Theor Appl Genet (1998) 97: 1370–1379 © Springer-Verlag 1998

S. N. Alam · M. B. Cohen

Detection and analysis of QTLs for resistance to the brown planthopper, *Nilaparvata lugens*, in a doubled-haploid rice population

Received: 10 May 1998 / Accepted: 4 June 1998

Abstract We used a mapping population of 131 doubled-haploid lines, produced from a cross between an improved *indica* rice variety (IR64) and a traditional *japonica* variety (Azucena), to detect quantitative trait loci (QTLs) for resistance to the brown planthopper (BPH), *Nilaparvata lugens*. We evaluated the parents and mapping population with six tests that measure varying combinations of the three basic mechanisms of insect host plant resistance, i.e., antixenosis, antibiosis, and tolerance. To factor-out the effect of the major resistance gene *Bph1* from IR64, the screening was done with two BPH populations from Luzon Island, The Philippines, that are almost completely adapted to this gene. A total of seven QTLs associated with resistance were identified, located on 6 of the 12 rice chromosomes. Individual QTLs accounted for between 5.1 and 16.6% of the phenotypic variance. Two QTLs were predominantly associated with a single resistance mechanism: one with antixenosis and one with tolerance. Most of the QTLs were derived from IR64, which has been shown to have a relatively durable level of moderate resistance under field conditions. The results of this study should be useful in transferring this resistance to additional rice varieties.

Key words Brown planthopper resistance · *Nilaparvata lugens* · QTL analysis · Rice · IR64

Communicated by R. Nelson

S. N. Alam[1] · M. B. Cohen (✉)
Entomology and Plant Pathology Division,
International Rice Research Institute, PO. Box 933,
1099 Manila, The Philippines
Tel.: + 63-2-845-0563
Fax: + 63-2-845-0606
E-mail: m.cohen@cgnet.com.

Present address:
[1]Entomology Division, Bangladesh Agricultural Research Institute, Gazipur 1701, Bangladesh

Introduction

The brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), was historically an occasional pest of rice in tropical Asia, but became a severe constraint to rice production following the introduction of high-yielding varieties in the 1960s (Gallagher et al. 1994; Way and Heong 1994). At the International Rice Research Institute (IRRI) in The Philippines, and other institutes throughout Asia, plant breeders and entomologists identified sources of major genes for BPH resistance and incorporated these genes into improved varieties. The first IRRI variety with BPH resistance, IR26, was released in 1973 and initially provided control of BPH over large areas. However, BPH populations adapted to the resistance gene in IR26, *Bph1*, in as few as 2 years (4–6 crop seasons and 12–18 *N. lugens* generations), and the outbreaks resumed. This pattern was repeated with subsequent varieties containing the *bph2* gene, first released in 1975. It was eventually determined that the principal cause of the outbreaks was over-use of broad-spectrum insecticides, which disrupt biological control of BPH by predators and parasitoids (Kenmore et al. 1984) and, at sublethal doses, can actually stimulate BPH reproduction (Heinrichs and Mochida 1984).

Although minimizing insecticide-use to promote biological control is the most important factor in BPH management in tropical rice, resistant varieties can play a complementary role (Way and Heong 1994). Resistant rice varieties often interact additively or synergistically with biological control and can also provide important "insurance" against BPH outbreaks caused by factors outside of farmers' control, such as unusual weather patterns or insecticide over-use in neighboring fields. In temperate rice-growing areas, BPH-resistant varieties are important in the control of outbreaks resulting from the annual mass immigration of BPH from the tropics.

It has long been proposed that moderate and/or polygenic resistance to insect pests, including BPH, should provide more durable resistance than single major genes (Heinrichs 1986; Bosque-Perez and Buddenhagen 1992). The development of crop varieties with polygenic insect resistance has been hindered by the added expense and difficulty of breeding for quantitative traits. However, the use of molecular-marker techniques in quantitative trait locus (QTL) analysis has opened new opportunities for working with quantitative traits. QTL analysis of insect resistance has been conducted on tomato (Maliepaard et al. 1995; Mutschler et al. 1996), potato (Bonierbale et al. 1994; Yencho et al. 1996), maize (Schon et al. 1993; Byrne et al. 1996; Bohn et al. 1996) and barley (Moharramipour et al. 1997).

The rice doubled-haploid population derived from a cross between an improved *indica* variety, IR64, and a traditional tropical *japonica* variety, Azucena, has been used for mapping and analyzing major genes and QTLs for numerous agronomic characters (Huang et al. 1997; Yadav et al. 1997). This mapping population also provides an excellent opportunity for characterizing QTLs for BPH resistance, because resistance in IR64 has been shown to be both polygenic and relatively durable. IR64 contains the *Bph1* gene, but apparently contains additional minor resistance genes as well. It shows moderate resistance to a greenhouse BPH colony fully adapted to *Bph1* (Khush 1989) and to BPH populations from Central Luzon, The Philippines, also adapted to *Bph1* (Cohen et al. 1997). IR64 has retained its moderate resistance to BPH in Central Luzon despite having been the most popular rice cultivar in this area for more than 10 years. In greenhouse selection experiments, we also found that BPH adapted more slowly to IR64 than to other resistant varieties (Alam and Cohen 1998).

Three mechanisms or 'modalities' of plant resistance to insects are generally recognized (Painter 1951; Kennedy et al. 1987): *antixenosis*, a quality that repels or disturbs insects, causing a reduction in colonization or oviposition; *antibiosis*, a quality that reduces insect survival, growth rate, or reproduction following the ingestion of host tissue; and *tolerance*, a capacity to produce a crop of high quality and yield despite insect infestation. These properties can be quantified by making relative comparisons among genotypes. Antixenosis, antibiosis, and tolerance have been detected, in various combinations, in rice germplasm resistant to BPH (Heinrichs et al. 1985). In the present study we have applied a series of screening techniques to identify QTLs for BPH resistance in the IR64 × Azucena mapping population. To determine whether the QTLs detected might confer resistance to BPH from different environments, we conducted these tests with BPH colonies established from two locations on Luzon Island, The Philippines.

Materials and methods

Plant material

We used a mapping population of 131 doubled-haploid lines generated at IRRI through in vitro anther culture (Guiderdoni et al. 1992) from a cross between the *indica* rice variety IR64 and *japonica* variety Azucena. We conducted one generation of seed increase, bagging the panicles at the flowering stage to prevent cross pollination of lines with low pollen fertility (Huang et al. 1997). Seeds were not available for four of the original 135 doubled-haploid lines. Genotyping work on this population is described in Huang et al. (1997). We used a total of 175 markers (8 isozymes, 14 RAPDs, 12 cloned genes, and 141 RFLPs) in our QTL analysis, with an average distance between pairs of markers of 11.5 cM.

Insects

Macropterous female BPH were collected from rice fields in two locations on Luzon Island, The Philippines: farmers' fields in Central Luzon (approximately 15°N, 120°E, 60 m above sea level) and the experiment farm at IRRI (14°12′N, 121°15′E, 22 m above sea level). Greenhouse colonies were established from each of the two locations and were maintained on variety IR22, which has no major genes for BPH resistance. All greenhouse phenotyping experiments were conducted within six generations of colony establishment.

Phenotyping

We conducted six phenotypic tests of BPH resistance. Three tests measured plant reaction to BPH feeding: seedbox screening, field screening, and tolerance. Three tests measured the reaction of BPH to the rice plants: feeding rate, antixenosis for settling, and antixenosis for oviposition. The 131 lines of the mapping population and IR64 and Azucena were included in all tests. All tests were conducted with both the IRRI and Central Luzon populations, with the exception of the field screening, which was done with only the IRRI population. The tests of feeding rate and antixenosis were conducted in a room maintained at 25–27°C. The other tests were conducted under ambient greenhouse conditions. During the period of testing (October 1996 to April 1997) noontime temperatures in the greenhouse ranged from 22 to 38°C. Relative humidity in the greenhouse and temperature-controlled room was 70–90%, and the natural photoperiod of approximately 12 h of light was used. Brief descriptions of the tests are provided below; additional details can be found in Heinrichs et al. (1985) and Cohen et al. (1997).

Greenhouse screening by the modified-seedbox test

Six pre-germinated seeds of each entry were sown in a seedbox (114 × 69 × 7 cm) in 10-cm rows, with a distance of 3.5 cm between the rows of each entry. We used a randomized complete block design with three replicates (seedboxes) for each BPH colony. Five days after sowing, the seedlings were thinned to four per row. The seedbox was covered with a fiberglass screen cage (112 × 67 × 75 cm) with nylon mesh at the top and at four side windows. At 10 days after sowing (DAS) the seedlings were infested with second- to third-instar nymphs at the rate of four per seedling. When all plants of the susceptible check variety, IR22, were dead, approximately 21 days after infestation (DAI), the entries were graded on a scale of 1 (= very slight damage) to 9 (= all plants dead).

Field screening using the insecticide-resurgence technique

Field screening was conducted on the IRRI experimental farm during the 1997 dry season (January to April). Three weeks prior to transplanting the test varieties, 20-day old plants of IR22 were transplanted in four 21-m strips spaced 1 m apart. Each strip consisted of four rows with a plant spacing of 20 cm within rows and 25 cm between rows. Twenty-day-old plants of the test entries were transplanted in a randomized complete block design with three replicates. Each block was located between two of the IR22 strips transplanted earlier, and consisted of one 1-m row of each test entry with 25-cm plant spacing within and between rows. Eighteen days after the test entries were transplanted, the strips of IR22 were sprayed with deltamethrin insecticide at a rate of 30 g of active ingredient per ha. [Deltamethrin is one of several broad-spectrum insecticides known to cause BPH population resurgence by stimulating BPH reproduction and by killing BPH predators and parasitoids (Heinrichs and Mochida 1984).] Two days later, these strips were infested with five second- and third-instar BPH nymphs per plant, with insects from the IRRI greenhouse colony. The insecticide application was repeated at 12 and 27 DAI. When the IR22 plants in the rows of test entries were beginning to die, the entries were graded for plant damage using the 1–9 scale.

Antixenosis

Pre-germinated seeds were sown in a $114 \times 69 \times 7$-cm seedbox and thinned after 1 week to one plant per entry, with 5×5 cm spacing. We used a randomized complete block design with four replicates (seedboxes) for each BPH colony. At 35 DAS the plants were trimmed to one tiller and the seedboxes were immersed in water and covered with a fiberglass screen cage ($112 \times 67 \times 75$ cm), with nylon mesh at the top and at four side windows. The cages were infested with gravid brachypterous BPH at a rate of four per seedling. The insects were able to move among the plants by swimming or walking. The number of insects on each plant was visually recorded at 24, 48, and 72 h after infestation. At 72 h, the plants were cut at soil level and dissected under a microscope to count the eggs deposited.

Feeding rate

Newly emerged adult females were held for 2 hours in a Petri dish with a moist cotton ball and then transferred singly to parafilm envelopes attached to the culm of 35 day-old plants, one envelope per plant. After 24 h the insects were removed and the weight of honeydew in each envelope was determined using a 0.1-mg sensitivity balance. The experiment was arranged in a randomized complete block design with three replicates (insects) per BPH colony.

Tolerance

Four pre-germinated seeds of the test entries were sown in pots and thinned to two per pot at 7 DAS. At 15 DAS, the plants were washed thoroughly and yellowing and outer leaf sheaths were cut to remove all arthropods, and a 13×90-cm cylindrical mylar cage was placed over each pot. Plants were infested with 35 or 70 first-instar BPH nymphs at 30 DAS. Control plants were left uninfested. The plants were arranged in a randomized complete block design, with three replicates (plants) of each test entry for each level of infestation. Due to the large numbers of plants in the experiment, one replicate was infested on December 5, 1996, and the second and third replicates on February 7, 1997. (Temperatures during December 1996 and February 1997 were similar, with average monthly temperatures recorded on the IRRI farm by the IRRI Climate Unit of 24.9 and 25.4°C, respectively.) When the susceptible check (IR22) was beginning to turn brown (14–17 DAI), the BPH on each were collected, dried at 60°C for 48 h, and weighed on a 0.1-mg sensitivity balance. The plants were removed from the pots, washed to clean the soil from the roots, dried at 75°C for 60 h, and weighed. We calculated a tolerance index (TI), using the formula of Dixon et al. (1990):

$$TI = [(W_c\text{-}W_t)/W_c)]/BPH,$$

where W_c is the weight of the uninfested control plant, W_t is the weight of the infested plant, and BPH is the weight of the *N. lugens* collected from the infested plant.

Statistical analysis

The data from the six tests were analyzed to determine the significance and chromosomal location of QTLs contributing to BPH resistance. The continuity and normality of the distributions were assessed graphically through frequency histograms of line means. Mapmaker QTL (Lander et al. 1987) was used for interval mapping (locating the most important QTL between flanking markers by maximum-likelihood estimation) and estimating the percentage of the phenotypic variance explained by each QTL. We considered a QTL to be significant if it exceeded a threshold LOD ($\log_{10}$-likelihood ratio) score of 1.5 in at least 2 of the 11 resistance tests, or of 3.0 in at least one test. (We conducted six tests with the IRRI population, and five with the Central Luzon population.)

Results

Statistical parameters of the mapping population

In all six tests of BPH resistance, Azucena and IR64 were well differentiated, with Azucena showing a higher phenotypic value, i.e., greater susceptibility (Table 1). Neither BPH population showed consistently higher levels of adaptation than the other, to either of the two parent varieties, across the resistance tests. However, in all tests, the mean phenotypic value of the doubled-haploid population was higher when screened with the CL than with the IRRI BPH colony. The lower adaptation of the IRRI population to the resistance genes in the mapping population was also reflected in the mean frequency histograms of variables graded in the resistance tests, which were generally skewed toward resistant scores (Fig. 1 b, c, e, g, i, and k). In contrast, the frequency distributions of scores with the CL population were more normally distributed (Fig. 1 a, d, f, h, and j). The frequency distributions of resistance scores for all tests are indicative of either oligogenic or polygenic control of the resistance traits.

Identification of QTLs associated with *N. lugens* resistance

We detected seven QTLs that exceeded the threshold criteria, on 6 of the 12 rice chromosomes (Table 2, Fig. 2). The peak LOD scores ranged from 1.51 to 3.69, and the percentage of phenotypic variance explained by any single QTL ranged from 5.1 to 16.6 (Table 2).

Table 1 Phenotypic values of parents and the IR64 × Azucena doubled-haploid population for brown planthopper resistance tests

Traits[a]	Parents[b]		Doubled-haploid population		
	IR64	Azucena	Mean	SD	Range
Seedbox screening[c] CL	4.67 ± 0.33	9.00 ± 0.00	6.30	1.05	4.33–9.00
Seedbox screening[c] IRRI	3.67 ± 0.67	9.00 ± 0.00	5.38	1.39	3.00–9.00
Field screening[c] IRRI	5.00 ± 0.00	9.00 ± 0.00	6.18	1.54	3.00–9.00
Feeding rate[d] CL	4.2 ± 1.0	36.2 ± 4.4	15.1	7.7	1.4–39.9
Feeding rate[d] IRRI	5.2 ± 1.1	36.4 ± 5.2	10.8	8.7	1.5–48.6
Antixenosis (settling)[e] CL	2.08 ± 0.30	8.75 ± 0.38	3.77	1.78	0.75–9.00
Antixenosis (settling)[e] IRRI	1.58 ± 0.08	9.50 ± 0.14	3.81	2.21	0.00–9.00
Antixenosis (oviposition)[f] CL	23.00 ± 5.52	254.50 ± 23.25	144.23	61.62	9.75–315.25
Antixenosis (oviposition)[f] IRRI	18.50 ± 4.66	245.25 ± 24.99	110.50	68.67	0.00–320.50
Tolerance[g] CL	0.64 ± 0.46	7.20 ± 0.91	7.69	3.65	0.14–17.72
Tolerance[g] IRRI	0.93 ± 0.36	7.68 ± 2.16	7.36	4.20	0.49–16.84

[a] CL, Central Luzon BPH population; IRRI, IRRI BPH population
[b] Mean ± SE
[c] Damage rating, 1–9 scale
[d] mg honeydew produced per female per 24 h
[e] BPH per plant
[f] Eggs laid per plant
[g] Tolerance index

These values indicate that the contribution of each QTL to BPH resistance is small. However, increased confidence in the association of these loci with BPH resistance is provided by the facts that all the QTLs were identified in at least two independent tests and that, for all tests detecting a particular QTL, the direction of the effect was the same (Table 2, Fig. 2). An additional five QTLs with LOD scores ≥ 1.5 were dectected in only a single resistance test, but the highest LOD score among these was only 2.38 (data not shown).

QTLs detected in 7 of the 11 tests of resistance mapped to a segment of chromosome 6 between the markers *Amp-3* and pRD10B. These tests were the four measuring antixenosis as well as the seedbox-screening tests for both BPH populations and the field-screening test conducted with the IRRI population. The QTL detected by field screening did not overlap with the region detected in three of the four antixenosis QTLs, suggesting that there may be two resistance-associated loci in this region of chromosome 6.

A QTL for all four antixenosis tests (to BPH settling and oviposition, for both the CL and IRRI populations) mapped to the same segment of chromosome 8 and shared the same peak interval (Table 2, Fig. 2). (Because the QTLs identified for BPH settling at 24, 48, and 72 h mapped to the same locations and explained similar percentages of variation, only the results for the 24-h time period are shown in Tables 1 and 2 and on Figs. 1 and 2.) This QTL on chromosome 8 was detected in only one other test, the seedbox test with the CL population. Another QTL for antixenosis to settling for both BPH populations was identified on chromosome 3, where resistance to the IRRI population as measured by feeding-rate was also mapped. This was the only significant QTL detected in the feeding-rate test with the IRRI population, and no significant QTLs were detected in the feeding-rate test with the CL population.

A QTL for plant tolerance to feeding by both BPH populations was detected on chromosome 1, with the peak interval in both cases between Amy1B and RZ276. Of the two levels of BPH infestation used in tolerance tests (35 and 70 insects per plant), the lower level proved more sensitive in detecting QTLs, possibly because even the more-resistant lines of the mapping population were heavily damaged at the higher infestation level. Thus, only the results for the level of 35 BPH per plant are shown in Tables 1 and 2 and on Figs. 1 and 2.

Huang et al. (1997), using the same IR64 × Azucena mapping population, mapped the major resistance gene *Bph1* between the markers *Sdh-1* and CDO334 on chromosome 12. We detected only one QTL exceeding a LOD score of 1.5 between these two markers. This was a QTL detected in the seedbox test, which had a peak LOD score of 1.61. Our results demonstrate that both the CL and IRRI populations were almost completely adapted to *Bph1*, and thus that the residual resistance of IR64 to these populations is attributable to other loci. Had we used BPH populations not adapted to *Bph1*, the effect of the major gene would have largely obscured the QTLs.

Transgressive segregation in both directions was observed in most of the resistance tests, indicating that neither parent contained all the alleles for resistance or susceptibility (Table 1). Exceptions included the three tests scored on the 1–9 scale (which are less quantitative than the other tests) in which Azucena was fully susceptible; and the test of antixenosis for settling with the CL

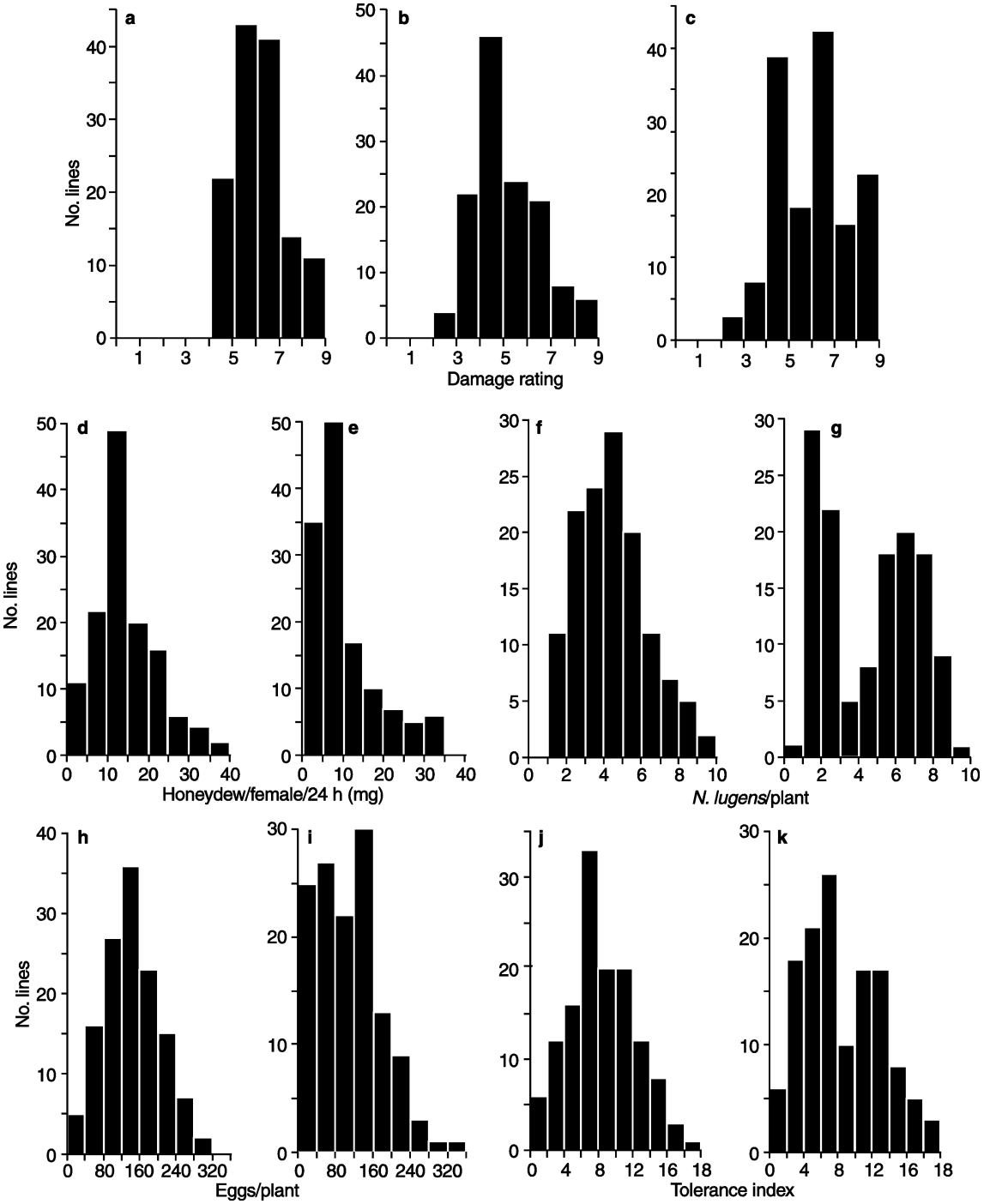

Fig. 1 Frequency distributions of phenotypic values from BPH resistance tests. **a** Seedbox screening with the Central Luzon (CL) BPH population, **b** seedbox screening with the IRRI BPH population, **c** field screening with IRRI, **d** feeding rate with CL, **e** feeding rate with IRRI, **f** antixenosis for settling with CL, **g** antixenosis for settling with IRRI, **h** antixenosis for oviposition with CL, **i** antixenosis for oviposition with IRRI, **j** tolerance index with CL, **k** tolerance index with IRRI

Table 2 Chromosomal locations and biometrical characteristics of QTLs for brown planthopper resistance

Trait	Peak interval		Chromosome	Peak LOD	% Variance explained	Additive[a]
Seedbox screening CL	W1	RG173	1	1.57	6.2	0.3223
	RZ144	RZ667	6	2.25	7.6	0.2921
	Amp-2	CDO99	8	1.56	5.6	0.2735
Seedbox screening IRRI	RG157	RZ318	2	1.88	8.0	0.4343
	RG143	RG620	4	3.01	10.1	− 0.4442
	Est-2	RZ144	6	2.27	7.8	0.3953
Field screening IRRI	RG157	RZ318	2	2.31	9.6	0.5244
	RG163	RZ590	4	3.52	16.6	− 0.6956
	Amp-3	*Est-2*	6	1.65	5.6	0.2811
Feeding rate IRRI	RG191	RZ678	3	3.69	13.0	3.5169
Antixenosis (settling) CL	RG191	RZ678	3	1.54	5.6	0.4710
	RZ144	RZ667	6	1.87	6.4	0.4515
	Amp-2	CDO99	8	2.20	8.1	0.5609
Antixenosis (settling) IRRI	RG191	RZ678	3	1.72	6.5	0.6134
	RZ667	Pgi-2	6	1.84	6.5	0.5685
	Amp-2	CDO99	8	1.99	7.6	0.6772
Antixenosis (oviposition) CL	W1	RG173	1	1.71	6.0	16.2100
	Pgi-2	pRD10B	6	1.83	6.4	16.6460
	Amp-2	CDO99	8	1.84	6.4	16.8750
Antixenosis (oviposition) IRRI	*Pgi-2*	pRD10B	6	1.87	6.4	17.4210
	Amp-2	CDO99	8	2.15	7.4	20.2470
Tolerance CL	Amy1B	RZ276	1	1.51	5.1	− 0.9317
Tolerance IRRI	Amy1B	RZ276	1	2.07	7.1	− 1.6920

[a] Additive: effect of Azucena alleles

population, where more BPH were found on Azucena than on any line in the mapping population.

As indicated by the negative value of the additive terms in Table 2, Azucena was the source of two QTLs for resistance. These were the QTL for tolerance detected on chromosome 1 with both the IRRI and CL populations, and a QTL mapped to chromosome 4 in the IRRI seedbox and field screening. Another QTL for resistance to the IRRI population in seedbox and field testing, originating from IR64, was identified on chromosome 2.

Discussion

Through QTL analysis of the IR64 × Azucena mapping population, we have established that several loci underlie the moderate and durable level of BPH resistance in IR64 that we detected in other studies (Cohen et al. 1997; Alam and Cohen 1998). Our results shed light on how diverse loci can contribute to diverse resistance factors in a plant variety, and should also be useful in breeding additional rice varieties with improved resistance to BPH.

The LOD scores for the QTLs we report in Table 2 and Fig. 2 are relatively low, but all these QTLs were identified in at least two independent resistance tests and showed the same direction of effect in all tests. Several other studies have determined, as we have, that moderate levels of insect resistance in plants can be the product of numerous loci, each explaining a relatively small amount of phenotypic variance. Yencho et al. (1996) phenotyped two *Solanum tuberosum* × *Solanum berthaultii* mapping populations for resistance to Colorado potato beetle, *Leptinotarsa decemlineata*, using tests of insect foliage consumption and egg production. Using threshold criteria similar to those of our study, these authors detected four QTLs for insect resistance. LOD scores for these QTLs ranged from 0.9 to 3.3, and the percentage of phenotypic variance explained by each QTL ranged from 3.4 to 12.6. Bonierbale et al. (1994) phenotyped the same mapping populations by measuring trichome density and biochemical tests related to trichome-mediated insect resistance. QTLs for several of these traits mapped to the same locations as the QTLs identified by Yencho et al. (1996) with insect-based bioassays, but the LOD scores obtained through the biochemical and morphological tests were generally higher (ranging as high as 20). Schon et al. (1993) phenotyped a maize mapping population for resistance to second-generation European corn borer, *Ostrinia nubilalis*, by quantifying tunnel length caused by larval feeding within stems. They identified seven QTLs, explaining from 3.4 to 15.7% of the phenotypic variance, with peak LOD scores of 2.3 to 9.1. Also in maize, Bohn et al. (1996) detected ten QTLs associated with resistance to feeding damage by first-generation sugarcane borer, *Diatraea saccharalis*. The LOD scores for these QTLs ranged from 2.5 to 5.8, with each QTL accounting for 7.2 to 15.4% of the phenotypic variance. Maliepaard et al. (1995) quantifed greenhouse whitefly, *Trialeurodes vaporariorum*, resistance in a

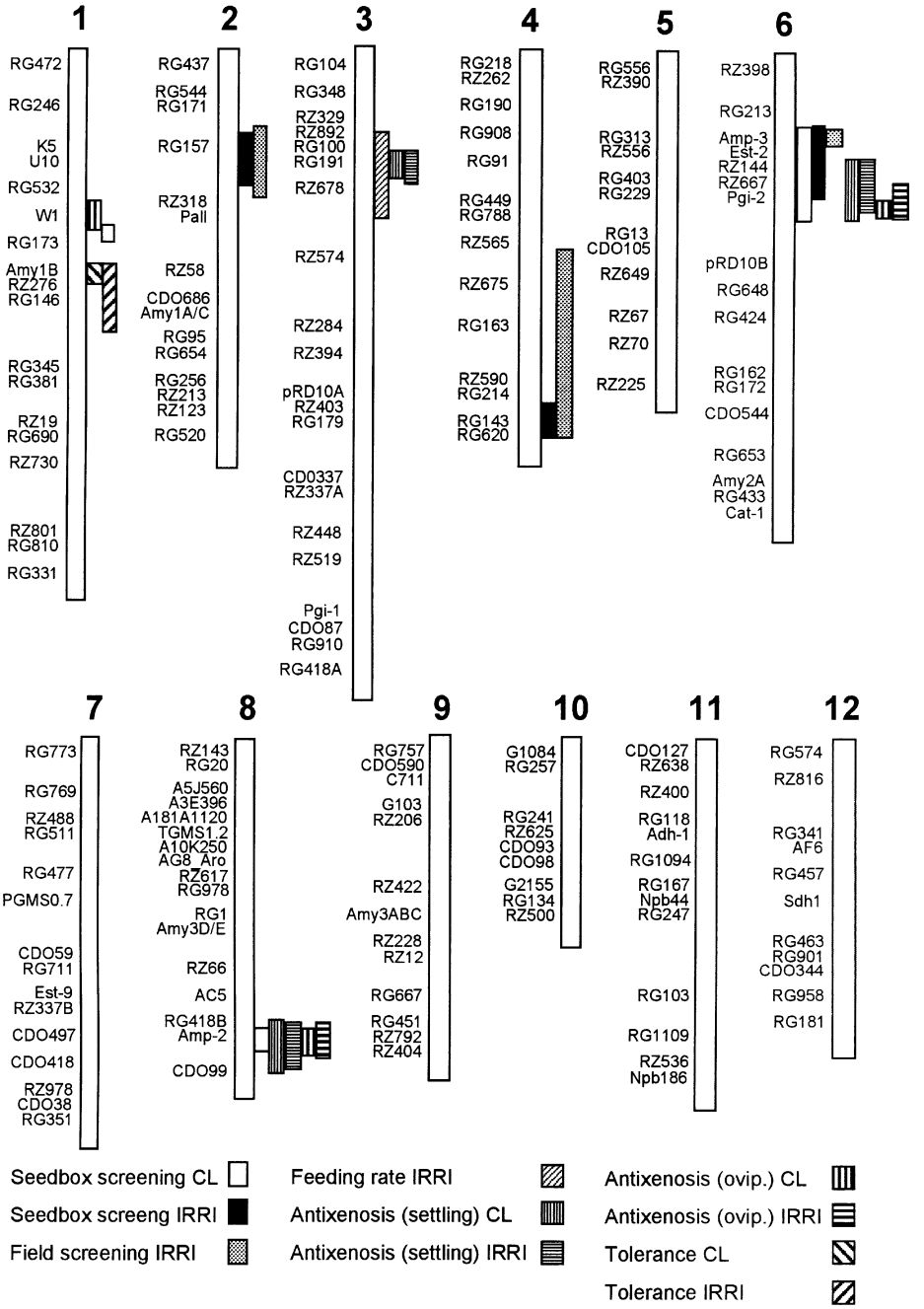

Fig. 2 QTLs identified for resistance to brown planthopper in an IR64 × Azucena doubled-haploid population (interval mapping with Mapmaker/QTL, LOD > 1.5), mapped onto the 12 rice chromosomes. The distances between markers on the figure are approximate

Lycopersicon esculentun × *L. hirsutum* f. *glaratum* mapping population. Two QTLs, with peak LOD scores of approximately 4 and 5, and explaining 6.4 and 8.0% of the phenotypic variance, were identified in one test measuring whitefly oviposition rate.

One of the objectives of our study was to determine the degree to which the three basic mechanisms of insect resistance (antixenosis, antibiosis, and tolerance), all of which have been detected in IR64 in relation to BPH (Cohen et al. 1997), would be influenced by different loci. One difficulty in addressing this question is that it is hard to precisely distinguish among the three resistance mechanisms. For example, the results of our test of antixenosis for settling might have been influenced by ingested antibiotic factors which later stimulated BPH to disperse after the manifestation of negative physiological effects. This situation is in fact suggested by the QTL on chromosome 3, which was detected in the feeding-rate test with the IRRI population and the antixenosis-for-settling test with both populations. Similarly, it has not been possible to develop techniques to entirely remove the effects of

antixenosis and antibiosis when quantifying tolerance (Reese et al. 1994). Because of the difficulty in measuring the effects of one resistance mechanism without interference from one or both of the other two, the pleiotropic effects of regulatory loci, and the effects that a single chemical might have on both antixenosis and antibiosis, we expected that the majority of QTLs would be detected in multiple tests designed to quantify different resistance mechanisms. Nonetheless, two QTLs were detected only, or predominantly, in tests of tolerance and antixenosis, suggesting that some QTLs confer resistance principally attributable to particular mechanisms. One QTL on chromosome 1 was detected only in the tolerance test with both BPH populations, and another on chromosome 8 was detected in all four antixenosis tests as well as in the seedbox-screening test with the CL population. Two other QTLs on chromosomes 2 and 4 were detected only in the seedbox and field-screening tests with the IRRI population, but these tests, which quantify plant damage following insect infestation, can be affected by all three resistance mechanisms.

Although IR64 was the more resistant parent in all phenotyping tests (Table 1), two alleles for resistance (on chromosomes 1 and 4) were derived from Azucena (Table 2). These QTLs were associated with tolerance to BPH feeding and with resistance in the seedbox and field screening tests, which can be highly influenced by plant tolerance. Traditional upland varieties such as Azucena might be expected to show substantial tolerance to insect feeding because of selection over many years for reliable yields under conditions of low fertilizer input, irregular water availability, and absence of protection by insecticides. However, it is somewhat surprising that additional tolerance-associated QTLs were not identified from IR64. In tolerance tests in the present study (Table 1) and in an earlier study with BPH from Central Luzon (Cohen et al. 1997), IR64 showed a greater level of tolerance than Azucena. The index we used to measure tolerance is derived from measurements of both plant and insect biomass and thus may be more difficult to accurately quantify, requiring a larger number of replicates to identify additional QTLs. Tolerance is a particularly advantageous mechanism of host-plant resistance because it does not exert selection for adaptation on pest populations, and therefore should theoretically be of unlimited durability (Kennedy et al. 1987). The QTL identified for tolerance from Azucena was identified only in this resistance test, which suggests that it may be possible to selectively breed for tolerance to BPH.

Three out of the seven QTLs identified were detected in tests with only one of the two BPH populations. The CL BPH population showed greater adaptation to the doubled-haploid population than did the IRRI population (Table 1), which may in part explain why the QTLs on chromosomes 2 and 4 were detected only with the IRRI population. There was, however, a QTL on chromosome 1 that was detected only with the CL population. Although there are some known differences between the environments of Central Luzon and the area surrounding IRRI, e.g., dry season temperatures in Central Luzon are higher and varietal diversity on the IRRI farm is much greater, we do not know the specific factors that account for the particular differences in QTLs detected with the two populations. Should future breeding efforts make use of the QTLs identified in this study, it may be most productive to focus on those QTLs that were associated with resistance to both BPH populations. We expect that some of the QTLs detected in this study would also be detected in tests with BPH populations from additional environments, because of the durability of minor gene BPH resistance in IR64 under field conditions in Central Luzon (Cohen et al. 1997) and in greenhouse selection studies with BPH populations from IRRI, Central Luzon, and Banaue, a distinct high-elevation site on Luzon (Alam and Cohen 1998).

To clone QTLs or make use of QTLs in marker-assisted breeding, it is helpful to identify markers tightly linked to them. Phenotyping additional recombinant lines to fine-map particular QTLs for BPH resistance identified in this study will be difficult, because each QTL accounts for a relatively small amount of phenotypic variation (5–17%; Table 2). An alternative strategy to facilitate cloning or marker-assisted breeding with QTLs is the "candidate gene approach," in which hypotheses are tested regarding the association of QTLs with known genes (Crandall 1996). For example, Byrne et al. (1996) determined that QTLs for corn earworm, *Helicoverpa zea*, resistance co-localize with genes associated with the metabolic pathway of maysin, a C-glycosyl flavone known to be an *H. zea* resistance factor in maize silks. To pursue this type of candidate gene approach, it is necessary that some of the chemical factors responsible for insect resistance in the parents of the mapping population be known. Apigenein-C-glycosides in phloem (Stevenson et al. 1996) and hydrocarbon- and carbonyl-containing fractions of the surface wax (Woodhead and Padgham 1988) have been shown to contribute to BPH resistance in some rice varieties. However, these studies were not conducted with IR64 or Azucena, and in fact no studies have been done to determine whether resistance in these varieties is phloem and/or surface-based. Thus, much additional work will be required before candidate genes associated with BPH resistance in IR64 or Azucena can be proposed.

A diversity of approaches is now available to rice breeders and entomologists for producing new BPH-resistant rice varieties. Several major genes continue to be used singly in traditional pedigree breeding. While monogenic resistance to BPH has often been short-lived, it has been suggested that in areas where insecticide over-use has declined and insecticide-induced BPH outbreaks are infrequent, major genes should

have increased durability (Gallagher et al. 1994). Alternatively, because several major BPH resistance genes have now been tagged and mapped (e.g., Ishii et al. 1994; Huang et al. 1997), pyramiding of major genes is also possible. However, two genes that have been tagged, *Bph1* and *bph2*, are no longer effective in some parts of Asia, (e.g. Gallagher et al. 1994; Cohen et al. 1997). In addition, pyramiding major genes will not necessarily provide more years of protection than sequential release (Kennedy et al. 1987). Genetic engineering is another option. At least one protein, a lectin from the snowdrop, *Galanthus nivalis*, has been found to confer resistance to BPH when produced in transgenic rice (Gatehouse et al. 1996), and other genes for transgenic resistance to the Homoptera are likely to become available. The cost, efficiency, and durability of marker-assisted selection with QTLs will have to be judged in comparison with these other available approaches for BPH resistance.

Acknowledgements We are grateful to Dr. B. Courtois for help in data analysis and interpretation, and to R. Abas for technical assistance. The experiments reported in this paper were conducted in compliance with the laws of the Republic of the Philippines.

References

Alam SN, Cohen MB (1998) Durability of brown planthopper, *Nilaparvata lugens*, resistance in rice variety IR64 in greenhouse selection studies. Entomol Exp Appl (in press)

Bohn M, Khairallah MM, Gozalez-de-Leon D, Hoisington DA, Utz HF, Deutsch JA, Jewell DC, Mihm JA, Melchinger AE (1996) QTL mapping in tropical maize. 1. Genomic regions affecting feeding resistance to sugarcane borer and other traits. Crop Sci 36:1352–1361

Bonierbale MW, Plaisted RL, Pineda O, Tanksley SD (1994) QTL analysis of trichome-mediated insect resistance in potato. Theor Appl Genet 87:973–987

Bosque-Perez NA, Buddenhagen IW (1992) The development of host-plant resistance to insect pests: outlook for the tropics. In: Menken SBJ, Visser JH, Harrewijn P (eds) Proc 8th Int Symp Insect-Plant Relationships. Kluwer Academic Publishers, Dordrecht, pp 235–249

Byrne PF, McMullen MD, Snooks ME, Musket TA, Theuri JM, Widstorm NW, Wiseman BR, Coe EH (1996) Quantitative trait loci and metabolic pathways: genetic control of the concentration of maysin, a corn earworm resistance factor, in maize silks. Proc Natl Acad Sci USA 93:8820–8825

Cohen MB, Alam, SN, Medina, EB, Bernal, CC (1997) Brown planthopper, *Nilaparvata lugens*, resistance in rice cultivar IR64: mechanism and role in successful *N. lugens* management in central Luzon, Philippines. Entomol Exp Appl 85: 221–229

Crandall KA (1996) Identifying links between genotype and phenotype using marker loci and candidate genes. In: Sobral BWS (ed) The impact of plant molecular genetics. Birkhauser, Boston, pp 137–157

Dixon AGO, Bramel-Cox PJ, Reese JC, Harvey, TL (1990) Mechanism of resistance and their interactions in 12 sources of resistance to biotype E greenbug (Homoptera: Aphididae). J Econ Entomol 83:234–240

Gallagher KD, Kenmore PE, Sogawa K (1994) Judicial use of insecticides deter planthoppers outbreak and extend the life of resistance varieties in southeast Asian rice. In Denno RF, Perfect JT (eds) Planthoppers: their ecology and management. Chapman and Hall, New York, pp 599–614

Gatehouse JA, Powell K, Edmonds H (1996) Genetic engineering of rice for resistance to homopteran insect pests. In: Khush GS (ed) Rice genetics III. Proc 3rd Int Rice Genet Symp 16–20 Oct 1995. International Rice Research Institute, Manila, The Philippines, pp 189–200

Guiderdoni E, Galinato E, Luistro J, Vergara G (1992) Anther culture of tropical japonica × indica hybrids of rice (*Oryza sativa* L.). Euphytica 62:219–224

Heinrichs EA (1986) Perspectives and directions for the continued development of insect-resistant rice varieties. Agric Ecosystems Environ 18:9–36

Heinrichs EA, Mochida O (1984) From secondary to major pest status: the case of insecticide-induced rice brown planthopper, *Nilaparvata lugens*, resurgence. Protection Ecol 7:201–218

Heinrichs EA, Medrano FG, Rapusas HR (1985) Genetic evaluation for insect resistance in rice. International Rice Research Institute, Manila, The Philippines

Huang N, Parco A, Mew T, Magpantay G, McCouch S, Guiderdoni E, Xu J, Subudhi P, Angeles ER, Khush GS (1997) RFLP mapping of isozymes, RAPD and QTLs for grain shape, brown planthopper resistance in a double-haploid population. Mol Breed 3:105–113

Ishii T, Brar DS, Multani DS, Khush GS (1994) Molecular tagging of genes for brown planthopper resistance and earliness introgressed from *Oryza autraliensis* into cultivated rice, *O. sativa*. Genome 37:217–221

Kenmore PE, Cariño FO, Perez CA, Dyck VA, Gutierrez AP (1984) Population regulation of the rice brown planthopper (*Nilaparvata lugens* Stål) within rice fields in the Philippines. J Plant Protection in the Tropics 1:19–37

Kennedy GG, Gould F, Deponti OMB, Stinner RE (1987) Ecological, agricultural, genetic and commercial considerations in the deployment of insect-resistant germplasm. Environ Entomol 16:327–338

Khush GS (1989) Multiple disease and insect resistance for increased yield stability in rice. In: Progress in irrigated rice research. International Rice Research Institute, Manila, The Philippines, pp 79–92

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincon SE, and Newburg L (1987) Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Maliepaard C, Bas N, van Heusden S, Kos J, Pet G, Verkerk R, Vrielink R, Zabel P, Lindhout P (1995) Mapping of QTLs for glandular trichome densities and *Trialeurodes vaporariorum* (greenhouse whitefly) resistance in an F_2 from *Lycopersicon esculentum* × *Lycopersicon hirsutum* f. *glabratum*. Heredity 75:425–433

Moharramipour S, Tsumuki H, Sato K, Yoshida H (1997) Mapping resistance to cereal aphids in barley. Theor Appl Genet 94:592–596

Mutschler MA, Doerge RW, Liu SC, Kuai JP, Liedl BE, Shapiro JA (1996) QTL analysis of pest resistance in wild tomato *Lycopersicon pennellii*: QTLs controlling acylsugar level and composition. Theor Appl Genet 92:709–718

Painter RH (1951) Insect resistance in crop plants. Macmillan, New York

Reese JC, Schwenke JR, Lamont PS, Zehr DD (1994) Importance and quantification of plant tolerance in crop pest management programs for aphids: greenbug resistance in sorghum. J Agric Entomol 11:255–270

Schon CC, Lee M, Melchinger AE, Guthrie WD, Woodman WL (1993) Mapping and characterization of quantitative trait loci affecting resistance against second-generation European corn borer in maize with the aid of RFLPs. Heredity 70:648–659

Stevenson PC, Kimmins FM, Grayer RJ, Raveendranath S (1996) Schaftosides from rice phloem as feeding inhibitors and

resistance factors to brown planthoppers, *Nilaparvata lugens*. Entomol Exp Appl 80 : 246–249

Way MJ, Heong KL (1994) The role of biodiversity in the dynamics and management of insect pests of tropical irrigated rice—a review. Bull Entomol Res 84 : 567–587

Woodhead S, Padgham DE (1988) The effect of plant surface characteristics on resistance of rice to the brown planthopper, *Nilaparvata lugens*. Entomol Exp Appl 47 : 15–22

Yadav R, Courtois B, Huang N, McLaren, G (1997) Mapping genes controlling root morphology and root distribution in a doubled-haploid population of rice. Theor Appl Genet 94 : 619–632

Yencho GC, Bonierbale MW, Tingey WM, Plaisted RL, Tanksley SD (1996) Molecular markers locate genes for resistance to the Colorado potato beetle, *Leptinotarsa decemlineata*, in hybrid *Solanum tuberosum* × *S. berthaultii* potato progenies. Entomol Exp Appl 81 : 141–154

View publication stats