ScienceAsia 31 (2005): **129-135**

Multiple Genes Determining Brown Planthopper (*Nilaparvata lugens* Stål) Resistance in Backcross Introgressed Lines of Thai Jasmine Rice 'KDML105'

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Received 26 Aug 2004
Accepted 3 Mar 2005

ABSTRACT: The brown planthopper (BPH), *Nilaparvata lugens* Stål, is one of the most serious insect pests in rice production worldwide. The BPH resistance genes in an indica cultivar 'Abhaya' were studied using 400 BC_4F_2 and F_3 backcross introgressed lines of KDML105 derived from a cross between Abhaya and KDML105. The BC_4F_2 plants were used for DNA analysis. Two local BPH populations collected from central and northeastern Thailand were used to evaluate the BPH resistance in the 400 BC_4F_3. Through bulked segregant analysis, four AFLP fragments were co-segregated with the BPH resistance. Linkage analysis revealed that these fragments were localized on rice chromosomes 6, 10 and 12. These map locations were in the same genomic regions where major BPH resistance genes or quantitative resistance loci were previously reported. These results indicated that multiple BPH resistance genes play major roles for BPH resistance in Abhaya. These genes may be a useful BPH resistance resource for rice breeding programs.

KEYWORDS: brown planthopper, Nilaparvata lugens, AFLP, bulked segregant analysis, BPH resistance gene.

INTRODUCTION

The widespread incidence of insect pests is one of the main constraints limiting rice yields in tropical environments. Among them, the brown planthopper (BPH), *Nilaparvata lugens* Stål (Homoptera: Delphacidae), is considered the most devastating insect. Excessive utilization of insecticides and a mono-culture of a single resistant cultivar are the main causes leading to an outbreak of BPH[1]. Four BPH biotypes are classified. Biotypes 1 and 2 are widely distributed in Southeast Asia, biotype 3 is a laboratory biotype produced in the Philippines and biotype 4 occurs in the Indian subcontinent. Although improving a durable and broad-spectrum resistance is necessary, little is known about the genetic control of the durable and broad-spectrum BPH resistance. To date, 22 major BPH resistance genes have been reported. Only 14 major effective BPH resistance genes in *indica* cultivars[2,3,4,5,6,7,8,9,10] and four wild relatives, *Oryza australiensis, O. officinalis, O. latifolia* and *O. eichingeri*[11,12,13,14,15] have been assigned to rice chromosomes.

Large efforts have been made to discover major BPH resistance genes from various sources. Four of these BPH resistance genes, *Bph1, bph2, Bph9* and *Bph10*, conferred resistant to different biotypes and were found to be located on rice chromosome 12[2,3,4,7,9,10,11]. The dominant *Bph1* gene was found to be closely linked with 6 DNA markers, RG463, *Sdh-1*, RRD7, RG457, C185 and XNpb248 in three rice cultivars including IR64, Gayabyeo and IR28[2,4]. The recessive *bph2* gene was linked to the *Bph1* and the estimated distance between the two BPH resistance genes was 10 cM[16]. Subsequently, a sequence tag site (STS) marker, which showed complete co-segregation with *bph2*, was found[7,16]. The dominant *Bph3* gene was linked to the recessive *bph4* gene, which has been mapped on rice chromosome 6[5]. A new dominant resistance gene has been mapped on rice chromosome 9 in Sanguizhan[6]. Recently, several quantitative trait loci (QTLs) for BPH resistance were identified on the 12 rice chromosomes[14,17,18,19]. We describe here the identification of the QTLs for BPH resistance from the resistant rice cultivar 'Abhaya' in a single desirable genetic background of KDML105.

Materials and Methods

Plant materials

Abhaya, KDML105 and ten differential rice varieties, IR64, ASD7, Rathu Heenati, Babawee, ARC10550, Swarnalata, T12, Chin Saba, Pokkali and IR65482-4-136-2-2 were screened against four BPH populations.

Four hundred BC_4F_2 plants were derived from a consecutive backcrossing between the recurrent parent, KDML105, and the donor parent, Abhaya. KDML105 is susceptible to BPH, while Abhaya is moderately resistance to BPH. The BC_4F_2 plants were used for DNA analysis to identify molecular markers tightly linked to BPH resistance. We conducted one more generation of seed propagation to increase the BC_4F_3 seeds that were used for phenotypic evaluations of BPH resistance.

BPH Bioassays

Four BPH populations collected from Pathum Thani (BPH-PTT), Ubon Ratchathani (BPH-UBN), Khon Kaen (BPH-KKN), and Pisanulok (BPH-PSL) were used to screen the 12 rice cultivars. The insects were fed on a susceptible rice cultivar, TN1, in the greenhouse. The experiment was carried out using the modified-seedbox screening method developed at IRRI[20]. Twenty-five seeds of each cultivar were sown as a single row in a 20-cm-long row in a standard seedbox (60x40x10 cm). The distance between rows was 2.5 cm. Two rows of TN1 were randomly planted among the 12 cultivars as control. Seven day old seedlings were infested with 2nd to 3rd-instar nymphs at a density of 8-10 insects per seedling. When all of the TN1 seedlings had died, plant reactions to BPH feeding were scored based on the degree of seedling damage. The scoring criteria were based on the Standard Evaluation System for Rice[21], with 1 indicating very slight damage and 9 indicating that the seedling was dead. The averages of damage scores (DS) were used to determine the degree of resistance for each cultivar.

Two of the BPH populations, BPH-PTT and BPH-UBN, were used for phenotypic evaluation for BPH resistance in the BC_4F_3 population. The phenotyping experiments were carried out using the modified-seedbox screening method (as described above) at Pathum Thani Rice Research Center (PRRC) and Ubon Ratchathani Rice Research Center (URRC) in 1997 and 1998, respectively.

Identification of Markers Linked with BPH-Resistant Genes by Bulked Segregant Analysis (BSA)

Bulked segregant analysis[22] was utilized to identify the linkage of molecular markers with BPH resistance genes. The twenty most resistant and twenty most susceptible BC_4F_3 were selected based on the DS. Total DNA was extracted from leaves of the BC_4F_2 population by the CTAB method as described by Doyle and Doyle[23]. Two bulks of DNA were made according to the strategy proposed by Michelmore et al[22]. DNA of the 20 most resistant and 20 most susceptible BC_4F_2 families were mixed with equal amounts of total genomic DNA to form the resistant bulk and susceptible bulk, respectively. The bulks and parental DNA were screened using 138 *EcoRI/MseI* primer combinations of amplified fragment length polymorphism (AFLP) and 11 rice microsatellite markers (RM). AFLP was performed as described by Vos et al[24] with minor modifications. The RMs were assayed as described by McCouch et al[25]. Two RMs on rice chromosome 4 (RM303 and RM317), four RMs on chromosomes 10 (RM244, RM216, RM239 and RM184) and five RMs on chromosomes 12 (RM83, RM101, RM179, RM277 and RM313) were localized in the genomic blocks where the BPH resistance genes have been reported[2,4,10,26]. All of PCR amplification products were fractionated by electrophoresis through 4.5% denaturing polyacrylamide gels for 1 h at 60 W and detected by sliver staining. The polymorphisms between the bulks and the parents were scored to identify the co-segregation of DNA fragments with the BPH resistance phenotype.

Localization of BPH Resistance Genes and Linkage Map Construction

AFLP fragments tightly linked with BPH resistance genes were mapped using 172 recombinant inbred lines (RIL) derived from a cross between FR13A and CT6241-17-1-5-1 as a reference population[27]. The map location was determined using JoinMap Version 2.0[28] and MAPMAKER Version 2.0[29]. The linkage map was calculated using a maximum recombination frequency of 0.3 and LOD scores greater than 6.0. Linkage analysis of 7 markers, including 3 RMs and 4 AFLP fragments, was also performed using BC_4F_2 individuals with JoinMap Version 2.0. The genetic linkage map was constructed based on LOD scores greater than 3.0. Map distances were calculated using the Kosambi function[30].

QTL Analysis

Seven markers linked to the BPH resistance genes were used to genotype the 400 BC_4F_2 individuals. Single-marker and multiple markers analyses, using the regression-based software STAT-GRAPHICS 2.1 and ANOVA, were used to determine the numbers and effects of QTLs and to detect two loci interactions of QTLs for BPH resistance.

Results and Discussion

Parental screening

In modified-seedbox screening test, 10 differential varieties and 2 parents showed different resistance reactions to the four BPH populations. Rathu Heenati carrying *Bph3*, Swarnalata carrying *Bph6*, and Abhaya were resistant to all BPH populations (Table 1). IR64 and T12, which carry *Bph1* and *bph7* genes, respectively,

Table 1. Reaction of twelve rice cultivars subjected to BPH feeding in four BPH populations, Pathum Thani (PTT), Ubon Ratchathani (UBN), Khon Kaen (KKN), and Phitsanulok (PSL). The experiment was conducted at UBN in 1999 using the modified-seedbox screening technique.

Variety	Resistance gene	Plant reaction			
		PTT	UBN	KKN	PSL
IR64	*Bph1*	MR	MS	S	-
ASD7	*bph2*	MS	S	S	S
Rathu Heenati	*Bph3*	R	R	R	R
Babawee	*bph4*	MS	S	MR	MR
ARC10550	*bph5*	MS	S	S	S
Swarnalata	*Bph6*	R	MR	MR	MR
T12	*bph7*	MR	S	S	S
Chin Saba	*bph8*	MS	MS	S	S
Pokkali	*Bph9*	MS	S	S	S
IR65482-4-136-2-2	*Bph10*	MS	MS	MS	S
Abhaya		MR	MR	MR	MR
KDML105		S	S	S	S

R=Resistance, MR=Moderated resistance, MS=Moderated susceptible, S=Susceptible, - = Missing data

were resistant to BPH-PTT but not resistant to BPH-UBN, BPH-KKN and BPH-PSL. The Babawee cultivar, which carries *bph4*, showed a specific resistance to BPH-KKN and BPH-PSL. KDML105 and cultivars that carried the recessive and dominant resistance genes *bph2, bph5, bph8, Bph9* and *Bph10* were susceptible to all BPH populations.

The donor, Abhaya, was resistant to all 4 BPH populations. Abhaya also showed a significant level of resistance to 8 BPH populations collected from various geographical areas of Thailand, according to the experiments conducted by the Department of Agriculture (DOA), Thailand during 1999-2001 (unpublished data). The adaptation rate of the BPH-UBN on Abhaya retained a significant level of resistance after 14 generations of the insect (unpublished data). Therefore, this cultivar should be a good source of BPH resistance for improving Thai rice.

Phenotypic Variations of Damage Scores (DS) in the BC_4F_3 Population

The parents differed significantly in their resistance to both BPH-PTT and BPH-UBN populations. The average DS was 3 for Abhaya and 9 for KDML105. Continuous distributions of the DS which were skewed toward susceptibility were observed in the BC_4F_3 population (Fig. 1). These data were not consistent

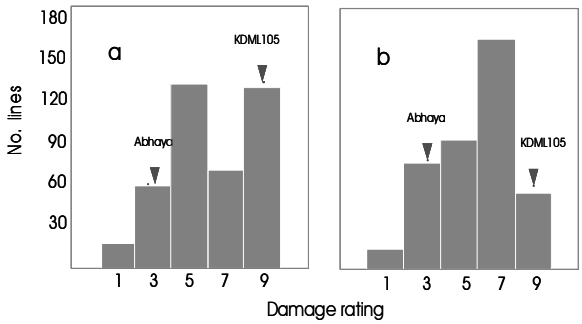

Fig 1. Frequency distributions of phenotypic values from BPH resistance tests. **a** Seedbox screening test of the 400 BC_4F_3, derived from KDML105 and Abhaya, with the Pathum Thani BPH population, **b** Seedbox screening test of the 400 BC_4F_3 with the Ubon Ratchathani BPH population. The damage ratings were based on the SES, with 1 indicating very slight damage and 9 indicating that the rice plant was dead.

with Mendelian analysis, suggesting that inheritance of the BPH resistance in Abhaya may be quantitative in nature. Transgressive segregation in the BC_4F_3 for the DS was not observed. This indicated that all resistance genes probably came from Abhaya.

Identification of DNA Markers Cosegregated with the BPH-Resistance Genes

The ultimate goal of this study is to identify the BPH resistance genes in Abhaya using BSA and QTL approaches. BSA has been extensively and successfully used for the identification of genes or DNA markers associated with qualitative and quantitative traits in many crops[3,31,32,33,34,35]. Although BSA is robust for the identification of a major gene, it is not for a minor gene with small effects[24,36].

In our study, a total of the 138 *EcoRI/MseI* primer combinations were used for the parental survey; 4 to 31% polymorphism between two parents were detected within 4,000 AFLP fragments. Thirty-six *EcoRI/MseI* primer combinations were excluded from the BSA because of the low quality of the fingerprinting (too dense or too weak) or one or more PCR amplification failures in four samples. The BSA identified the 2 RM markers, RM216 and RM277, and 36 AFLP markers

showing a polymorphism between the bulks and the parents. This indicated a co-segregation of these RM and AFLP markers with the BPH resistance genes in the BC_4F_2 population.

Forty BC_4F_2 individuals used to make the bulks were genotyped with 36 AFLP markers. Of the 36 AFLP markers, two (E4/M2-1 and E1/M13-1) completely co-segregated with the DS (Fig.2). Another two, E4/M15-1 and E5/M3-3, displayed nearly complete co-segregation with the DS. This indicated that these four markers were closely linked candidates. They were further used to identify the map location.

Localization of Candidate AFLP Markers and Linkage Analysis

Four AFLP markers, E4/M2-1, E1/M13-1, E4/M15-1 and E5/M3-3, were mapped on the existing linkage map of the RIL population. This 1,310 cM-linkage map of the RIL was previously constructed using 183 markers. E4/M15-1 was mapped near RM50 on

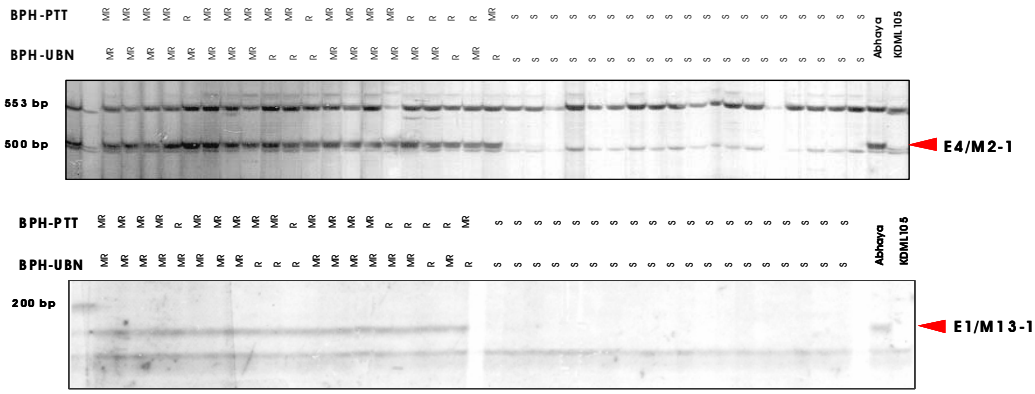

Fig 2. AFLP marker E4/M2-1 and E1/M13-1 linked to BPH resistance. The markers were identified in individual lines of the BC_4F_2 cross between susceptible KDML105 and resistant Abhaya, which were used to construct the bulks. The arrows indicate the polymorphic fragments, E4/M2-1 and E1/M13-1. The reaction of individual lines to BPH from Pathum Thani (BPH-PTT) and Ubon Ratchathani (BPH-UBN) were shown. R, MR, and S refer to resistance, moderate resistance, and susceptible, respectively.

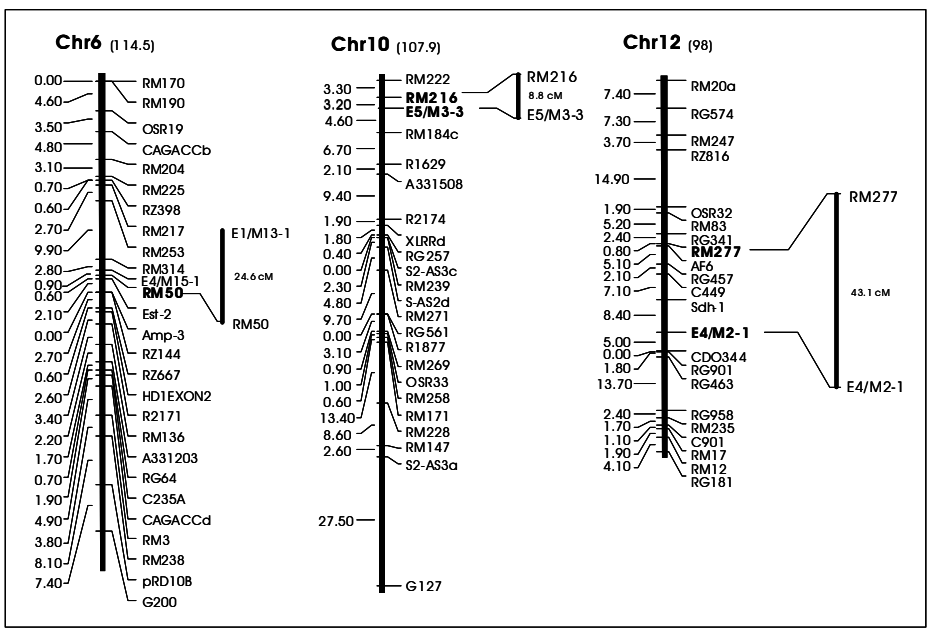

Fig 3. QTLs identified for resistance to BPH on rice chromosomes 6, 10 and 12. Mapping was done on an RIL population derived from a cross between FR 13 A and CT6241-17-1-2-1. The AFLP marker E4/M2-1 linked to BPH resistance is shown on chromosome 12. Other AFLP fragments, E4/M15-1 and E5/M3-3 markers derived from BSA, were located on chromosomes 6 and 10, respectively. The black bars on the right indicate linkage groups analyzed by JoinMap software. Genetic distances, in centiMorgans, are shown to the left of the respective maps.

Table 2. Putative QTLs detected in the BC_4F_2 population derived from a cross between Abhaya and KDML105.

BPH Population	Locus	Chromosomal Location	Effect	R-squared	*P*-value
BPH-UBN	E4/M2-1	12	-0.9886	48.7	0.0000
	RM277	12	-0.6892	15.4	0.0000
	E5/M3-3	10	-0.5536	12.9	0.0001
	RM216	10	-0.5962	12.3	0.0000
	RM50	6	-0.7881	21.6	0.0000
	E1/M13-1	6	-0.8748	32.0	0.0000
BPH-PTT	E4/M2-1	12	-1.3418	28.0	0.0000
	RM277	12	-0.9302	9.9	0.0000
	E5/M3-3	10	-0.6135	6.4	0.0068
	RM216	10	-0.5990	4.1	0.0001
	RM50	6	-0.7435	6.9	0.0000
	E1/M13-1	6	-1.1165	19.2	0.0000

BPH-UBN and BPH-PTT represent BPH populations collected from Ubon Ratchathani and Pathum Thani respectively.

chromosomes 6. E5/M3-3 was mapped on chromosome 10 with a 3.2 cM distance from RM216. E4/M2-1 was mapped on chromosome 12 between markers *Sdh-1* and CDO344. This marker was approximately 23.5 cM distance from RM277 (Fig. 3). E1/M13-1 could not be mapped using this population, because there was no polymorphism between the parents. These results might indicate three possible BPH-resistance QTLs, designated as *Qbph6, Qbph10* and *Qbph12*. Three linkage groups were also constructed based on the BC_4F_2 individuals. This result reconfirmed the linkage of these markers. Moreover, the E1/M13-1 unmapped in the RIL-linkage map was located in the same linkage group with E4/M15-1 and RM50.

QTL Analysis

Simple regression showed a significant ($P<0.0001$) association between 7 markers on chromosomes 6, 10 and 12, and the DS (Table 2). Phenotypic variance explained (PVE) by individual markers ranged from 12.3 to 48.7% of the DS at URRC and 4.1 to 28.0% at PRRC. Abhaya alleles of all loci resulted in lower DS. Two AFLP fragments, E4/M2-1 and E1/M13-1, which closely linked with BPH resistance genes, individually explained 48.6% and 32.0% of phenotypic variance at URRC and 28% and 19.2% of phenotypic variance at PRRC, respectively. When all significant markers were included in the multiple regression model, only E4/M2-1 and E1/M13-1 linked with *Qbph6* and *Qbph12* were significant at $P<0.0001$. The E5/M3-3 linked with *Qbph10* was not a significant explanatory genetic factor for the DS. This lends some support to the concept of two major QTL with one minor QTL underlying BPH resistance in Abhaya. Because the *Qbph10* contributed to such a low proportion of the genetic variation, its effect might be obscured by the presence of *Qbph6* or *Qbph12*. The *Qbph6* and *Qbph12* QTLs jointly showed a significant reduction of 2.2 and 3.3 of the DS at URRC and PRRC, respectively (Table 3).

Three mechanisms, antixenosis or non-preference, antibiosis, and tolerance, are generally recognized to function in quantitative resistance to BPH[17,19]. Some BPH resistance genes or QTLs confer resistance principally attributable to a particular mechanism. Since the DS measured in this experiment was designed to provide an overall evaluation on different resistance mechanisms, we found that *Qbph6* was located in a genomic location similar to where a QTL for antixenosis, feeding rate and DS mapped in a doubled haploid population of IR64 x Azucena[17]. Abhaya retained a significant level of resistance in more than 14 generations of selection with BPH (data not shown). It is possible that antibiosis is one of the resistance mechanisms of the *Qbph6* in reducing insect survival, growth rate, or reproduction following the ingestion of host tissue. Because of the coincidental location of *Qbph6* and the QTL for antixenosis, *Qbph6* may also confer antixenosis by repelling or disturbing insects,

Table 3. Comparison of QTL combinations for damage score (DS) within the BC_4F_3 population derived from a cross between Abhaya and KDML105.

QTL combination	Damage score (DS)*			
	BPH-UBN	Reduction of DS	BPH-PTT	Reduction of DS
Qbph6 + *Qbph12*	4.5±0.2[a]	2.2	4.2±0.2[a]	3.3
Qbph12	5.4±0.2[b]	1.3	5.5±0.3[b]	2.0
Qbph6	6.1±0.2[c]	0.6	7.2±0.4[c]	0.3
No QTL	6.7±0.1[d]	0.0	7.5±0.2[c]	0.0

*Values are expressed as Mean ± SE.
Damage score based on 1-9 scale by the modified-seedbox screening test.
BPH-UBN and BPH-PTT represent BPH populations collected from Ubon Ratchathani and Pathum Thani respectively.
The means scores with the same letter are not significantly different (LSD, $P>0.05$).

causing a reduction in colonization or oviposition. However, further independent experiments for a specific mechanism need to be carried out to address its function.

The average DS of the BC_4F_3 lines containing only *Qbph12* at URRC and PRRC was 5.4±0.2 and 5.5±0.3, while those of the BC_4F_3 lines containing only *Qbph6* was 6.1±0.2 and 7.2±0.4, respectively. The BC_4F_3 lines without these two loci were susceptible with average DS of 6.7 to 7.5 (Table 3). This provides clear evidence that Abhaya has at least two BPH resistance genes.

Acknowledgements

This research was a part of the human resource development for biotechnology project supported by the Rockefeller Foundation. We thank Ubon Ratchathani, Pathum Thani and Phitsanulok Rice Research Centers, and Khon Kaen Rice Experiment Station for the BPH screening. We also thank the Rice Gene Discovery Unit and DNA Technology Laboratory in providing lab space for the DNA and data analysis.

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