

Mapping quantitative trait loci and expressed sequence tags related to brown planthopper resistance in rice

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With 2 figures and 1 table

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Abstract

To map genes responsible for brown planthopper (BPH) resistance in rice, a rice genetic map was constructed based on a recombinant inbred line population from a cross between a BPH-resistant line 'B5' and a susceptible variety 'Minghui 63'. Four quantitative trait loci (QTLs) for BPH resistance were detected. ESTs differentially regulated by BPH feeding were isolated by suppressive subtractive hybridization (SSH) and assigned to chromosomes based on RFLP mapping and searches of the rice genome database. The distribution of ESTs showed some clustering, and some ESTs are related to known QTLs and known BPH resistance genes. These findings suggest that the mapping of differentially induced ESTs may be a useful strategy for the identification of candidate plant defence genes, which could be beneficial in the development of a BPH-resistant rice variety.

Key words: *oryza sativa* — brown planthopper — expressed sequence tag — phloem-feeding insect — quantitative trait locus

The brown planthopper (BPH) feeds specifically on rice, using stylet-like mouthparts to penetrate the plant tissues and suck assimilates from the phloem. Among all the major diseases and insects, BPH infestation causes the largest estimated average annual yield loss in Chinese rice crops (Liu and Wu 1992), and the insects also transmit several viral diseases that cause additional crop damage (Ou 1985). Thus, researchers are currently seeking to identify BPH-resistance genes and use them to breed resistant cultivars that help guard against the negative impacts of BPH infestation.

To date, at least 10 major BPH-resistance genes have been identified and mapped in rice (Athwal et al. 1971, Huang et al. 2001, Yang et al. 2002). However, the BPH resistance conferred by these major genes is not durable; for example, *Bph1* and *bph2* can be overcome by BPH biotypes 2 and 3 (Pathak and Heinrichs 1982, Panda and Khush 1995). In contrast, quantitative trait loci (QTLs) were found to confer more durable BPH resistance in cultivar 'IR64' (Cohen et al. 1997). Further analysis of recombinant inbred lines (RIL) and double haploid lines (DHL) identified QTLs for BPH resistance on several chromosomes (Alam and Cohen 1998, Su et al. 2002, Xu et al. 2002), providing valuable information for future map-based cloning of BPH-resistance genes and marker-assisted selection of stably resistant varieties.

However, further expansion of this work has been complicated by the lack of a simple, direct strategy for characterizing QTLs in rice. As genome sequence information has become available, more groups have turned to identification of new genes using the candidate-gene strategy, where QTL map

positions are compared with genomic sequences and used to select candidate genes, which are then tested for possible association with the desired trait. Genetic mapping with markers derived from expressed sequence tags (ESTs) or known genes whose functions are possibly related to the phenotypic traits is an effective method for identifying QTL-associated genes. Using this strategy, expressed sequence tags (ESTs) and functional genes were used for mapping of QTLs for carbohydrate metabolism and transport, and sucrose content, yield and quality (Chen et al. 2001, Schneider et al. 2002). Linkage analysis of expressed disease-resistance gene analogues of sugar beet has identified alleles associated with resistance for rhizomania and *Cercospora* (Hunger et al. 2003). In rice, Ishimaru et al. (2001) constructed a function map for 23 important physiological and agronomic characters in rice. Xiong et al. (2002) has mapped a number of pathogen-induced defence-responsive genes in resistance QTL regions for different rice diseases. The ESTs became candidates if their map positions coincided with those of significant QTLs. Thus, a combinatorial approach using EST mapping and candidate-gene strategy seems appropriate for the identification of new QTL-associated genes in rice (Ishimaru et al. 2004).

In general, plants use constitutive and inducible defences to protect themselves against attacks by herbivores; these defence mechanisms must balance survival from immediate and subsequent attacks against long-term plant vitality, longevity and reproduction. Inducible defences play a major role in conferring resistance against phytophagous insects and their effects can include increased toxicity, delay of larval development, or increased attack by insect parasitoids (Baldwin and Preston 1999). Genes activated by herbivore attack are strongly correlated with the mode of herbivore feeding and the degree of tissue damage at the feeding site (Walling 2000). Previously, hybridization analysis of differentially displayed mRNA was used to identify infection-induced genes responsible for disease resistance QTLs in rice (Wang et al. 2001, Xiong et al. 2002, Zhou et al. 2002). Applying this approach seems likely to provide a new strategy for elucidating the genetic basis of BPH resistance in rice.

Rice is not only a staple food crop worldwide, but also an excellent model organism. The interaction between rice and BPH should prove an excellent model system for studying the genetic basis of plant defence against phloem-feeding insects. In addition, this work will help to fine-tune the publicly available, high-density EST rice linkage maps, which will become more useful as additional ESTs are associated with phenotypes or

QTLs. In this study, a population of 187 recombination inbred lines (RILs), was used produced from a cross between a BPH-resistant line 'B5' and a BPH-susceptible variety 'Minghui 63', to construct a linkage map and identify new QTLs for BPH resistance. ESTs that were differentially expressed following BPH feeding were incorporated into this map either by restriction fragment length polymorphism (RFLP) analysis or database search, allowing correlations between ESTs and QTLs, and the identification of possible new candidate genes for potentially durable BPH resistance in rice.

Materials and Methods

Plant materials and insects: A total of 187 RILs of rice, *Oryza sativa* L. from a cross between BPH-resistant indica rice line 'B5' (Huang et al. 2001) and a BPH-susceptible indica variety 'Minghui 63' were used for genetic mapping. The BPH insects used for infestation were maintained in the Genetics Institute of Wuhan University by feeding on rice strain Taichung Native 1, which is highly susceptible to BPH. Resistance to BPH was evaluated in all RILs using a standard seedling box screening (SSBS) technique (Huang et al. 2001). From these data, 10 RILs extremely susceptible to BPH were selected and designated as the susceptible bulk (S-bulk) for later experiments.

Genetic mapping and QTL analysis: Genomic DNA was prepared from the parental and RIL rice seedlings using the CTAB method described by Murray and Thompson (1980). RFLP analysis was performed as previously described (Huang et al. 2001), using five restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV and *Hind*III) and RFLP landmarks kindly provided by the Japanese Rice Genome Research Project and Cornell University (New York City, NY, USA). A total of 302 SSR primer pairs were used to search for simple sequence length polymorphisms (SSLP) between 'B5' and 'Minghui 63', and 52 pairs showing differences between the parents were used in RIL genotyping. PCR was performed with 20 ng of genomic DNA in a total volume of 12.5 μ l containing 0.2 μ M of each primer, 200 μ M of each dNTP, 0.5 U of *Taq*DNA polymerase and the buffer supplied (Promega, Madison, WI, USA). Samples were amplified at 94°C for 5 min, followed by 35 cycles of 1 min at 94°C, 0.5 min at 55°C and 2 min at 72°C, with a final extension at 72°C for 5 min (PCR System 9700; Perkin Elmer, Boston, MA, USA). The resulting PCR products were denatured, separated by 6% sequencing gel containing 7 M urea, and visualized by silver staining (Panaud et al. 1996). These RFLP and SSR markers were assembled into a genetic linkage map using Mapmaker/Exp 3.0 at LOD (log 10 likelihood ratio) 3.0 (Lincoln et al. 1992), and QTLs were detected using QtlMapper 1.01 b (Wang et al. 1999) at LOD 2.4. The phenotypic scores used to represent RIL BPH resistance were the averages of three independent experiments.

Isolation and mapping of BPH-induced ESTs: The BPH-induced ESTs were previously isolated by X. Wang and H. Yuan (unpublished data). Briefly, three-leaf stage seedlings of 'B5' and the RIL S-bulk were subjected to infestation by BPH, and BPH-responsive genes were isolated by suppressive subtractive hybridization (SSH) (Diatchenko et al. 1996) using the PCR-Select cDNA subtraction kit (Clontech, Palo Alto, CA, USA). Forward subtractive hybridization was performed using poly (A)⁺ RNA from infested 'B5' plants as the tester, and that from both the infested S-bulk and the uninfested 'B5' as the driver. Conversely, reverse subtractive hybridization used the S-bulk/uninfested 'B5' RNA pool as the tester and that of the infested 'B5' as the driver. PCR-amplified inserts from the resulting SSH cDNA library were arrayed in duplicate on Hybond-N⁺ membranes (Amersham-Pharmacia, Piscataway, NJ, USA), using a Q-Pix robot (Genetix, New Milton, Hampshire, UK). The membranes were probed with cDNA from the infested 'B5' and infested S-bulk seedlings, respectively. The cDNA for the ubiquitin gene, shown by Northern blot to be unaffected by BPH infestation, was used as an internal

control for normalization of signal differences between the duplicate membranes. Clones showing twofold or greater differences in signal intensity between the probes were considered to be BPH-responsive.

The BPH-responsive ESTs were sequenced on ABI 377 DNA sequencer (Perkin Elmer). BLAST compared with cDNA and protein sequences in the major public databases (<http://www.ncbi.nih.gov>) and then used to search the rice genome database for chromosomal localization based on the rice genetic linkage map (<http://www.gramene.org>). All BPH-responsive ESTs were assessed for use as RFLP probes, and those showing polymorphisms between the parents were mapped in the RILs.

Results

Linkage map and QTL detection

A total of 187 RILs were employed to construct a rice linkage map including RFLP, SSR and EST markers (Fig. 1). A total of 242 molecular markers were mapped to 12 linkage groups, covering 1478 Kosambi cM at a frequency of approximately one marker per 6.1 cM. Though the map contained some gaps and deletions, most of the markers were evenly distributed among the chromosomes, and the marker orders mapped were in good agreement with previously published rice maps (Causse et al. 1994, Harushima et al. 1998).

Four QTLs for BPH resistance were detected, with one each on chromosomes 2, 3, 4 and 9 (Fig. 1). These QTLs accounted for 62.6% of the variation in BPH resistance observed in the RIL population. The loci detected on chromosomes 3 and 4 coincided with *Qbp1* (between R1925 and G1318, *Bph14*) and *Qbp2* (between C820 and R288, *Bph15*), which were previously detected in a 'Minghui 63'/'B5' F₂ population (Huang et al. 2001). The LOD scores for these two loci were 24.7 and 17.2, respectively, and their effects accounted for 30.5 and 22.1%, respectively, of the variation in BPH resistance observed in the RIL population. These two loci showed markedly additive effects. In addition, two minor QTLs were detected and designated *Qbp3* and *Qbp4* on chromosomes 2 and 9; these loci had not been identified previously. With LOD scores of 3.7 and 5.8, respectively, *Qbp3* and *Qbp4* accounted for 3.4 and 6.7% of the total variation of resistance.

Detection of differentially expressed ESTs

Suppressive subtractive hybridizations were used to find the rice genes that were regulated by BPH feeding. PCR-generated products from subtractive hybridizations were inserted into pGEM-T vector and the forward and reverse subtractive cDNA libraries were constructed. The inserted sizes were estimated to be from 160 to 1100 bp (data not shown). The PCR-amplified inserts of 1200 clones from the libraries were arrayed on to membranes and differential screening was performed using cDNAs from the infested 'B5' and infested S-bulk seedlings as probes. A total of 112 clones showed differences exceeding double the signal intensity between the probes in three independent experiments, and they were considered as genes differentially regulated by BPH feeding. Sequence analysis revealed that of the 112 positive clones, 98 were unique sequences and 14 were the duplicated ones. Homology search with the BLAST programme in the Gene bank database showed 49 clones sharing sequence similarity with previously described genes. According to their functions, the genes could be divided into 10 groups including those related to carbon metabolism, cell maintenance and growth,

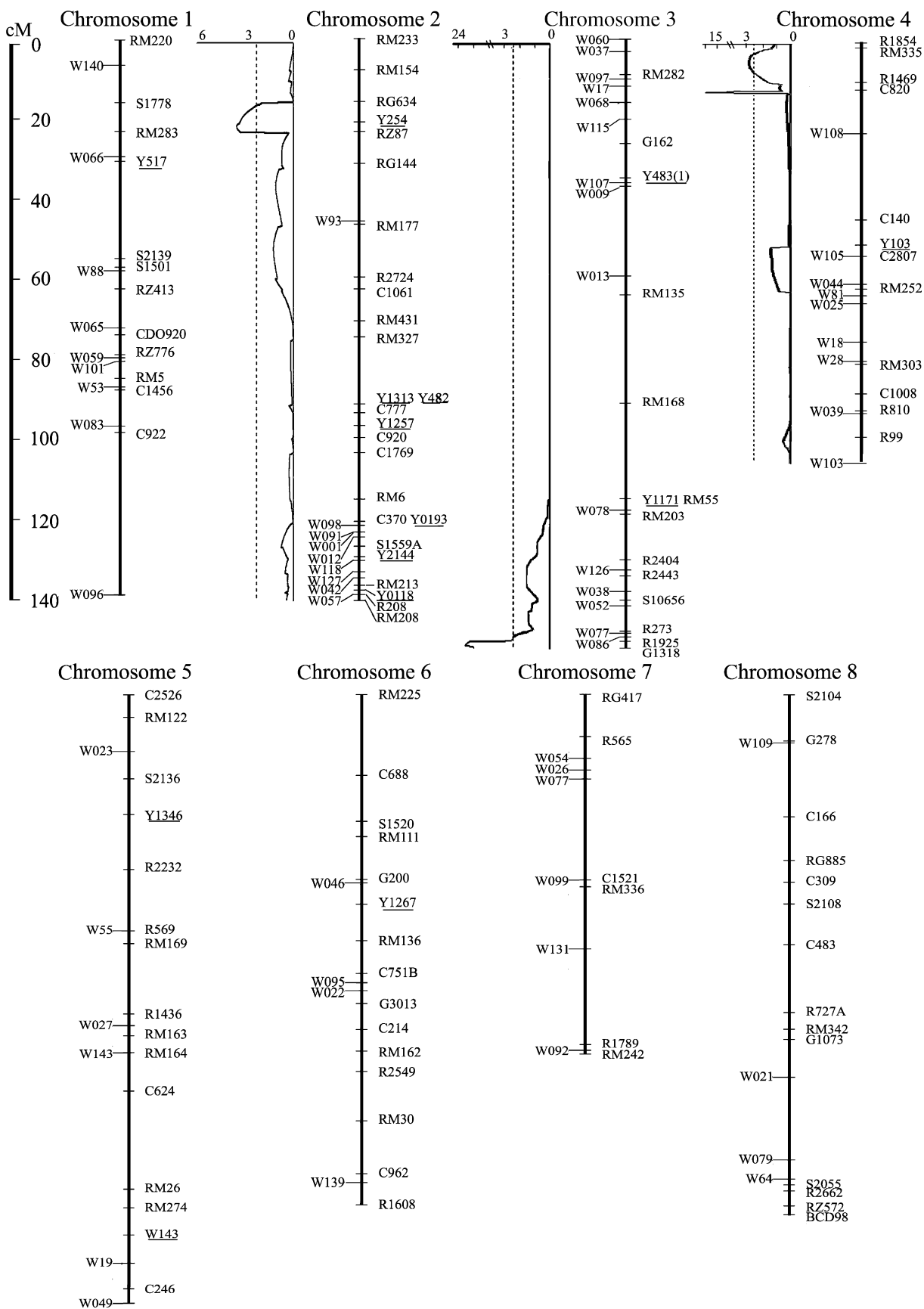


Fig. 1: Positions of brown planthopper (BPH)-responsive expressed sequence tags (ESTs) on the rice linkage map by RFLP analysis. The EST positions on rice chromosomes are shown as a composite map based on the 'Minghui 63'/'B5' RIL population. EST markers mapped through comparison against genomic sequences are on the left side of the linkage groups, and those mapped by RFLP analysis are on the right side and underlined. LOD value curves (left) indicate the BPH resistance QTLs. The threshold line is LOD 2.4

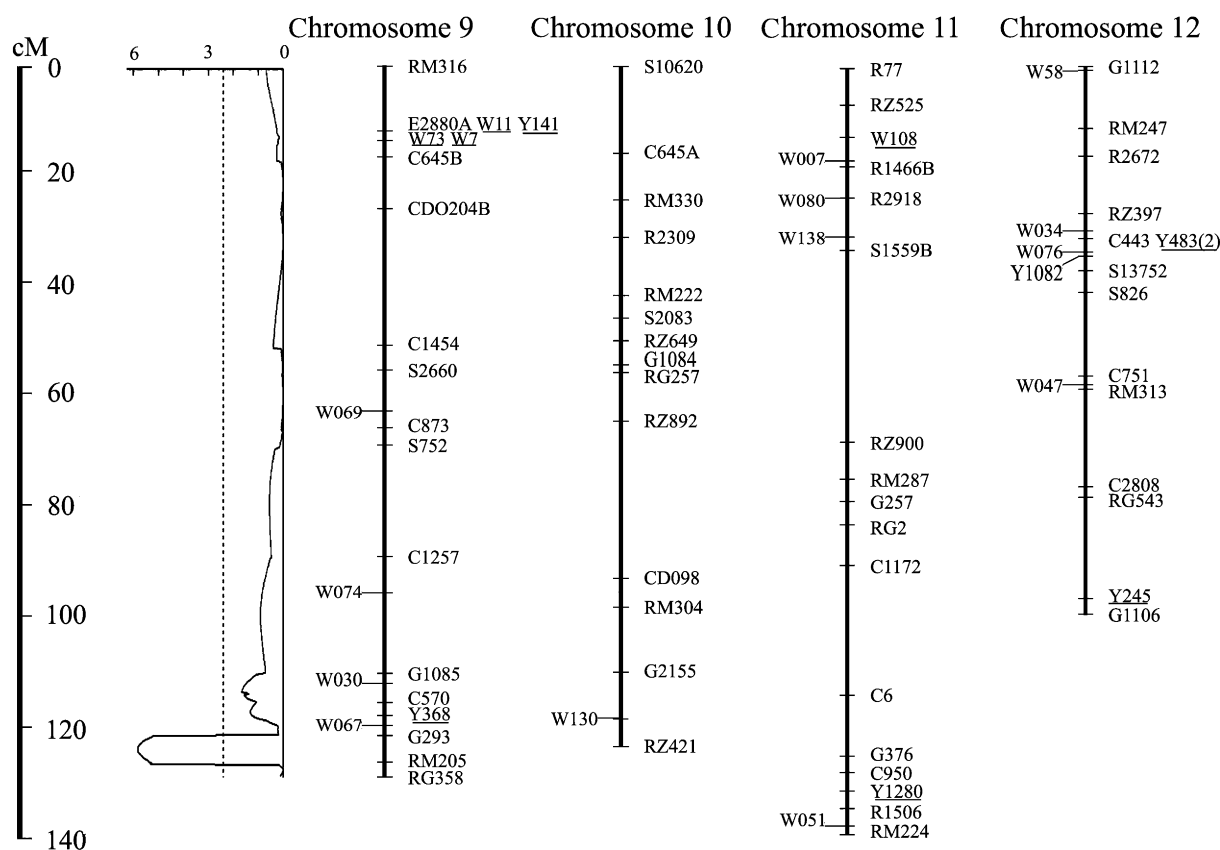


Fig. 1: Continued

signalling, electron transport, cytoskeleton, and response to stress, wounding or invasion of pathogens. Fifty-one clones were homologous to unknown rice or Arabidopsis proteins, ESTs or to rice scaffold sequences; and two did not match any sequence. Detailed analysis of the expression profile of genes regulated by BPH feeding will be reported elsewhere.

Mapping of ESTs

These ESTs were next examined for their utility as RFLP probes. Approximately 20% of them were polymorphic between the parental lines, and could thus be used for mapping in the RIL population. There was below average polymorphism in these ESTs when compared with common RFLP markers, as coding sequences of genes tend to be conserved between varieties belonging to the same subspecies of rice. Twenty-three ESTs were mapped on nine chromosomes through RFLP analysis (Fig. 1). Sequence similarity search on NCBI using BLAST allowed the biological functions to be assigned to the mapped ESTs (Table 1).

On chromosome 1, clone Y517 was mapped at 31 cM, this EST shares high homology with a gene induced by cold stress in wheat seedlings. Seven ESTs were mapped on chromosome 2. Y1257 encoding a *Xa21* family member A1-like protein was located at 98.2 cM. *Xa21* is a well-established rice resistance gene family (Song et al. 1995). Y118 was found to be homologous to a gene-encoding inosine monophosphate dehydrogenase, which catalyzes the rate-limiting step in the *de novo* biosynthesis of guanine nucleotides (Weber 1983), and could be involved in the ROS/NO (Reactive Oxygen Species/

Nitric Oxide) pathways (Patricia et al. 2002). It located at 138.9 cM. The other five ESTs, Y254, Y1313, Y482, Y193 and Y2144 had no similar sequence with genes of known function in the database. They were located at 21.2, 92.7, 92.7, 122.7 and 131.6 cM of chromosome 2 respectively. Y483 is identical to *Mycobacterium tuberculosis* CDC1551. Two loci were detected by Y483, one was at 26.3 cM of chromosome 3, and the other was at 31 cM of chromosome 12. Y1171 representing a cDNA-encoding activator of tyrosine mono-oxygenase, which is related to external stresses, is also mapped on chromosome 3. A novel sequence Y103 was located at 52.3 cM of chromosome 4. Two ESTs, Y1346 and W143 were located at 30.6 and 137.8 cM of chromosome 5, respectively. EST Y1346 is homologous to a gene encoding rice aspartic proteinase (EC 3.4.23), which was reported to be chilling sensitive. The aspartic proteinase appears to be the major enzyme processing the lectin in seeds (Mutlu et al. 1998). W143 is similar to a rice leaf cDNA that could be induced by *M. grisea* infection. On chromosome 6, a novel sequence Y1267 was mapped at 49.1 cM. Four EST clones, W73, Y141, W11 and W7 were found clustered on the short arm of chromosome 9. W73 encodes a GTP binding signal recognition particle receptor-like protein. Y141 encodes a NADH dehydrogenase subunit 1. W7 and W11 could not be assigned to any function by a similarity search in the database. A putative multispanning membrane protein gene (Y368) was mapped at 114.7 cM of chromosome 9. W108, a gene for zinc finger protein, was mapped at 12.1 cM of chromosome 11. Clone Y1280 encoding a putative dormancy-associated protein, was located at 129.6 cM of chromosome 11. Three clones, Y483, Y0182 and

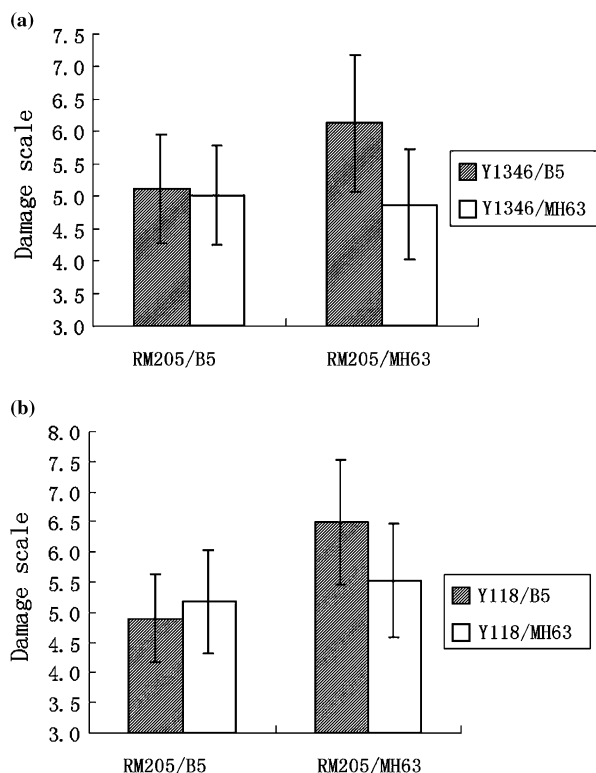


Fig. 2: Epistasis between *Qbp4* and two mapped ESTs ($P < 0.05$). The genotypes of *Qbp4* were indicated by the genotypes of SSR marker (RM205). (a) Epistasis between *Qbp4* and Y1346. (b) Epistasis between *Qbp4* and Y118.

Y245 were located on chromosome 12 at 31, 39.1 and 95.9 cM, respectively. Clone Y0182 encodes a fatty acid alpha-oxidase. Y245 did not show homology to any known sequence. RFLP analysis did not located EST on chromosome 7, 8 or 10.

Among the mapped 23 ESTs, Y254, a novel gene, was found to be covered in the *Qbp3* LOD peak on chromosome 2. A significant epistatic effect ($P < 0.05$) was observed between *Qbp4* and two mapped ESTs, Y1346 and Y118 (Fig. 2). When the genotype of *Qbp4* involved the 'B5' allele, the effect of Y1346 was totally masked, and no difference between the damage scales for alleles of Y1346 was found. However, when *Qbp4* 'Minghui 63' allele, the 'B5' allele of Y1346 showed a scale 1.2 grades higher than that of the 'Minghui 63' allele ($P = 0.044$, Fig. 2a). The effect of Y0118 was masked when the genotype of *Qbp4* involved the 'B5' allele, and the average damage scales of both genotypes were low. But when *Qbp4* involved the 'Minghui 63' allele, the scale of the 'B5' allele of Y118 was 1.0 grade lower than that of the 'Minghui 63' allele ($P = 0.023$, Fig. 2b).

The rest ESTs were mapped through sequence comparison against rice genomic sequences (<http://www.gramene.org>) The ESTs identified were not evenly distributed amongst the 12 chromosomes within the rice genome (Fig. 1). Most (19.7%) were located on chromosome 3, while only one was located on chromosome 10, and some BPH-responsive ESTs were clustered on one arm of chromosomes 2, 3 and 4.

Discussion

The rice 'B5' is highly resistant to BPH, so crossing this line with the susceptible variety 'Minghui 63' allowed the

generation of an RIL population in which it was possible to conduct repeated experiments for evaluation of resistance phenotypes, and be assured of a relatively low random error rate. This sensitivity allowed the detection of two minor novel QTLs, *Qbp3* and *Qbp4*, in addition to *Qbp1* and *Qbp2* previously detected in the 'Minghui 63'/'B5' F_2 population (Huang et al. 2001). In the RIL population, *Qbp1* and *Qbp2* accounted for a greater proportion of the variation (30.5 and 22.1%) than in the F_2 population (26.4 and 14.3 %). It is reasonable to believe that the four QTLs detected in this study account for the majority of BPH resistance seen in 'B5' cultivars. Thus, these results provide valuable data for the continued genetic improvement of BPH-resistance in cultivated rice.

Using the SSH technique, 98 unique genes were identified differentially responding to BPH-feeding. Twenty-three ESTs were mapped on the linkage map through DNA hybridization. Sequence similarity searches allowed one to assign presumptive functions to the 23 mapped ESTs (Table 1). The genes could be grouped into four classes: disease-inducible genes, abiotic stress-inducible genes, signal-transduction pathway genes and miscellaneous genes. The disease-inducible group included seven ESTs (Y1257, Y118, Y1280, Y182, Y2144, W143 and Y368) homologous to genes induced by various plant pathogens. The abiotic stress-inducible group included three clones (Y1171, Y1346 and Y517), the signal-transduction pathway group two clones (W108 and W73), and the miscellaneous group 11 ESTs without known functions. There was no evidence of any gene involved in octadecanoid pathway and volatile production in all the sequenced ESTs (Walling 2000). It is suggested that the molecular response of rice to BPH would be quite different from that of plants to chewing insects.

Among the ESTs mapped by RFLP analysis, one clone, Y254 was found within the LOD peak of *Qbp4* on chromosome 2 (Fig. 1). Two clones were found to have epistatic effect with QTL for BPH resistance (Fig. 2). A large number of genes and QTLs conferring resistance to BPH had been previously mapped in different rice populations; these reports were used to identify associations between the identified ESTs and various resistance genes/QTLs. It was found that more ESTs can be associated with the known BPH resistance genes and QTLs when the rest of the ESTs were mapped through comparison with rice genomic sequences (Fig. 1). In this way, two ESTs were located in regions identified as being QTLs for BPH resistance. EST W077 (BU572377), encoding an AMP-binding protein, was located within the *Qbp1* LOD-supported region on chromosome 3; and EST W067 (BU572367), encoding an ascorbate free radical reductase, was mapped to the *Qbp4* LOD-supported region on chromosome 9. BPH resistance gene *Bph13* (Liu et al. 2001) located near two novel BPH-responsive ESTs, W118 (BQ529315) and Y0193 (BQ529298), on the long arm of chromosome 2. Similarly, BPH-resistance gene *Bph3* (Yan et al. 2002) located near a chitin-binding motif-like family member gene W040 (BU572341), on chromosome 4, and *bph2* (Murata et al. 1997) is associated with both an O-methyltransferase gene W076 (BU572376), and a putative fatty acid alpha-oxidase gene Y0182 (BQ529316) on chromosome 12. In total 12 previously reported QTLs or genes for BPH resistance were found to be near the BPH-responding ESTs in this study. These findings suggest that mapping of differentially induced ESTs may be a useful strategy for the identification of candidate BPH-resistance genes.

Table 1: Summary of differentially expressed sequence tags responding to brown plant hopper infection

Clone	Length	Chro no.	GeneBank accession no.	Sequence homology/match	E-value
Y517	571	1	BQ529322	cDNA wheat cold-stressed seedling from library (BE490440)	0.0
Y254	337	2	BQ529312	No significant similarity	—
Y1313	546	2	BQ529303	Stem library from <i>Oryza sativa</i> (3–5 leaf stage) <i>Oryza sativa</i> (BI805545)	0.0
Y482	228	2	BQ529314	No significant similarity	—
Y1257	300	2	BQ548081	Protein kinase Xa21 (EC 2.7.1.-) A1 (T10725)	8e ⁻²⁰
Y193	141	2	BQ529298	Rice root <i>Oryza sativa</i> cDNA clone (AU184765)	2e ⁻⁴⁶
Y2144	766	2	BQ529308	<i>Oryza sativa</i> mature leaf library induced by <i>M. grisea</i> <i>Oryza</i> (BI807939)	e ⁻¹³³
Y118	322	2	BQ529315	cDNA from subtracted rice blast infection Stage cDNA library (AW070134), inosine monophosphate dehydrogenase (AE005036)	0.0 4e ⁻¹⁰
Y483	318	3	BQ548082	<i>Mycobacterium tuberculosis</i> CDC1551 (AE006953)	e ⁻¹⁶⁴
Y1171	243	3	BQ529309	cDNA encoding activator of tyrosine monooxygenase and tryptophan monooxygenase (E05577)	e ⁻¹¹⁸
Y103	167	4	BQ529299	No significant similarity	—
Y1346	157	5	BQ529317	<i>Oryza sativa</i> RAP mRNA for aspartic proteinase (AB028888)	e ⁻⁵¹
W143	372	5	BQ548086	cDNA from <i>Oryza sativa</i> mature leaf library induced by <i>M. grisea</i> <i>Oryza</i> (BI808637)	e ⁻¹⁴⁸
Y1267	177	6	BQ529310	No significant similarity	—
W073	504	9	BQ548084	Signal recognition particle receptor-like protein (A29440)	9e ⁻⁴³
Y141	504	9	BQ529323	<i>Nilaparvata lugens</i> country Philippines 16S ribosomal RNA gene (AF158038)	e ⁻⁹⁷
W007	193	9	BQ548085	Upogebia affinis NADH dehydrogenase subunit 1 gene (AF436047)	e ⁻²⁵
W011	265	9	BQ579829	Rice root <i>Oryza sativa</i> cDNA (AU095430)	e ⁻¹⁰⁶
Y368	283	9	BQ529313	cDNA from rice immature seed cDNA Library (AA751881)	e ⁻¹³⁴
				cDNA of <i>Anthracoze</i> -infected leaves from 2-week-old sorghum plants (BE597690)	e ⁻⁸⁸
				Putative multispinning membrane protein similar to MURA (MUDR protein A) transposase (m02191)	e ⁻¹⁷
W108	461	11	BQ548083	<i>Oryza sativa</i> mRNA for Dof zinc finger protein (AB028132)	e ⁻¹⁵³
Y1280	181	11	BQ529319	<i>Oryza sativa</i> putative dormancy-associated protein mRNA induced by <i>M. grisea</i> (AF467730)	e ⁻¹⁰⁰
Y182	566	12	BQ529316	<i>Oryza sativa</i> mature leaf library induced by <i>M. grisea</i> (BI811405), <i>Oryza sativa</i> fatty acid alpha-oxidase (AF229813)	0.0
Y245	140	12	BQ529320	Rice root <i>Oryza sativa</i> cDNA (AU091703)	7e ⁻⁷¹

At present, the functions of most rice ESTs contained in the JRGP map are unknown (Harushima et al. 1998). Here, the identification and mapping of a number of BPH-responsive ESTs, is reported thus adding to the saturation of the rice gene map and deepening our understanding of plant defence. The co-localization between some BPH-responding ESTs and resistance genes or QTLs provides some hints for the role of these ESTs in resistance, although fine mapping and further polymorphic analysis in the regions will be required, and possible transformation experiments aimed at conferring resistance phenotypes on to susceptible varieties. Thus, these results illustrate the potential of using insect-induced ESTs to identify candidate genes involved in plant defence against insects.

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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.

Athwal, D. S., M. D. Pathak, E. H. Bacalangco, and C. D. Pura, 1971: Genetics of resistance to brown planthoppers and green leafhoppers in *Oryza sativa* L. *Crop Sci.* **11**, 747–750.

Baldwin, I. T., and C. A. Preston, 1999: The eco-physiological complexity of plant responses to insect herbivores. *Planta* **208**, 137–145.

Causse, M. A., T. M. Fulton, Y. G. Cho, S. N. Ahn, J. Chunwongse, K. Wu, J. Xiao, Z. Yu, P. C. Ronald, S. E. Harrington, G. Second, S. R. McCouch, and S. D. Tanksley, 1994: Saturated molecular map of the rice genome based on an interspecific backcross population. *Genetics* **138**, 1251–1274.

Chen, X., F. Salamini, and C. Gebhardt, 2001: A potato molecular-function map for carbohydrate metabolism. *Theor. Appl. Genet.* **102**, 284–295.

Cohen, M. B., S. N. Alam, E. B. Medina, and C. C. Bernal, 1997: Brown planthopper, *Nilaparvata lugens*, resistance in rice cultivar IR64, mechanism and role in successful *N. lugens* management in Central Luzon, Philippines. *Entomologia Experimentalis et Applicata* **85**, 221–229.

Diatchenko, L., Y. F. C. Lau, A. P. Campbell, A. Chenchik, F. Moqadam, Huang, B., K. Lukyanov, N. Gurskaya, E. Sverdlov, and P. D. Siebert, 1996: Suppression subtractive hybridization, a method for generating differentially regulated or tissue-specific cDNA probes. *Proc. Natl. Acad. Sci. USA* **93**, 6025–6030.

Harushima, Y., M. Yano, A. Shomura, M. Sato, T. Shimano, Y. Kuboki, T. Yamamoto, S. Lin, B. A. Antonio, A. Parco, H. Kajiyu, N. Huang, K. Yamamoto, Y. Nagamura, N. Kurata, G. S. Khush, and T. Sasaki, 1998: A high-density rice genetic linkage map with 2275 markers using a single F₂ population. *Genetics* **148**, 479–494.

- 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.
- Hunger, S., G. D. Gaspero, S. Möhring, D. Bellin, R. Schäfer-Pregl, D. C. Borchardt, C. Durel, M. Werber, B. Weisshaar, F. Salamini, and K. Schneider, 2003: Isolation and linkage analysis of expressed disease-resistance gene analogues of sugar beet (*Beta vulgaris* L.). *Genome* **46**, 70—82.
- Ishimaru, K., M. Yano, N. Aoki, K. Ono, T. Hirose, S. Y. Yin, L. Monna, T. Sasaki, and R. Ohsugi, 2001: Toward the mapping of physiological and agronomic characters on a rice function map: QTL analysis and comparison between QTLs and expressed sequence tags. *Theor. Appl. Genet.* **102**, 793—800.
- Ishimaru, K., K. Ono, and T. Kashiwagi, 2004: Identification of a new gene controlling plant height in rice using the candidate-gene strategy. *Planta* **218**, 388—395.
- Lincoln, S., M. Daly, and E. S. Lander, 1992: Constructing Genetic Maps with MAPMAKER/EXP 3.0. Whitehead Institute, Cambridge, MA, USA.
- Liu, C., and R. Wu, 1992: Studies on genetic mechanism and phenotypic stability of resistance to BPH in rice varieties. *J. Huanan Agric. Univ.* **13**, 20—25.
- Liu, G. Q., H. H. Yan, Q. Fu, Q. Qian, Z. T. Zhang, W. X. Zhai, and L. H. Zhu, 2001: Mapping of a new gene for brown planthopper resistance in cultivated rice introgressed from *Oryza eichingeri*. *Chinese Sci. Bull.* **48**, 1459—1462.
- Murata, K., M. Fujiwara, C. Nakamura, N. Mori, and C. Kaneda, 1997: Linkage analysis of brown planthopper resistance genes, *bph2* and *Bph9*, in rice. *Breed. Sci.* **47**(Suppl. 2), 168.
- Murray, M. G., and W. F. Thompson, 1980: Rapid isolation of high molecular-weight plant DNA. *Nucleic Acids Res.* **8**, 4321—4325.
- Mutlu, A., J. E. Pfeil, and S. Gal, 1998: A probarley lectin processing enzyme purified from *Aravidopsis thalianaseeds*. *Phytochemistry* **47**, 1453—1459.
- Ou, S. H., 1985: Rice Diseases. 2nd edition. 360. Commonwealth Mycological Institute, UK.
- Panaud, O., X. Chen, and S. R. McCouch, 1996: Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). *Mol. Gen. Gent.* **252**, 597—607.
- Panda, N., and G. S. Khush, 1995: Host Plant Resistance to Insects. CAB International, UK.
- Pathak, P. K., and E. A. Heinrichs, 1982: Selection of biotype populations 2 and 3 of *Nilaparvata lugens* by exposure to resistant rice varieties. *Environ. Entomol.* **11**, 85—90.
- Patricia, S. R., G. D. James, and A. F. Richard, 2002: Anoxia-induced changes in purine nucleoside metabolism of *in vitro* aged human fibroblasts. *Life Sci.* **70**, 1369—1382.
- Schneider, K., R. Schäfer-Pregl, D. C. Borchardt, and F. Salamini, 2002: Mapping QTLs for sucrose content, yield and quality in a sugar beet population fingerprinted by EST-related markers. *Theor. Appl. Genet.* **104**, 1107—1113.
- Song, W. Y., G. L. Wang, L. L. Chen, K. S. Kim, L. Y. Pi, T. Holsten, J. Gardner, B. Wang, W. X. Zhai, L. H. Zhu, C. Fauquet, and P. C. A. Ronald, 1995: Receptor kinase-like protein encoded by the rice disease resistance gene Xa21. *Science* **270**, 1804—1806.
- Su, C. C., X. N. Cheng, H. Q. Zhai, and J. M. Wan, 2002: Detection and analysis of QTL for resistance to the brown planthopper, *Nilaparvata lugens* (Stål), in rice (*Oryza sativa* L.), using backcross inbred lines. *Acta Genetica Sinica* **29**, 332—338.
- Walling, L. L., 2000: The myriad plant responses to herbivores. *J. Plant Growth Regulat.* **19**, 195—216.
- Wang, D. L., J. Zhu, Z. K. Li, and A. H. Paterson, 1999: Mapping QTLs with epistatic effects and QTL x- Environment interactions by mixed linear model approaches. *Theor. Appl. Genet.* **99**, 1255—1264.
- Wang, Z., G. Taramino, D. Yang, G. Liu, S. V. Tingey, G. H. Miao, and G. L. Wang, 2001: Rice ESTs with disease-resistance gene- or defense-response gene-like sequences mapped to regions containing major resistance genes or QTLs. *Mol. Genet. Genomics* **265**, 302—310.
- Weber, G. 1983: Biochemical strategy of cancer cells and design of chemotherapy. *Cancer Res.* **43**, 3466—3492.
- Xiong, M., S. Wang, and Q. Zhang, 2002: Coincidence in map positions between pathogen-induced defense-responsive genes and quantitative resistance loci in rice. *Sci. China (Ser. C)* **45**, 518—526.
- 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, H. M., R. Qin, W. W. Jin, G. C. He, and Y. C. Song, 2002: Comparative physical mapping of Bph3 with BAC-FISH in *Oryza officinalis* and *O. sativa*. *Acta Botanica Sinica* **44**, 583—587.
- Yang, H., X. Ren, Q. Weng, L. Zhu, and G. He, 2002: Molecular mapping and genetic analysis of a rice brown planthopper (*Nilaparvata lugens* Stål) resistance gene. *Hereditas* **136**, 39—43.
- Zhou, B., K. M. Peng, Z. H. Chu, S. P. Wang, and Q. F. Zhang, 2002: The defense-responsive genes showing enhanced and repressed expression after pathogen infection in rice (*Oryza sativa* L.). *Sci. China (Ser. C)* **45**, 449—467.

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