaiR54 plants using ten pre-weighed, second-instar nymphs. Ten replicates of 35-day-old seedlings were established for each genotype treatment of pre-NIL and BaiR54. Four days after the treatment, the surviving nymphs on each plant were collected and the weight was recorded. The population growth rate (PGR) of surviving nymphs was calculated according to Klingler et al. (2005) and Qiu et al. (2010).

Statistical analysis

The Chi-square test for goodness-of-fit was performed with MS-excel; and the resistance data were analyzed using one-way ANOVA and comparing the LSD test at a 5 % significance level.

Results

Genetic analysis and development of mapping population of *BPH27*

In BPH-resistance bioassay, the average resistance scores of Ptb33, GX2183, TeQing, BaiMao, BaiR54 and TN1 were 1.8, 3.3, 8.9, 9.0, 9.0 and 9.0, respectively. In the BC_1F_2 populations of ME488 and ME1458, of all 6,010 individual plants bioassayed, 46 were with score 1, 300 with score 3, 150 with score 5, 584 with score 7, and 4,930 were with score 9. In the genetic analysis, if the individual plants with scores from 0 to 3 were designated as resistant and those with scores from 5 to 9 as susceptible, the segregation of resistant (*R*, scores 0–3) and susceptible (*S*, scores 5–9) individuals was 346 *R*:5,664 *S* that well fitted a ratio of 1:15 ($\chi_c^2 = 2.4 < \chi_c^2 = 3.84$). However, if we considered the plants with scores within the ranges 0–7.0 and 7.1–9.0 as resistant and susceptible, respectively (Huang et al. 2001; Yang et al. 2002; Qiu et al. 2012), and thus based on the resistance score of the 6,010 BC_1F_2 plants, the segregation of the resistant (*R*, scores ≤ 7) to susceptible (*S*, scores > 7) plants, 1,080 *R* and 4,930 *S*, was not fitted a ratio of 1:15 ($\chi_c^2 = 1{,}407.1 > \chi_c^2 = 3.84$) or 1:3 ($\chi_c^2 = 158 > \chi_c^2 = 3.84$). These results indicate that possibly more than one gene/QTL controls the BPH resistance in the BC_1F_2 populations.

To construct mapping population of *BPH27*, a total of 346 resistance extremes including 80 from 1,500 plants of ME488 population and 266 from 4,510 plants of ME1458 population were selected and employed for the fine-mapping analysis.

Confirmation of the *BPH27* gene

In our earlier study, *BPH27* had been mapped to a 17-cM region flanked by SSR markers RM273 and RM471 (Fig. 2a). To confirm this result and to further detect whether there are other BPH-resistance genes in the studied rice line, a total of 183 SSR markers, which were polymorphic between GX2183 and BaiMao/BaiR54 and distributed on 12 rice chromosomes, were used for the BSA survey. Consequently, only markers, RM471, RM16846 and RM417, from a contiguous region on chromosome 4, were different between the resistant and susceptible bulks, indicating that one BPH-resistance gene is located in this region. Therefore, ten polymorphic SSR markers, derived from the target region, were applied to test all 346 resistance extremes (Table 1; Fig. 2a). No chromosomal segments of GX2183 were detected with markers RM16820, RM3839, RM17148, and RM17150 from the individuals tested, indicating that *BPH27* does not exist in the region encompassed by these markers. A total of 47 recombinants

were identified from the 346 plants by other six markers. Among them, 34 were detected by RM17044 and 13 by RM16835 (Fig. 2a). Among the 34 recombinants detected by RM17044, 10, 11 and 33 recombinants were detected by RM16888, RM16903 and RM17033, respectively (Fig. 2a). Among the 13 recombinants detected by RM16835, two recombinants, ME1458-29 and ME488-42, were detected by RM16846 (Figs. 2b, 3a, b). Actually, these recombinants were identified to be overlapped. Thus, one linkage map was constructed according to the recombinant events among the markers, and the location of *BPH27* was defined by markers RM16846 and RM16888 (Fig. 2a).

Fine mapping of *BPH27*

Four polymorphic markers, RM16852, RM16853, RM16 858, and RM16874, in the region flanked by RM16846 and RM16888 were developed to test the 346 resistance extremes for fine mapping of *BPH27* (Table 1; Fig. 3a). Among the ten recombinants detected by RM16888, six could be detected by RM16874, three by RM16858, and only one (ME488-14) by RM16853 (Fig. 3a, b, c), while no recombinant could be detected by RM16852, a marker completely co-segregated with *BPH27* (Fig. 3a, d). According to the recombinant events among the markers, one linkage map was constructed and the *BPH27* was mapped to an 86.3-kb interval defined by markers RM16846 and RM16853, on BAC clone OSJNBa005B21 of *japonica* rice Nipponbare (Fig. 3a). In *indica* rice cultivar 93-11, this region corresponds to a 92.9-kb region that covers scaffold000971 and scaffold001498 (Fig. 3a).

Candidate genes in the target region

There are 12 predicted genes in the 86.3-kb region of Nipponbare according to the database of Rice Genome Annotation Project (TIGR, http://www.rice.plantbiology.msu.edu/overview.shtml). Of these genes, nine were predicted by FGENESH in both 93-11 and Nipponbare (Supplementary Table S1). The corresponding region in 93-11 is 92.9-kb, which is 6.6-kb longer than that in Nipponbare. By FGENESH prediction, there are only 12 predicted genes in the 86.3-kb region of Nipponbare, while there are 15 in the 92.9-kb region in 93-11. Comparison analysis indicates that ten of these genes are similar between 93-11 and Nipponbare with identities ranging from 83 to 100 % (Supplementary Table S1). Most of the ORFs are supported with rice full-length cDNAs or ESTs, indicating that they are transcriptionally active. In FGENESH prediction, three distinguished genes found in 93-11 are ORF10, ORF13, and ORF12, encoding a hypothetical protein of 152 amino acids (aa), a hypothetical protein of 67 aa, and a TGF beta receptor associated protein-like

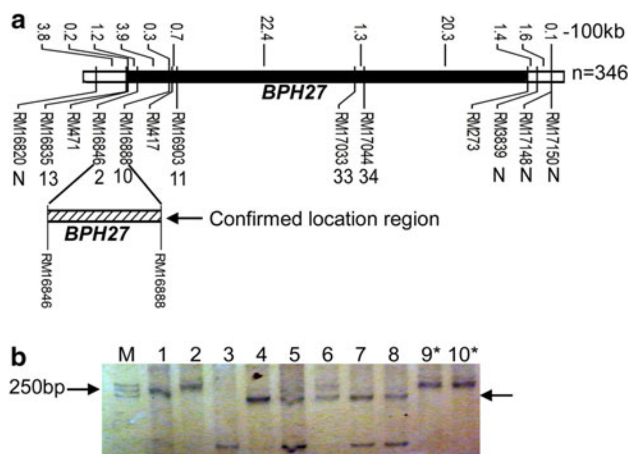


Fig. 2 Confirmation of *BPH27*. **a** Confirmation of locus *BPH27*. Ten new markers specific to the wild rice were developed to analyze 346 resistance extremes. Each marker was used independently to test all extremes. The positions of RM471, RM417 and RM273, markers that were used in the previous research, are also shown. *Numbers* on the linkage map indicate genetic distances between adjacent markers. The number of recombinants between markers and *BPH27* locus is indicated under the linkage map (see Luo et al. 2008). The genetic distance between detected marker and *BPH27* is positively correlated with the number of detected recombinants, i.e., fewer recombinant events indicate shorter genetic distance between detected marker and the locus of *BPH27*. *N* represents no chromosomal segments of GX2183 detected. Previous location of *BPH27* on the region (*filled bar*) flanked by RM273 and RM471. *Lowercase letter n* indicates the number of plants tested. *Oblique filled bar* indicates the confirmed location region defined by RM16846 and RM16888. **b** Recombinants detected by RM16846. *Lane M*, marker; *lanes 1, 2, 3 and 4* indicate TeQing, BaiMao and BaiR54 and GX2183, respectively; and *lanes 5, 6, 7, 8, 9 and 10* indicate ME1458-85, ME488-24, ME1458-14, ME488-14, ME1458-29 and ME488-42, respectively. *Arrow* on *left* indicates fragment size of the marker, and *arrow* on the *right* shows specific fragments of GX2183. *Asterisk* indicates recombinants detected. From all 346 resistance extremes, two recombinants, ME1458-29 and ME488-42, were detected by RM16846

protein (also annotated as succinate-semialdehyde dehydrogenase protein) of 423 aa, respectively (Supplementary Table S1). The only distinguished ORF in the target region in Nipponbare is ORF2, which encodes a nodulin protein of 140 aa (Supplementary Table S1). By functional category, the ORFs are annotated as 11 types of proteins, including putative nodulin protein (LOC_Os04g31924), DNAJ domain containing protein (LOC_Os04g31940), LSM domain containing protein (LOC_Os04g31950), acetolactate synthase protein (both LOC_Os04g31960 and LOC_Os04g32010), putative retrotransposon protein (LOC_Os04g31984), putative myb transcription factor (LOC_Os04g3200), TGF beta receptor associated protein-like protein (ORF12 of 9311), 2-oxoglutarate dehydrogenase E1 component (LOC_Os04g32020), heavy metal-associated domain containing protein (LOC_Os04g32030), expressed protein (LOC_Os04g31010 and LOC_Os04g31971) and

Table 1 SSR markers polymorphic between resistant parent and all the three susceptible parents

Marker	Product size (bp)	Forward primer (5′–3′)	Reverse primer (5′–3′)
RM16820	174	CCCTGCACCTGGATTCTCTCTCC	ATTGACGCACAGACAAGAACAAGACG
RM16835	127	CATTGCCAACCCGTAAAGCTACC	GGTGAGCTGAAGATGTTCTTTCATGG
RM471[a]	198	AGAAATGGATCGGACTGAACATGC	AGACACTCGGACGCACAAGC
RM16846	256	CTACAAGCAACACAGTATCACAGC	GGTAACTGGTGCTTATTTAGCC
RM16852[b]	97	GTAGCCTTGCACTCGACCGTACC	ACCAACTCTGGCAATGCATCC
RM16853[b]	170	CTCCCATCCTTCATTTCATCTCG	CTTTCTGCAAGACACTGCAAACG
RM16858[b]	122	ACGATAACGGCTCTGTTTCTTCG	CGTATCTCGTGGTTGCAGATCG
RM16874[b]	157	TAGCAAGCTTGGAGAAGTGATGG	CAGAAGAAGTCAGCTCTATGCTTGG
RM16888	98	AGGGAATTCCAGCAAAGGAACC	GTTGCATTGCATAGCGACTCAGG
RM417[a]	266	CGGATCCAAGAAACAGCAG	TTCGGTATCCTCCACACCTC
RM16903	484	TTCTCCTCCTCCTGCTACACTGC	GCATACACAAGAGCACCGAGAGG
RM17033	159	GAACCCAATCCCTAGCTACAGACC	GGTACGACTGGTCGTGCTTCC
RM17044	207	TCCTCTCCTCTCACCATTTGACC	GCCACAAGATTTAGGGTGATTTGC
RM273[a]	210	GAAGCCGTCGTGAAGTTACC	GTTTCCTACCTGATCGCGAC
RM3839	217	AATGGGACCAGAAAGCACAC	AAAAAGAGCATGGGGGCTAC
RM17148	297	CACAAGCTGTTCCTGCTCTCG	ATATCTGACGCCAAGCACATCG
RM17150	87	CACAGTTCAGTCATCCAGTGTGG	CAGGTTCCAGCACACAGATCG

[a] Markers used in the previous research

[b] New markers developed for fine mapping

hypothetical protein (LOC_Os04g32004) (Supplementary Table S1).

BPH-resistance evaluation of *BPH27* pre-NILs

In the BC_3F_2 progenies, plants homozygous for BaiR54 *BPH27* region (pre-NILS) were highly susceptible to BPH with an average resistance score of 8.7 (BaiR54, score 9.0) in the seedling bulk tests (Fig. 4). The plants heterozygous for the GX2183 *BPH27* region (pre-NILH) also showed susceptible to BPH (average score of 7.4). The pre-NILR plants that were homozygous for the GX2183 *BPH27* region and GX2183 plants were both resistant to BPH (average scores of 4.2 and 3.2, respectively). The results indicate that the *BPH27* gene functioned in a BaiR54 genetic background.

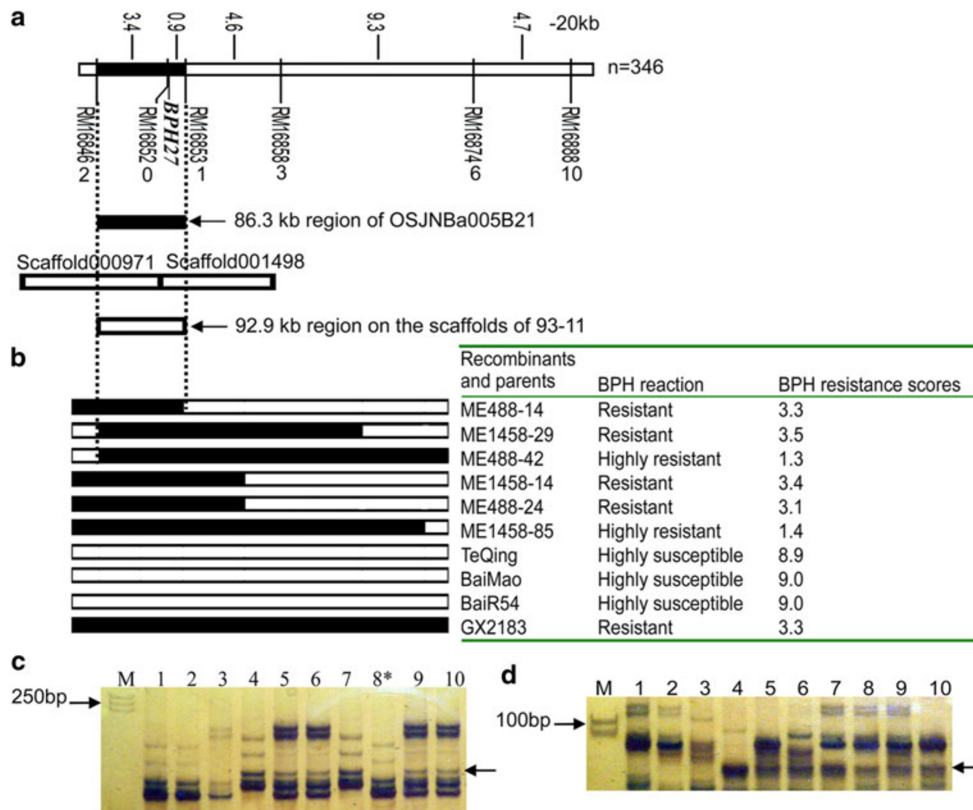
Antixenotic effect of *BPH27* toward BPH insects

The selected BC_3F_1 individual, which has been self-pollinated to produce pre-NILs, has 88.75 % identity to the recurrent parent BaiR54 as detected by using 82 SSR markers. Accordingly, the pre-NIL plants were used to detect the host preference of the BPHs to test the antixenosis in the BPH resistance conferred by *BPH27*. The settled BPHs on the pre-NIL plants were more than that on the BaiR54 plants over the 6-h observation period in the BPH host preference test. After that, the BPHs remained relatively constant at the 12- and 24-h time points, and decreased from the observation period of 48–120 h on the pre-NIL plants, whereas the BPHs on BaiR54 plants increased over the observation period of 12–120 h. One-way ANOVA analysis showed that the BPH insects had significant preference for the pre-NIL and BaiR54 plants at 96 h ($F = 14.38$, $P = 0.004$) and 120 h ($F = 26.41$, $P = 0.0004$) after release (Fig. 5). This result indicated that antixenotic factors were present in the pre-NIL plants.

BPH performance on pre-NIL plants

The BPH PGR on the pre-NIL and BaiR54 plants was compared to determine whether the pre-NIL plants affected BPH growth and development. By the fourth day after treatment, a 50.7 % reduction in PGR of the BPHs was observed on the pre-NIL plants (0.0323 mg/BPH/day) compared to BaiR54 (0.0637 mg/BPH/day), reflecting a significant difference between them ($F = 13.46$, $P = 0.002$; Fig. 6a). The result indicates that the BPH growth and development were significantly inhibited on the pre-NIL plants.

We measured the BPH survival rates on the pre-NIL and BaiR54 plants every day for 9 days to test whether antibiosis was a component of the BPH resistance conferred by *BPH27*. As shown in Fig. 6b, the average number of surviving BPHs on the pre-NIL and BaiR54 plants decreased gradually with the days of BPH infestation. However, the average number of surviving BPHs on the pre-NIL plants decreased more quickly than that on the susceptible plants



Recombinants and parents	BPH reaction	BPH resistance scores
ME488-14	Resistant	3.3
ME1458-29	Resistant	3.5
ME488-42	Highly resistant	1.3
ME1458-14	Resistant	3.4
ME488-24	Resistant	3.1
ME1458-85	Highly resistant	1.4
TeQing	Highly susceptible	8.9
BaiMao	Highly susceptible	9.0
BaiR54	Highly susceptible	9.0
GX2183	Resistant	3.3

Fig. 3 Fine mapping of *BPH27*. **a** Fine mapping of the *BPH27* on an 86.3-kb region (*filled bar*) in the BAC clone OSJNBa005B21 of Nipponbare, and a 92.9-kb region (*blank bar*) in the scaffold000971 and scaffold001498 of 93-11, flanked by RM16846 and RM16853. *Numbers* on the linkage map indicate genetic distances between adjacent markers. The number of recombinants between markers and *BPH27* locus is indicated under the linkage map. *Lowercase letter n* indicates the number of plants tested. **b** Graphical presentation of phenotypes of the recombinants. *Filled bars* denote chromosome segment originating from GX2183, and *blank bars* denote chromosome segment originating from TeQing, BaiMao or BaiR54. **c** Detection of recombinant ME488-14. Only one recombinant ME488-14 was detected by RM16853 from all 346 resistance extremes. **d** No recombinant was detected by RM16852. *Lane M*, marker; *lanes 1, 2, 3 and 4* indicate TeQing, BaiMao, BaiR54 and GX2183, respectively; and *lanes 5, 6, 7, 8, 9 and 10* indicate ME1458-85, ME488-24, ME1458-14, ME488-14, ME1458-29 and ME488-42, respectively. *Arrows* on the *left* indicate fragment size of the marker; *arrows* on the *right* show special fragments of GX2183. *Asterisk* indicates recombinants detected

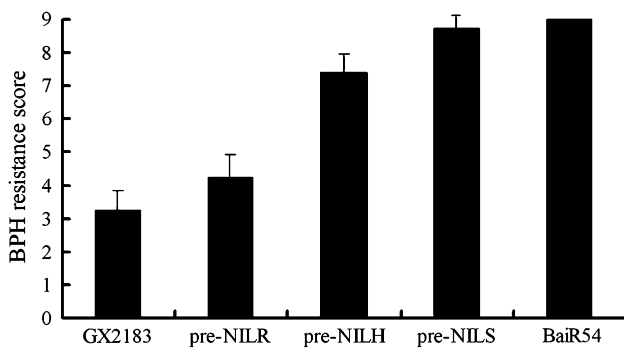


Fig. 4 BPH-resistance phenotype in pre-NILs and parents as measured using the seedling bulk test. *Bars* represent means of four to six replicates. *Error bars* represent the SD. pre-NILR, pre-NILH and pre-NILS denote the BC_3F_2 plants that were homozygous for GX2183 and BaiR54 or heterozygous at the *BPH27* region in BaiR54 genetic background. GX2183 and BaiR54 were the parents

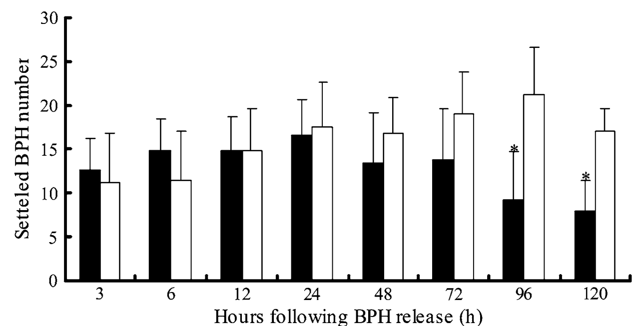


Fig. 5 Results of BPH host choice test on pre-NIL and BaiR54. *Black bars* pre-NIL; *white bars* BaiR54. *Bars* represent means of six replicates. *Error bars* represent the SD. Means labeled with *asterisks* are significantly different ($P < 0.01$)

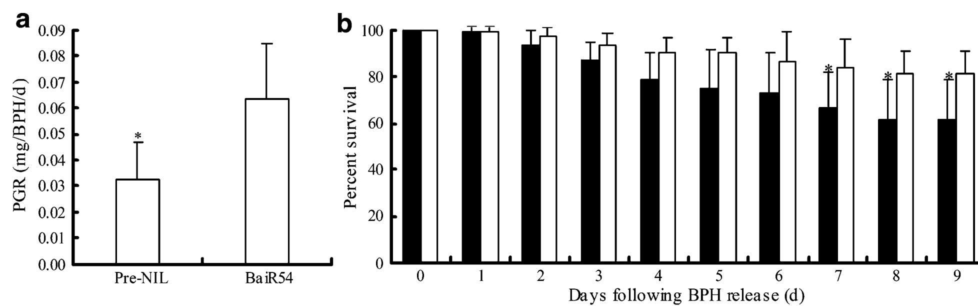


Fig. 6 Effects of plant genotype on the BPH population growth rate (mg/BPH/day, PGR) and BPH survival. **a** PGR of the pre-NIL and BaiR54. PGR was measured as described by Edwards (2001) and Qiu et al. (2010). **b** BPH survival rates on pre-NIL and BaiR54. *Black bars* pre-NIL; *white bars* BaiR54. *Bars* represent means of 11 replicates for **a**, and ten replicates for **b**. *Error bars* represent the SD. Means labeled with *asterisks* are significantly different ($P < 0.05$)

and showed a significant difference in number compared with BaiR54 at 7, 8 and 9 days after release ($F = 4.7$, $P = 0.05$ at 7 days; $F = 5.4$, $P = 0.04$ at 8 days; $F = 5.6$, $P = 0.03$ at 9 days). These findings suggest that the BPH insects had an effect on the antibiotic factors in the pre-NIL plants.

Discussion

BPH27—a novel gene conferring resistance to BPH

In the present study, we used two BC_1F_2 populations derived from advanced introgression lines to construct high-resolution genetic map of a recessive BPH resistance gene *BPH27*, and located the locus within an 86.3-kb region on the long arm of the chromosome 4, in which six other BPH-resistance genes had been identified (Supplementary Figure S1). *BPH6* was mapped to the region flanked by markers RM6997 and RM5742 (Qiu et al. 2010), *BPH12* to the region flanked by markers RM16459 and RM1305 (Qiu et al. 2012), *Bph15* to the region defined by markers RG1 and RG2 (Yang et al. 2004), *Bph17* to the middle of the region defined by RM8213 and RM5953 (Sun et al. 2005), and *Bph20*(*t*) on the region flanked by the markers B42 and B44 (Rahman et al. 2009). For *BPH6*, the other four genes (*BPH12*, *Bph15*, *Bph17* and *Bph20*(*t*)) are distributed in a narrow region between RM8213 and RM5953 (Supplementary Figure S1). The recessive resistance gene *bph16* is located in the region flanked by markers G271 and R93 on the long arm of chromosome 4 (Hirabayashi et al. 1998). Interestingly, *BPH27* was also mapped to the long arm of chromosome 4, about 1 Mb upstream the *bph16* region according to located markers and cv. genome sequence (Supplementary Figure S1). Fine mapping in the present research indicated that *O. rufipogon*-derived *BPH27* is different from the *O. officinalis*-derived *bph16*, and might be a novel BPH-resistance gene. However, additional genetic analyses such as gene cloning and transformation study are needed to clarify the relationship of *BPH27* and other BPH-resistance genes in the region. In addition, the locations of dominant and recessive BPH-resistance genes on chromosome 4 support the assumption that BPH-resistance genes also tend to be present in cluster in the particular regions of chromosomes (Rahman et al. 2009; Qiu et al. 2010).

Previously, several BPH-resistance rice varieties/lines were identified to be carrying more than one resistance gene/QTL. For instance, Cohen et al. (1997) detected that the rice cultivar IR64 carried the major gene *Bph1* and seven other minor QTLs for the BPH resistance by using 175 RFLP markers. Moreover, the rice line B5 was also detected to carry two major BPH-resistance genes (*Bph14* and *Bph15*) (Huang et al. 2001). A recent study by Myint et al. (2012) also showed that two major BPH-resistance genes (*BPH25* and *BPH26*) were identified in the rice line ADR52. In the present study, we also found that there possibly was more than one BPH-resistance gene/QTL in the rice line GX2183 based on the genetic analysis of the resistance scores. However, except for *BPH27*, the other resistance genes/QTLs have not been detected by applying the traditional introgression lines or BSA analysis. It is probable that the genetic backgrounds of the mapping populations, at least carrying the introgression fragments of wild rice species and variety TeQing, were too complex and it was difficult to locate the resistance genes/QTLs when a BSA analysis was performed. Therefore, more advanced backcrossing lines were needed to be constructed and applied to resistance evaluation in further study. In this way, we can apply the resistance lines carrying a single gene/QTL to detect and locate the other resistance genes/QTLs. Furthermore, we also can study the genetic effects among the different resistance genes/QTLs using these rice lines.

Possible nature of *BPH27*

It is now known that the responses of rice to BPH feeding are most possibly similar to pathogen-defense responses

(Wang et al. 2008; Du et al. 2009). For example, *Bph14* is a member of the coiled-coil, nucleotide-binding, and leucine-rich repeat (CC-NB-LRR) disease resistance gene family and provides resistance to BPH in a mechanism fundamentally similar to defense mechanisms against pathogens by activating an SA-dependent pathway (Du et al. 2009). The chromosome region containing *BPH27* is rich in genes involved in disease response. Three predicted genes, LOC_Os04g31924, LOC_Os04g32000 and ORF12 of 93-11, are likely involved in response to disease and thus are considered the primary candidates of the *BPH27* gene. Among the three predicted genes, LOC_Os04g31924 encodes a putative nodulin protein which may play a role in nodule architectural modification (Wilson et al. 1994), sugar-signaling pathway (Guzzo et al. 2005) and membrane transport (Rivers et al. 1997). LOC_Os04g32000 encodes a homolog of MYB30 and MYB96 (Supplementary Table S1). Both MYB30 and MYB96 are transcription factors in Arabidopsis and MYB96 is a key transcriptional activator of the HR in response to bacterial pathogens (Daniel et al. 1999; Vailleau et al. 2002; Raffaele et al. 2006). MYB96-mediated ABA signals enhance plant disease resistance by inducing SA biosynthesis (Seo and Park 2010). ORF12 encodes a homolog of TGF-β receptor interacting protein-like protein. The TGF-β family of polypeptide growth factors regulates proliferation, lineage determination, differentiation and death of cells in fruit fly to humans (Massague 1998). The rice gene *OsTVLP1*, which encodes a TGF-β receptor interacting protein-like protein, is activated specifically in disease resistance response, and may play a role in rice disease resistance response against pathogen infection (Hu et al. 2006).

Among the seven remaining genes with putative function, LOC_Os04g31940 encodes a putative DnaJ protein, LOC_Os04g31950 encodes an Lsm (like-Sm) protein, both LOC_Os04g31960 and LOC_Os04g320010 encode acetolactate synthase related proteins, LOC_Os04g31984 encodes a putative retrotransposons protein, LOC_Os04g32020 encodes a putative 2-oxoglutarate dehydrogenase E1 component, and LOC_Os04g32030 encodes a heavy metal-associated domain-containing protein (Supplementary Table S1). Although these genes might contribute to BPH resistance, the possibility is far more remote than the three primary candidates.

Resistance mechanism of *BPH27*

Understanding the mechanisms underlying rice resistance to BPH is essential for developing appropriate breeding strategies. The most widely accepted classification of insect-resistance mechanisms viz. antixenosis (non-preference), antibiosis and tolerance is proposed by Painter (1951). According to the tests of the BPH host preference and performance on the pre-NIL and BaiR54 plants, *BPH27*-mediated resistance involves both antixenosis and antibiosis in BaiR54 genetic background. In detail, the settled BPHs on the pre-NIL plants decreased from the observation period of 48–120 h, whereas the BPHs on BaiR54 plants increased over the observation period of 12–120 h (Fig. 5). Moreover, BPH growth and development were significantly inhibited on the pre-NIL plants, and the average number of surviving BPHs on the pre-NIL plants decreased more quickly than that on the susceptible plants at 7, 8 and 9 days after release (Fig. 6b). We previously found that the BPH-resistance genes (e.g., *BPH6*, *BPH12*) also confer antixenosis and antibiosis to the BPH insects. *BPH6* and *BPH12* deterred the BPHs from settling on the NIL plants within 120 h of release. The decreased survival rates of the BPHs on NIL-*BPH6* or NIL-*BPH12* compared to Nipponbare were significantly different at the eighth or ninth day after the BPH infestation (Qiu et al. 2010, 2012).

Potential exploitation of BPH-resistance genes for rice improvement

Compared with dominant resistance genes to BPH, little progress has been made in recessive resistance genes. Fine mapping of *BPH27* on chromosome 4, thus, provides useful information for understanding the molecular mechanisms underlying the recessive gene-mediated resistance. In future studies, we will validate the candidate genes in the fine-mapped region by sequencing parental lines and transforming susceptible parent with cloned *BPH27* candidate genes. In addition, a number of simple molecular markers will be exploited to assist in the development of rice cultivars that are resistant to multiple BPH biotypes.

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