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Detection of quantitative trait loci (QTL) associated with resistance to whitebacked planthopper (*Sogatella furcifera*) in rice (*Oryza sativa*)

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Abstract

Whitebacked planthopper (WBPH) is an important insect pest of rice. In this study, we report quantitative trait loci (QTL) associated with resistance to WBPH using a doubled-haploid (DH) mapping population derived from the cross IR64/Azucena. We evaluated a set of 91 DH lines using various screening tests which measure seedling resistance, antibiosis and tolerance to WBPH. QTL analysis involving a RFLP map of 175 markers detected a significant QTL on chromosome 7 (RG511-RG477) associated with seedling resistance to WBPH. In addition, QTL analysis involving available defence related candidate genes as markers on a sub set of 60 DH lines showed significant association of genomic regions on chromosome 1 (W1-pMRF1), 2 (XLRfrI7-RG157) and 7 (RG711-CDO418) with resistance to WBPH. Several suggestive QTL were detected on chromosomes 2, 3, 6, 7, 8 and 11 showing the possibility of their association with resistance to WBPH. The phenotypic contribution of the QTL ranged from 8.4% to 32.1%. Some of the WBPH resistance QTL detected in this study showed similar map positions with the QTL reported for resistance to brown planthopper (BPH) in the same mapping population. These results would be useful for attempts to trace the genes associated with resistance to planthoppers in rice.

Key words: host plant resistance — molecular markers — QTL — rice — whitebacked planthopper

Introduction

The whitebacked planthopper (WBPH), *Sogatella furcifera* (Horvath) is a serious insect pest of rice throughout rice growing regions of the world. It often co-occurs with the brown planthopper (BPH), another insect species, which is the most destructive pest of rice. It feeds on phloem sap and causes complete death of rice plants, the symptom known as hopperburn. Outbreaks of WBPH occur regularly resulting in complete loss of crop (Gunathilagaraj and Ganeshkumar 1997, Ambikadevi et al. 1998). Host plant resistance has been successfully exploited for developing varieties with improved resistance to WBPH in rice. Attempts to resolve the genetic basis of resistance to WBPH in rice have resulted in the identification of many major genes: *Wbph1*, *Wbph2*, *Wbph3*, *wbph4*, *Wbph5* (Khush and Brar 1991), *Wbph6*(*t*) (Ma et al. 2001, Li et al. 2004), *Wbph7*(*t*) and *Wbph8*(*t*) (Tan et al. 2004). Sidhu et al. (2005) reported some new sources of major genes conferring resistance to WBPH population prevalent in northern India.

It has long been proposed that quantitative and/or polygenic resistance to insect pests should provide more durable resistance than single major genes. For instance, some of the known major genes for WBPH resistance appear to be not effective against WBPH population in northern India (Sidhu et al. 2005). However, genetic basis of quantitative resistance to WBPH has not been explored in detail.

Molecular markers have been widely used to locate quantitative trait loci (QTL) associated with quantitative resistance to insects in many crop plants (Yencho et al. 2000). Only a few reports on QTL associated with WBPH resistance in rice are available. Yamasaki et al. (1999) detected QTL associated with antibiosis, based on ovicidal response, to WBPH using recombinant inbred lines produced from the cross 'Asominori'/'IR24'. Yamasaki et al. (2003) reported the progress towards development of QTL-near isogenic lines and fine-mapping of such QTL.

The rice doubled-haploid (DH) population derived from a cross between an improved *indica* variety, 'IR64', and a traditional tropical *japonica* variety, 'Azucena', has been used for mapping and analysing major genes and QTL for numerous agronomic traits (Huang et al. 1997). Alam and Cohen (1998), Ramalingam et al. (2003) and Soundararajan et al. (2004) reported several QTL associated with resistance to BPH in this mapping population using a series of screening techniques quantifying three mechanisms of plant resistance to insects: antixenosis, antibiosis and tolerance (Painter 1951).

In our earlier study, using the 'IR64'/'Azucena' DH population, we reported a significant QTL associated with tolerance to WBPH (Kadirvel et al. 1999). The objective of the present study was to conduct more detailed phenotypic analysis for resistance to WBPH in the same mapping population to detect if there are more QTL. We evaluated the DH lines with additional phenotypic tests: standard seedbox screening test (SSST) to measure seedling resistance, population increase (PI) to measure antibiosis and days to wilt (DW), at different plant ages, to measure tolerance to WBPH. We also used an available linkage map of 'IR64'/'Azucena' augmented with candidate gene markers (Ramalingam et al. 2003) for QTL analysis to detect if there is any association of candidate gene markers with resistance to WBPH.

Materials and methods

Plant material: A population of 135 DH lines was generated at the International Rice Research Institute (IRRI) through *in vitro* anther culture (Guiderdoni et al. 1992). It was derived from a F_1 hybrid

between 'IR64', an *indica* variety adapted to irrigated conditions and 'Azucena', a traditional upland *japonica* variety from the Philippines. A subset of 91 DH lines was used in this study.

Insects: The WBPH was mass reared on the susceptible rice variety 'Taichung Native 1' (TN1) following the method of Heinrichs et al. (1985). Initial WBPH population was collected from the rice fields at the Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore, India. The adults were confined on 35 day-old potted plants of TN1 placed in oviposition cages ($45 \times 45 \times 60$ cm) having wooden frames, glass top, door and wire mesh sidewalls. The ovipositing insects were removed 3 days later and plants with eggs were taken out of cages, placed in separate cages for the nymphs to emerge. The emerged nymphs were then transferred to 15-day-old TN1 seedlings raised in the germination trays, which in turn were placed in galvanized iron trays ($62 \times 47 \times 15$ cm) containing 5 cm depth of water to increase humidity and to avoid watering daily. The seedling trays were changed as and when necessary. Using this technique, a continuous culture of the WBPH was maintained during the period of study.

Phenotyping: The phenotyping experiments were conducted in the Genetics greenhouse of the Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India (10°10′ and 11°30′ of the northern longitude and 76°46′ and 77°30′ of eastern latitude). The DH lines along with 'IR64' and 'Azucena' were evaluated for seedling resistance, antibiosis and tolerance to WBPH. The screening parameters *viz*., SSST (Heinrichs et al. 1985), PI (Heinrichs et al. 1985) and DW (Soundararajan et al. 2004) were used to measure seedling resistance, antibiosis and tolerance, respectively.

Standard seedbox screening test: The pregerminated seeds of test lines were sown 3 cm apart in 20 cm rows in $50 \times 50 \times 10$ cm wooden boxes. Each line was planted in three replications across the width of the seedbox in such a way that at least 15 plants were maintained per row. One row each of the susceptible check TN1 and the resistant check, PTB33 were also sown at random in all the seedboxes. On the seventh day of seeding, the wooden seedboxes were transferred to galvanized iron trays ($62 \times 47 \times 15$ cm) filled with 5 cm of water. Ten days after seeding, the seedlings were infested with first to third instar nymphs of WBPH at the rate of approximately 5–8 nymphs per seedling. After infestation, the wooden seedling boxes with seedlings were covered with wire mesh wooden cages. The test plants were observed daily for damage by WBPH. Damage rating of the test lines was done on a row basis when 90% of the plants in the susceptible check row were killed. The test lines were graded using 1–9 scale of the Standard Evaluation System for Rice (SES) scale (IRRI 1998).

Population increase: The PI of WBPH on different DH lines was studied by releasing 10 first instar nymphs on 35-day-old caged plants. The adults oviposited on the plants and the nymphs developed after hatching. When the susceptible DH plants started to wilt, the experiment was terminated and the population of hoppers was counted. The experiment was replicated twice.

Days to wilt: Days to wilt was used as a measure of tolerance where the damage by WBPH population on each DH line was estimated by counting the number of days required to kill the plants after infestation. DW was measured at three plant age levels *viz*., 30, 45 and 60 days after sowing with an insect load of 50 first and second instar nymphs per plant, hereafter referred as DW30, DW45 and DW60 respectively. For DW30 and DW45, 15-day-old seedlings were transplanted in 15 cm dia. clay pots and caged with cylindrical mylar sheet cage (13×75 cm). For DW60, the seedlings were transplanted in 30 cm dia. clay pots and caged with mylar cage (25×90 cm). The nymphs were released on the plants and allowed to feed. The day on which the plant wilted completely was recorded. The experiment was replicated twice.

Data analysis: Mean, range and SD estimates for the phenotypic values of resistance to WBPH were obtained using standard Excel programme of windows. For QTL analysis, the linkage map data of 'IR64'/'Azucena' DH population with 175 marker loci [8 isozymes + 14 randomly amplified polymorphic DNA (RAPD) + 12 cloned genes + 141 restriction fragment length polymorphism (RFLP)] developed by Huang et al. (1997) and the phenotypic data of 91 DH lines were used. A candidate gene map of 'IR64'/'Azucena' DH population with a set of defence related candidate gene markers (115 marker loci) published by Ramalingam et al. (2003) was also used for QTL analysis using a subset of 60 DH lines.

QTL analyses were carried out based on simple interval mapping (SIM) by MAPMAKER/QTL (Lander et al. 1987) and composite interval mapping (CIM) (Zeng 1994) by Windows QTL cartographer 2.5 application (Wang et al. 2007). Permutation test (1000 iterations) was used to establish an experiment wise significant value at the 0.05 confidence level defined as a minimum logarithm of odds (LOD) threshold for each trait in CIM (Doerge and Churchill 1996). LOD values were computed from likelihood ratio (LR) values based on the empirical relationship of 1 LOD is equal to 0.217LR. For each form of interval analysis, the maximum LOD value associated with the most closely linked marker, the weight value associated with additive marker allele effects, and the proportion of the phenotypic variance attributable to the QTL were tabulated. QTL detected with LOD values higher than the empirical threshold values were considered as significant and the QTL detected with LOD values of more than 2 but less than the threshold values were considered as suggestive.

Results

Phenotypic trait mean and frequency distribution

'IR64' was moderately resistant (damage rating of 5) and 'Azucena' was highly susceptible (damage rating of 7.7) to WBPH in SSST (Table 1). PI was lower on 'IR64' (32.5 WBPH/plant) and higher on 'Azucena' (784.0 WBPH/plant). DW after WBPH infestation at different growth stages was longer for IR64 than Azucena. Thirty-days-old plants of 'IR64' (DW30) survived up to 88 days after infestation whereas 'Azucena' plants survived only up to 18.5 days. When 45 and 60 days old plants were infested (DW45 and DW60, respectively), WBPH could not kill 'IR64' plants even after 90 days after infestation but 'Azucena' plants wilted quickly (26.5 and 28.0 days, respectively). The DH lines exhibited considerable quantitative variation for these resistance traits to WBPH. In SSST, DH lines showed damage ratings ranging from 3.7 to 9.0 with the mean of 5.7. PI of WBPH on DH lines ranged

Table 1: Phenotypic values of parents and DH lines of 'IR64'/'Azucena' cross for resistance to WBPH in rice

	Parents[1]		DH population[1]		
Traits	IR64	Azucena	Mean	Range	SD
Standard seedbox screening test (SSST)[2]	5	7.7	5.7	3.7–9.0	1.5
Population increase[3]	32.5	784.0	547.7	0–1423	364.3
Days to wilt (30 DAS)[4]	88.0	18.5	28.9	9–>90	18.8
Days to wilt (45 DAS)	>90.0	26.5	54.1	11–>90	29.3
Days to wilt (60 DAS)	>90.0	28.0	48.5	14–>90	23.5

DH, doubled-haploid; DAS, days after sowing; SES, standard evaluation system.
[1]Mean of two or three replications.
[2]SES (1–9 scale).
[3]WBPH per plant.
[4]Days from infestation to complete wilting of plant; >90.0, not wilted at 90th day after infestation.

from 0 to 1423 WBPH per plant with the mean of 547.7. Similarly, the lines with shorter DW of 9, 11 and 14 days after infestation at DW30, DW45 and DW60 respectively as well as the lines that did not wilt even after 90 days after infestation were also observed. The resistance levels of some of the DH lines exceeded the parents indicating the presence of transgressive variation for WBPH resistance. Frequency distribution of DH lines for the phenotypic values of resistance traits clearly displayed the spectrum of quantitative variation for resistance to WBPH present in the mapping population (Fig. 1).

Detection of QTL

QTL analysis detected 10 genomic regions (four significant and six suggestive) associated with resistance to WBPH in the 'IR64'/'Azucena' DH population. QTL results obtained using two linkage maps (RFLP and candidate gene markers) in two analytical methods (SIM and CIM) are presented in Tables 2 and 3 and Fig. 2.

QTL detected in different analytical methods

In QTL analysis involving RFLP map of 175 markers, SIM detected three QTL: on chromosome 3 (RG179-CDO337), (RG100-RZ678) and 7 (RG511-RG477) with LOD scores of more than 2 but less than 3, whereas, CIM detected six QTL on chromosome 1 (W1-RG173), 3 (RZ892-RZ678), 6 (Pgi2-RG648, RG172-RG653), 7 (RG511-RG477) and 11 (RZ400-RG1094). The QTL on chromosome 7 (RG511-RG477) was significant as it was detected with LOD value of 4.2 exceeding the threshold value of 3.1. The other QTL detected with LOD values of more than 2 but less than 3 were considered as suggestive QTL. The QTL on chromosome 3 (RG100-RZ678) and 7 (RG511-RG477) were detected consistently in both analytical methods (Table 2).

In QTL analysis involving a linkage map of candidate gene markers, SIM detected five QTL on chromosome 3 (RZ892-RG100, RG179-CDO337), 7 (RG511-XLRin12I1), 11 [r3(NBS-LRR)-XLRfrA6, CDO127-RZ638] with LOD values of more than 2 but less than 3 (Table 3). CIM detected seven QTL on chromosomes 1(W1-pMRF1), 2 (XLRfrI7-RG157), 3 (RZ892-RZ678), 6 (NLRfrA1-RG424), 7 (RG711-CDO418), 8 (XLRfrI1-S2AS3I4) and 11 (RG167-RG103). The QTL on chromosome 1 (W1-pMRF1), 2 (XLRfrI7-RG157) and 7 (RG711-CDO418) were significant and the other QTL were suggestive (Table 3). Only the regions on chromosome 3 (RZ892-RZ678) and 11 (RG167-RG103) were common between the SIM and CIM. The other QTL were detected

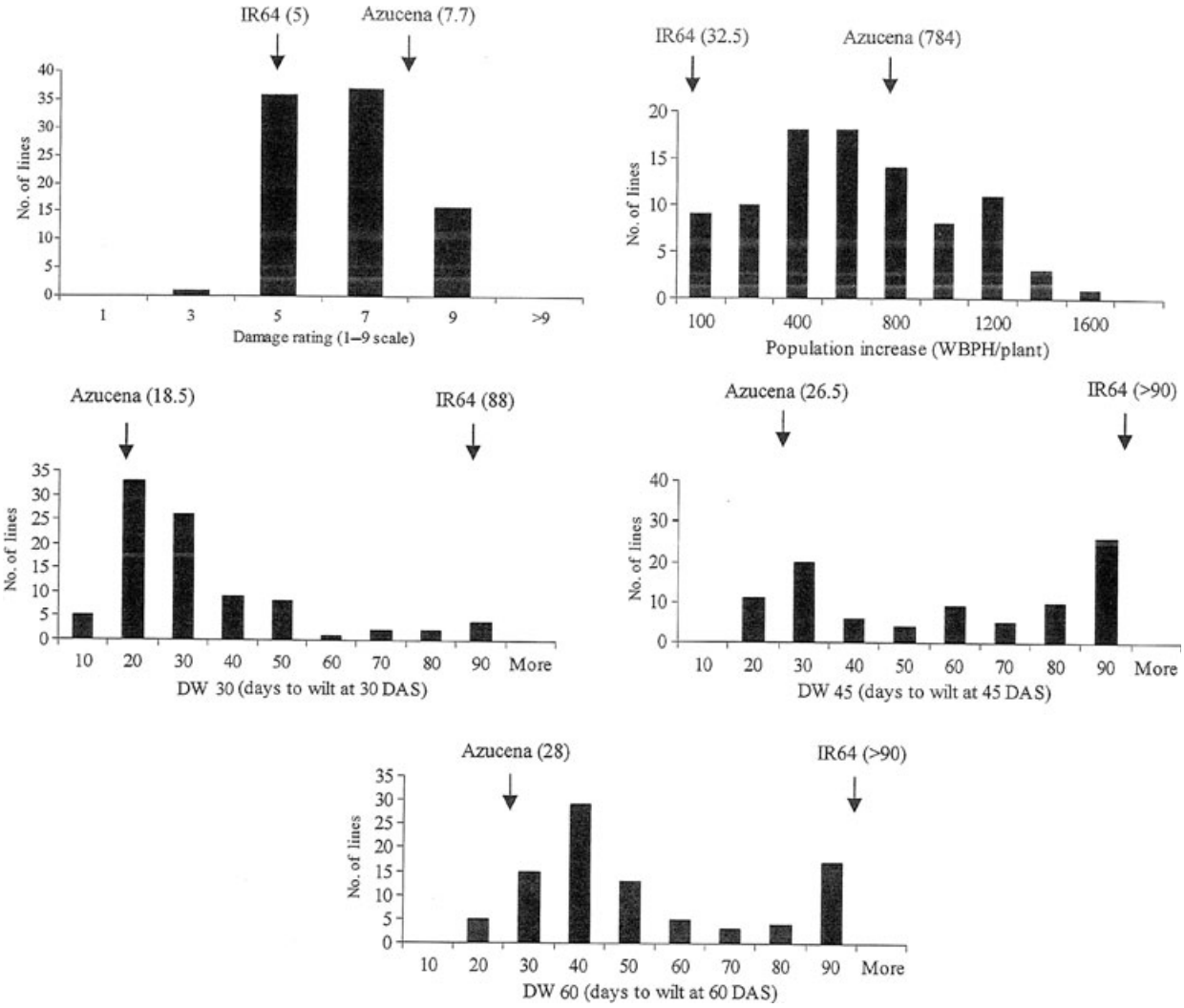

Fig. 1: Frequency distribution of phenotypic values for resistance to whitebacked planthopper in IR64/Azucena DH population in rice

Table 2: QTLs associated with resistance to WBPH using RFLP map of IR64/Azucena DH population in rice

	RFLP map (91 DH lines)									
	Simple interval mapping[1]					Composite interval mapping[2]				
Trait	Marker interval	Chromosome	LOD	Variance	Additive[3]	Marker interval	Chromo-some	LOD[4]	Variance	Additive[5]
SSST	RG179-CDO337	3	2.6	16	0.6	RG511-RG477	7	4.2 (3.1)	18.2	−0.6
	RG511-RG477	7	2.3	12.6	0.5					
DW30						W1-RG173	1	2.5 (3.5)	13.4	8.0
						Pgi2-RG648	6	2.3 (3.5)	8.4	−5.7
						RZ400-RG1094	11	2.6 (3.5)	9.9	7.1
DW45	RG100-RZ678	3	2.2	11.7	−11.4	RZ892-RZ678	3	2.3 (3.5)	9.6	10.2
DW60						RG172-RG653	6	2.7 (3.3)	10.5	7.8

QTL, quantitative trait loci; WBPH, whitebacked planthopper; DH, doubled-haploid; SSST, standard seedbox screening test.
[1]Using Mapmaker/QTL 1.1.
[2]Using Windows QTL Cartographer V2.5.
[3]Effect of 'Azucena' allele.
[4]Values in parenthesis indicate empirical threshold LOD values determined by permutation tests (1000 iterations).
[5]Effect of 'IR64' allele.

Table 3: QTLs associated with resistance to WBPH using candidate gene map of IR64/Azucena DH population in rice

	Candidate gene map (60 DH lines)									
	Simple interval mapping[1]					Composite interval mapping[2]				
Trait	Marker interval	Chromosome	LOD	Variance	Additive[3]	Marker interval	Chromo-some	LOD[4]	Variance	Additive[5]
SSST	RZ892-RG100	3	2.0	15.8	0.6	RZ892-RZ678	3	2.2 (2.8)	15.2	0.6
						XLRfrI1-S2AS3I4	8	2.4 (2.8)	12.1	−0.6
PI	r3 (9NBS-LRR)-XLRfrA6	11	2.2	14.2	145.7	NLRfrA1-RG424	6	2.7 (3.3)	14.3	146.2
DW30	CDO127-RZ638	11	2.5	16.5	−8.2	W1-pMRF1	1	4.5 (3.1)	21.1	12.3
						XLRfrI7-RG157	2	3.4 (3.1)	15.4	8.5
						RG167-RG103	11	2.2 (3.1)	17.3	−9.8
DW45	RG179-CDO337	3	2.8	24.9	−17.0	XLRfrI1-S2AS3I4	8	3.0 (3.5)	16.7	14.4
DW60	RG511-XLRin12I1	7	2.6	19.3	−16.4	RG711-CDO418	7	5.4 (3.5)	32.1	−19.5

QTL, quantitative trait loci; WBPH, whitebacked planthopper; DH, doubled-haploid; SSST, standard seedbox screening test; PI, population increase.
[1]Using Mapmaker/QTL 1.1.
[2]Using Windows QTL Cartographer 2.5.
[3]Effect of 'Azucena' allele.
[4]Values in parenthesis indicate empirical threshold LOD values determined by permutation tests (1000 iterations).
[5]Effect of 'IR64' allele.

only in CIM (Table 3). As there were differences in QTL detection between SIM and CIM, the results obtained from CIM were considered for reporting and further discussion.

In summary, the QTL on chromosomes 1 (W1-pMRF1), 2 (XLRfrI7-RG157) and 7 (RG511-RG477, RG711-CDO418) were significant whereas the QTL on chromosomes 3 (RZ892-RZ678), 6 (Pgi2-RG648, RG172-RG653), 8 (XLRfrI1-S2AS3I4), 11 (RZ400-RG1094, RG167-RG103) were suggestive for resistance to WBPH in IR64/Azucena DH population.

QTL associated with different mechanisms of resistance to WBPH

The significant QTL on chromosome 7 (RG511-RG477) detected in SSST was associated with seedling resistance to WBPH (Table 2). The phenotypic contribution of this QTL was 18.2%. Two more suggestive QTL on chromosomes 3 (RZ892-RZ678) and 8 (XLRfrI1-S2AS3I4) were also detected in SSST (Table 3).

No significant QTL associated with antibiosis based on PI was detected. However, one suggestive QTL on chromosome 6 (NLRfrA1-RG424) was detected when candidate gene marker data was used for QTL analysis (Table 3).

Three significant QTL and six suggestive QTL were detected for tolerance to WBPH based on DW at 30, 45 and 60 day old plants. The QTL on chromosomes 1 (W1-pMRF1) and 2 (XLRfrI7-RG157) were found to be significantly associated with DW30 (Table 3). The QTL on chromosomes 6 (Pgi2-RG648) and 11 (RZ400-RG1094, RG167-RG103) were found to be suggestive (Table 2 & 3). Two suggestive QTL on chromosomes 3 (RZ892-RZ678) and 8 (XLRfrI1-S2AS3I4) were found to be associated with DW45 (Tables 2 and 3). The QTL on chromosome 7 (RG711-CDO418) was found to be significantly associated with DW60 (Table 3). A suggestive QTL on chromosome 6 (RG172-RG653) was also detected for DW60 (Table 2).

Co-localization of candidate gene markers and QTL

The candidate gene markers *viz*., XLRin12I4, pMRF1 on chromosome 1, XLRfrI7, PK1K2I1 and NLRin12I3 on chromosome 2, Thaumatin2, NLRfrI4, NLRfrA1, NLRfrI1 and PK1K2I3 on chromosome 6, Thaumatin1 and XLRin12I1

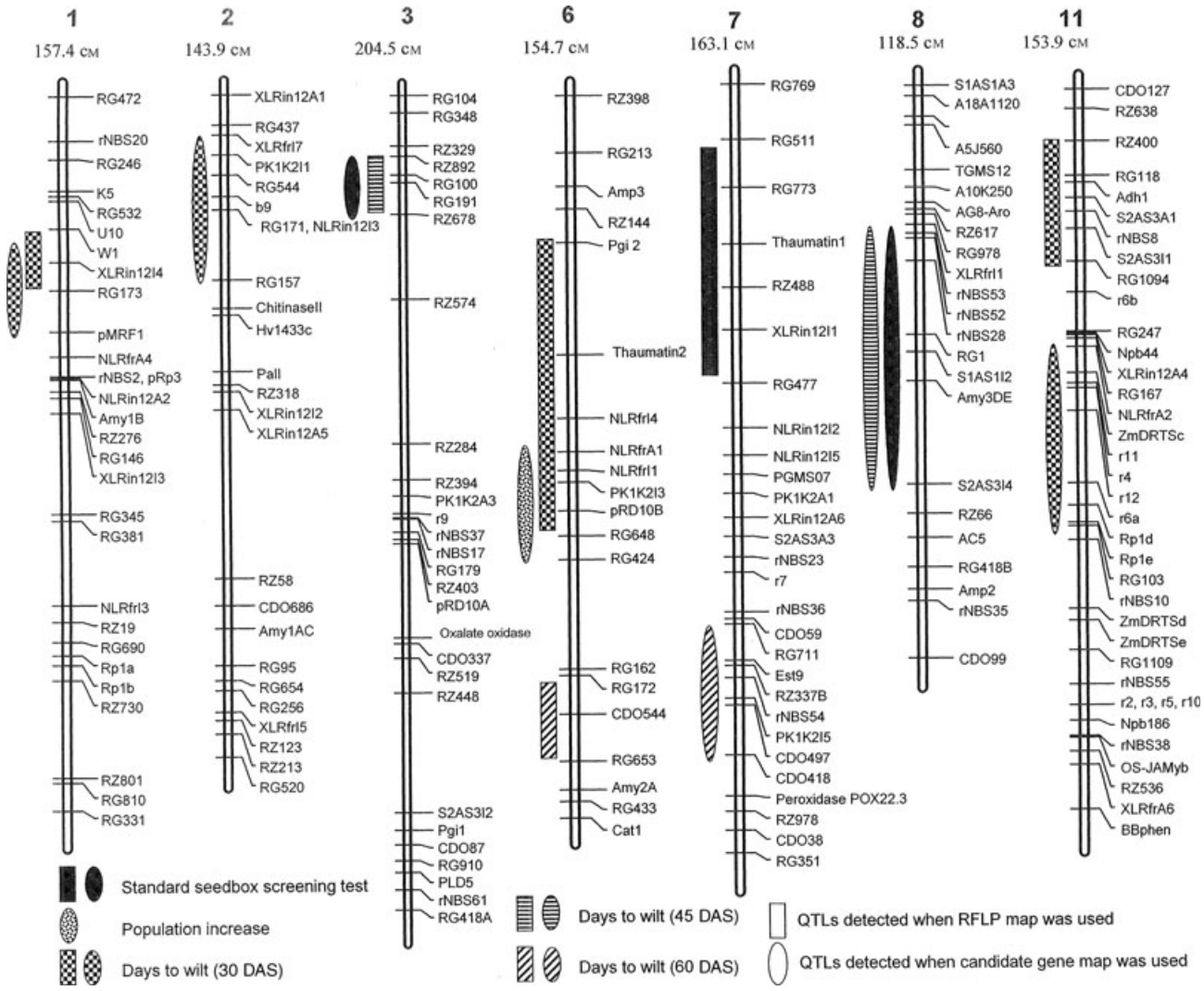

Fig. 2: Map locations of the detected quantitative trait locis associated with resistance to whitebacked planthopper in IR64/Azucena DH population. The linkage map was modified from Ramalingam et al. (2003)

on chromosome 7, XLRfrI1, rNBS53, rNBS52, rNBS28, S1AS1I2 and S2AS3I4 on chromosome 8, S2AS3A1, rNBS8, S2AS311, XLRin12A4, NLRfrA2, ZmDRTSc, r11, r4, r12, r6a, Rp1d, Rp1e on chromosome 11 were located in the QTL regions (Fig. 2).

Discussion

The results of the present study showed that quantitative resistance in 'IR64' to WBPH was due to antibiosis and tolerance mechanisms and the QTL on chromosomes 1 (W1-pMRF1), 2 (XLRfrI7-RG157), 3 (RZ892-RZ678), 6 (Pgi2-RG648, RG172-RG653), 7 (RG511-RG477, RG711-CDO418), 8 (XLRfrI1-S2AS3I4) and 11 (RZ400-RG1094, RG167-RG103) were contributing to this resistance. Antixenosis was not determined in this study. Alam and Cohen (1998) and Soundararajan et al. (2004) also reported that host plant resistance to BPH in 'IR64' was due to combination of antixenosis, antibiosis and tolerance. 'IR64' might have inherited quantitative resistance to insects from several land races (>20) that were involved during its development (Khush and Virk 2005).

In this study, we employed two analytical methods for QTL detection: SIM and CIM in order to minimize the QTL false positives. The use of several marker-trait association analysis techniques provides the means to assess the robustness of QTL detection in a single experimental design. Lot of discrepancies were observed while comparing the QTL results obtained using SIM and CIM. In general, SIM detected less number of QTL with relatively low LOD values whereas CIM detected extra QTL with relatively higher LOD values. However, some of the QTL were consistently detected in both SIM and CIM. For example, the QTL on chromosome 7 (RG511-RG477) associated with seedling resistance and 3 (RG100-RZ678) associated with DW45 were detected in both SIM and CIM (Table 2).

Although different QTL were detected in different phenotypic tests, it was difficult to assign QTL specific to a particular mechanism of resistance. In some cases, the same QTL were detected in more than one phenotypic test. It is possible because the resistance trait is very complex and the phenotypic tests might not be able to clearly differentiate the mechanisms. However, in some cases, QTL for specific mechanism of resistance, for instance, antixenosis in soybean, antibiosis in maize and potato (Yencho et al. 2000) have been clearly established.

The significant QTL on chromosome 11 (RG167-RG103) detected earlier, based on PDLOSS as a measure of tolerance (Kadirvel et al. 1999), appeared as a suggestive QTL when DW30 was used as a measure of tolerance in this study (Table 3). The previous study involved the same set of DH lines (94 lines) but the QTL analysis was carried out using the linkage map of 135 RFLP markers. It appears that the use of different phenotypic test and linkage map with varying number of markers might have affected the significance of the QTL detected.

Thus, the results supported that the population size, number of markers, nature of phenotypic tests and the analytical method used for QTL analysis are some of the important factors that might influence QTL detection (Asíns 2002). However, a high degree of congruence between the results of QTL across mapping populations, phenotypic tests and analytical methods should provide the basis to prioritize the selection of putative marker-QTL combination for further fine-mapping studies.

There are only a few reports on QTL for WBPH resistance in rice. Also, these studies have used different set of markers for mapping QTL. This situation limits the comparative analysis of the QTL on multiple mapping populations. Sogawa et al. (2001) reported two QTL for resistance to WBPH on chromosome 6 and 11 in a DH population from 'Zaiyeqing 8' (an *indica* variety) × 'Jingxi 17' (a *japonica* variety) cross. A minor QTL on chromosome 11 was mapped between RG167 and CT442. The *indica* parent 'Zaiyeqing 8' was the source for this QTL. It is interesting to note that RG167 showed its association with resistance to WBPH in both 'IR64'/'Azucena' and 'Zaiyeqing 8'/'Jingxi 17' populations.

It appears that none of the QTL reported in this study co-localized with the major genes for WBPH resistance except for the QTL on chromosome 1 (W1-RG173), which was detected near *Wbph1* (McCouch 1990). It is expected because there is no report on major gene resistance to WBPH in 'IR64'.

Another important observation from this study was that some of the WBPH resistance QTL detected in this study co-localized with the BPH resistance QTL reported in earlier studies in the same mapping population. The QTL on chromosome 2 (XLRfrI7-RG157), 3 (RZ892-RZ678), 6 (Pgi2-RG648), 7 (RG511-RG477) and 11 (RG167-RG103) co-localized with BPH resistance QTL reported by Alam and Cohen (1998), Soundararajan et al. (2004) and Ramalingam et al. (2003), respectively. This observation suggests the possibility of common loci conferring resistance to both the planthoppers in rice. Accumulation of QTL information for both the planthoppers in the same mapping population would help to verify this hypothesis. Tan et al. (2004) reported that two WBPH resistance genes in rice share the same loci with those for BPH resistance.

Fine-mapping some of these QTL to identify tightly linked markers would be helpful for marker-assisted breeding. However, it might be difficult to fine-map them as the phenotypic effect contributed by these QTL were small and could hardly be detected. Similar observations were made by Alam and Cohen (1998) and Soundararajan et al. (2004) while mapping QTL for BPH resistance using the same IR64/Azucena DH population.

Candidate gene approach is proposed as an alternative strategy to identify genes underlying QTL. Our preliminary results showed that the candidate gene markers derived from NBS-LRR regions, thaumatin, rust resistance gene family (Rp1), S-adenosyl methionine synthetase (pMRF1) and dihydrofolate reductase thymidylate synthase (ZmDRTS) co-located with WBPH resistance QTL on chromosome 1, 2, 6, 7, 8 and 11 (Fig. 2). The details of these candidate genes are provided by Ramalingam et al. (2003). They reported the association of these candidate genes with QTL mapped for bacterial blight, blast and BPH resistance in rice. The present study strengthens this QTL database in 'IR64'/'Azucena' population by adding information on association of candidate genes with WBPH resistance in rice. Further studies would require establishing the association and role of these candidate genes with WBPH resistance in rice.

In conclusion, the analysis presented here provides an insight into the genetic control of resistance to WBPH in the rice variety 'IR64'. The results showed that 'IR64' could be a potential source of quantitative resistance to WBPH. Many QTL contributed by IR64 for resistance to WBPH were detected in different phenotypic tests measuring seedling resistance, antibiosis and tolerance mechanisms, using 'IR64'/'Azucena' DH population. A preliminary association of the defence related candidate genes with such QTL was made. The results also shed light on the possibility of common loci conferring resistance to both BPH and WBPH in 'IR64'. These observations should be further validated through fine-mapping and candidate gene analysis to make use of them in breeding rice cultivars with improved resistance to planthoppers.

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