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RFLP-facilitated investigation of the quantitative resistance of rice to brown planthopper (*Nilaparvata lugens*)

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Abstract Quantitative trait loci (QTLs), conferring quantitative resistance to rice brown planthopper (BPH), were investigated using 160 F_{11} recombinant inbred lines (RILs) from the Lemont/Teqing cross, a complete RFLP map, and replicated phenotyping of seedbox inoculation. The paternal indica parent, Teqing, was more-resistant to BPH than the maternal japonica parent, Lemont. The RILs showed transgressive segregation for resistance to BPH. Seven main-effect QTLs and many epistatic QTL pairs were identified and mapped on the 12 rice chromosomes. Collectively, the main-effect and epistatic QTLs accounted for over 70% of the total variation in damage scores. Teqing has the resistance allele at four main-effect QTLs, and the Lemont allele resulted in resistance at the other three. Of the main-effect QTLs identified, *QBphr5b* was mapped to the vicinity of *gl1*, a major gene controlling leaf and stem pubescence. The Teqing allele controlling leaf and stem pubescence was associated with resistance, while the Lemont allele for glabrous stem and leaves was associated with susceptibility, indicating that this gene may have contributed to resistance through antixenosis. Similar to the reported BPH resistance genes, the other six detected main-effect QTLs were all mapped to regions where major disease resistance genes locate, suggesting they might have contributed either to antibiosis or tolerance. Our results indicated that marker-aided pyramiding of major resistance genes and QTLs should provide effective and stable control over this devastating pest.

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Introduction

The brown planthopper (*Nilaparvata lugens*, BPH) has been one of the most-devastating pests to rice crops in Asia, and susceptible rice cultivars often suffer severe yield loss up to 60% from its attacks (Khush 1979; Panda and Khush 1995). The estimated average annual yield loss from BPH is approximately 10% in some South China provinces in recent years (Liu and Wu 1992). Breeding resistant cultivars has proven to be one of the most-efficient ways to control this pest (Pathak 1969; Pathak and Saxena 1980).

Since the discovery of four major BPH resistance genes, *Bph1* and *Bph2* (Athwal et al. 1971), *Bph3* and *Bph4* (Lakshminarayana and Khush 1977), at least ten major genes conferring resistance to BPH have been reported (Ikeda and Kaneda 1981; Panda and Khush 1995). Two of these BPH resistance genes, *Bph1* and *Bph–10*(*t*), were found to be closely linked with two RFLP markers, C185 and RG457 on rice chromosome *12* (Jena et al. 1992; Ishii et al. 1994; Hirabayashi and Ogawa 1996). However, rice resistance to BPH conferred by major genes is not stable. The breakdown of *Bph1* and *bph2* by BPH biotypes 2 and 3 has been reported in several cases (Gallun and Khush 1980; Pathak and Saxena 1980; Panda and Khush 1995) and the other major resistance genes are facing the same problem (Khush and Brar 1991; Liu and Wu 1992; Medina et al. 1996). As an alternative source of resistance, the value of the quantitative resistance to BPH in rice has been recognized, but remains poorly understood because genetic characterization of this type of resistance is difficult.

Recent advances in DNA marker technology and molecular biology have greatly facilitated studies to understand the genetic basis of complex phenotypes. Genes contributing to quantitative trait variation, or quantitative trait loci (QTLs) related to a wide range of complex phe-

notypes including growth and plant height, grain yield components, resistance to bacterial blight, etc., have been mapped in rice (Li 2001). Using RFLP markers and 123 doubled-haploid lines from the cross IR64/Azucena, Alam and Cohen (1998) first reported the mapping of seven QTLs associated with the quantitative resistance of rice to two Philippine populations of BPH.

We report here a study to map QTLs and epistatic loci associated with the quantitative resistance of rice plants to BPH using 160 recombinant inbred lines and a complete RFLP linkage map.

Materials and methods

Materials and genotyping

A subset of 160 F_{11} recombinant inbred lines (RILs) from the RI population derived from the cross between Lemont (*japonica*) and Teqing (*indica*) were used as the mapping population (Li et al. 1999). An *indica* variety, Taichung Native 1 (TN1), was used as the susceptible check. Teqing is moderately resistant to BPH while Lemont is highly susceptible to BPH. All RILs were genotyped with 182 well-distributed RFLP markers and a complete molecular linkage map was constructed, as described previously (Li et al. 1999).

Phenotyping experiments

The BPH populations used in this study belonged to BPH biotype 2 and were collected from the experimental paddy fields at Guangxi Academy of Agricultural Sciences, China. The insects were fed on the susceptible rice cultivar, Shanyou 63, in the greenhouse for several weeks to produce a sufficiently large population required for the phenotyping experiment. Phenotyping the quantitative resistance of the 160 RILs to BPH biotype 2 was conducted in a replicated greenhouse experiment in the summer of 1997, using the standard method of the seedling bulk test developed at IRRI (Khush and Brar 1991). The experiment was repeated three times (as three replications) in the greenhouse in Nanjing Agricultural University. The first experiment was carried out from July 3–16, the second from August 6–20, and the third from September 9–23, 1997. Two treatments were adopted. In treatment 1 (bulk test), about 20 seeds of each RIL were sown as a single row in a standard seedbox (60×40×40 cm) with ten RILs and the susceptible check (TN1) randomly arranged in each of the seedboxes. There were 16 seedboxes in each of the replications. In treatment 2 (the independent test), seeds of each RIL as well as TN1 were separately planted in a single plastic pot. In both the bulk and independent tests, at the 2-leaf stage or 12 days after seeding, the seedlings were infested with the pre-cultured BPH nymphs at the rate of five first- or second-instar nymphs/seedling; seedboxes were covered with a nylon-net immediately after the inoculation. Three days after inoculation, the seedlings were rated daily for the degree of seedling damage based on the standard 10 damage scores (DS) with 0 indicating no symptom in all seedlings and 9 indicating all seedlings dead. The survival rate of the BPH nymphs on the seedlings of each RIL was recorded daily from the second day after infestation until all seedlings of the susceptible check died. Moreover, the survival numbers of seedlings were also counted day by day, and the total survival number of each RIL in 12 days was used as the resistance index (RI).

Data analyses

The mean damage index of each line was used as the input data, while the resistance index (seedling survival numbers) and seedling damage scale gave parallel results (see Table 1). The mean values of three experiments in the bulk treatment were used for the further QTL analyses using the computer software 'QTLMAPER V 1.0a' (Wang et al. 1999) to interval-map QTLs associated with BPH resistance based on a mixed model approach. The threshold was 2.0 LOD for detecting main-effect QTLs and 2.4 for claiming digenic epistatic QTLs.

Results

Phenotypic variation of resistance to BPH

In the bulk test, the parents differed significantly in their resistance to BPH. The average RI and DS were 35.3 and 9.0 for Lemont, and 80.1 and 5.6 for Teqing, respectively (Table 1). Lemont was even more susceptible than the

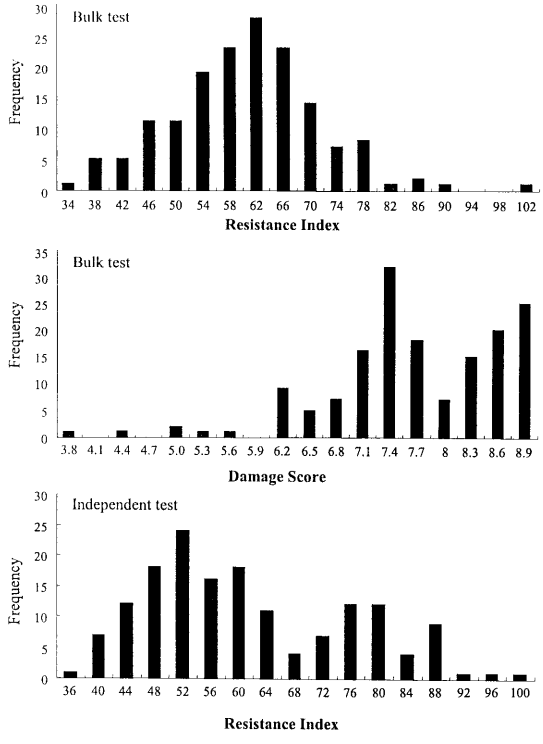

Fig. 1 Frequency distribution of 160 Lemont/Teqing recombinant inbred lines for resistance and damage indices to brown planthopper tested in bulk and independent treatments

Table 1 Quantitative resistance to brown planthopper measured as the resistance index (RI) and damage score (DS) of 160 Lemont/Teqing recombinant inbred lines (RILs) and their parents

Trait	CK (TN 1) mean	Lemont mean	Teqing mean	RILs	
				Mean±SD	Range
RI (bulk test)	46.0	35.3	80.1	61.0±11.1	35.0–101.0
DS (bulk test)	8.8	9.0	5.6	7.7±1.0	3.6–9.0
RI (independent test)	41.0	38.0	78.0	61.9±14.5	38.0–99.9

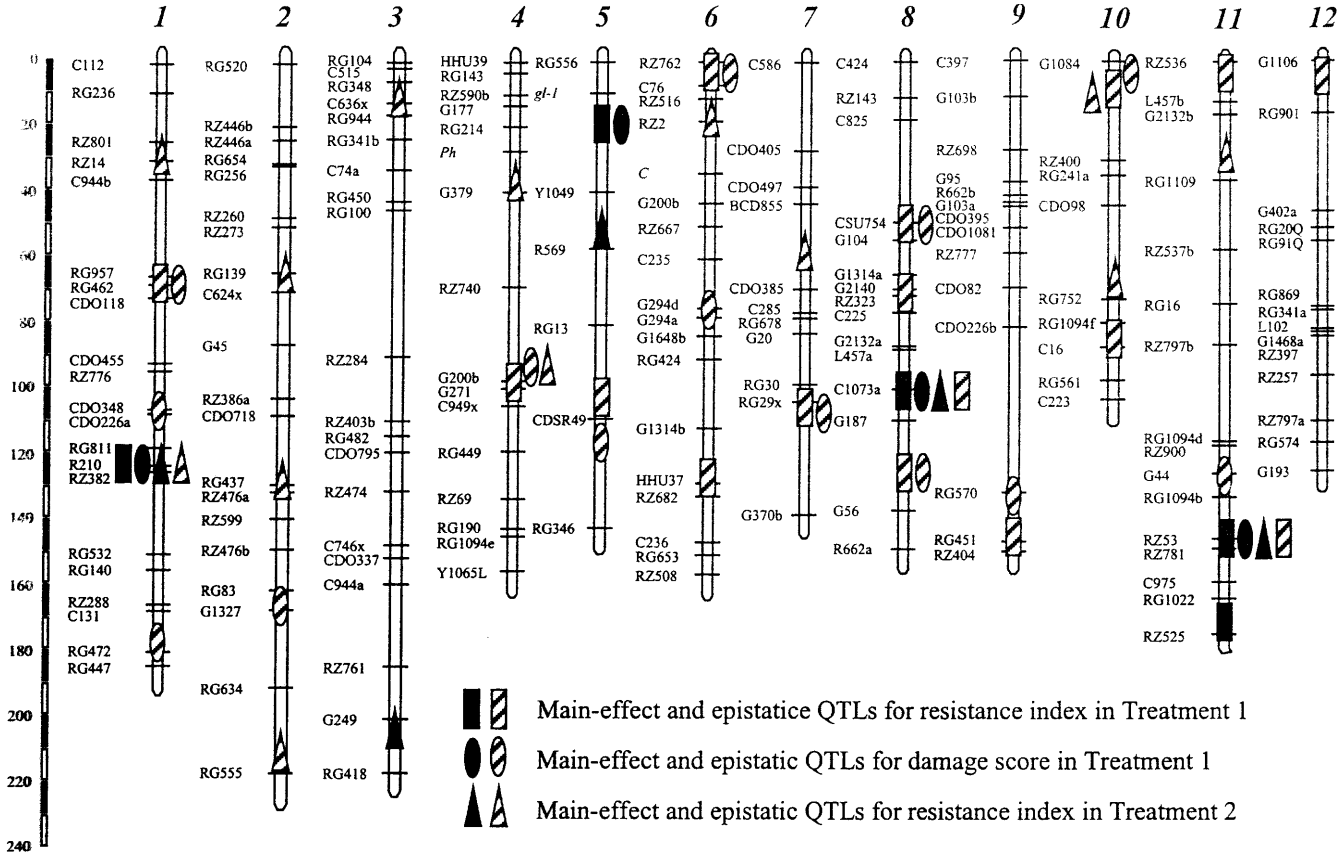

Fig. 2 Main-effect and epistatic QTLs affecting BPH resistance detected in the Lemont/Teqing recombinant inbred population

susceptible check, TN 1. The average RI of the RILs to BPH gave a continuous distribution but DS in the bulk test and RI in the independent test showed a skewed distribution with two peaks at the high value regions (Fig. 1). Transgressive segregation in the RILs for RI or DS was present but not frequent. The average value was 61.1, ranging from 35 to 101 for RI, and 7.69 with a range from 3.6 to 9.0 for DS. RI was highly correlated with DS (r=–0.95). Similar results were obtained in the independent test. The RI obtained in the independent test was also correlated with RI (r=0.57, P<0.0001) and DS (r=–0.59, P<0.0001) in the bulk test.

Main-effect resistance QTLs

In the bulk test, five main-effect QTLs were identified and mapped to chromosomes 1, 5, 8 and 11 (Table 2, Fig. 2). All five QTLs except *QBphr11b* were associated with both RI and DS (Table 1). Collectively, these main-effect QTLs explained 54.6% and 50.5% of the total phenotypic variation in RI and DS. Three QTLs, *QBphr1*, *QBphr5a* and *QBphr11a*, had relatively large effects and individually explained 13.7%, 16.9% and 12.1% of the variation of DS in the bulk test. The Teqing allele was associated with resistance at four of the QTLs (*QBphr3*, *QBphr5a*, *QBphr11a* and *QBphr11b*), and the Lemont allele with the remaining three (*QBphr1*, *QBphr5b* and *QBphr8*). Data from the independent test allowed detection of five main-effect QTLs for RI, which explained 39.5% of the total trait variation. Of these, three (*QBphr1*, *QBphr8* and *QBphr11a*) were mapped to the same locations as in the bulk test. Two additional ones, *QBphr3* and *QBphr5b*, were identified, though *QBphr5b* was detected with a marginal LOD of 1.95 and closely linked to *QBphr5a* detected in the bulk test.

Digenic epistatic resistance QTLs

In the bulk test, 12 pairs of epistatic loci associated with RI or DS were detected and mapped on rice chromosomes 1, 2, 4, 6, 7, 8, 9 and 10 (Table 3, Fig. 2). Collectively, these epistatic QTL pairs explained 32.4% and 27.4% of the total trait variation in RI and DS, respectively. Two interacting loci near CDO348 on chromosome 1 and G44 on chromosme 11 also had highly significant main effects on DS. A significant interaction was also detected between two main-effect QTLs, *QBphr8* and *QBphr11b*. Resistance resulting from the recombinant-type interaction was observed at seven of the interacting QTL pairs, while the parental-type interaction at the remaining five QTL pairs resulted in resistance. In the independent test, seven pairs of epistatic loci associated with RI were identified, which explained 30% of the total trait variation. The main-effect QTL, *QBphr1*, was involved in two of these interactions,

Table 2 Main-effect QTLs associated with quantitative resistance to brown planthopper detected in the Lemont/Teqing recombinant inbred population

QTL	Trait.[a]	Treat.[b]	Chr.	Marker intervalc	LOD	*A* [d]	R^2 (%)
QBphr1	RI	1	1	R210–RZ382	5.44	3.41	13.2
	DS	1			4.51	–0.31	13.7
	RI	2			3.35	3.93	9.1
QBphr3	RI	2	3	G249–RG418b	3.38	–3.82	8.7
QBphr5a	RI	1	5	*gl1*–Y1049	5.06	–3.87	16.9
	DS	1			2.23	0.25	9.0
QBphr5b	RI	2	5	Y1049–R569a	1.95	2.97	5.2
QBphr8	RI	1	8	C1073a–G187	2.10	1.75	3.5
	DS	1			1.84	–0.16	3.9
	RI	2			1.65	2.34	3.3
QBphr11a	RI	1	11	RZ53–RZ781	4.82	–2.82	9.0
	DS	1			5.04	0.29	12.1
	RI	2			5.74	–4.73	13.2
QBphr11b	RI	1	11	RG1022–RZ525a	2.02	–1.80	3.7

[a] RI and DS are the resistance index and the damage score
[b] Treatments 1 and 2 represent the bulk test and the independent test for phenotyping
[c] The underlined are markers closer to the LOD peaks
[d] The QTL effect was due to substitution of the Lemont allele by the Teqing allele

Table 3 Digenic epistatic QTL pairs associated with the BPH damage index detected in the Lemont/Teqing RI population

Trait	Chr.	Marker interval *i*[a]	Chr.	Marker interval *j*[a]	LOD	*Ai*	*Aj*	Aaij	R^2 (%)
		Bulk test							
RI	1	RG462 – CDO118	8	G187–G56a	4.72			2.78	4.3
DS					2.46			–0.17	2.1
RI	4	G200b – G271	7	RG29–G370b	5.12			–2.95	4.8
DS					3.82			0.29	6.3
RI	5	RG13–CDSR49	10	G1084–RZ400	6.56	–1.73**		–4.16	9.6
RI	6	RZ762–C76	8	C825a–G104	3.67			2.90	4.6
DS					3.73			–0.28	5.7
RI	6	HHU37–RZ682	11	RZ536a–L457b	5.11			–2.92	4.7
RI	8	G2140–RZ323a	10	RG1094f–C16	3.10			2.11	2.5
RI	8	C1073a–G187	11	RZ53–RZ781	9.01	1.72**	–2.72***	–2.25	2.8
RI	9	RG451–RZ404	12	G1106–RG901a	2.86			2.35	3.1
DS	1	CDO348–CDO226a	11	G44–RG1094b	6.16	0.27***	0.24***	0.20	2.9
DS	1	C131–RG472	6	G294d–G294a	3.39			0.24	4.3
DS	2	RG83–G1327	9	RG570a–RG451	2.74			–0.26	4.9
DS	5	CDSR49–RG346	10	G1084–RZ400	3.56		0.13*	0.21	3.2
		Individual test							
	1	RZ801–RZ14	10	CDO98–RG752	4.89			4.54	4.8
	1	R210–RZ382	4	*Ph*–G379	5.78	4.18****		3.37	2.7
	1	R210–RZ382	11	G2132b–RG1109	4.72	4.41****		3.51	2.9
	2	RG139–C624x	2	RG437–RZ476a	5.16			4.13	4.0
	2	RG634–RG555	3	C636x–RG944	4.07		2.10*	4.00	3.8
	4	G200b–G271	6	RZ2–*C*	6.82			–5.20	6.3
	7	BCD855–CDO385	10	G1084–RZ400	2.78			–4.80	5.4

*, **, ***, **** Represent significance levels of $P \leq 0.05$, 0.01, 0.001 and 0.0001, respectively
[a] The underlined are markers closer to the LOD peaks

and the remaining interactions occurred between complementary loci. Resistance resulting from the parental-type interaction was observed at five of the interacting QTL pairs, while the recombinant-type interaction at the remaining two QTL pairs resulted in resistance.

Discussion and conclusions

It is known that the quantitative resistance to BPH may result from different mechanisms, such as non-preference or antixenosis, antibiosis and tolerance (Sogawa and Pathak 1970; Cohen et al. 1997; Alam and Cohen 1998). The resistance index and damage score on the bulk test (treatment 1) in this study were designed to provide an overall evaluation on different resistance mechanisms, though the former tended to weigh more on the non-preference and antibiosis, and the latter would also include tolerance. In contrast, the independent test in treatment 2 was expected to measure primarily antibiosis and tolerance, since antixenosis was eliminated in the independent test. Using the 160 RILs and a complete

linkage map, we were able to identify seven main-effect QTLs and many epistatic QTLs associated with the quantitative resistance to BPH. Of the seven main-effect QTLs, *QBphr5a* appeared to be associated with non-preference or antixenosis. This was suggested from the following observations. First, this QTL had a very large effect on RI (LOD=5.06, R^2=16.9%), and a much smaller effect on DS (LOD=2.23, R^2=9.0%). Second, this QTL was not identified in the independent test, as expected. Interestingly, this QTL was mapped to the vicinity of *gl1*, which controls the leaf pubescence (leaf hairs). It was the Teqing allele (the dominant one) that results in leaf and stem pubescence and was associated with resistance, while the Lemont allele that causes glabrous leaves and stems was associated with susceptibility. Third, an additional linked QTL, *QBphr5b*, was detected only in the individual test with a marginal LOD score of 1.95. This QTL had the opposite effect with *QBphr5a*, indicating that they represented different genes. It appeared to make sense that the BPH nymphs did not prefer the pubescent plants since leaf/stem hairs could have created a physical barrier for the sucking nymphs to settle. In fact, the association of leaf pubescence or hairs with insect resistances to the leaf chewing and sucking insects is well known in several plant species including cotton, soybean and wheat. Our results suggest that leaf pubescence of rice plants might have also contributed to the quantitative resistance to BPH. Similarly, five of the remaining six main-effect QTLs except *QBphr11b* appeared to have contributed to both antibiosis and/or tolerance as they were detected under both tests. The strong associations of these QTLs with DS and their close vicinities to the blast or bacterial blight resistance genes/QTLs segregating in the same population (Li et al. 1999; Tabien et al. 2000; Zhong et al. 2000, unpublished data), further strengthen this inference. It was noted that none of the resistance QTLs were detected in the regions of chromosomes 4 and 12 where several major BPH resistance genes, including *Bph10(t)*, *Bph2* and *Bph3*, reportedly locate (Jena et al. 1992; Ishii et al. 1994; Hirabayashi and Ogawa 1996; Murai et al. 2000). Comparing our results with those reported by Alam and Cohen (1998), we found that three main-effect QTLs, *QBph1a* (near CDO348), *QBph1b* and *QBph8*, were mapped in similar genomic locations with the BPH resistance QTLs segregating in the IR64/Azucena DH population.

It was not surprising that the susceptible allele at most main-effect QTLs was from Lemont since no BPH is present in Southern US where Lemont was a leading commercial cultivar for many years. However, the Lemont allele conferring resistance at three QTLs (*QBphr1*, *QBphr5b* and *QBphr8*) and the presence of many epistatic QTL pairs in which the recombinant-type interaction resulted in resistance, provided an adequate explanation of the transgressive segregation for the BPH resistance observed in the RI population. In fact, five RILs had damage scores lower than 5.5 with the lowest value of 3.6, which would provide good protection for rice plants from the pest under field conditions. These results suggest that the number of resistance QTLs in rice germplasm is very large, and the level of this quantitative resistance to BPH can be further raised by the pyramiding of different resistance genes/QTLs either through marker-aided selection or by conventional breeding.

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