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Mapping of a broad-spectrum brown planthopper resistance gene, *Bph3*, on rice chromosome 6

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Abstract The brown planthopper (BPH) is one of the most destructive insect pests of rice in Thailand. We performed a cluster analysis that revealed the existence of four groups corresponding to the variation of virulence against BPH resistance genes in 45 BPH populations collected in Thailand. Rice cultivars Rathu Heenati and PTB33, which carry *Bph3*, showed a broad-spectrum resistance against all BPH populations used in this study. The resistant gene *Bph3* has been extensively studied and used in rice breeding programs against BPH; however, the chromosomal location of *Bph3* in the rice genome has not yet been determined. In this study, a simple sequence repeat (SSR) analysis was performed to identify and localize the *Bph3* gene derived from cvs. Rathu Heenati and PTB33. For mapping of the *Bph3* locus, we developed two backcross populations, BC_1F_2 and BC_3F_2, from crosses of PTB33 × RD6 and Rathu Heenati × KDML105, respectively, and evaluated these for BPH resistance. Thirty-six polymorphic SSR markers on chromosomes 4, 6 and 10 were used to survey 15 resistant (R) and 15 susceptible (S) individuals from the backcross populations. One SSR marker, RM190, on chromosome 6 was associated with resistance and susceptibility in both backcross populations. Additional SSR markers surrounding the RM190 locus were also examined to define the location of *Bph3*. Based on the linkage analysis of 208 BC_1F_2 and 333 BC_3F_2 individuals, we were able to map the *Bph3* locus between two flanking SSR markers, RM589 and RM588, on the short arm of chromosome 6 within 0.9 and 1.4 cM, respectively. This study confirms both the location of *Bph3* and the allelic relationship between *Bph3* and *bph4* on chromosome 6 that have been previously reported. The tightly linked SSR markers will facilitate marker-assisted gene pyramiding and provide the basis for map-based cloning of the resistant gene.

Keywords Biotype · *bph4* · BPH resistance gene · Broad-spectrum · *Nilaparvata lugens* · SSR marker

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Introduction

The brown planthopper (BPH), *Nilaparvata lugens* Stål, is one of the most serious insect pests of rice. The damage caused by BPH feeding has

the greatest effect on the growth and crop yield of the susceptible rice plant through the removal of assimilates and the reduction in photosynthetic rate of leaves. Plant death can occur if the amount of energy supplied is less than that required for tissue maintenance (Watanabe and Kitagawa 2000; Yuan et al. 2005). The BPH not only feeds on the rice plant directly but also transmits viruses that cause severe diseases of rice (Heinrichs 1979). The application of BPH resistance genes has been recognized as the most economic, effective measure for BPH management and the most environmentally friendly. To date, up to 20 resistance genes and several quantitative trait loci (QTL) associated with BPH resistance have been reported and reviewed (Alam and Cohen 1998; Chen et al. 2006; Huang et al. 1997, 2001; Ishii et al. 1994; Jairin et al. 2005; Jena et al. 2003, 2006; Jeon et al. 1999; Kawaguchi et al. 2001; Liu et al. 2001; Mei et al. 1996; Murai et al. 2001; Murata et al. 1998, 2001; Renganayaki et al. 2002; Sharma et al. 2003a, b; Soundararajan et al. 2004; Su et al. 2002; Sun et al. 2005; Wang et al. 2001; Xu et al. 2002; Yan et al. 2002; Yang et al. 2002). Among these, *Bph1, bph2, Bph3* and *bph4* have been used extensively in Thai breeding programs. Improved rice cultivars carrying *Bph1, bph2* and *bph4*, however, have lost their ability against BPH in most of the rice growing areas in Thailand, and rice cultivars carrying *Bph3* have shown a higher degree and broader-spectrum of resistance against the BPH.

The rice cultivars PTB33 and Rathu Heenati demonstrate resistance to all BPH biotypes identified at IRRI and in some field populations in Asia, including India, Philippines, Vietnam, China, Bangladesh, Laos and Thailand (Angeles et al. 1986; Jairin et al. 2005; Khush 1984; Li et al. 2002; Soundararajan et al. 2004; Velusamy et al. 1995). The dominant BPH resistance gene *Bph3* was first identified in cvs. Rathu Heenati acc. no. 11730 and PTB33 acc. no. 19325 (Ikeda 1985; Lakshminarayana and Khush 1977). PTB33 was found to carry two BPH resistance genes, *bph2* and *Bph3*, and the inheritance of the digenic control of the resistance to BPH in PTB33 has been confirmed (Angeles et al. 1986). The gene *Bph3* was reported to be tightly linked to a recessive resistance gene, *bph4*, in cv. Babawee (Ikeda and Kaneda 1981; Sidhu and Khush 1979), and this allelic relationship has also been confirmed (Angeles et al. 1986). These two allelic resistance genes were first assigned to rice chromosome 10 based on trisomic analysis (Ikeda and Kaneda 1981); however, a recent fluorescence in situ hybridization (FISH) study found that *Bph3* was physically localized on rice chromosome 4 (Yan et al. 2002). Moreover, a major BPH resistance gene (*Bph*17) in cv. Rathu Heenati was also assigned to rice chromosome 4 (Sun et al. 2005). However, *bph4* from cv. Babawee has been assigned to the short arm of rice chromosome 6 (Kawaguchi et al. 2001).

The aim of this study was to clarify and confirm the map position of the *Bph3* locus on the rice linkage map using two backcross populations and simple sequence repeat (SSR) markers.

Materials and methods

Plant materials

A differential set of ten BPH-resistant cultivars with known resistant genes for BPH, including Mudgo [acc. no. 6663 (*Bph1*); Athwal et al. 1971], ASD7 [acc. no. 6303 (*bph2*); Athwal et al. 1971], Rathu Heenati [acc. no. 11730 (*Bph3*); Lakshminarayana and Khush 1977], PTB33 (acc. no. 19325 (*bph2* and *Bph3*); Lakshminarayana and Khush 1977], Babawee (acc. no. 8978 (*bph4*); Lakshminarayana and Khush 1977], ARC10550 [acc. no. 12507 (*bph5*); Khush et al. 1985], Swarnalata [acc. no. 33964 (*Bph6*); Kabir and Khush 1988], T12 (acc. no. 56989 (*bph7*); Kabir and Khush 1988], Chin Saba [acc. no. 33016 (*bph8*); Nemoto et al. 1989] and Pokkali (*Bph9*; Nemoto et al. 1989), were used to identify the variation of virulence among the BPH populations collected from rice fields in Thailand.

To map the *Bph3* locus, we obtained 208 progenies of a BC_1F_2 population obtained from a cross between the donor parent PTB33 (acc. no. 19325) and the recurrent parent RD6 and 333 progenies of a BC_3F_2 population obtained from a cross between Rathu Heenati (acc. no. 11730), the donor parent, and the recurrent parent KDML105

and used these as mapping populations. The BC_1 generation resulted from the backcrossing of the F_1 generation with the recurrent parents. Only a resistant BC_1 line was selected to generate BC_1F_2 of the cross of PTB33 × RD6. For the cross of Rathu Heenati × KDML105, the second backcross (BC_2) and the third backcross (BC_3) generations were derived from the cross of selected resistant BC_1 and BC_2 plants, respectively, to the recurrent parent. RD6 and KDML105 are susceptible to BPH, whereas PTB33 and Rathu Heenati show a high degree of resistance to BPH. All BPH resistance cultivars used in this study were obtained from the International Rice Research Institute (IRRI), Philippines.

BPH populations and bioassay for BPH resistance

Forty-five BPH populations were collected from rice fields in 31 provinces of the northeastern, northern, central and southern regions of Thailand. The insect populations were reared on rice cultivar TN1 in a temperature-controlled room maintained at a light regime of 15/9-h light/dark and day/night temperatures of 26–28°C. The BPH colonies were employed for BPH bioassays after four to six generations of the insects. The resistant cultivars with specific resistance genes were screened to identify the variation of virulence among BPH populations using the standard seedbox screening (SSBS) technique according to Heinrichs et al. (1985). To determine the variation among BPH population, the resistance or susceptibility of the differential set of ten BPH resistance cultivars was converted into binary data. Similarity matrices were calculated with Dice's coefficient and the SIMQUAL program of NTSYS-PC. Cluster analysis was carried out within the SAHN program using the UPGMA method.

The modified mass tiller screening (MMTS) technique, which was modified from the modified seedbox screening technique (Velusamy et al. 1986) and the tiller seedbox screening technique (Wang et al. 2001), was used to evaluate the BPH resistance of the 208 BC_1F_2 and 333 BC_3F_2 progenies. First, the seeds of TN1, a susceptible cultivar, PTB33, RD6, Rathu Heenati, KDML105 and each BC_1F_2 and BC_3F_2 progeny was separately sown in the seedling plots. The seedlings were transplanted into 7×24-m^2 plots when the seedlings had three to four tillers (approximately 20–25 days). Ten days after transplanting the seedlings were infested with third to fourth instar nymphs of the BPH at a density of ten insects per tiller. One of the most virulent BPH populations, collected from Ubon Ratchathani (UBN) province, was used for the infestation. We let the insects feed, mate, lay eggs and hatch freely. Until TN1 and the susceptible recurrent parents died, we evaluated the severity scores of each BC_1F_2 and BC_3F_2 individuals according to the standard evaluation system (International Rice Research Institute 1996) with minor modifications. All tests were conducted under greenhouse condition.

Determining the map location of BPH resistance gene

A rapid CTAB DNA isolation technique (Chen and Ronald 1999) was used with minor modifications for extracting total DNA from young rice leaves. Based on the results of the BPH bioassays from the MMTS, we generated two groups of 15 resistant (R) and 15 susceptible (S) progenies from each of the backcross populations, BC_1F_2 and BC_3F_2, derived from crosses of PTB33 × RD6 and Rathu Heenati × KDML105, respectively. Thirty-six polymorphic SSR markers, including 13 markers covering a genetic distance of 5.4–151.1 cM on chromosome 4, 14 markers covering a genetic distance of 2.3–105.1 cM on chromosome 6 and nine markers covering a genetic distance 17.6–113.0 cM on chromosome 10 (McCouch et al. 1997, 2002), were selected to identify the individual progenies in the R and S groups. Seven previously reported SSR markers (RM8213, RM261, RM6487, RM401, RM190, RM469, RM204) tightly linked to *Bph3* and *bph4* (Ikeda and Kaneda 1981; Kawaguchi et al. 2001; Sun et al. 2005; Yan et al. 2002) were the target loci of the BPH resistance genes. A STS marker, KAM4, which completely co-segregated with *bph2* (Murai et al. 2001), was used to

determine the *bph2* locus in the PTB33 and the R/S individuals from the BC_1F_2 progenies of the PTB33 × RD6 cross.

A linkage analysis was performed using the 208 BC_1F_2 and 333 BC_3F_2 individuals. Recombination values were calculated by JoinMap ver. 3.0 (Van Ooijen and Voorrips 2001) with LOD scores greater than 3.0. Map distances were calculated using the Kosambi function (Kosambi 1944). The genetic contribution to the phenotypic resistance by the chromosome region was analyzed using MapQTL ver. 5 at LOD threshold 3.0 (Van Ooijen 2004).

Results

In order to investigate genetic variation in the BPH populations in Thailand, we identified 45 BPH populations based on the differential set of ten resistant cultivars using SSBS. We found that variations in virulence occurred among BPH populations against resistant cultivars carrying specific resistance genes (Table 1), indicating that variations of virulence do exist among BPH populations from different geographic locations. We subsequently classified the 45 BPH populations into four major groups based on a similarity relationship of more than 0.88 (Fig. 1). Only two resistance cultivars, Rathu Heenati and PTB33, both carrying *Bph3*, showed a broad-spectrum of resistance against all BPH populations used in this study (Table 1). Therefore, we attempted to determine the *Bph3* locus on the rice linkage map. For this, we obtained two backcross populations, BC_1F_2 and BC_3F_2, from crosses of PTB33 × RD6 and Rathu Heenati × KDML105, respectively. Both PTB33 and Rathu Heenati expressed a strong resistance to BPH biotypes in Thailand, while RD6 and KDML105 were completely susceptible to the BPH. The F_1 plants of PTB33 × RD6 and Rathu Heenati × KDML105 showed resistance to the BPH, indicating that BPH resistance in PTB33 and Rathu Heenati might be controlled by a dominant gene (Table 2). The resistance score of the 208 BC_1F_2 and 333 BC_3F_2 lines infested with the BPH population showed a continuous distribution (Fig. 2). We then studied the segregation of BPH resistance in both backcross populations by directly assaying the phenotypes of the BC_1F_2 and BC_3F_2 individuals and found that resistant and susceptible BC_1F_2 and BC_3F_2 plants segregated in a 3:1 segregation ratio (χ^2=1.17, P>0.28; χ^2=0.03, P>0.86, respectively), which indicated the presence of a major dominant gene conferring resistance to BPH.

Table 1 Reaction of a differential set of resistant cultivars with specific resistance genes to some BPH populations collected in Thailand using the standard seedbox screening technique. The BPH populations were randomly selected from a total of 45 populations

Cultivar	R gene[a]	Reaction of rice cultivar to brown planthopper populations[b]																
		YST	NST	KLS	UBN	CYP	NAN	CMI	ANJ	SKW	NKM	ATG	BRR	SPR	CCS	SRN	SRNI	CNT
TN1	none	8.9	9.0	9.0	9.0	9.0	8.7	9.0	8.6	9.0	9.0	9.0	9.0	8.6	9.0	9.0	9.0	9.0
Mudgo	*Bph1*	7.6	4.4	9.0	8.6	7.2	6.6	8.4	7.0	4.5	5.5	6.2	6.6	6.1	6.3	9.0	7.5	8.3
ASD7	*bph2*	7.8	9.0	9.0	9.0	7.0	3.8	7.9	6.0	8.4	1.4	6.2	7.1	6.0	8.1	7.2	6.1	9.0
Rathu Heenati	*Bph3*	3.0	3.0	1.0	2.1	3.0	2.6	4.4	1.3	1.3	1.2	4.3	4.1	1.0	3.0	3.0	1.0	1.3
PTB33	*bph2, Bph3*	3.0	1.0	2.1	2.6	2.5	2.5	3.6	3.0	1.1	3.3	4.4	4.3	1.2	3.4	2.8	3.0	2.4
Babawee	*bph4*	3.9	6.4	7.8	6.7	6.4	7.3	7.6	3.8	6.9	6.3	7.7	3.6	2.0	6.1	8.4	4.1	6.5
ARC10550	*bph5*	8.7	9.0	9.0	9.0	6.5	9.0	7.0	9.0	7.2	9.0	8.3	4.0	7.0	6.3	9.0	8.1	9.0
Swarnalata	*Bph6*	3.8	4.0	2.6	8.6	3.7	1.3	8.0	1.2	1.1	1.5	1.7	1.0	1.8	1.0	5.6	9.0	4.4
T12	*bph7*	8.3	7.9	8.2	9.0	4.0	4.3	8.3	7.6	8.0	3.0	6.1	7.0	3.7	4.3	7.4	6.4	6.8
Chin Saba	*bph8*	7.4	9.0	8.7	9.0	9.0	8.5	9.0	6.7	9.0	9.0	8.5	8.3	6.9	6.4	9.0	8.0	9.0
Pokkali	*Bph9*	9.0	8.9	9.0	9.0	9.0	9.0	8.5	9.0	7.5	9.0	8.8	8.2	8.7	9.0	9.0	9.0	9.0

[a] R gene, BPH resistance gene

[b] Average damage score: scale 1–9 (1 = most resistance, 9 = most susceptible)

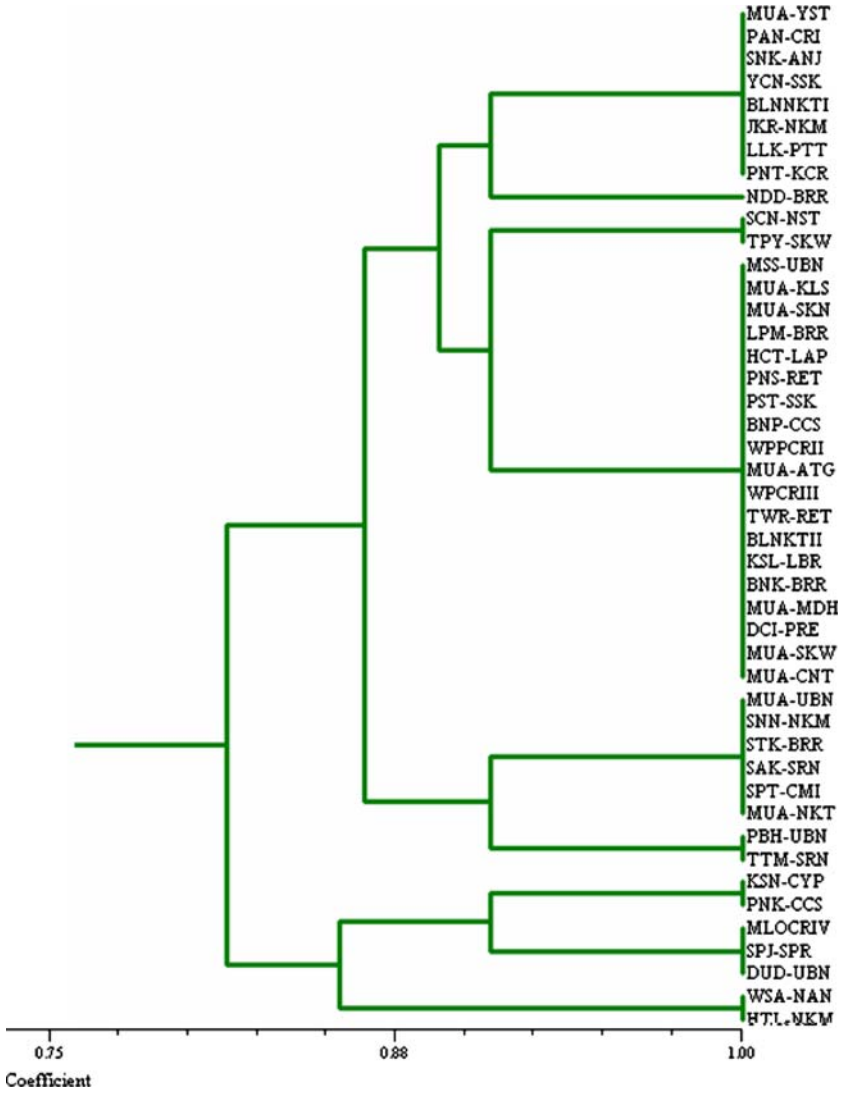

Fig. 1 Cluster analysis of 45 BPH populations collected from the rice fields in Thailand, based on the damage scores of the differential set of ten BPH resistance cultivars. The dendrogram was constructed using UPGMA based on Dice similarity coefficients. Scale of the dendrogram is the Dice coefficient of similarity

Table 2 Average damage score (scale: 1–9) of the parents and F_1 population to the BPH population collected from Ubon Ratchathani (UBN) province, Thailand

Cultivar	SSBS[a]	MMTS[b]
TN1	9.0	9.0
KDML105	9.0	9.0
RD6	9.0	9.0
PTB33	2.6	1.0
Rathu Heenati (Acc No.11730)	2.1	1.0
F_1 (PTB33 × RD6)	3.1	–
F_1 (Rathu Heenati × KDML105)	3.0	–

[a] SSBS, Standard seedbox screening technique

[b] MMTS, Modified mass tiller screening technique

To determine the map location of the BPH resistance gene, we assayed BC_1F_2 individuals in the R and S groups with 36 polymorphic SSR markers on chromosome 4, 6, and 10 in order to determine which of the SSR markers were associated with resistance/susceptibility. This analysis showed that SSR marker RM190 on chromosome 6 was strongly associated with the resistance/susceptibility (Fig. 3) and that none of the SSR markers tested were significantly associated with BPH resistance on chromosomes 4 and 10. These results indicated that the BPH resistance gene from PTB33 was linked to RM190 on chromosome 6. Furthermore, RM190 was used to identify R and S groups of the BC_3F_2 population from the cross of Rathu Heenati × KDML105. This analysis revealed that RM190 was also strongly asso-

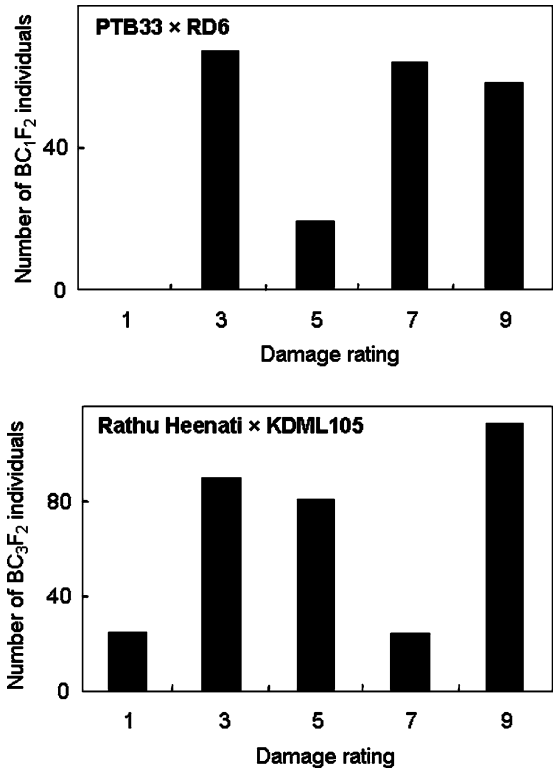

Fig. 2 Frequency distribution of BPH damage rating of two backcross populations using the modified mass tiller screening technique. The damage severity scores of the donor parents, PTB33 and Rathu Heenati, were 1 while that of the recurrent parents, RD6 and KDML105, was 9

ciated with the R and S groups of the BC_3F_2 (data not shown). The BPH resistance gene in PTB33 and Rathu Heenati was therefore also located in the same region on chromosome 6.

To further confirm the chromosome location of the resistant gene, we employed additional SSR markers surrounding the RM190 locus. Of 20 SSR markers tested, only six showed polymorphism between the parents. RM190 and six additional SSR markers on chromosome 6 were used to assay 208 BC_1F_2 and 333 BC_3F_2 progenies. A linkage map was constructed with LOD scores greater than 3.0 based on the segregation data. In the linkage map constructed for chromosome 6, the order of all SSR markers agreed with that of the standard SSR map (McCouch et al. 2002). However, the estimated distances of some markers were larger than those of the standard map. The BPH resistance locus detected from the BC_1F_2 and BC_3F_2 populations was mapped between two flanking markers RM589 and RM588 on the short arm of chromosome 6 within 0.9 and 1.4 cM of these markers, respectively (Fig. 4). The tightly linked marker RM589 and RM586 explained 59.8 and 57.4% of the phenotypic variance of BPH resistance with high LOD scores of 41.1 and 61.6 in the BC_1F_2 and BC_3F_2 populations, respectively (Table 3).

Discussion

We report the localization of the major resistance gene *Bph3* to the short arm of rice chromosome 6 based on analyses utilizing SSR markers previously located near the *bph4* locus. The tightly linked SSR markers identified in this study should clarify role of the *Bph3* locus carried by the resistant donors PTB33 and Rathu Heenati and should be useful in maker-assisted breeding programs aimed at developing improved BPH resistance cultivars.

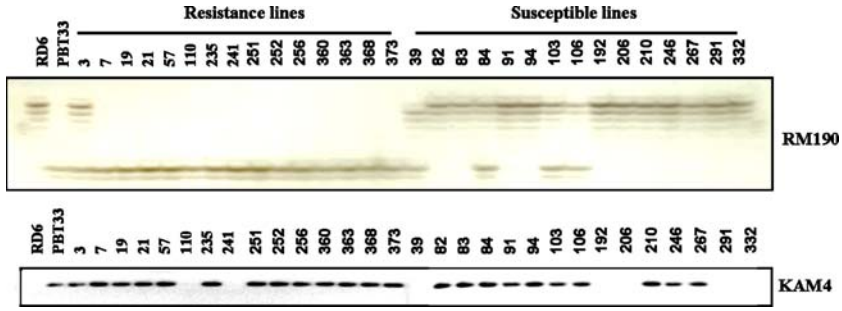

Fig. 3 The simple sequence repeat (SSR) marker RM190 and the sequence-tagged site (STS) marker KAM4 linked to BPH resistance gene *Bph3* and *bph2*, respectively. The markers were identified in resistance and susceptible individual lines of the BC_1F_2 population, which were derived from a cross between PTB33 and RD6

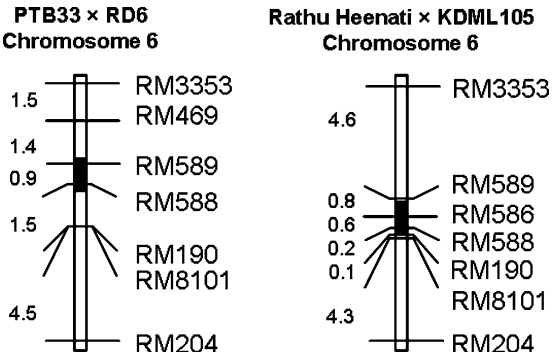

Fig. 4 Linkage maps of the BPH resistance gene *Bph3* on short arm of chromosome 6. Marker names are listed on the *right* of the chromosome. The distance between markers is in centiMorgans. The *solid bars* indicate the location of the BPH resistance gene *Bph3*

The resistant gene *Bph3* has been used extensively in rice breeding programs in Asia since 1980 (Khush 1984). This is also the case in Thai breeding programs, and in Thailand *Bph3* is still effective against BPH populations. Based on cluster analysis, we found that the field BPH populations in Thailand demonstrated varying levels of virulence and, consequently, were able to classify four different groups of BPH populations based on a similarity relationship of more than 0.88 (Fig. 1). This indicated that at least four different biotypes of BPH are present in Thailand. PTB33 and Rathu Heenati had been reported earlier to carry *Bph3* and confer a high level of broad-spectrum resistance against BPH (Angeles et al. 1986; Kabir and Khush 1988; Khush 1984; Khush et al. 1985; Li et al. 2002; Nemoto et al. 1989; Sidhu and Kush 1979). Our study also found that PTB33 and Rathu Heenati showed resistance against all of the BPH populations tested, indicating the broad-spectrum of resistance carried by these cultivars against the BPH biotypes found in Thailand. As well as the importance of resistant genes in a breeding program showing a broad spectrum of resistance, the durability of resistant genes is also important as a longer durability will slow down the appearance of virulent biotypes (Heinrichs 1986). Based on our unpublished data from the selection experiments, PTB33 retains a resistance to the BPH collected from UBN of at least 30 generations.

Because SSR markers are co-dominant, multiallelic and available at a high density in the rice genome, which is approximately one SSR every 157 kb (McCouch et al. 2002), these markers can be used to scan and identify the target regions associated with interested traits. To find molecular markers tightly linked to the *Bph3* locus, we used SSR markers surrounding the target regions that had been identified in previous studies. Applying this approach, we were able to detect markers associated with the major resistance gene. Based on the SSR analysis and linkage analysis, we assigned the major resistance gene *Bph3* to the short arm of rice chromosome 6. It should be noted that *Bph3* from PTB33 and Rathu Heenati was a major resistance gene against BPH populations, which was used in this study, since the tightly linked marker RM589 and RM586 could explain 59.8 and 57.4% of the phenotypic variance, respectively (Table 3). However, according to the frequency distribution of the damage rating of the two backcross populations, BPH resistance in PTB33 and Rathu Heenati is likely to be controlled by a major and other minor resistance genes.

Although *Bph3* has been reported to be present on chromosomes 4 and 10 (Ikeda and Kaneda 1981; Sun et al. 2005; Yan et al. 2002), our study did not detect any selected SSR markers on those chromosomes that were associated with the R and S groups. The resistant gene *Bph3* on rice chromosome 4 assigned by Yan et al. (2002) was

Table 3 The SSR markers associated to the BPH resistance gene on rice chromosome 6 in two backcross mapping populations

Backcross population	Chromosome	Source	Marker	LOD	PVE (%)	Additive	*P*
BC_1F_2 (PTB33 × RD6)	Chr6	PTB33	RM589	41.1	59.8	– 2.259	>0.0001
	Chr6	PTB33	RM588	35.3	54.3	– 2.283	>0.0001
BC_3F_2 (Rathu × KDML)	Chr6	RATHU	RM589	57.5	54.9	– 2.641	>0.0001
	Chr6	RATHU	RM586	61.6	57.4	– 2.665	>0.0001
	Chr6	RATHU	RM588	61.5	57.3	– 2.673	>0.0001

derived from *Oryza officinalis*; however, this resistant gene was later designated as a new resistance gene, *Bph15* (Yang et al. 2004). The resistant gene *Bph12* from *O. latifolia* was also reported in the same region on the short arm of chromosome 4 (Yang et al. 2002). Recently, a new major resistance gene, tentatively designated as *Bph17*, derived from Rathu Heenati has been reported in the same region of *Bph15* and *Bph12* (Sun et al. 2005). Our study did not detect any significant *Bph17*-tightly linked markers (RM8213, RM6487, RM401) on chromosome 4 as reported earlier by Sun et al. (2005). The result obtained in our study showed that the major resistance gene carried by Rathu Heenati was tightly linked to *bph4* on chromosome 6, which has been reported earlier by Kawaguchi et al. (2001).

Several studies have detected major BPH resistance genes using different BPH biotypes. BPH biotypes 1 and 2 were used previously to identify *Bph3* and determine the allelic relationship between *Bph3* and *bph4* (Angeles et al. 1986; Heinrichs et al. 1985; Ikeda and Kaneda 1981; Lakshminarayana and Khush 1977; Sidhu and Khush 1978). Sun et al. (2005) also used a mixture of biotypes 1 and 2 to identify the major resistance gene in Rathu Heenati on chromosome 4; however, the resistant gene *bph4* was assigned to chromosome 6 (Kawaguchi et al. 2001). We suggest that the resistant gene detected by Sun et al. (2005) must be a new BPH resistance gene. The present study and that of Sun et al. (2005) detected two different major resistance genes in Rathu Heenati, perhaps because different germplasm sources of Rathu Heenati was used. These observations may provide insights into some of the issues concerning germplasm sources of BPH resistance in mapping studies. *Bph3* was first identified in Rathu Heenati accession no. 11730 against BPH biotypes 1, 2, 3, 4 and some biotypes in Thailand (Angeles et al. 1986; Heinrichs et al. 1985; Jairin et al. 2005; Lakshminarayana and Khush 1977). The same accession number of Rathu Heenati provided by IRRI was also used to determine the *Bph3* locus in the present study.

PTB33 has been reported to carry two major resistance genes, *bph2* and *Bph3*. Since the BPH population used in this study was completely adapted to the *bph2* gene, we could only detect the *Bph3* locus associated with the R and S individuals. Another resistant cultivar, ASD7, carrying *bph2* was also susceptible to the BPH population collected from UBN (Table 1). To investigate this, we employed the PCR-based STS marker, KAM4, which showed complete co-segregation with *bph2* on rice chromosome 12 (Murai et al. 2001), to survey ASD7 and R and S groups of BC_1F_2 derived from the PTB33 × RD6 cross. Since the KAM4 is a dominant STS marker, we found that the amplified fragment appeared only in PTB33 and ASD7 but did not appear in RD6. The amplified fragments were detected in progenies from both R and S individuals (Fig. 3). This result probably indicates that the *bph2* gene in PTB33 is not effective against the BPH population used in this study. However, the interaction between two major resistance genes in PTB33 would require further investigation.

Kawaguchi et al. (2001) reported that the resistance gene *bph4* from Babawee was located near restriction fragment length polymorphism marker C76A on the short arm of chromosome 6. According to the standard linkage map of SSR constructed by McCouch et al. (2002), the tightly linked marker C76A is located in the same position as the RM190 locus. Our study indicated that the distance between RM190 and RM589, the tightly linked marker to *Bph3* locus, is about 2.4 cM. Therefore, this study clarifies the location of the broad-spectrum resistance gene *Bph3* and confirms the genetic analysis by classical genetic approach of Ikeda and Kaneda (1981) and Sidhu and Khush (1979) that *Bph3* in Rathu Heenati and PTB33 is tightly linked to the *bph4* in Babawee. These results also confirm those of Kawaguchi et al. (2001) using two backcross mapping populations that *Bph3* and *bph4* are localized on the short arm of rice chromosome 6. The resistance gene *Bph3* detected in the present study should be further validated through high-resolution mapping and candidate gene analysis. The tightly linked markers to *Bph3* gene will facilitate marker-assisted breeding to improve BPH resistance of rice cultivars as well.

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