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Detection of Brown Planthopper Resistance Genes from Different Rice Mapping Populations in the Same Genomic Location

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ABSTRACT: Three rice mapping populations of 208 BC_1F_2, 333 BC_3F_2 and 335 F_2 lines derived from crosses of PTB33 × RD6, Rathu Heenati × KDML105 and IR71033-121-15 × KDML105, respectively, were used to detect brown planthopper (BPH) resistance genes. The modified mass tiller screening (MMTS) method was applied to evaluate the BPH resistance of all mapping population lines at the tillering stage. The BPH resistance genes detected from the BC_1F_2, BC_3F_2 and F_2 populations were mapped in the same genomic region on the short arm of chromosome 6. The tightly linked markers RM589 and RM586 could explain 59.8%, 28.2% and 57.4 % of the phenotypic variance of the BPH resistance from the BC_1F_2, F_2 and BC_3F_2, respectively. The tightly linked SSR markers identified from this study should be useful in marker-assisted breeding to produce BPH resistant cultivars.

KEYWORDS: BPH resistance gene, modified mass tiller screening (MMTS), *Nilaparvata lugens*, *Oryza minuta*, SSR marker.

INTRODUCTION

Rice (*Oryza sativa* L.) is the primary food source for nearly half of the world's population. Economically, rice is among the top three export commodities in Thailand. The widespread damage caused by insect pests is one of the main biotic constraints on rice production. Brown planthopper (BPH), *Nilaparvata lugens* (Stål), a destructive monophagous insect pest, is considered to be the most devastating insect in the rice-producing areas in Thailand. In addition to feeding on rice plants directly, BPH also causes indirect damage by acting as a vector for the viruses that cause ragged and grassy stunt diseases.

The application of resistant rice cultivars has been recognized as the most economic and effective measure for BPH management. The genetic basis of qualitative and quantitative BPH resistance has been well studies and up to 19 major resistance genes (*Bph1* to *bph19*) have been discovered from cultivated rice and wild relatives. Of these genes, 15 resistance genes (*Bph1* to *bph4* and *Bph9* to *bph19*) have been assigned to rice chromosomes. According to previous studies, some of BPH resistance genes derived from native indica cultivars and wild species of *Oryza* tend to be clustered in particular regions of rice chromosomes. There are four main clusters of BPH resistance genes, which are found on chromosomes 3, 4, 6 and 12. Two major resistance genes and two QTLs are clustered together on chromosome 3[1-3], as are three major resistance genes on chromosome 4[4,5], three major resistance genes and two QTLs on chromosome 6[1,3,6] and five major resistance genes and one QTL on chromosome 12[7-10].

Although, up to 19 major BPH resistance genes have been discovered, more than half of them could not be used against some BPH populations found in Thailand[11]. Breeding resistant cultivars with major resistance genes has been highly successful, however, BPH itself has also successfully adapted to feed on the resistant cultivars by changes in its biotypes. Some Thai rice cultivars carrying BPH resistance genes, such as RD1, RD21, CNT1, SPR1, SPR2 and SPR90, have been bred and released, but the resistance was broken down by the occurrence of biotypes virulent to the resistant cultivars within a few years. The occurrence of new virulent biotypes has been a serious problem in breeding rice cultivars resistant against BPH. Therefore, selection of BPH resistance genes for improving resistant cultivars needs to be considered carefully.

Two resistance rice cultivars, Rathu Heenati, a local variety in Sri Lanka, and PTB33, an Indian local variety, were found to confer a strong and broad-spectrum resistance to BPH in Thailand[11]. BPH resistance in these two local varieties was concluded to be controlled by

a single dominant gene, *Bph3*[12]. By a classical genetic approach, *Bph3* was shown to be closely linked to *bph4* in the rice cultivar Babawee, because no recombinants between these genes were observed[13]. The chromosomal locations of these two resistance genes were first assigned to rice chromosome 10 based on trisomic analysis[14]. Later, it was suggested that *Bph3* was located on chromosome 4[15] and *bph4* was located on chromosome 6[16].

O. minuta, a wild species of rice, has been considered to be one of the most important resources for BPH resistance. Several introgression lines having genes for BPH resistance from the wild species have been developed at the International Rice Research Institute (IRRI). Seven BPH resistance genes from *O. australiensis*, *O. oûcinalis*, *O. latifolia* and *O. eichingeri* have been tagged with molecular markers,[3,8,17] while the resistance genes from *O. minuta* have yet to be located on a rice chromosome. Therefore, an *O. minuta*-derived resistance line, IR71033-121-15, was used as the donor to determine the location of its BPH resistance locus.

In this study, we attempted to identify the map location of BPH resistance genes from three different mapping populations. The tightly linked markers identified from this study should be useful in marker-assisted selection in Thai breeding programs.

Materials and Methods

Plant Materials

Three different populations, BC_1F_2, BC_3F_2, and F_2, derived from crosses of PTB33 × RD6, Rathu Heenati × KDML105, and IR71033-121-15 × KDML105, respectively, were used as mapping populations. KDML105 and RD6 were used as recurrent parents, while PTB33 (acc. no. 19325), Rathu Heenati (acc. no. 11730) and *O. minuta*-derived resistance line IR71033-121-15 were used as donor parents of BPH resistance genes.

The BC_1 from the cross of PTB33 × RD6 resulted from the backcrossing of the F_1 generation with the recurrent parents, RD6. Only one resistant BC_1 line was selected to generate BC_1F_2. 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, KDML105. The F_2 population was generated from F_1 from a cross between IR71033-121-15 and KDML105. All donor parents were obtained from IRRI.

Evaluation of BPH Resistance

The BPH population was collected from rice fields in Ubon Ratchathani province, Thailand. The insect population was cultured on rice cultivar TN1 in a temperature-controlled room under 15/9-h light/dark and day/night temperatures of 26-28°C. The BPH colony was employed for BPH bioassays after 6 generations of the insects. All donor and recurrent parents were screened for BPH resistance using three bioassays, the standard seedbox screening (SSBS) method, MMTS method and semi-field screening (SMFS) method.

The SSBS method was used to measure the levels of resistance of parents and progenies at the seedling stage. The pre-germinated seeds of test lines were sown 5 cm apart in 20 cm rows in seedboxes. The susceptible check, TN1, was sown at random in all the seedboxes. Seven days after sowing, the seedlings were infested with 1st to 2nd instar nymphs of BPH at the rate of ten to fifteen nymphs per seedling. Damage rating of the test lines was done when 90% of the plants in the susceptible check row were killed. The test lines were graded on a scale of 1 (very slight damage) to 9 (all plants dead) using the standard evaluation system (SES) of rice[18].

The SMFS method was conducted in the rice field. Twenty-day-old plants of the test entries were transplanted in the field with a plant spacing of 10 cm within rows and 20 cm between rows. The field was covered with nylon mesh (20×20×2 m). Fifteen days after transplanting, the seedlings were infested with 4th to 5th instar nymphs of BPH at the rate of ten to fifteen nymphs per hill. When TN1 and the susceptible parents died, we evaluated the severity scores of the test entries according to the SES.

The MMTS method was modified according to Wang et al[3] and was used to evaluate the BPH resistance of 208 BC_1F_2, 333 BC_3F_2 and 335 F_2 individuals under greenhouse conditions. In brief, the seeds of TN1, PTB33, IR71033-121-15, Rathu Heenati, RD6, KDML105 and the BC_1F_2, BC_3F_2 and F_2 progenies were separately sown in the seedling plots. When the seedlings had 3-4 tillers (approximately 20-25 days), tillers with similar growth conditions were separated and were transplanted in 7x24 m^2 plots. Before transplanting, the rice leaves of each individual progeny were cut and prepared for the DNA extraction. Ten days after transplanting, the seedlings were infested with 3rd to 4th instar BPH nymphs at the rate of 10 insects per tiller. Then, we let the insects feed, mate, lay eggs and hatch freely. When TN1 and the susceptible recurrent parents died, we evaluated the severity scores of each BC_1F_2, BC_3F_2 and F_2 individuals according to the SES.

Determining the Map Location of BPH Resistance Genes

A rapid CTAB DNA isolation technique[19] was used 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 mapping populations.

Thirty-six polymorphic SSR markers[20], including 13 markers on chromosome 4, 14 markers on chromosome 6 and 9 markers on chromosome 10, were selected based on previous studies[14-15] to identify the individual progenies in the R and S groups. Of the 36 SSR markers tested, seven previously reported SSR markers (RM8213, RM261, RM6487, RM401, RM190, RM469, RM204) tightly linked to *Bph3* and *bph4* were the target loci of the BPH resistance genes in the BC_1F_2 and BC_3F_2 populations.

Fifty-five polymorphic SSR markers covering 12 chromosomes (approximately 4-5 SSR markers to each chromosome) were selected as anchor markers and were identified in the individual progenies in the R and S groups derived from the F_2 population.

A linkage analysis was performed using 208 BC_1F_2, 333 BC_3F_2 and 335 F_2 individuals. Recombination values were calculated by JoinMap ver. 3.0[21] with LOD scores greater than 3.0. Map distances were calculated using the Kosambi function[22]. The genetic contribution to the phenotypic resistance by the chromosome region was analyzed using MapQTL ver. 5[23] at a LOD threshold of 3.0.

Results

Screening for BPH Resistance in the Parents and Progenies

PTB33, Rathu Heenati and IR71033-121-15 expressed strong resistance to BPH collected in Ubon Ratchathani province, while RD6 and KDML105 were completely susceptible to the BPH (Table 1). The F_1 plants of PTB33 × RD6, Rathu Heenati × KDML105 and IR71033-121-15 × KDML105 showed resistance to the BPH (Table 1) indicating that BPH resistance in PTB33, Rathu Heenati and IR71033-121-15 might be under dominant gene control. The mean frequency histogram distributions of variables scores in the resistance tests are shown in Fig. 1. Segregations of BPH resistance in all populations were studied by directly assaying the phenotypes of BC_1F_2, BC_3F_2 and F_2 individuals. A plant was classified as resistant if the average severity score was less than 7, and as susceptible if the average severity score was greater than 7. The resistant and susceptible BC_1F_2, BC_3F_2 and F_2 plants showed a 3:1 segregation ratio (χ^2=1.17, χ^2=0.03 and χ^2=0.72, respectively).

Table 1. Average damage score of the parents and F_1 populations exposed to the BPH.

Cultivar	SSBS[a]	MMTS[b]	SMFS[c]
TN1	9.0	9.0	9.0
KDML105	9.0	9.0	9.0
RD6	9.0	9.0	9.0
PTB33	2.6	1.0	1.4
Rathu Heenati (acc no.11730)	2.1	1.0	1.1
IR71033-121-15	3.0	1.1	2.0
F_1 (PTB33 × RD6)	3.1	-	-
F_1 (Rathu Heenati × KDML105)	3.0	-	-
F_1 (IR71033-121-15 × KDML105)	3.5	-	-

[a] SSBS, Standard seedbox screening.
[b] MMTS, Modified mass tiller screening.
[c] SMFS, Semi-field screening.

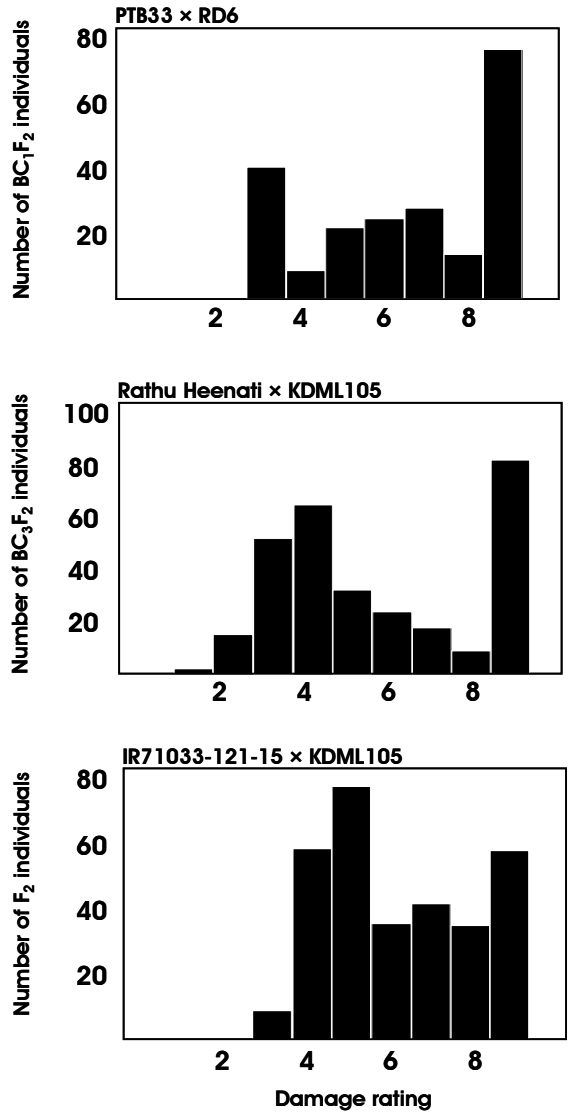

Fig 1. Frequency distribution of BPH damage rating of three mapping populations using the modified mass tiller screening technique. The damage severity scores of the donor parents, PTB33, Rathu Heenati, and IR71033-121-15 were 1, while those of the recurrent parents, RD6 and KDML105, were 9.

Detection of the BPH Resistance Genes

To determine the map location of the BPH resistance

gene in PTB33, thirty-six polymorphic SSR markers on chromosomes 4, 6, and 10 were used to assay the BC_1F_2 individuals in the R and S groups to find SSR markers associated with the R/S phenotype. The analysis showed that SSR marker RM190 on chromosome 6 was associated with the R/S phenotype. We did not detect any significant SSR marker associated with BPH resistance on chromosomes 4 and 10. The result indicated that the BPH resistance gene from PTB33 was linked to RM190 on chromosome 6. Furthermore, when RM190 was used to identify the R and S groups of the BC_3F_2 from the cross of Rathu Heenati × KDML105, RM190 was also strongly associated with the R and S groups of the BC_3F_2 (data not shown). This indicates that the BPH resistance genes in PTB33 and Rathu Heenati are located in the same region on chromosome 6.

To determine the locus of BPH resistance in IR71033-121-15, fifty-five polymorphic SSR markers spanning the twelve rice chromosomes were selected and used to identify R and S groups of F_2 from the cross of IR71033-121-15 × KDML105. Only markers near the RM190 locus on the short arm of chromosome 6 were shown to be positive. We did not detect any SSR marker associated with BPH resistance on the other chromosomes.

Mapping of the BPH Resistance Genes

To further confirm the chromosome location of the resistance genes, we employed additional SSR markers surrounding the RM190 locus. Of twenty SSR marker tested, only six showed polymorphism between the parents of the crosses PTB33 × RD6 and Rathu Heenati × KDML105 and eight showed polymorphim between the parents of the cross IR71033-121-15 × KDML105. RM190 and the six additional SSR markers on chromosome 6 were used to assay 208 BC_1F_2 and 333 BC_3F_2 progenies and the eight polymorphic SSR markers were used to assay 335 F_2 progenies. Linkage maps were constructed with LOD scores greater than 3.0 based on the segregation data. In the linkage maps constructed for chromosome 6, the order of all SSR markers agreed with that of the standard SSR map[20]. The BPH resistance loci detected from the BC_1F_2, BC_3F_2 and F_2 populations were mapped between flanking markers RM469-RM588, RM589-RM588 and RM469-RM586 on the short arm of chromosome 6, which were within 2.3, 1.4 and 5.5 cM, respectively (Fig. 2). The physical distance between the flanking markers RM469-RM588 was approximately 0.8 Mb base on the Nipponbare genomic sequence from Gramene database (http://www.gramene.org/). The tightly linked marker RM589 and RM586 explained 59.8, 28.2 and 57.4% of the phenotypic variance of BPH resistance with high LOD scores of 41.1, 24.1 and 61.6 in the BC_1F_2, F_2 and BC_3F_2 populations, respectively (Table 2). It was interesting to find that the resistance genes from different donors were mapped in the same location on the short arm of chromosome 6 using the MMTS technique, which we have developed to evaluate the BPH resistance at the tillering stage of rice.

Discussion

In this study, we report the molecular tagging of BPH resistance genes that might cluster together on the short arm of rice chromosome 6 using three different mapping populations. Based on analyses utilizing SSR markers near the BPH resistance loci that have been previously reported[14-16], we were able to localize the major resistance genes in PTB33 and Rathu Heenati. It should be noted that the resistance genes from PTB33 and Rathu Heenati were major resistance genes against BPH, since the tightly linked markers RM589 and RM586 could explain 59.8% and 57.4% of the phenotypic variance, respectively (Table 2). According to the frequency distribution of the damage ratings of the two mapping populations, the BPH resistance in PTB33 and

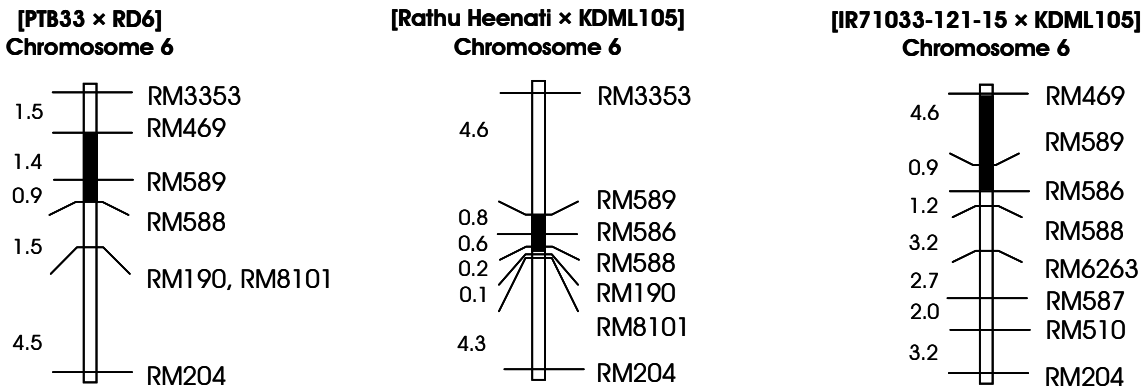

Fig 2. Linkage maps of the BPH resistance genes on the short arm of chromosome 6. Marker names are listed on the right of the chromosomes. The distance between markers is in centiMorgans. The solid bars indicate the location of the BPH resistance genes.

Table 2. SSR markers associated with the BPH resistance genes on chromosome 6 in three different mapping populations.

Backcross population	Marker	LOD	PVE (%)[a]	Additive[b]	*P*
BC_1F_2 (PTB33 × RD6)	RM469	38.6	57.5	-2.253	0.000
	RM589	41.1	59.8	-2.259	0.000
	RM588	35.3	54.3	-2.283	0.000
BC_3F_2 (Rathu Heenati × KDML105)	RM589	57.5	54.9	-2.641	0.000
	RM586	61.6	57.4	-2.665	0.000
	RM588	61.5	57.3	-2.673	0.000
F_2 (IR71033-121-15 × KDML105)	RM469	20.0	24.1	-1.118	0.000
	RM589	24.1	28.2	-1.259	0.000
	RM586	22.3	26.5	-1.255	0.000

[a] Percentage of phenotypic variation explained (%).
[b] Additive effect of donors allele.

Rathu Heenati are likely to be controlled by major and other minor resistance genes. Unlike PTB33 and Rathu Heenati, the resistance gene from IR71033-121-15 on chromosome 6 could explain only 28.2% of the phenotypic variance, indicating the presence of other possible resistance genes that was not detected in this study. The failure to identify the resistance genes in *Oryza minuta*-derived resistance line IR71033-121-15 may due to the limited numbers of SSR markers that were used in this study. So further study with more markers is needed. The tightly linked SSR markers identified from this study should be useful in marker-assisted breeding to improve BPH resistance in cultivars.

Recently, there has been great progress in identifying chromosomal regions that influence qualitative and quantitative resistance to BPH from native indica cultivars and wild species of rice. According to the information that has been reported about their locations so far, some of the BPH resistance genes and QTLs associated with BPH resistance tend to be clustered in regions of rice chromosomes, particularly on chromosomes 3, 4, 6 and 12 (Fig. 3). Five BPH resistance genes, *Bph1*, *bph2*, *Bph9*, *Bph10* and *Bph18,* and one QTL were first found to be clustered on the long arm of chromosome 12[8,17,24-25]. The cluster of BPH resistance genes on chromosome 3 has been described by Chen et al[7]. They found that two major resistance genes and two QTLs, which were derived from four different BPH resistance donors, were mapped in the same region on chromosome 3. Three resistance genes, designated as *Bph12*, *Bph15* and *Bph17* from *O. officinalis*, *O. latifolia* and Rathu Heenati, respectively, were mapped in one particular region of chromosome 4[5,15]. A recessive gene, *bph4,* from Babawee and *O. rufipogon* and two QTLs from IR64 and Teqing were reported in the same genomic location on the short arm of chromosome 6[1,16,26]. The result obtained from our work was also mapped the resistance genes from different donors in the same region on chromosome 6 near the *bph4* locus[16]. Our study did not detect any significant *Bph17*-tightly linked markers on chromosome 4 as reported earlier by Sun et al[15]. The present study and that of Sun et al[15] detected different major resistance genes in Rathu Heenati, perhaps because different germplasm sources of Rathu Heenati or different BPH populations were used[11]. Although the resistance genes were located in the same region, no evidence has yet been obtained as to whether they share the same genomic sequence or they are different loci but tightly linked to each other. The answers of these questions could be provided in the near future when the BPH resistance genes have been cloned.

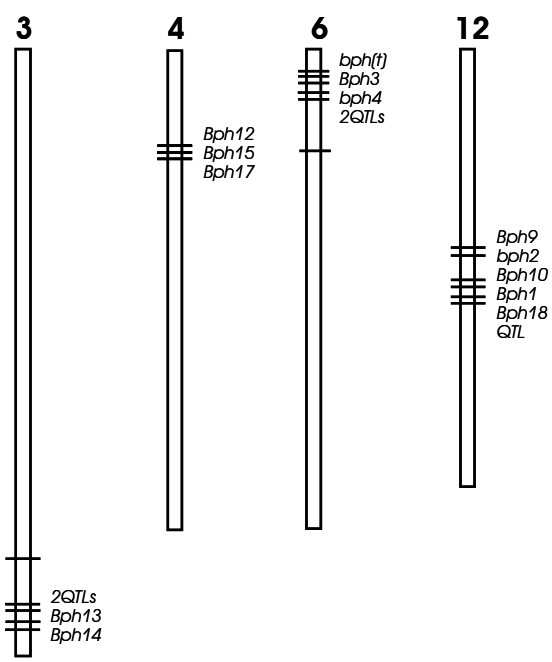

Fig 3. Locations of the BPH resistance genes and QTLs clustered on rice chromosomes 3, 4, 6, and 12, based on the standard genetic map of SSR markers of McCouch et al[20].

Although BPH resistance genes have been intensively studied and discovered throughout the rice genome, until recently none of the BPH resistance genes

have been cloned in rice and our current knowledge about insect resistance genes in rice plants is still limited. Therefore, cloning BPH resistance genes and elucidating resistance mechanisms in rice is necessary. Cloning of the genes would eventually lead to the elucidation of the differences and the evolution of the BPH resistance genes in rice.

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