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Detection of a brown planthopper resistance gene *bph4* at the same chromosomal position of *Bph3* using two different genetic backgrounds of rice

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The brown planthopper (BPH) resistance gene *bph4* has previously been assigned on the short arm of rice chromosome 6. However, the map position of the gene could not be determined. To detect the *bph4* locus, 15 polymorphic simple sequence repeat (SSR) markers covering genetic distance of 0.0–63.4 cM on chromosome 6 were used to survey 15 BPH resistant (R) and susceptible (S) individuals from each of the 95 and 78 F_2 populations derived from crosses of TN1/Babawee and Babawee/KDML105, respectively. One SSR marker, RM586, was associated with the R and S from the F_2 populations. Additional markers surrounding the RM586 locus were examined to define the location of *bph4*. From the genetic linkage map and QTL analysis of 95 and 78 F_2 individuals, the *bph4* locus was mapped at the same chromosomal region of *Bph3* between two flanking markers RM589 and RM586. Markers linked to the resistance gene explained 58.8–70.1% of the phenotypic variations and can be used for marker-assisted selection in BPH-resistant breeding programs. In addition, our experiment provides evidence that a recessive gene could behave as a dominant gene under different genetic backgrounds.

Key Words: rice, brown planthopper, *Nilaparvata lugens*, *bph4*, Babawee, SSR marker.

Introduction

The brown planthopper, *Nilaparvata lugens* Stål (Homoptera: Delphacidae), is one of the most significant factors leading to substantial decrease in rice yield (Duck and Thomas 1979, Watanabe and Kitagawa 2000). Excessive use of insecticides is a major cause of BPH outbreaks in rice fields (Chelliah and Heinrichs 1980, Yin *et al*. 2008). The concept of utilizing resistance genes to deal with the BPH problem has been considered as a promissing approach to control this insect pest (Khush 1979). Currently, up to 26 major BPH resistance genes and several QTL associated with BPH resistance have been identified from cultivated and wild rice species (Alam and Cohen 1998, Jairin *et al.* 2007b, Phi *et al.* 2009, Rahman *et al*. 2009, Rongbai *et al.* 2006, Soundararajan *et al*. 2004, Su *et al*. 2002, Sun *et al.* 2005, Zhang 2007). Among them *Bph1*, *bph2*, *Bph3* and *bph4* have been widely used in breeding programs (Khush and Virk 2005, Voramisara and Sa-nguansaj 1994).

The recessive gene *bph4* can resist against BPH biotypes 1–4 identified at IRRI and some field populations in Thailand (Jairin *et al*. 2007a). The *bph4* gene was first identified in rice cultivar Babawee (Sidhu and Khush 1979), and it was reported to be closely linked to a dominant gene *Bph3* in cultivars Rathu Heenati and PTB33 (Ikeda and Kaneda 1981, Sidhu and Khush 1979). From the study of genetic analysis by the classical genetic approach, *Bph3* was shown to be closely linked to *bph4* as no recombinants between these genes were observed among nearly 1,200 of F_3 progenies (Sidhu and Khush 1979). The allelic relationship of the genes was later confirmed (Angeles *et al*. 1986).

Previous studies found that *Bph3* was physically localized on rice chromosome 6 (Jairin *et al*. 2007a) and *bph4* was assigned to the short arm of chromosome 6 (Kawaguchi *et al*. 2001). However, the precise location of the *bph4* gene could not be determined, and it needs to be investigated. To clarify and confirm the map position, we attempted to determine the *bph4* locus on the rice linkage map using two small mapping populations and SSR markers.

Materials and Methods

Plant materials

A total of 95 and 78 F_2 progenies derived from crosses of TN1/Babawee and Babawee/KDML105 respectively were used as mapping populations and to evaluate the BPH resistance. TN1 and KDML105 are susceptible to the BPH, whereas Babawee shows resistance to the BPH. Rathu Heenati and TN1 were used as resistant and susceptible controls, respectively.

Insect population and bioassay for BPH resistance

One BPH population (biotype 4), which was collected from the rice field in Ubon Ratchathani province, was used

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to screen the mapping populations. The insects were reared on rice cultivar TN1. The BPH colony was employed for BPH bioassays after 15 generations of rearing without introgression of wild-caught individuals. The modified mass tiller screening (MMTS) technique was used to evaluate the BPH resistance of F_1 and F_2 individuals at tillering stage of rice plants according to our previous study (Jairin *et al*. 2007a). Briefly, the seeds of TN1, Babawee, Rathu Heenati, KDML105 and each F_2 progenies were separately sown in the seedling plots. When the seedlings had 4–5 tillers, three tillers of each plant were transplanted in a 2×12-m plot. The main tiller of each plant was transplanted into the separate plot for collecting seeds. Ten days after transplanting, the seedlings were infested with 3rd to 4th instar nymphs of the BPH at the density of 10 insects per tiller. Then, 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 F_2 individual following the Standard Evaluation System for Rice proposed by International Rice Research Institute (IRRI 1996).

Tagging and mapping of the 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 result of the BPH bioassay from the MMTS, we generated two groups of 15 resistant (R) and 15 susceptible (S) progenies from each F_2 population. Fifteen polymorphic SSR markers covering genetic distance of 0.0–63.4 cM on the short arm of chromosome 6 (McCouch *et al*. 2002) were selected to identify the individual progenies in R and S groups.

The genetic linkage map was canculated by JoinMap 4 (Van Ooijen and Voorrips 2001) using genotype data of 95 and 78 F_2 individuals derived from crosses of TN1/Babawee and Babawee/KDML105, respectively. Genetic distances were calculated using the Kosambi function (Kosambi 1944). The resistance gene position on the linkage map was analyzed by interval mapping in MapQTL 5 (Van Ooijen 2004).

Results

Evaluate of the BPH resistance

The donor parent Babawee and a referent cultivar Rathu Heenati expressed strong resistance to the BPH population used in this study, while TN1 and KDML105 were completely susceptible to the BPH. TN1 was more susceptible than KDML105 to the BPH. The F_1 plants of the cross of TN1/Babawee were susceptible, whereas the F_1 plants from the Babawee/KDML105 cross were moderately resistant to the BPH (Table 1). The distribution of the resistance score of the 95 F_2 progenies from the cross of TN1/Babawee was skewed towards susceptibility (Fig. 1). The segregation of genotypes at the *bph4* region and phenotypes of the F_2 followed the 1RR : 2RS : 1SS and 1R : 3S ratio respectively, which indicated a major recessive resistance gene (Table 2).

Table 1. Segregation of phenotypes in 95 and 78 F_2 individuals derived from crosses of TN1/Babawee and Babawee/KDML105, respectively

Mapping populations	Number of F_1 and F_2 individuals		χ^2 value		P
	Resistant	Susceptible			
F_1 TN1/Babawee[a]	0	19			
F_1 Babawee/KDML105	20	0			
F_2 TN1/Babawee	24	71	1:3	0.01	0.95
F_2 Babawee/KDML105	56	22	3:1	0.43	0.51

[a] The average damage scores of F_1 individual plants from crosses of TN1/Babawee and Babawee/KDML105 were 9 and 4.9 respectively.

Table 2. Segregation of genotypes in 95 and 78 F_2 individuals derived from crosses of TN1/Babawee and Babawee/KDML105, respectively

Mapping populations	F_2 genotype[a]	Number of F_2 individuals		
		Resistant	Susceptible	Total
F_2 TN1/Babawee	RR[b]	17	1	18
	RS	6	44	50
	SS	1	26	27
	Total	24	71	95
F_2 Babawee/KDML105	RR[c]	15	0	15
	RS	38	2	40
	SS	3	20	23
	Total	56	22	78

[a] Genotypes were analyzed using SSR marker (RM589) linked to resistance gene *bph4*. R refers to Babawee allele and S refers to TN1 or KDML105 alleles.
[b] χ^2 of 1RR: 2RS: 1SS = 1.97 (p = 0.37).
[c] χ^2 of 1RR: 2RS: 1SS = 1.69 (p = 0.43).

The resistance score of the 78 F_2 individuals of the Babawee/KDML105 cross showed a continuous distribution (Fig. 1). The genotypes and phenotypes of the F_2 individual plants segregated in 1RR : 2RS : 1SS and 3R : 1S ratio, respectively (Table 1 and Table 2). The segregations indicated the presence of a major dominant gene conferring resistance to the BPH.

Detection of BPH resistance gene bph4

To detect the map position of the resistant locus, we assayed F_2 individuals in the R and S groups with 15 polymorphic SSR markers on chromosome 6 in order to determine which of the SSR markers were associated with R/S groups. This analysis showed that SSR marker RM586 was the most strongly associated with the R/S groups from TN1/Babawee and Babawee/KDML105. This result indicated that the BPH resistance gene from Babawee was linked to RM586 in both F_2 populations. We employed additional SSR markers surrounding the RM586 locus. Of 35 SSR markers tested, only 5 and 9 markers showed clearly distinguishable polymorphisms between the parents of TN1/Babawee and Babawee/KDML105 crosses, respectively. The additional markers were used to assay 95 and 78 F_2 progenies. The segregation

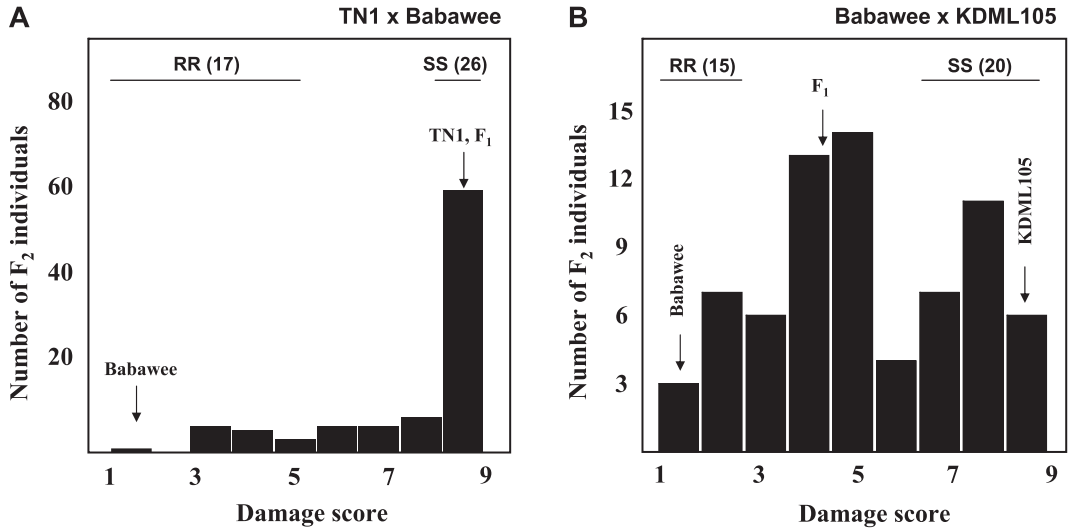

Fig. 1. Frequency distribution of BPH damage scores of 95 and 78 F_2 progenies derived from crosses of TN1/Babawee (A) and Babawee/KDML105 (B) respectively. The damage severity scores of the donor parent Babawee was 1.5 while the recurrent parents KDML105 and TN1 were 9. RR: homozygous resistant, SS: homozygous, all the others were heterozygous.

of most SSR loci in the F_2 populations fitted the expected 1 : 2 : 1 genetic ratio, whereas three loci (RM133, RM19320 and RM469) showed slight distortion segregation biased towards the KDML105 parent. A genetic linkage map was constructed based on the segregation data (Fig. 2). 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) and the public database released by Gramene (http://www.gramene.org/). However, the estimated distances of some markers were larger than those of the standard map. To determine the resistance gene position, QTL analysis was performed using the MapQTL 5 software. The resistance gene was detected in the interval between two flanking markers, RM589 and RM586 on chromosome 6 (Table 3 and Fig. 2), with 58.8% and 70.1% of phenotypic variation of BPH resistance in the TN1/Babawee and Babawee/KDML105 crosses, respectively. The resistant locus had the respective additive effect indicating that the allele responsible for BPH resistance was from the donor parent Babawee (Table 3).

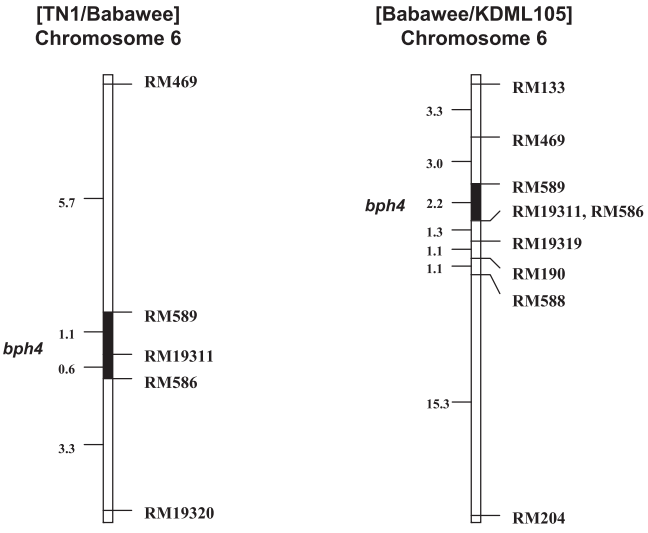

Fig. 2. Mapping of the BPH resistance gene *bph4* on the short arm of chromosome 6. Marker names are listed on the right of the chromosome. The distance between markers is in centimorgan. The solid bars indicate the location of a *bph4* gene.

Discussion

The BPH resistance gene *bph4* was first identified in the Sri Lankan *indica* rice cultivar Babawee (Lakshminarayana and Khush 1977). We found that *bph4* from Babawee introgressed into the cultivar TN1 behaved as a major recessive gene, whereas it behaved as a major dominant gene when introgressed into KDML105. According to previous studies, recessive resistance genes can behave as dominant genes under different genetic backgrounds or with different insect biotypes (Murai *et al*. 2001). In the present study, we used only one insect population (biotype 4) to screen the F_2

Table 3. The SSR markers associated to BPH resistance gene on the rice chromosome 6 in F_2 populations

Rice population	Source	Marker interval	LOD	PVE (%)	Additive	*P*
F_2 (TN1/Babawee)	Babawee	RM589-RM586	18.2	58.8	–2.507	< 0.001
F_2 (Babawee/KDML105)	Babawee	RM589-RM586	19.1	70.1	–2.905	< 0.001

populations. The result revealed that the dominance or recessiveness of *bph4* depends on the different genetic backgrounds. A similar result was obtained with another recessive BPH resistance gene. The *bph2* gene was first found in a cultivar ASD7 and behaved as a recessive gene in a cross of TN1/ASD7 (Athwal *et al*. 1971). However, the studies on the resistance segregation in a large number of F_2 and F_3 progenies from crosses of ASD7/C418 and Tsukushibare/Norin-PL4, respectively confirmed the dominant nature of *bph2* (Murai *et al*. 2001, Murata *et al*. 1998).

Although *bph4* was first detected on the short arm of chromosome 6 using two mapping populations, the map position of the gene could not be determined (Kawaguchi *et al*. 2001). In this study, we present genetic mappings of the *bph4* on chromosome 6 between two flanking markers RM589 and RM586. *Bph3* was reported to be closely linked to *bph4*. The allelic relationship between *Bph3* and *bph4* has been previously reported in several studies (Angeles *et al*. 1986, Ikeda and Kaneda 1981). Sidhu and Khush (1979) found that no recombinants between *Bph3* and *bph4* genes were observed among the large number of F_3 progenies. Not surprisingly, our result mapped *bph4* in the same region as *Bph3* on chromosome 6. It is possible that these BPH resistance genes may share the same genomic sequence or resistant mechanisms, or they are different loci but very closely linked to each other. Further study is needed to investigate and prove these hypotheses.

The large number of BPH resistance genes identified so far has provided sources for marker-assisted breeding to develop BPH-resistant cultivars. Successful MAS for BPH resistance genes had already been reported in rice. Sharma *et al*. (2004) succeeded in performing a molecular marker-assisted pyramiding of two BPH resistance genes, *Bph1* and *bph2*, into a *japonica* line. Our recent study was also successful in introducing *Bph3* into a Thai aromatic rice cultivar KDML105 using the MAS approach (Jairin *et al*. 2009). The SSR markers linked to the *bph4* locus in this study would also be useful for MAS in BPH-resistant breeding programs.

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