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A new locus for resistance to brown planthopper identified in the *indica* rice variety DV85

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Abstract

The brown planthopper (BPH) is one of the most destructive insect pests of rice. Resistant varieties have proved to be one of the most economic and effective measures for BPH management. In this study, an *indica* rice 'DV85' showed resistance to biotype 2 of BPH by bulked seedling test, and a recombinant inbred line (RIL) population derived from a cross between a susceptible rice 'Kinmaze' and 'DV85' was phenotyped to map genetic factors conferring BPH resistance in 'DV85'. Composite interval mapping revealed that one quantitative trait locus (QTL) with a LOD score of 10.1 was detected between *XNpb202* and *C1172* on chromosome 11. This QTL was designated as *Qbph11*. *Qbph11* explained 68.4% of the phenotypic variance of BPH resistance in this population. The allele from the resistant parent 'DV85' at *Qbph11* reduced the damage caused by BPH feeding and would be very useful in breeding resistant rice varieties via marker-assisted selection.

Key words: *Oryza sativa* — *Nilaparvata lugens* — brown planthopper resistance — quantitative trait locus analysis — recombinant inbred lines

The brown planthopper (BPH), *Nilaparvata lugens* Stål, is one of the most serious insect pests of rice (*Oryza sativa* L.) in Asian rice growing areas. BPH causes direct damage by sucking plant sap, and it also transmits several viral diseases such as rice grassy stunt (Rivera et al. 1966) and rugged stunt (Ling et al. 1978).

Using insecticides to control BPH insects are not only costly in terms of labour and money, but also cause environmental damage. In addition, resurgence, a phenomenon of pest population increase after application of insecticides (Heinrich et al. 1982), has also been reported. To solve the problem, naturally evolved resistance systems should provide a promising and readily acceptable means of control.

Utilization of host resistance has been recognized as one of the most economic and effective measures for BPH management. So far, 13 BPH resistance genes have been reported, of which *Bph1*, *bph2*, *Bph9* and *Bph10*(t) were mapped on chromosome 12 (Ishii et al. 1994, Hirabayashi and Ogawa 1995, Murata et al. 1998, 2000). Four additional resistance genes *bph4*, *bph11*(t), *bph12*(t) and *Bph13*(t) were assigned to chromosomes 6, 3, 4 and 2, respectively (Hirabayashi and Ogawa 1999, Kawaguchi et al. 2001, Liu et al. 2001). Studies of quantitative trait loci (QTL) contributing to BPH resistance in 'IR64', 'Kasalath' and wild rice, *O. officinalis*, have also been carried out (Alam and Cohen 1998, Huang et al. 2001, Su et al. 2002).

In this paper, a bulk seedling test was applied to identify QTL for BPH resistance in a mapping population from 'Kinmaze' and 'DV85'. The aims were to map BPH resistance loci in the resistant variety 'DV85', and to find markers linked to these loci that could be helpful in marker-assisted selection programmes for breeding BPH-resistant varieties.

Materials and Methods

Plant materials: The genetic material was a set of recombinant inbred lines (RILs) of rice, *Oryza sativa* L., derived from a cross between the *japonica* rice variety 'Kinmaze' and the *indica* rice variety 'DV85' (Ikeda et al. 1998). 'Rathu Heenati', carrying *Bph3* (Lakshminarayana and Khush 1977), and 'Taichung Native 1' (TN1) with no resistance genes were used as resistant and susceptible controls, respectively.

Insects: The BPH population used for infestation was biotype 2, which were first collected from a rice field at Hangzhou, China, and have been maintained on 'Mudgo' in a greenhouse for 10 generations. In this experiment, insects were maintained on 'TN1' under natural conditions in a greenhouse of the Nanjing Agriculture University, Nanjing, China.

Evaluation of BPH resistance: Bulked seedling tests with modification were conducted to phenotype the reaction to BPH feeding in parental and control varieties and in 81 RIL lines. To ensure all seedlings were at the same growth stage for insect infestation, seeds were first germinated in Petri dishes.

In the bulked seedling tests, 17 germinated seeds were sown in a row of 20 cm length in a plastic box. The distance between rows was 2.5 cm. A total of five lines of 'DV85', five lines of 'Kinmaze', four lines of 'Rathu Heenati' and four lines of 'TN1' were randomly planted in the same box.

To evaluate BPH resistance in the RIL population, about 65 seeds of each RIL, parental and control varieties were sown in a plastic pot 10 cm-diameter with a hole in the base. Generally, 28 pots, together with one pot of parents and control varieties, were placed in a $68 \times 42 \times 16$ cm plastic seed-box. About 2-cm depth of water was maintained in the seed-box.

At the second-leaf stage, 15 days after sowing, the seedlings were infested with second to third-instar BPH nymphs at six insects per seedling. When all seedlings of 'TN1' had died, the plants of each entry were given a score of 0, 1, 3, 5, 7 or 9 according to Athwal et al. (1971). The resistance level of each entry was then calculated based on the weighted average of the seedlings examined.

Mapping QTLs for BPH resistance: A linkage map constructed from the RIL population was used for mapping QTL resistance to BPH (Ikeda et al. 1998). The linkage map comprised 138 restriction fragment length polymorphism (RFLP) markers and covered 1386.2 cM of the rice genome with an average marker interval of 10.1 cM. QTL analysis of the BPH resistance was conducted with Windows QTL Cartographer 1.13a (North Carolina State University, Raleigh, NC, USA) (Basten et al. 1999) at an LOD threshold of 3.0.

Results

Reaction of two parental varieties to BPH feeding

In bulked seedling tests, the resistance scores of 'DV85' and 'Kinmaze' were 2.1 and 7.3, respectively, which indicated that 'DV85' was resistant to biotype 2 of BPH while 'Kinmaze' was susceptible. It was inferred that 'DV85' was less resistant than 'Rathu Heenati' whose resistance score was 0.4 (Table 1).

Segregation of resistance to BPH in RIL population

The resistance scores of 81 RILs infested with biotype 2 showed a bimodal distribution (Fig. 1). If RILs with a resistance score lower than 5 were considered as resistant while those above 5 were considered as susceptible, 42 RILs were resistant and 39 RILs were susceptible. The result fitted the expected ratio of 1:1 ($\chi^2 = 0.111$, $\chi^2_{0.05-1} = 3.84$, $P = 0.05$). Therefore, a major genetic factor should be governing the resistance segregation in this RIL population. Transgressive individuals were found in the RIL population.

QTL analysis of BPH resistance

First, co-segregation of RFLP markers with BPH resistance by using MAPMAKER/3.0 software (Lander et al. 1987) was attempted, but the resistance gene was not mapped, successfully even when segregants with a resistance score of 4–6 were removed. So QTL analysis was carried out and one major QTL with a LOD score of 10.1 was detected between *X202* and *C1172* on chromosome 11 by composite interval mapping (Table 2 and Fig. 2) and was designated as *Qbph11*. The *t*-tests (Table 3) showed the resistance score in the RIL population were

Table 1: The scores of brown planthopper (BPH) resistance in parental and control varieties

Varieties[1]	Number of seedlings tested	Resistance score (0–9)
DV85	81	2.1
Kinmaze	79	7.3
Rathu Heenati	60	0.4
TN1	65	9.0

[1] 'Rathu Heenati' and 'Taichung Native 1' (TN1) were used as resistant and susceptible controls for BPH biotype 2, respectively.

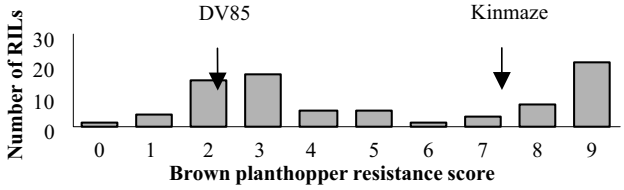

Fig. 1: Distribution of brown planthopper (BPH) resistance scores of the 81 recombinant inbred lines (RILs), The scores of the parental varieties 'Kinmaze' and 'DV85' are indicated

Table 2: Quantitative trait locus (QTL) identified for brown planthopper resistance using an recombinant inbred line (RIL) population from 'Kinmaze'/'DV85'

QTL	Interval	Chromosome	LOD score	PVE (%)[1]	Additive[2]
Qbph11	*X202-C1172*	11	10.1	68.4	−2.78

[1] Percentage of variance explained (%).
[2] Additive effect of 'DV85' allele.

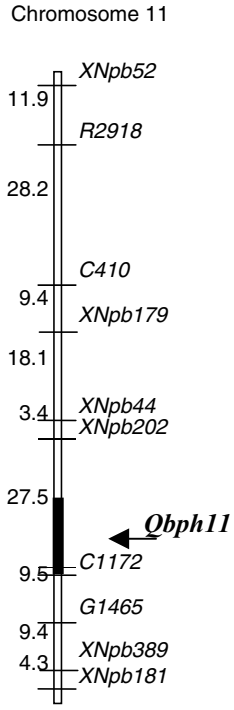

Fig. 2: Location of *Qbph11* on chromosome 11. Marker names are listed on the right side of the chromosome with distances (cM) on the left. The solid bar indicates the location of *Qbph11*

Table 3: Distribution of resistance score by genotypes of markers *C1172* and *XNpb202* in recombinant inbred line (RIL) population

		Number of RILs in resistance score class					
Marker	Genotype[1]	0–3	3–6	6–9	Total[2]	Mean	*t*-value[3]
C1172	1/1	8	7	20	35	6.06	3.95**
	2/2	29	4	11	44	3.46	
XNpb202	1/1	11	7	22	40	5.63	3.47**
	2/2	28	4	8	40	3.35	

[1] 1/1 denotes genotype of 'Kinmaze', 2/2 denotes genotype of 'DV85'.
[2] Genotypes of two RILs at *C1172* and one at *XNpb202* were missing.
[3] Significant difference between mean values of two genotype at $P = 0.01$.
[4] represent the significance at $P \leq 0.01$.

significantly differentiated by genotypes at markers *C1172* and *X202*, which further indicated that this QTL for BPH resistance was linked to these two markers. This QTL was near to *C1172* and explained 68.4% of the phenotypic variance of BPH resistance in this population and thus should be a QTL

with major effect. As indicated by the additive effect estimated, this QTL was derived from the resistant parent 'DV85' and reduced the damage caused by BPH feeding (Table 2).

Discussion

In this study, a genetic factor was found for BPH resistance in *indica* rice 'DV85' using an RIL mapping population derived from 'Kinmaze' and 'DV85'. This QTL was assigned to chromosome 11 and designated as *Qbph11*. This QTL was detected at a high LOD (10.1) and explained a large percentage of phenotypic variance (68.4%). Since no BPH resistance genes have been located on chromosome 11, *Qbph11* should be a new BPH resistance locus.

A pair of dominant resistance genes of 'DV85' with complementary expression to green rice leafhopper (GRH) and green leafhopper (GLH) have already been mapped on chromosomes 3 and 11 and designated as *Grh4* and *Grh2*, respectively (Fukuta et al. 1998, Yazawa et al. 1998, Yasui and Yoshimura 1999). The resistance gene on chromosome 11 was tightly linked to the marker *G1465*. On the linkage map used in the present study, *G1465* was only 9.5 cM distance from *C1172*, which was found to be near to *Qbph11* (Fig. 2). The chromosome region around *G1465* and *C1172* might be related to rice's resistance to such types of insect, or genes giving resistance to sucking insects might cluster in this chromosome region of 'DV85'. However, no resistance QTL was detected on chromosome 3.

The first BPH resistance variety 'IR26' with *Bph1* was released in 1973 and initially provided control of BPH over large areas. However, the BPH population adapted to 'IR26' in as little as 2 years. This pattern was repeated with subsequent varieties containing the *bph2* gene. At present, four BPH biotypes are known. Biotype 1 and biotype 2 are wildly distributed in Southeast Asia, biotype 3 is a laboratory biotype produced in the Philippines, and biotype 4 occurs in the Indian subcontinent (Khush and Brar 1991). To cope with such a problem of resistance breakdown associated with outbreaks of new biotypes, additional BPH resistance genes are required for widening genetic base. So the BPH resistance gene in the locus recognized here should be useful to another BPH resistance source in marker-assisted selection.

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