Mapping of Quantitative Trait Loci Associated with Resistance to Brown Planthopper in Rice by Means of a Doubled Haploid Population

R. P. Soundararajan, P. Kadirvel, K. Gunathilagaraj, and M. Maheswaran*

ABSTRACT

The brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), is one of the major insect pests of rice (*Oryza sativa* L.). Many major genes are now available to manage this pest through host-plant resistance. In this study, we mapped quantitative trait loci (QTLs) associated with resistance to BPH using a doubled haploid (DH) population derived from the cross IR64/Azucena. We evaluated a set of 94 DH lines using a series of phenotypic tests that cover seedling resistance and resistance mechanisms: antixenosis, antibiosis, and tolerance. QTL analysis detected six QTLs on chromosomes 1, 2, 6, and 7 associated with resistance to BPH in this mapping population. The QTLs on chromosome 7 (Est9–RZ337B) and 2 (RG157–RZ318) showed their association with seedling resistance and antibiosis, respectively. Four QTLs on chromosomes 1 (RG146–RG345), 6 (RG213–Est2; Pgi2–pRD10B), and 7 (RG773–Est2) showed their association with tolerance. The phenotypic contribution of the QTLs ranged from 10.4 to 17.6%. The study confirmed the presence of QTLs on chromosomes 1, 2, and 6 that have been previously reported for resistance to BPH populations of the Philippines. In addition, two additional QTLs were detected on chromosome 7 (Est7-RZ337B and RG773-CDO59) in the same mapping population. The results showed that detailed phenotypic analyses of plant resistance would help in improving the efficiency of QTL detection and in understanding the quantitative resistance to insect pests in crop plants.

The brown planthopper, a historically minor pest of rice, emerged as a major pest in the tropical Asia during green revolution of the 1960s (Heinrichs and Mochida, 1984; Gallagher et al., 1994). In managing this pest, host-plant resistance (HPR) has long been used as a viable alternative to chemical control methods. In general, plant resistance to BPH is recognized as a qualitative as well as quantitative trait. The genetic basis of qualitative resistance has been well established and 14 major genes for resistance to BPH have been discovered from rice germplasm including nine from cultivated varieties (Khush and Brar, 1991) and five from wild relatives: one from *O. australiensis* (Ishii et al., 1994), three from *O. officinalis* (Hirabayashi et al., 1997; Renganayaki et al., 2002), and one from *O. latifolia* (Yang et al., 2002).

Breeding resistant rice cultivars with some of these major genes was highly successful (Khush, 1989). However, in some cases, this major gene resistance was short-lived because of the adaptation of the BPH population to the highly resistant varieties, harboring any one of these major genes (Gallagher et al., 1994; Ketipearachchi et al., 1998). It has long been suggested that quantitative resistance should be more durable (Heinrichs, 1986; Bosque-Perez and Buddenhagen, 1992). Until recently, the genetic basis of quantitative resistance to insect pests could not be explained owing to the inherent complexity of the trait and the limitations of conventional genetic tools. With the advent of new molecular genetic tools, the search for genes involved in complex traits has become a rapidly developing area of research. In the past decade, there has been great progress in identifying chromosomal regions that influence quantitative resistance to insects in many plants (Yencho et al., 2000).

In rice, the genetic basis of quantitative resistance to BPH has been established in the widely grown rice cultivar IR64 through QTL analysis. The availability of IR64/Azucena doubled haploid (DH) mapping population (Guiderdoni et al., 1992) and saturated molecular marker linkage map (Huang et al., 1997) led to identification of several QTLs underlying various resistance mechanisms to BPH populations of the Philippines (Alam and Cohen, 1998). Furthermore, Huang et al. (2001) reported BPH resistance QTLs from wild species. Recently, Xu et al. (2002) reported several main-effect and epistatic QTLs associated with quantitative resistance to BPH using the Teqing/Lemont recombinant inbred (RI) population, and Su et al. (2002) detected QTLs on chromosomes 2, 10, and 12 in a population of backcross inbred lines derived from Nipponbare (*japonica*)/Kasalath(*indica*)//Nipponbare. Thus, several QTLs have been reported for resistance to BPH across rice mapping populations. Our hypothesis is that successful detection of additional QTLs and validation of detected QTLs requires further dissection of plant resistance to BPH. It is well known that plant resistance to insects has evolved as a complex trait, and it results basically from three mechanisms: antixenosis, antibiosis, and tolerance (Painter, 1951). Here, we report our attempt in dissecting a complex resistance phenotype into various resistance mechanisms and identifying sensitive phenotypic screens that can detect QTLs associated with these mechanisms to BPH in the DH population derived from the cross IR64/Azucena.

MATERIALS AND METHODS

Plant Material

We used a mapping population of 94 DH lines obtained from the International Rice Research Institute (IRRI), Manila, the Philippines. It was derived from an F_1 hybrid between IR64, an *indica* variety adapted to irrigated conditions and

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Abbreviations: BPH, brown planthopper; DH, doubled haploid; HPR, host plant resistance; QTL, quantitative trait loci.

Azucena, a traditional upland *japonica* variety through anther culture (Guiderdoni et al., 1992).

Phenotyping

The phenotyping experiments were performed in the Toxicology Unit Glasshouse, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, from April 1998 to April 2000. The mean temperature during the study period ranged from 26.1 to 32.9°C and the relative humidity from 76.0 to 93.5%. BPH was mass cultured in the glasshouse on the susceptible rice variety Taichung Native 1 (TN1) as per the method of Heinrichs et al. (1985). The nymphs and adults obtained from stock culture were used in a series of phenotypic tests, to probe the three mechanisms of resistance. In each experiment, the parents, IR64 and Azucena, the standard susceptible check, TN1 and the resistant check, PTB33 were included. All the experiments were replicated thrice.

Seedling Screening

The standard seedbox screening test (SSST) was used to measure the levels of resistance of parents and DH lines at seedling stage (Heinrichs et al., 1985). The pre-germinated seeds of test lines were sown 3 cm apart in 20-cm rows in 60- by 50- by 10-cm wooden boxes. Each line was planted in three replications across the width of the seedbox with at least 15 plants per row. One row each of the susceptible check, TN1 and the resistant check, PTB33 was sown at random in all the seedboxes. Ten days after sowing (DAS), the seedlings were infested with first to third instar nymphs of BPH at the rate of five to eight nymphs per seedling. After infestation, the wooden seedboxes with seedlings were covered with wire mesh wooden cages (70 by 60 by 50 cm). The seedlings were observed daily for damage by BPH. 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 the Standard Evaluation System (SES) of Rice (IRRI, 1996).

Antixenosis

Antixenosis (AX) (preference of nymphs on seedlings) was assessed by means of a conventional seedbox test (Heinrichs et al., 1985). Seedlings were raised as in SSST but in 15-cm rows in wooden boxes (60 by 40 by 10 cm). At 10 DAS, approximately 1000 second instar BPH nymphs were released on the seedlings. The number of nymphs on each seedling was counted at 12, 24, 48, and 72 h after infestation. The seedlings were disturbed after each count for reorientation of nymphs on seedlings.

Antibiosis

Antibiosis was measured in terms of nymphal survival, developmental period, adult longevity, population increase and feeding rate. Fifteen-day-old seedlings were transplanted in 10-cm diameter clay pots. The potted plants were then covered with cylindrical mylar sheet cages (13 by 90 cm).

To study nymphal survival (NS), 10 first instar BPH nymphs were released on 35-d-old caged plants. The number of nymphs that reached adulthood was counted and the percentage of nymphal survival was calculated (Heinrichs et al., 1985). Developmental period (DP) of nymphs was studied by releasing three first instar nymphs on 35-d-old caged plants. The nymphs were observed daily for ecdysis and the number of days taken for the nymphs to reach the adult stage was recorded (Pongprasert and Weerapat, 1979). Growth index (GI) of BPH on each line was computed from the data obtained from the experiments on nymphal survival and developmental period as per cent of nymphs survived divided by the developmental period of nymphs (Panda and Heinrichs, 1983).

Adult longevity (AL) of BPH males (ALM) and females (ALF) was studied by releasing three pairs of newly emerged adults on 35-d-old caged plants. The adult BPHs were observed daily for their survival on each line. The population increase (PI) of BPH (Heinrichs et al., 1985) was studied by releasing 10 first instar nymphs on 35-d-old caged plants. The form (macroptery and brachyptery) and sex (female and male) of emerging adult BPHs were also observed during the development. The adults then oviposited on the plant and the nymphs developed after hatching. During the development of first generation nymphs, the plants of test lines started to wilt. Therefore, the experiment was stopped at different times and the population of hoppers was counted at the time when the plants started to wilt.

Feeding rate (FR) of BPH on each line was assessed by estimating the honeydew produced by the adult BPHs. Honeydew area (HDA) and weight (HDW) were used as measures to represent the feeding rate. The feeding chamber developed earlier (Sogawa and Pathak, 1976) was used for measuring the honeydew produced. Five freshly emerged female BPHs, prestarved for 6 h, were released in the chamber and after 24 h, the preweighed filter papers were removed and sprayed with 0.01% (w/v) ninhydrin-acetone solution. The honeydew stains appeared as violet or purple spots. The spots were traced on tracing paper and squares were counted over a millimeter square graph paper. The area of honeydew spot was expressed as mm^2. The filter paper was weighed and the weight of honeydew was calculated and expressed as mg.

Tolerance

To study the level of tolerance, 15-d-old seedlings were transplanted in 30-cm diameter clay pots. Thirty-five-day-old plants were caged with cylindrical mylar sheet cages (30 by 90 cm) and 50 first instar nymphs were released on each plant. A control plant without insects was maintained for each line. When the TN1 plants (susceptible check) started to wilt, the experiment was stopped and the BPHs were collected from all the lines, oven dried for 48 h and weighed. However, some of the DH lines wilted earlier than TN1. In such cases, we collected the insects before the wilting of TN1 plants. The infested and uninfested plants were removed from the pots along with roots, washed thoroughly, air dried for 3 h, then dried in an oven at 70°C for 60 h and weighed. The level of tolerance of DH lines was calculated using the following parameters such as functional plant loss index (FPLI), tolerance index (TI) and plant dry weight loss per mg of BPH dry weight produced (PDLOSS) as described by Panda and Heinrichs (1983).

$$\text{FPLI} = [1 - (\text{Dry weight of infested plant}/\text{Dry weight of uninfested plant})] \times 100$$

$$\text{TI} = \text{BPH dry weight on test line}/\text{BPH dry weight on susceptible check, TN1}$$

$$\text{PDLOSS} = (\text{Dry weight of uninfested plant} - \text{Dry weight of infested plant})/\text{Dry weight of BPH progeny on infested plant}$$

We used days to wilt (DW) as another measure of tolerance where the damage by BPH population on each line was estimated by counting the number of days required to kill the plants. Two different aged plants 30 and 60 d old were used for the experiment with two levels of insect load 25 and 50

Table 1. Phenotypic performance of standard checks, parents and DH lines of the cross IR64/Azucena for resistance to BPH.

Trait	Mean ± SE†					Range	LSD‡
	PTB33	TN1	IR 64	Azucena	DHLs		
Seedling resistance							
Seedbox screening (SSST) (damage rating of 1–9 scale)	3.7 ± 0.7c	8.3 ± 0.7a	5.7 ± 0.7b	9.0 ± 0.0a	7.1 ± 0.1	3–9	1.3
Antixenosis							
Antixenosis (AX) (BPH nymphs/seedling at 72 h after infestation)	2.4 ± 0.1c	7.1 ± 0.8a	4.3 ± 0.4b	6.8 ± 0.4a	4.2 ± 0.1	2.6–6.7	1.6
Antibiosis							
Nymphal survival (NS) (%)	63.3 ± 3.3b	83.3 ± 3.3a	73.3 ± 3.3b	76.7 ± 3.3ab	66.1 ± 0.9	30.0–86.7	19.2
Developmental period (DP) (days)	20.2 ± 0.3a	10.8 ± 0.3c	13.6 ± 0.6b	13.1 ± 0.4b	13.7 ± 0.1	9.2–18.0	1.3
Adult longevity (female) (ALF) (days)	7.2 ± 0.3b	12.0 ± 0.7a	11.3 ± 0.2a	11.8 ± 0.4a	8.9 ± 0.1	2.9–13.0	1.2
Adult longevity (male) (ALM) (days)	6.4 ± 0.1c	10.3 ± 0.3a	9.1 ± 0.4b	8.9 ± 0.4b	7.6 ± 0.1	2.7–11.5	1.3
Population increase (PI) (BPH nymphs/plant)	37.3 ± 4.7c	182.3 ± 5.5a	120.3 ± 5.8b	131 ± 13.7b	82.6 ± 1.4	26.3–172.0	19.3
Form (macroptery) (%)	23.3 ± 3.3a	0.0 ± 0.0b	23.3 ± 3.3a	3.3 ± 3.3b	7.7 ± 0.5	0–26.7	14.5
Form (brachyptery) (%)	56.7 ± 3.3b	96.7 ± 3.3a	66.7 ± 3.3b	93.3 ± 3.3a	81.5 ± 0.6	60–93.3	15.4
Sex (female) (%)	40 ± 11.5a	33.3 ± 3.3a	53.3 ± 3.3a	43.3 ± 3.3a	37.8 ± 0.6	16.7–56.7	16.0
Sex (male) (%)	40 ± 10.0b	63.3 ± 3.3a	36.7 ± 3.3b	53.3 ± 3.3ab	50.8 ± 0.7	26.7–73.3	16.1
Growth index (GI)	3.1 ± 0.2c	7.8 ± 0.4a	5.4 ± 0.4b	5.8 ± 0.1b	4.9 ± 0.1	2.8–7.4	1.5
Honeydew area (HDA) (mm^2)	36 ± 4.4c	682 ± 31.8a	448.3 ± 19.8b	614 ± 36.8a	367.4 ± 6.9	121.0–550.0	44.5
Honeydew weight (HDW) (mg)	3.1 ± 0.1b	9.2 ± 0.3a	7.2 ± 0.8a	8.7 ± 0.6a	6.0 ± 0.1	2.9–9.0	0.9
Tolerance							
Functional plant loss index (FPLI)	26.5 ± 1.4d	84.5 ± 0.4b	48.1 ± 2.4c	95.9 ± 0.5a	58.7 ± 0.8	33.6–93.9	10.0
Tolerance index (TI)	0.28 ± 0.0c	1.0 ± 0.0a	0.51 ± 0.0b	0.27 ± 0.0c	0.38 ± 0.0	0.18–0.71	0.1
Plant dry weight loss per mg of BPH dry weight produced (PDLOSS) (mg)	107.2 ± 16.8b	78.6 ± 0.4bc	60.8 ± 3.0c	304.6 ± 1.7a	89.8 ± 3.2	23.9–337.3	17.0
Days to wilt (30 d and 25 nymphs/plant) (DW1)	18.7 ± 0.3a	8.3 ± 0.3c	16.7 ± 0.3b	7.3 ± 0.3c	14.1 ± 0.1	8.7–19.7	2.2
Days to wilt (30 d and 50 nymphs/plant) (DW2)	12.7 ± 0.7a	5.7 ± 0.3b	12.7 ± 2.2a	5.3 ± 0.9b	9.0 ± 0.1	6.3–13.0	2.0
Days to wilt (60 d and 25 nymphs/plant) (DW3)	29.3 ± 0.7a	17.3 ± 0.7b	27.0 ± 0.6a	16 ± 1.2b	23.2 ± 0.2	14.3–34.7	1.9
Days to wilt (60 d and 50 nymphs/plant) (DW4)	21.3 ± 0.9a	9.3 ± 0.3c	17.7 ± 0.7b	11.3 ± 0.9c	14 ± 0.1	8.67–19.7	1.9

† Comparisons of means of PTB33, TN1, IR64, and Azucena based on *t* tests (LSD at 5% level); Means with the same letter are not significantly different.
‡ LSD of DHL means at 5% level of significance.

per plant (DW1, DW2, DW3 and DW4 respectively). At 15 DAS, the seedlings were transplanted in 30-cm diameter clay pots and caged with cylindrical mylar sheet cage (30 by 90 cm). The first instar nymphs were released on the plants and allowed to feed. The day on which the plant wilted completely was recorded.

Data Analysis

The data were analyzed by using the SAS package (SAS Institute, 1985). The mean and standard error of the phenotypic data were obtained by using the PROC MEANS procedure. The means of parents of the mapping population and standard checks were compared by *t* tests (Least square difference, LSD at 5% level of significance) and the LSD of DH line means at 5% level of significance were obtained by using the PROC GLM procedure.

The mean phenotypic values of the traits were used for QTL analysis. The marker data (175 markers comprising eight isozymes, 14 RAPD, 12 cloned genes, and 141 RFLP) developed by Huang et al. (1997) was used for mapping the putative QTLs associated with resistance to BPH using the computer software Mapmaker/QTL (Lander and Botstein, 1989). The threshold LOD score of 2.5 was fixed to claim the putative QTL. The LOD score of more than 2 and less than 2.5 was used to consider possible QTLs.

RESULTS AND DISCUSSION

The parents, IR64 and Azucena, differed significantly for 12 traits, whereas the standard checks, PTB33 and TN1, differed significantly for all 21 resistance traits used in the study, which represented antixenosis, antibiosis, and tolerance to BPH (Table 1). DH lines showed quantitative variation for the traits (Fig. 1). Six QTLs associated with resistance to BPH in this mapping population were detected (Table 2). The detected QTLs were mapped on chromosomes 1, 2, 6, and 7 (Fig. 2). The LOD scores ranged from 2.1 to 3.1 and the phenotypic variance explained by each QTL ranged from 10.4 to 17.6%. Though we used many phenotypic tests, only six of them were sensitive enough to detect QTLs associated with resistance to BPH. Seedbox screening test (SSST) and feeding rate based on honeydew production (HDW) detected single QTLs on chromosomes 7 and 2, respectively. Tolerance tests (FPLI, TI, DW2, and DW4) detected four QTLs on chromosomes 1, 6, and 7. Many QTLs with LOD scores between 1.5 and 2.0 were found in other tests but were not considered because of their low significance levels (data not shown). Thus, the results of this study showed that plant resistance to BPH in rice is a complex quantitative trait that is governed by many genes.

The quantitative resistance to BPH in IR64 has been studied since the observations that it shows moderate resistance to BPH, which might be due to presence of minor genes in addition to a major gene *Bph1* (Khush, 1989). Through greenhouse screening, Cohen et al.

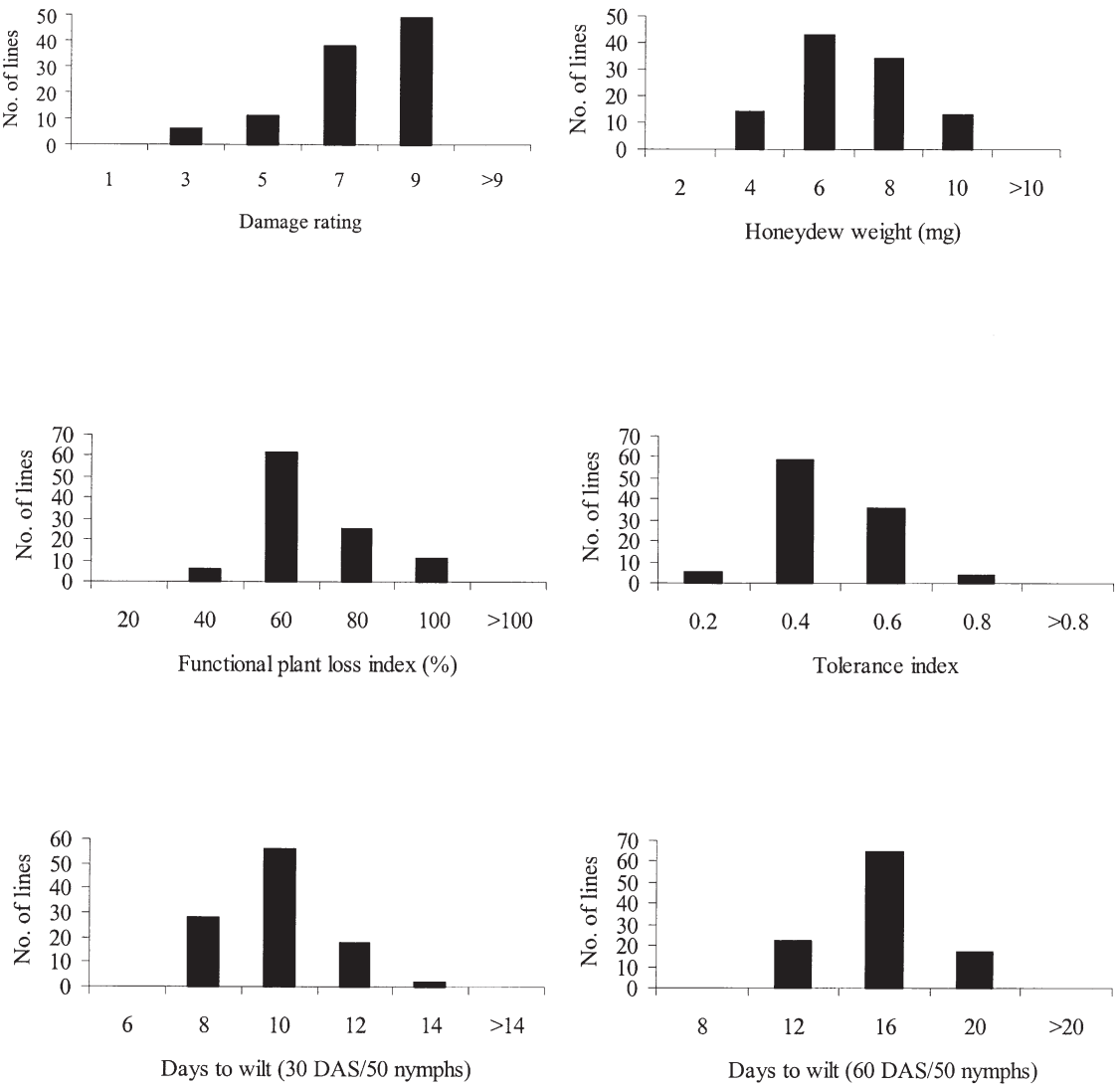

Fig. 1. Frequency distribution of phenotypic values of DH lines for various traits for resistance to BPH in the cross IR64/Azucena.

(1997) established that the major gene *Bph1* is no longer effective against two BPH populations of the Philippines and the moderate resistance results from minor genes associated with the three resistance mechanisms. Marker-based genetic analyses using the mapping population derived from the cross IR64/Azucena, resulted in detection of several QTLs associated with various resistance mechanisms to BPH (Alam and Cohen, 1998; Ramalingam et al., 2003).

We used the same mapping population to test our hypothesis that detection of additional QTLs and validation of the detected QTLs for resistance to BPH would require further phenotypic analyses of plant resistance. The study confirmed the presence of some of the QTLs previously reported and also found two additional QTLs for resistance to BPH in rice. Alam and Cohen (1998) reported seven significant QTLs for resistance to BPH that were mapped on chromosomes 1, 2, 3, 4, 6, and 8 using 175 markers. The peak LOD scores ranged from 1.5 to 3.7. Individual QTL accounted for between 5.1 to 16.6% of the phenotypic variance. Further, Ramalingam et al. (2003) reported four additional QTLs (one on chromosome 11 and three on chromosome 12) associated with resistance to BPH with LOD scores of 2.5 to

Table 2. The putative and possible QTLs detected for various traits associated with resistance to BPH in IR64/Azucena DH population.

Trait	Marker interval	Chromosome	LOD†	Variance (%)	Additive‡
Seedbox screening (SSST)	Est9–RZ337B	7	2.3	10.5	0.57
Honeydew weight (HDW)	RG157–RZ318	2	2.7	12.8	−0.61
Functional plant loss index (FPLI)	RG213–Est2	6	2.3	10.7	4.75
Tolerance index (TI)	RG146–RG345	1	2.1	10.4	0.06
Days to wilt (DW2) (30 d and 50 nymphs/plant)	Pgi2–pRD10B	6	2.5	14.3	−0.52
Days to wilt (DW4) (60 d and 50 nymphs/plant)	RG773–CDO59	7	3.1	17.6	1.1

†Putative QTL (LOD score of >2.5); possible QTL (LOD score of >2.0 and <2.5).
‡Effect of Azucena allele.

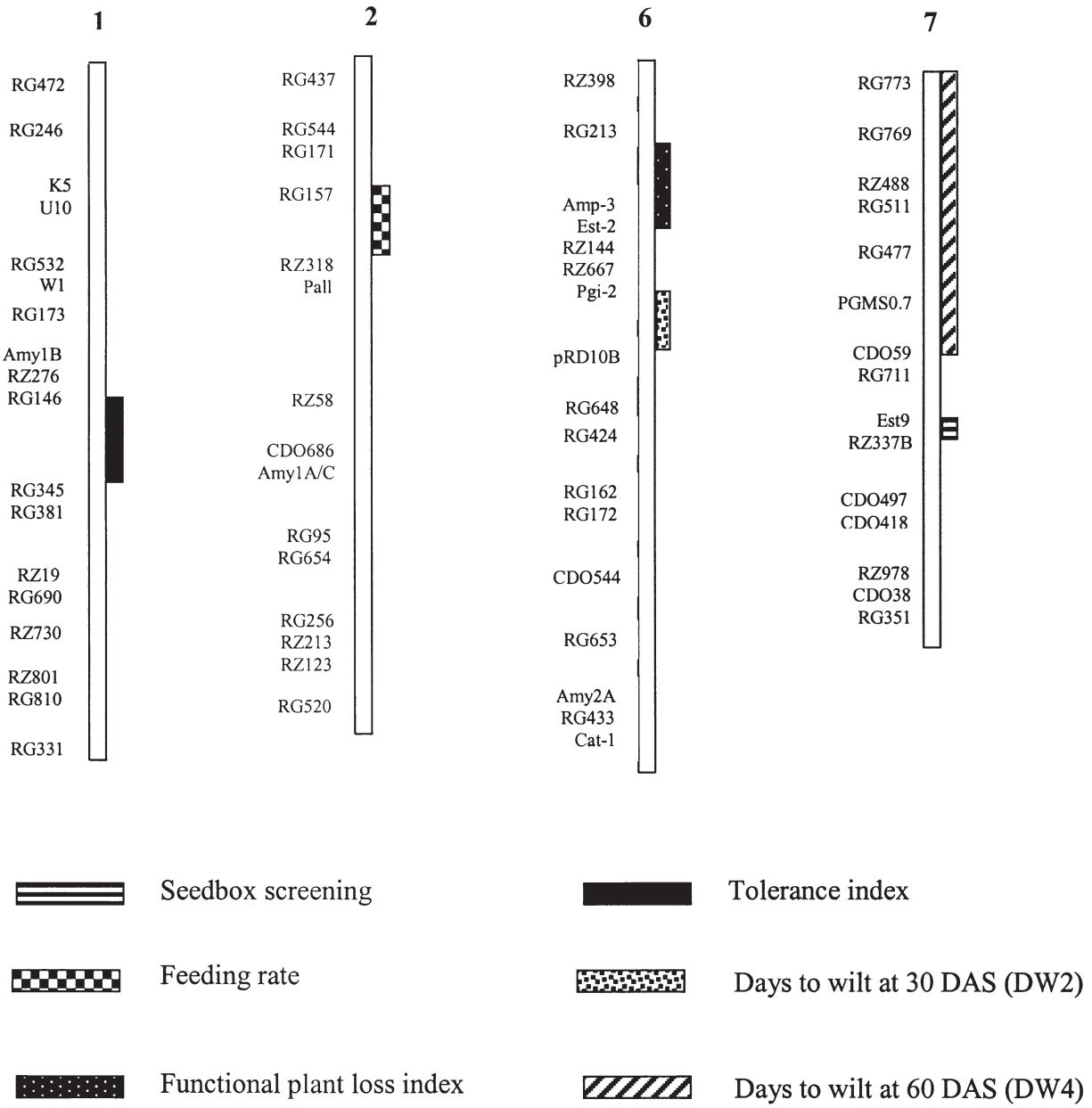

Fig. 2. Linkage map showing chromosomal locations of putative QTLs detected for resistance to BPH in IR64/Azucena DH population.

4.5 using additional 105 candidate gene markers in the same population.

The tolerance index (TI) QTL on chromosome 1 (RG146–RG345) detected in the present study was also reported by Alam and Cohen (1998). The QTL for antibiosis on chromosome 2 (RG157–RZ318) detected in the present study was shown to be associated with seedling resistance as well as for field resistance to BPH (Alam and Cohen, 1998). They detected a significant QTL on chromosome 6 in seedbox screening, field screening and antixenosis tests, but in our work the same genomic region was detected for tolerance based on FPLI and DW2. The noteworthy finding of the present study is that it detected two additional QTLs on chromosome 7 associated with seedling resistance as well as plant resistance at the age of 60 DAS. Our work did not detect significant QTLs on chromosomes 3, 4, 8, 11, and 12 as reported earlier by Alam and Cohen (1998) and Ramalingam et al. (2003). The results obtained in our work were based on only 94 DH lines and 175 markers. Alam and Cohen (1998) used 131 DH lines and 175 markers for their work. Ramalinagam et al. (2003) used only 96 DH lines but with additional 105 candidate gene markers in the same mapping population. Therefore, small population size and less number of markers might have resulted in missing of some of the QTLs that were detected in those studies.

These observations provide insights into some of the issues concerning genetic analysis of quantitative resistance to insects. First, designing new screening tests will aid in detection of additional QTLs and also validation of previously reported QTLs. In our work, in addition to the standard screening tests, we employed a new screening test, days to wilt (DW) as a measure of the

plant's reaction to BPH at two different age levels 30 and 60 DAS with two levels of insect load, 25 and 50 per plant (DW2 and DW4, respectively). Interestingly, two significant QTLs were detected for DW2 and DW4 on chromosomes 6 and 7, respectively.

Second, evaluation of a mapping population in different environments might detect common QTLs that operate across environments and also detect QTLs for specific environments. The present work and that of Alam and Cohen (1998) detected common QTLs on chromosomes 1, 2, and 6 against BPH populations of the Philippines and India. Two additional QTLs were detected on chromosome 7 from the experiment in India and not detected from the experiments in the Philippines suggesting that comparative mapping of QTLs across environments might improve the efficiency of QTL detection and increase the confidence with which QTLs are claimed.

Third, growth stage specific phenotypic analyses might result in detecting QTLs associated with specific plant ages. The detection of two different and significant QTLs on chromosome 6 (Pgi2–pRD10B) and chromosome 7 (RG773–CDO59) for DW at 30 and 60 DAS (DW2 and DW4), respectively indicates the possibility of two different loci controlling the resistance during different stages of plant growth. The influence of plant age on the level of resistance has been reported in a number of cases (Russell, 1978). In rice, it was observed that the levels of resistance expressed by the cultivars were dependent on plant age (Velusamy et al., 1986). For plant height and tiller number in rice, it was shown that the QTL expression dynamics varied with developmental stages of plants using the same IR64/Azucena mapping population (Yan et al., 1998; Wu et al., 1999). Most of the QTL mapping studies have been limited in analyzing the performance of a trait observed at a fixed time or stage of ontogenesis. Therefore, it is suggested that the genetic studies on quantitative resistance to insects must involve phenotypic analysis at the appropriate growth stages.

Fourth, though common QTLs were detected for resistance to BPH in both the studies, they were not common for a particular mechanism except for the tolerance QTL on chromosome 1. For example, the QTL on chromosome 6 detected for tolerance in our work was found to be associated with seedling screening, field screening and antixenosis in the study of Alam and Cohen (1998). It is also possible that the same QTL for tolerance might have contributed for resistance in field screening as detected by Alam and Cohen (1998). Similarly, the QTL on chromosome 2 for feeding rate detected in our work was found to be associated with seedbox screening and field screening. Other studies have already detected QTLs for specific mechanisms, for example, antixenosis in soybean (Rector et al., 1999) and antibiosis in maize and potato (Yencho et al., 2000). In rice, a QTL for resistance to BPH has been mapped near the marker *gl-1*, which controls the leaf pubescence (Xu et al., 2002). Such QTLs for different resistance mechanisms would be useful in breeding cultivars with a specific mechanism of resistance (Kennedy et al., 1987).

Conventional resistance breeding has been complemented with novel biotechnological tools such as molecular markers in the past decade. A number of genes and QTLs governing BPH resistance have been identified and mapped on available genetic linkage maps; however, the absence of tightly linked markers limits their practical utility for marker-assisted selection. It has to be realized that identification and mapping of major genes and QTLs is only the beginning of a molecular breeding process. After the QTL has been mapped, progress should be made toward fine mapping and development of near isogenic lines (NILs) for specific QTLs. The QTL analyses using segregating populations such as F_2, F_2–derived F_3 lines, RI lines, and DH lines can detect QTLs on the basis of statistical analyses but cannot clearly confirm whether the QTLs exist. Moreover, precise mapping and determining the actual genetic effect of a single QTL among multiple QTLs is difficult because the genetic parameters of the QTL are often affected by simultaneous segregation of other QTLs (Tanksley, 1993). Near isogenic lines are useful to overcome these difficulties and allow precise determination of the effect and location of a single QTL, resulting in fine mapping of QTLs as single Mendelian factors and positional cloning (Yamamoto et al., 1998; Yano et al., 2000; Yamasaki et al., 2003). Candidate gene approach is also expected to speed up the process of identifying genes underlying complex plant traits (Pflieger et al., 2001). Byrne et al. (1996) demonstrated the candidate gene approach to trace QTLs for resistance to European corn borer (*Ostrinia nubilalis* Hübner) in maize (*Zea mays* L.). So far, no such information is available in rice. However, Ramalingam et al. (2003) reported the association of defense related candidate genes with major genes and QTLs governing insect and disease resistance in rice using the same IR64/Azucena mapping population. Particularly, they showed two of the BPH QTLs colocalizing with candidate genes such as thaumatin and dihydrofolate reductase thymidylate synthase. These QTLs also colocalized with blast QTLs. These observations provide a starting point for exploring the candidate gene approach for insect resistance in rice. Thus, the QTLs detected in the present study should be further validated through fine mapping and candidate gene analysis to make use of them in marker assisted breeding to improve BPH resistance of rice cultivars.

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REFERENCES

Alam, S.N., and M.B. Cohen. 1998. Detection and analysis of QTLs for resistance to the brown planthopper, *Nilaparvata lugens*, in a doubled-haploid rice population. Theor. Appl. Genet. 97:1370–1379.

Bosque-Perez, N.A., and I.W. Buddenhagen. 1992. The development of host plant resistance to insects: Outlook for the tropics. p. 235–249. *In* S.B.J. Menken et al. (ed.) Proceedings of 8th International

Symposium on Insect-Plant relationships. Kluwer Academic Publishers, Dordreht, the Netherlands.

Byrne, P.F., M.D. McMullen, M.E. Snook, T.A. Musket, J.M. Theuri, N.M. Widstrom, B.R. Wiseman, and E.H. Coe. 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, M.B., S.N. Alam, E.B. Medina, and C.C. Bernal. 1997. Brown planthopper, *Nilaparvata lugens*, and resistance in rice cultivar IR 64: Mechanism and role in successful *N. lugens* management in Central Luzon, Philippines. Entomol. Exp. Appl. 85:221–229.

Gallagher, K.D., P.E Kenmore, and K. Sogawa. 1994. Judicial use of insecticides deter planthopper outbreaks and extend the life of resistant varieties in Southeast Asian rice. p. 599–614. *In* R.F. Denno and J.T. Perfect (ed.) Planthoppers: Their ecology and management. Chapman and Hall, New York.

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

Heinrichs, E.A. 1986. Perspectives and directions for the continued development of insect-resistant rice varieties. Agric. Ecosyst. Environ. 18:9–36.

Heinrichs, E.A., and O. Mochida. 1984. From secondary to major pest status: The case of insecticide-induced rice brown planthopper, *Nilaparvata lugens* resurgence. Prot. Ecol. 7:201–218.

Heinrichs, E.A., F.G. Medrano, and H.R. Rapusas. 1985. Genetic evaluation for insect resistance in rice. International Rice Research Institute, Manila, Philippines.

Hirabayashi, H., R. Kaji, T. Ogawa, D.S. Brar, A.R. Angeles, and G.S. Khush. 1997. Mapping of a new intorgression gene for brown planthopper (BPH) resistance from *O. officinalis* using RFLP markers in rice. p. 401–402. *In* Harmonizing agricultural productivity and conservation of biodiversity: Breeding and ecology. Proceedings 8th SABRAO Congress and Annual Meeting of Korean Breeding Society, Seoul Parktel, Seoul. 24–28 Sept. 1997. Korean Breeding Society and the Society for Advancement of Breeding Researches in Asia and Oceania.

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

Huang, Z., G. He, L. Shu, X. Li, and Q. Zhang. 2001. Identification and mapping of two brown planthopper resistance genes in rice. Theor. Appl. Genet. 102:929–934.

International Rice Research Institute. 1996. Standard evaluation system for rice. 4th ed. International Rice Research Institute, Manila, Philippines.

Ishii, T., D.S. Brar, D.S. Multani, and G.S. Khush. 1994. Molecular tagging of genes for brown planthopper resistance and earliness introgressed from *Oryza australiensis* into cultivated rice, *O. sativa.* Genome 37:217–221.

Kennedy, G.G., F. Gould, O.M.B. de Ponti, and R.E. Stinner. 1987. Ecological, agricultural, genetic and commercial considerations in the deployment of insect-resistant germplasm. Environ. Entomol. 16:327–338.

Ketipearachchi, Y., C. Kaneda, and C. Nakamura. 1998. Adaptation of the brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae) to BPH resistant rice cultivars carrying *bph8* or *Bph9.* Appl. Entomol. Zool. 33:497–505.

Khush, G.S. 1989. Multiple disease and insect resistance for increased yield stability in rice., p. 79–92 *In* IRRI (ed.) Progress in irrigated rice research. International Rice Research Institute, Manila, Philippines.

Khush, G.S., and D.S. Brar. 1991. Genetics of resistance to insects in crop plants. Adv. Agron. 45:223–274.

Lander, E.S., and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185–199.

Painter, R.H. 1951. Insect resistance in crop plants. Macmillan, New York.

Panda, N., and E.A. Heinrichs. 1983. Levels of tolerance and antibiosis in rice varieties having moderate resistance to the brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) Environ. Entomol. 12:1204–1214.

Pflieger, S., V. Lefebvre, and M. Causse. 2001. The candidate gene approach in plant genetics: A review. Mol. Breed. 7:275–291.

Pongprasert, S., and P. Weerapat. 1979. Varietal resistance to the brown planthopper in Thailand. p. 273– 284. *In* IRRI (ed.) Brown planthopper: Threat to rice production in Asia. The International Rice Research Institute, Manila, Philippines.

Ramalingam, J., C.M. Vera Cruz, K. Kukreja, J.M. Chittoor, J.-L. Wu, S.W. Lee, M. Baraoidan, M.L. George, M.B. Cohen, S.H. Hulbert, J.E. Leach, and H. Leung. 2003. Candidate defense genes from rice, barley and maize and their association with qualitative and quantitative resistance in rice. Mol. Plant Microb. Interact. 16: 14–24.

Rector, B.G., J.N. All, W.A. Parrott, and H.R. Boerma. 1999. Quantitative trait loci for antixenosis resistance to corn earworm in soybean. Crop Sci. 39:531–538.

Renganayaki, K., A.K. Fritz, S. Sadasivam, S. Pammi, S.E. Harington, S.R. McCouch, S. Mohankumar, and A.S. Reddy. 2002. Mapping and progress toward map-based cloning of brown planthopper biotype-4 resistance gene introgressed from *Oryza officinalis* into cultivated rice, *O. sativa.* Crop Sci. 42:2112–2117.

Russell, G.E. 1978. Plant breeding for pest and disease resistance. Butterworth, New York.

SAS Institute. 1985. SAS user's guide: Statistics, 5th ed. SAS Institute, Cary, NC.

Sogawa, K., and M.D. Pathak. 1976. Mechanism of brown planthopper resistance in Mudgo variety in rice (Hemiptera: Delphacidae). Appl. Entomol. Zool. 5:145–158.

Su, C.C., X.N. Cheng, H.Q. Zhai, and J.M. Wan. 2002. Molecular mapping and genetic analysis of a rice brown planthopper (*Nilaparvata lugens* Stål) resistance gene. Acta Genet. Sinica 29:332–338.

Tanksley, S.D. 1993. Mapping polygenes. Annu. Rev. Genet. 27:205–233.

Velusamy, R., E.A. Heinrichs, and F.G. Medrano. 1986. Greenhouse techniques to identify field resistance to the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae) in rice cultivars. Crop Prot. 5:328–333.

Wu, W.R., W. Li, D. Tangi, H. Lu, and A.J. Worland. 1999. Time related mapping of quantitative trait loci underlying tiller number in rice. Genetics 151:297–303.

Xu, X.F., H.W. Mei, L.J. Luo, X.N. Cheng, and Z.K. Li. 2002. RFLP-facilitated investigation of the quantitative resistance of rice to brown planthopper (*Nilaparvata lugens*). Theor. Appl. Genet. 104: 248–253.

Yan, J., J. Zhu, C. He, M. Benmoussa, and P. Wu. 1998. Molecular dissection of developmental behaviour of plant height in rice (*Oryza sativa* L.). Genetics 150:1257–1265.

Yang, H., X. Ren, Q. Weng, L. Zhu, and G. He. 2002. Detection and analysis of QTL for resistance to brown planthopper, *Nilaparvata lugens* (Stal), in rice (*Oryza sativa* L.), using backcross inbred lines. Hereditas 136:39–43.

Yamamoto, T., Y. Kuboki, S.Y. Lin, T. Sasaki, and M. Yano. 1998. Fine mapping of quantitative trait loci *Hd-1*, *Hd-2* and *Hd-3*, controlling heading date of rice as single Mendelian factors. Theor. Appl. Genet. 97:37–44.

Yamasaki, M., A. Yoshimura, and H. Yasui. 2003. Genetic basis of ovicidal response to whitebacked planthopper (*Sogatella furcifera* Horvath) in rice (*Oryza sativa* L.). Mol. Breed. 12:133–143.

Yano, M., Y. Katayose, M. Ashikari, U. Yamanouchi, L. Monna, T. Fuse, T. Baba, K. Yamamoto, Y. Umehara, Y. Nagamura, and T. Sasaki. 2000. *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene *CONSTANS.* Plant Cell 12:2473–2484.

Yencho, G.C., M.B. Cohen, and P.F. Byrne. 2000. Applications of tagging and mapping insect resistant loci in plants. Annu. Rev. Entomol. 45:393–422.