Applications of DNA-Markers to Analyze Rice Planthopper Resistance Genes

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应用 DNA 标记分析稻飞虱的抗性基因

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摘　要：简要地回顾了水稻抗飞虱的遗传位点定位和作图的新进展。来自具有不同基因组的野生稻渗入系的 4 个抗褐飞虱基因 *Bph 1*、*bph 2*、*bph 4* 和 *Bph 9*，以及 4 个暂定名抗褐飞虱基因 *Bph 10(t)*、*bph 11(t)*、*bph 12(t)* 和 *Bph 13(t)*，目前已被定位于水稻 12 条染色体中的 5 条。其中，*Bph 1*、*bph 2*、*Bph 9* 和 *Bph 10(t)* 在水稻第 12 染色体的长臂上形成 1 个连锁区段，位于 *bph 2* 位点附近约 25 cM。检测出几个对田间抗性和杀卵作用有影响的 QTL。抗白背飞虱基因 *Wbph 1*、*Wbph 2* 和 *Wbph 6(t)* 已经或暂时定位了。粳稻中对白背飞虱具有杀卵抗性的 QTL 已进行了详细的分析，在第 6 染色体的短臂上检测到有效的 QTL，在同一位点鉴定出 1 个显性的杀卵基因 *Ovc*。在杀卵基因 *Ovc* 存在时，第 1 染色体上的 1 个 QTL 和第 5 染色体上的 2 个 QTL 增加白背飞虱的卵死亡率。用 DNA 标记进行 QTL 作图可以加深人们对作物抗虫性中复杂的生理和遗传机理的理解。标记辅助选择可以加速培育具多基因抗虫性的作物，还可以将野生种中的有利抗虫特性转入改良品种中，增加作物抗虫性的持久性和遗传多样性。

关键词：水稻；品种抗性；抗性基因；褐飞虱；白背飞虱；田间抗性；数量性状位点；分子标记

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Abstract: Recent achievements in molecular tagging and mapping of genetic loci for rice planthopper resistance in rice were briefly reviewed. Four rice genes for the brown planthopper (BPH) resistance, *Bph 1*, *bph 2*, *bph 4* and *Bph 9*, and four putative BPH-resistance genes, *Bph 10(t)*, *bph 11(t)*, *bph 12(t)* and *Bph 13(t)*, introgressed from wild rice species with different genomes have so far been mapped onto 5 of 12 rice chromosomes. Of them, *Bph 1*, *bph 2*, *Bph 9* and *Bph 10(t)*, have been found forming a linkage block on the long arm of rice chromosome 12, in the vicinity of about 25 cM from the *bph 2* locus. Several QTLs affecting field resistance and ovicidal activities have also been detected. The whitebacked planthopper (WBPH) resistance genes, *Wbph 1*, *Wbph 2* and *Wbph 6(t)* have been tagged or tentatively mapped. QTLs for ovicidal resistance to WBPH in japonica rice have been analyzed in detail. The effective QTL has been detected on the short arm of chromosome 6, and a dominant ovicidal gene *Ovc* has been identified at the locus. One QTL on chromosome 1 and two QTLs on chromosome 5 increased WBPH egg mortality in the presence of ovicidal gene *Ovc*. QTL mapping with DNA-markers will increase our understandings of complicated physiological and genetic mechanisms in varietal resistance in crop plants. Marker-assisted selection will facilitate to develop insect resistant crops with polygenic basis, and to introduce valuable insect resistance traits from wild relatives into improved crop varieties in order to increase durability and genetic diversity of insect resistance in the crops.

Key words: rice; varietal resistance; resistance gene; brown planthopper; whitebacked planthopper; field resistance; quantitative trait loci; molecular marker

The brown planthopper (BPH), *Nilaparvata lugens*, and the whitebacked planthopper (WBPH), *Sogatella furcifera*, are typical rice-monophagous *r*-strategic herbivores associated with paddy ecosystems in Asia[54]. Prior to 1970, these planthoppers were not regarded as economic pests of rice in the traditional low input sustainable paddy ecosystems. However, BPH has dramatically emerged as a major pest, causing massive losses in tropical rice production under the "Green Revolution" with high-yielding IR varieties[29]. Likewise, WBPH was a secondary pest of rice in monsoonal East Asia but became an outbreak-prone pest following the introduction of high-yielding hybrid rice, the so-called "East Miracle Rice" in China[57]. It has been well documented that outbreaks of the rice planthoppers were induced by pest mis-management with insecticides as well as genetic vulnerability of high yielding rice varieties[9,29,58,66].

Outbreaks of BPH prompted the initiation of breeding programs for the development of resistant rice varieties. International Rice Research Institute (IRRI) established a standard seedbox-screening test (SSST) to facilitate screen-

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ing and selection of insect resistant germplasm[10]. Nine major genes for resistance to BPH have been identified from indica varieties by SSST[4,26,32,47]. Of them, four resistance genes were successfully incorporated into IR varieties. IR26 was first released as a BPH resistant variety in 1973. However, IR26 was defeated within as short as two years after release by the development of BPH biotypes virulent to IR26 and allied varieties with the same resistance gene[8,48,62]. Similar varietal breakdown of BPH resistance was repeated when new IR varieties having a different major gene for BPH resistance were released to cope with the varietal resistance-breaking biotype[5,7]. The rapid virulence shifts of BPH biotypes had a continuous threat to the sustainable BPH management by sequential releases of resistant varieties conferred by a single major gene. However, a few IR varieties, for example, IR64, were noticed to maintain moderate resistance to BPH even after breakdown of the major gene resistance[1,6]. It could be possible that the minor gene(s) contribute to moderate but durable field resistance to BPH. It was also suggested that a high level of host plant resistance was not necessary for the BPH management in the sound paddy ecosystems[1,6,9,39].

Five major genes conferring resistance to WBPH have been identified from indica rice varieties by SSST at IRRI[3,12,46,53,68]. Attempts have been made to introduce these genes into breeding lines, but none of the IR varieties have been bred with the objective of incorporating resistance to WBPH so far. It has been, however, pointed out that 16 of 27 IR varieties showed field resistance to the planthopper[63]. Besides, the level of resistance to WBPH varies significantly among the varieties with the same major resistance gene[11]. These evidences indicate that practical field resistance to WBPH in rice could be more commonly dependent on polygenic properties that cannot be detected by the conventional SSST.

It has been long recognized that polygenic minor gene resistance might be more durable and viable for insect pest management than a single major gene resistance. However, the difficulties in recognition and screening of such complex polygenic traits, and breeding for polygenic resistance have hampered the application of this approach. These problems could be overcome by mapping and tagging techniques for quantitative trait loci (QTL) based on high-density DNA molecular marker linkage maps of rice chromosomes, and by marker-assisted selection (MAS) for those genomic regions that improve insect resistance without adversely affecting important agronomic traits[41]. These molecular marker technologies could also be useful for a better understanding of the mechanisms of plant resistance to insect herbivores since complicated phenotypic traits could be broken up into their genotypic components by QTL mapping[76].

We briefly reviewed recent research work on molecular tagging and mapping of resistance and tolerance loci associated with the rice planthoppers, BPH and WBPH, with special references to field resistance in rice to these insect pests.

1 Mapping of brown planthopper resistance loci

Four rice genes for BPH resistance, *Bph 1*, *bph 2*, *bph 4* and *Bph 9*, and four putative BPH-resistance genes, *Bph 10(t)*, *bph 11(t)*, *bph 12(t)* and *Bph 13(t)*, introgressed

Table 1. BPH resistance genes tagged or mapped.

Gene	Chromosome	Marker		Distance /cM	Reference
		Type	Name		
Bph 1	12	RFLP	C185	−11.7	Hirabayashi & Ogawa(1996)[17]
		RFLP	XNpb248	10.9	
	12	RFLP	RG634	−2.9	Jeon *et al.* (1999)[25]
		RAPD	RRD-7	0.0	
		RFLP	RG457	2.9	
bph 2	12	RFLP	G2140	3.5	Murata *et al.* (1998)[44]
	12	AFLP	KAM-3	−0.2	Murai *et al.* (2001)[43]
		AFLP	KAM-4	0.0	
		AFLP	KAM-5	0.8	
bph 4	6	RFLP,SSR		Distal region of short arm side	Kawaguchi *et al.* (2001)[28]
Bph 9	12	RAPD	OPR04	8.8	Murata *et al.* (2000)[45]
Bph 10(t)	12	RFLP	RG457	3.7	Ishii *et al.* (1994)[24]
bph 11(t)	3	RFLP	G1318	12.4	Hirabayashi *et al.* (1995)[14]
bph 12(t)	4	RFLP	G271	2.4	Hirabayashi *et al.* (1999)[13]
		RFLP	R93	4.0	
Bph 13(t)	2	SSR	RM240	−6.1	Liu *et al.* (2001)[35]
		SSR	RM250	5.5	

from wild rice species with different genomes have so far been mapped onto 5 of 12 rice chromosomes (Table 1).

Of nine designated major genes for BPH resistance in rice, a dominant *Bph 1* gene had been first identified from local indica rice Mudgo[4], and incorporated into high-yielding IR varieties. *Bph 1* gene was located on chromosome 4 based on trisomic analysis, and by using an ebisu dwarf gene, *d-2*[20,21]. However, linkage analysis between *Bph 1* gene and restriction fragment length polymorphic (RFLP) markers disclosed that *Bph 1* gene from IR28 was located on chromosome 12, using F_3 progenies from the cross of IR28 and a susceptible japonica variety Koshihikari. *Bph 1* gene was linked with RFLP marker XNpb248 at the recombination value of 10.7%[16-18]. Possible *Bph 1* gene from IR64 was also mapped on chromosome 12 near the RFLP marker RG463 and an isozyme marker *Sdh 1* using a doubled haploid (DH) rice population derived from a cross between IR64 and japonica variety Azucena[19]. Localization of *Bph 1* gene was further defined using different plant materials. The *Bph 1* gene, which was introduced from Mudgo to a Korean variety Gayabyeo, showed complete co-segregation with a RAPD (random amplified polymorphic DNA) marker RRD-7, which was mapped to the chromosome 12 region flanked by two RFLP markers RG634 and RG457 being 5.8 cM apart[25].

The second BPH resistance gene, *bph 2*, was identified in a local indica variety, Karsamba red ASD7, which was reportedly recessive and either allelic or closely linked to *Bph 1*[4]. However, segregation analyses of BPH resistance in the progenies from crosses between a *bph 2* introgression japonica line Norin-PL4 and a susceptible japonica variety indicated that the *bph 2* gene was a dominant one[44,61]. Six RFLP markers on a large segment of chromosome 12 of Norin-PL4 was co-segregated with BPH resistance, and *bph 2* gene was mapped at 3.5 cM from the closest RFLP marker G2140 on chromosome 12. The position of *bph 2* gene was located about 30 cM apart from *Bph 1* gene[44]. A high-resolution linkage map of *bph 2* gene was constructed by using an advanced mapping population derived from a cross of Norin-PL4 and a susceptible japonica variety Tsukushibare. The *bph 2* gene was located within a 3.2 cM region containing eight amplified fragment length polymorphism (AFLP) markers[42]. One AFLP marker KAM-4 that completely co-segregated with *bph 2* gene was further converted into a PCR-based sequence-tagged-site (STS) marker[43]. A *bph 2* -linked PCR marker was also established, which was specified by the STS-specific primers synthesized based on a genomic DNA clone derived from the *bph 2* introgression japonica line Norin-PL4[60].

A recessive BPH resistance gene *bph 4* was first identified in an indica rice Babawee in Sri Lanka[32]. The *bph 4* gene was reported to be either allelic or closely linked to a dominant BPH resistance gene *Bph 3*[32]. These two genes were first assigned to chromosome 10 based on trisomic analysis[22]. Through bulked segregation analysis and linkage analysis by using RFLP and microsatellite markers, the locus of *bph 4* was assigned to the short arm of chromosome 6[28]. However, the position of *bph 4* could not be determined in the linkage maps, due to the significant deviations in the BPH resistance segregation in the two mapping populations, which were derived from the two cross combinations between Babawee carrying *bph 4* gene and susceptible varieties, either indica IR24 or japonica Tsukushibare. Significant deviations from the ratio expected for the single recessive gene model suggested that the BPH resistance in Babawee might be controlled by the major gene *bph 4* as well as by some other minor genes[28].

A dominant BPH resistance gene, *Bph 9*, which was first identified in a Sri Lankan variety Pokkali[47]. The *Bph 9* gene was mapped in the segment, which was delimited by two RFLP markers, R617 and R1709, on the long arm of chromosome 12. A RAPD marker, OPR04, was found to be closest with a map distance of 8.8 cM from the *Bph 9* locus[45].

So far four BPH resistance genes, *Bph 1*, *bph 2*, *Bph 9* and *Bph 10 (t)* have been found forming a linkage block on the long arm of rice chromosome 12 (Fig. 1). They are located in the vicinity of 25 cM from the *bph 2* locus on the standard Nipponbare/Kasalath map[44,45].

A Chinese indica rice Sanguizhan was reported to have a single dominant resistance gene to BPH[34], which was non-allelic to *Bph 1* and *Bph 3* genes, and was estimated to be located near the end of chromosome 9[40].

Wild relatives of rice are potential sources of new resistance genes to cope with the varietal resistance-breaking BPH

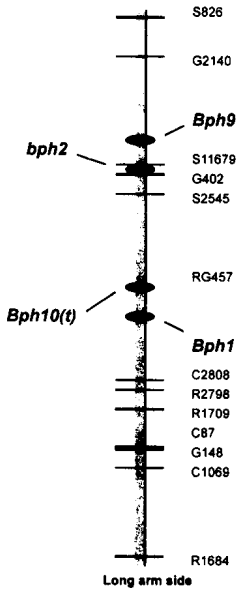

Fig. 1. Cluster of BPH resistance genes, *Bph 1*, *bph 2*, *Bph 9* and *Bph 10 (t)* on the long arm of rice chromosome 12 (modified from Murai *et al.*, 2001).

biotypes[22,65,67]. DNA markers provide a unique opportunity to monitor and tag alien genes introgressed from wild rice species to cultivated rice varieties. The BPH resistance in an introgressive line, IR65482-2-4-136-2-2, was controlled by a single dominant alien gene that was introgressed from chromosome 12 of wild rice, *Oryza australiensis* [24]. Co-segregation for BPH resistance and RFLP markers, which detected introgression from *O. australiensis* into the introgression line, indicated that the alien gene for BPH resistance is linked closely with RG457 with a distance of (3.68±1.29) cM[24].

Two recessive BPH resistance genes, *bph 11(t)* and *bph 12(t)*, were identified in indica introgression lines IR742-23-19-16-12-3 and GSK185-2, respectively, into which BPH resistance genes were introgressed from *O. officinalis* [13,14]. The linkage analysis between RFLP markers and BPH resistance showed that *bph 11(t)* was linked with G1318 at a distance of 12.4 cM on chromosome 3[14]. The other BPH resistance gene, *bph 12(t)*, was located at a small *O. officinalis* segment flanked by the RFLP markers, G271 being 2.4 cM apart and R93 being 4.0 cM apart, on chromosome 4[13]. A putative BPH resistance gene *Bph 13(t)*, which was introgressed from a wild rice *O. eichingeri*, has been mapped on chromosome 2[35].

Recombinant inbred lines derived from a cross between an introgression line M1635-7, into which BPH-resistance was introgressed from *O. minuta*, and a susceptible japonica variety Koshihikari were subjected to QTL analysis for the introgressed BPH-resistance loci [15]. Based on the seedbox screening test, four QTLs for BPH resistance were detected in the vicinities of RFLP markers C112, C1230, R902 and C751 on chromosomes 1, 5, 8 and 12, respectively. Among them, the QTL on chromosome 12 was the most effective, which explained 24.8% of phenotypic variance with a LOD score of 4.02. One of the BPH-resistance QTLs was located on a Koshihikari segment of chromosome 1.

It has long been proposed that moderate and polygenic resistance to insect pests could be more durable and stable than a single major genic resistance. The breeding of rice varieties with polygenic insect resistance has been hindered by difficulty in manipulations of quantitative traits. The DH mapping population derived from a cross between an improved indica variety IR64 and a traditional tropical japonica variety Azucena provided an interesting material to analyze QTLs for BPH resistance. IR64 has been estimated to have additional minor resistance genes as well as a major resistance gene, *Bph 1*, because it retains moderate and durable resistance to the BPH biotypes virulent to the resistant varieties with *Bph 1* gene under the field conditions[1]. Interactions between BPH and DH lines were measured by three phenotypic traits to quantify BPH responses (feeding rate, preference response, and oviposition) and three tests to evaluate rice plant reactions to BPH infestations (seedbox screening, field screening and tolerance). Totally, seven QTLs that were associated with field resistance to BPH in IR64 were mapped on 6 chromosomes[2] (Fig. 2). Their peak LOD scores ranged from 1.51 to 3.69, and the percentages of phenotypic variance ranged from 5.1% to 16.6%. These values showed that the contribution of each QTL to moderate levels of BPH resistance in IR64 was small. However, all the QTLs were identified in at least two independent phenotypic tests. No definite QTL was detected at the same map position of the major resistance gene *Bph 1* on chromosome 12, because the BPH populations employed in these experiments were almost completely adapted to the varieties with *Bph 1* gene. Therefore, the QTL analysis detected residual genetic traits conferring to moderate and durable resistance performance in IR64.

A major QTL was mapped to a segment of chromosome 6 between the markers Amp-3 and pRD10B, where seven resistance-associated phenotypes measured by BPH preference responses as well as seedbox- and field-screening tests were concentrated. Two QTLs were predominantly associated with antixenosis and tolerance. The antixenosis QTLs phenotyped by BPH settling and oviposition were mapped to the same segment near the distal end of chromosome 8, sharing the same peak interval. The tolerance QTLs based on functional plant weight loss due to BPH infestations were localized to chromosome 1. In addition, a significant QTL for BPH feeding rate, which was quantified by honeydew measurement, was identified on chromosome 3. These results indicated that a combination of diverse loci contribute to moderate and durable resistance to BPH in IR64 under the field conditions.

QTLs for moderate or quantitative resistance to BPH in a Chinese semi-dwarf indica rice Teqing were analyzed by using a set of 160 recombinant inbred (RI) lines from a cross between an American japonica Lemont and Teqing[69,70]. Chromosomal positions of moderate BPH-resistance QTLs were estimated by linkage analyses between 178 RFLP markers and damage scores of rice seedlings infested with biotype 2 BPH nymphs in the standard and modified seedbox screening tests. Five possible QTLs were detected in chromosomes 5, 9, 10 and 11. Of them, two QTLs co-segregated with RFLP markers Y1049 and R569a on chromosome 5 were the most effective. These QTLs are entirely different from those detected in the DH population from IR64 and Azucena[2].

Induction of ovicidal response in japonica rice is one of the defense mechanisms against the rice planthopper infestations. The ovicidal response to BPH in Japanese rice has been reported, although it was lower than that to WBPH[31]. QTLs for ovicidal response to BPH were analyzed by using a set of recombinant inbred lines derived from a cross between a japonica variety Asominori with ovicidal response and an indica variety IR24 without ovicidal response. Two QTLs were mapped on the short arm of chromosome 6 and the long arm

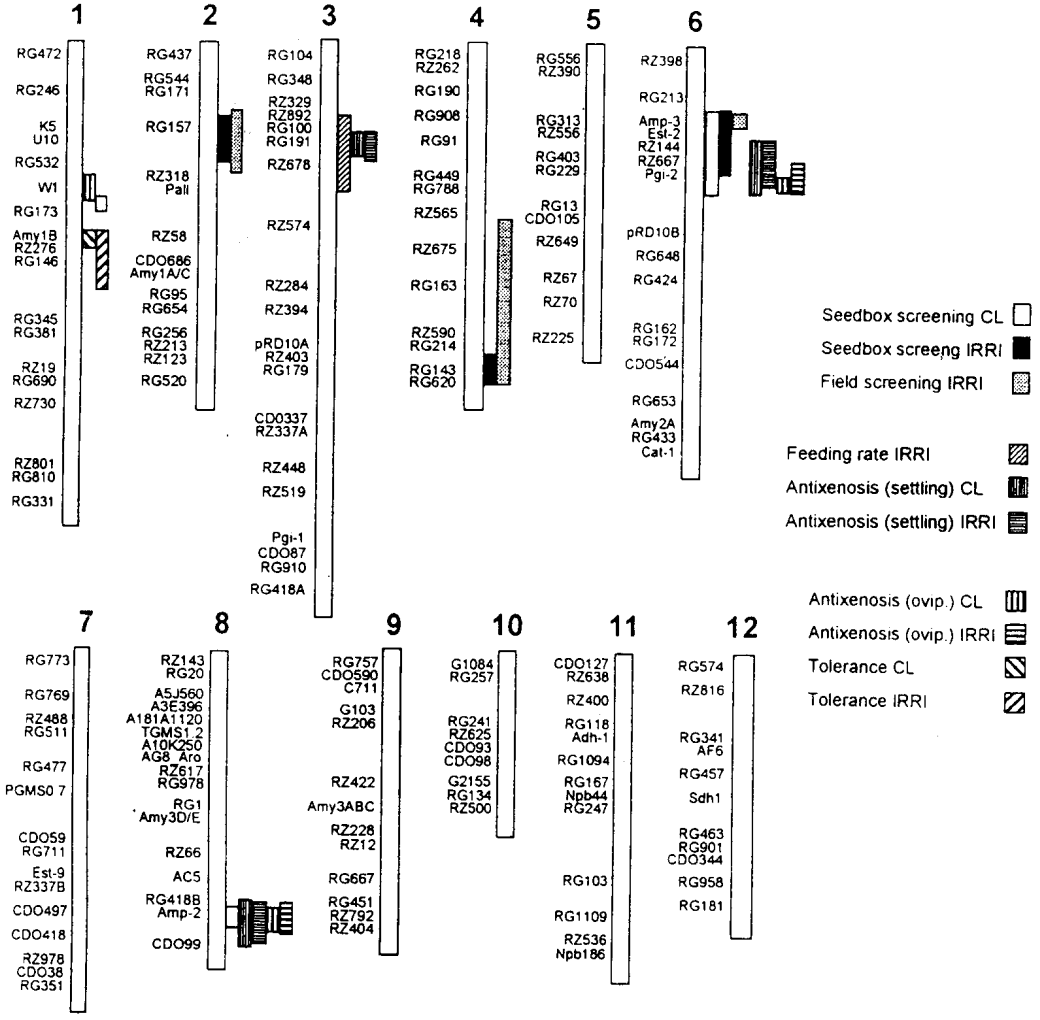

Fig. 2. QTLs for field resistance to BPH in the doubled haploid population derived from a cross between IR64 and Azucena.

CL and IRRI indicate the BPHs collected in Central Luzon and International Rice Research Institute, respectively (after Alam and Cohen, 1998).

of chromosome 1 by linkage analysis with RFLP markers[75]. The QTL linked tightly to a RFLP marker R1954 on chromosome 6 was essential for the ovicidal response, and explained 72.1% and 85.1% of the phenotypic variations for grade of watery lesions (ovicidal symptom) and egg mortality, respectively.

2 Mapping of whitebacked planthopper resistance loci

WBPH resistance genes detected by seedbox screening test, *Wbph 1*, *Wbph 2* and *Wbph 6(t)*, and a dominant ovicidal gene *Ovc* have been tagged or tentatively mapped on rice chromosomes (Table 2).

A dominant *Wbph 1* gene conferring resistance to WBPH in an indica variety N22 was tagged with two RFLP markers RG146 and RG445, although the chromosomal location of the gene remains unclear[38]. *Wbph 2*, a WBPH-resistance gene harbored in an indica variety ARC10239, was previously reported to be linked with marker genes *lg* (leguleless) and *Ph* (phenol staining) on chromosome 4 with recombination

Table 2. WBPH resistance genes tagged or mapped.

Gene	Chromosome	Marker		Distance/cM	Reference
		Type	Name		
Wbph 1	?	RFLP	RG146	0—5.2	McCouch *et al.* (1991)[38]
			RG445		
Wbph 2	6	RFLP	RZ667	25.6	Liu *et al.* (2001)[36]
Wbph 6(t)	11	SSLP	RM167	21.2	Ma *et al.* (2002)[37]
Ovc	6	RFLP	R1954	Tightly linked	Yamasaki *et al.* (1999)[73]

values of 30.8% and 37.8%, respectively[52]. However, the same gene was roughly mapped on chromosome 6 by RFLP linkage analysis, where *Wbph 2* was linked to RFLP markers RZ667, RG64 and RG265 at a distance of 25.6, 27.8 and 36.4 cM, respectively[36]. A dominant *Wbph 6 (t)* gene for WBPH-resistance has been identified in a Chinese indica rice Guiyigu[33]. A F_3 population from a cross between a WBPH-susceptible indica TN1 and Guiyigu was used for linkage analysis between RFLP and SSLP (simple sequence length polymorphism) markers. *Wbph 6 (t)* was mapped on the short arm of chromosome 11 at a distance of 21.2 cM from a SSLP marker RM167[37]. In addition to the WBPH-resistance genes mentioned above, two dominant *Wbph 3* and *Wbph 5* and one recessive *wbph 4* genes have been identified in indica germplasm accessions, but their chromosomal location has remained obscure.

A Chinese japonica variety Chunjiang 06 is highly resistant to WBPH[56]. The WBPH resistance in Chunjiang 06 is mediated by two independent genetic mechanisms, sucking inhibitory resistance and ovicidal resistance. A single dominant gene governs the sucking inhibitory resistance. We found that a putative sucking inhibitory gene is located at the position 18 cM apart from a CASP (cleaved amplified sequence polymorphism) marker P167 on chromosome 11, using F_2 populations from a reciprocal cross between Chunjiang 06 and a WBPH-susceptible indica TN1.

WBPH resistance in japonica rice is characterized by ovicidal response[55,59]. This is an induced resistance to WBPH infestation, in which WBPH eggs suffer high mortality by an ovicidal substance, benzyl benzoate, due to watery lesions in the oviposited plant tissues[51]. Ovicidal QTLs were studied in detail by using RI lines derived from a cross between an

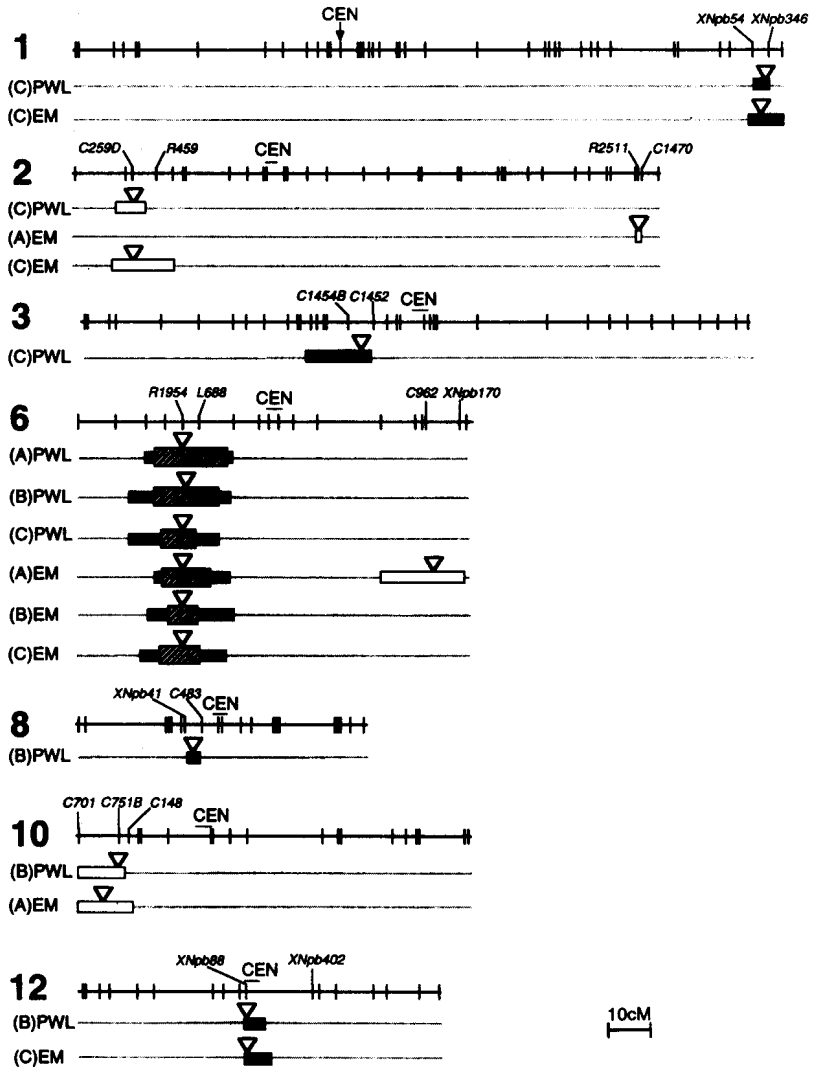

Fig. 3. Putative QTLs for ovicidal resistance to WBPH in the recombinant inbred(RI) populations derived from a cross between Asominori and IR24.

PWL: Percentage of watery lesions; EM: Egg mortality; (A),(B) and (C) indicate F_8, F_9 and F_{10} RI populations, respectively. Black and white bars indicate loci from Asominori and IR24, respectively. Hatched bars indicate the presence of indicative QTLs with LOD scores over the genome-experimentwise thresholds. Triangles indicate the position of the peak LOD scores (after Yamasaki *et al.*, 1999).

ovicidal japonica variety Asominori and a non-ovicidal indica variety IR24[71,72]. A total of 10 putative QTLs for ovicidal response have been detected on 7 of the 12 chromosomes with 292 RFLP markers by composite interval mapping (Fig. 3). Five of seven positive alleles for ovicidal response came from Asominori, while the other two were from the non-ovicidal IR24. Accumulation of QTLs from both parents was the genetic basis of the transgressive segregation in the RI populations. The QTL flanked by the RFLP markers R1954 and L688 on the short arm of chromosome 6 was most significantly associated with the ovicidal response, and accounted for 69.9% of phenotypic variance for percentage of watery lesions and 46.0% of phenotypic variance for egg mortality. A major gene *Ovc* for ovicidal response was identified at the QTL region on chromosome 6 by using a near-isogenic line, which was heterozygous only for the QTL region[73]. The ovicidal gene was dominant, and was tightly linked to RFLP marker R1954 on chromosome 6. One QTL on chromosome 1 and two QTLs on chromosome 5 were designated as *qOVA-1-3*, *qOVA-5-1* and *qOVA-5-2*, and tagged by the RFLP markers C112, R3313 and C1268, respectively. The Asominori alleles at these QTLs showed complete dominance effect, and increased egg mortality in the presence of ovicidal gene *Ovc*[74]. The IR24 allele at the QTL on chromosome 4, which was designated as *qOVA-4* and linked to the RFLP markers R2373 and R1854, also increased egg mortality in the presence of *Ovc* [71,72]. Based on the above results, it was concluded that *Ovc* is essential for ovicidal response to WBPH, and the four ovicidal QTLs enhanced the ovicidal response.

We examined QTLs affecting the WBPH performance to host plant by using a DH mapping population. The DH lines were established by anther culture of F_1 plants from the cross between Zhaiyeqing 8 (ZYQ-8, indica) and Jingxi 17 (JX-17, japonica)[77]. The QTL analysis according to the maximum scores of ovicidal symptoms for each DH line revealed two major QTLs, which localized very close to each other on the short arm of chromosome 6, and accounted for 55.3% of phenotypic variance for ovicidal symptoms. These two suggestive QTLs could be identical to the ovicidal gene *Ovc* that was previously detected by using japonica/indica RI lines[72,73]. We also found that the differential combinations of small QTLs were concerned with the expression of ovicidal symptoms at different growth stages of DH lines. Four to five small QTLs were detected in the DH lines at early- to mid-tillering stage.

In addition to the ovicidal QTLs, we detected one QTL for honeydew excretion, three QTLs in relation to the density of second-generation nymphs and three QTLs for the levels of final damages due to WBPH infestation. These minor QTLs may play certain roles in the expression of differential field resistance and/or tolerance to WBPH in rice varieties that have no major genes for WBPH resistance.

A RFLP marker-based QTL analysis using a set of DH lines derived from IR64/Azucena indicated that a major QTL associated with tolerance to WBPH was located on chromosome 11[27]. The QTL was flanked by RG103 and RG167, and explained 79% of the phenotypic variance for the tolerance parameter (plant dry weight loss per milligram of WBPH dry weight produced) with a LOD score of 7.31.

3 Applications of molecular markers to develop rice varieties with durable field resistance

Since the discovery of BPH-resistant indica local variety Mudgo at IRRI in 1967[50], breeding studies on high-yielding IR varieties with BPH-resistance have been initiated. Establishment of SSST has enabled efficient mass screening of rice germplasm accessions for insect resistance. Several genes for resistance to the rice planthoppers have been identified in the seedboxes, where the rice varieties are simply phenotyped by damage scores of seedlings exposed to preferential infestations with newly hatched nymphs of the planthoppers. Such "seedbox genes" for BPH-resistance were immediately utilized to cope with the BPH outbreaks in high-yielding IR varieties in tropical Asia[30]. IR varieties incorporated with BPH-resistance "seedbox genes" have shown dramatic effects in preventing BPH epidemics. However, their varietal resistance was not durable in the fields, and was readily defeated by the prevalence of virulent biotypes of BPH. The "seedbox genes" have not provided a thorough solution for BPH problems in high yielding paddy ecosystems.

Field resistance to insects in crop plants is most often a quantitatively inherited trait as has been exemplified in the case of resistance to the rice stemborers[30]. Field resistance to the stem borers is of a moderate level and appears to be under polygenic or QTL control. Complicated combinations of resistance mechanisms, antixenosis, antibiosis and tolerance express moderate but durable field resistance to the stem borers. TKM6 has been widely used as a source of resistance to the striped stem borer. Many IR varieties inherit more or less their moderately resistant nature against the stem borer from TKM6. Although the stem borers have continuously been endemic, the moderately resistant IR varieties have effectively suppressed destructive damages due to outbreaks or virulence shifts of the stem borers. On the contrary, it has been thought that the planthopper resistance is a typical qualitative trait governed by a single major gene. This concept largely depends upon the results obtained by internationally adopted SSST established at IRRI. In this screening system, the planthopper and rice plant interactions are over simplified to be a consequence of the intimate artificial interaction between newly hatched nymphs of planthopper and the newly germinated seedlings of rice plants. The insect-plant interaction in the seedbox screening system does not display the actual behav-

ioral and physiological processes in the host plant selection by rice planthoppers under the field conditions. Therefore, the "seedbox genes" are not necessarily the major functional genes mediating field resistance to the planthopper populations in the paddy habitats. As has been demonstrated with IR46, IR64, Triveni and Utri Rajapan, field resistance to BPH is governed by complex genetic traits other than the major genes found in the seedbox[6,49,64]. Likewise, field resistance to WBPH in several IR varieties is also expressed without the major genes that were identified in SSST[63]. We should, therefore, pay much more attention to, and understand such genetic systems that mediate durable field resistance to the rice planthoppers based on the ecological interactions between the insect pests and rice varieties under the field conditions.

QTL analysis using DNA-molecular markers increases our understanding of complicated physiological and genetic mechanisms of varietal resistance in crop plants, and genetic interactions between resistant crop varieties and host resistance-breaking insect biotypes. Detection of QTLs and major gene loci mediating specific insect and plant responses is a meaningful approach for dissecting complex over-all resistance phenomena in crop plants into individual physiological and genetic components[76]. Identification and precise chromosomal localization of functional QTLs facilitate the breeding process for more durable resistant varieties by MAS. Tagging and mapping alien genes for BPH resistance introgressed from wild rice species are now being employed to breed BPH resistant varieties with unique genetic background[13,14,15,24,35]. QTL mapping and MAS breeding could also play innovative roles in breeding insect-resistant crop plants with quantitative and polygenic basis, and in facilitating introgression of valuable insect resistance traits from wild relatives into improved varieties to increase the genetic diversity and durability of insect resistance in crops.

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