Plant Breeding **129**, 63—67 (2010)

doi:10.1111/j.1439-0523.2009.01648.x

QTL analysis for the resistance to small brown planthopper (*Laodelphax striatellus* Fallén) in rice using backcross inbred lines

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With 3 figures and 3 tables

Received August 21, 2008/Accepted February 5, 2009
Communicated by Z. K. Li

Abstract

Small brown planthopper (SBPH) is a serious pest of rice (*Oryza sativa* L.) in China. An *indica* variety 'Kasalath' is highly resistant to SBPH. A mapping population consisting of 98 BC_1F_9 lines, derived from a backcross of 'Nipponbare'/'Kasalath'//'Nipponbare', was applied to detect quantitative trait loci (QTL) for resistance to SBPH. In the modified seedbox screening test, three QTLs for SBPH resistance were mapped on chromosomes 3 and 11, explaining 49.9% of the phenotypic variance. In the antixenosis test, a total of three QTLs conferring antixenosis against SBPH were detected on chromosomes 3, 8 and 11, which accounted for 36.4% of the total phenotypic variance. In addition, two QTLs expressing antibiosis to SBPH were detected on chromosomes 2 and 11, explaining 13.8% and 14.7% of the phenotypic variance, respectively. *Qsbph11e*, *Qsbph11f* and *Qsbph11g* were located in the region between S2260 and G257 on chromosome 11, indicating that the locus is significant in conferring resistance to SBPH in 'Kasalath'. The molecular markers linked to these QTLs should be useful in the development of varieties with horizontal resistance to SBPH.

Key words: rice — resistance — small brown planthopper — BIL population — quantitative trait locus

The small brown planthopper (SBPH), *Laodelphax striatellus* Fallén (Homoptera: Delphacide), is an economically important pest in rice (*Oryza sativa* L.) and is distributed widely in China. It not only causes direct damage by sucking plant sap, but also transmits several viral diseases such as *Rice stripe virus* (RSV) and *Rice black-streaked dwarf virus*, which often cause major yield losses (Gray 1996, Ding et al. 2005). In recent years, the damage caused by SBPH feeding and the diseases transmitted by this planthopper have been increasing in China. When the outbreak occurred in Jiangsu and Anhui Provinces in 2004 and 2005, the insect density of SBPH was as high as 450 million per hectare, and 3.4 million hectare of rice were infested, which caused serious yield reduction (Gu et al. 2005, Tai et al. 2005, Zhang et al. 2005). The widespread of SBPH also resulted in the prevalence of rice stripe disease, which is one of the most serious diseases in rice in China. Rice yield was reduced by 30–40% in heavily infected fields, and in some of the most infected fields, no harvest was possible (Wei 2003, Gu et al. 2005).

The rice stripe disease will be effectively controlled once damage by SBPH feeding is reduced markedly or even avoided. The stripe virus disease became serious along with the increment in the numbers of SBPH and the severity of symptom of rice stripe is directly related to the amount of virus in plant transmitted by SBPH (Xu et al. 2005). Therefore, it is important to control SBPH. Protection against SBPH has depended mostly on insecticides in the past years, which has led to enhanced resistance of SBPH to chemicals, natural enemy death and environmental pollution, and, in turn, caused pest resurgence (Sone et al. 1995, Endo and Tsurumachi 2000, Endo et al. 2002, Lin et al. 2005). Host resistance has been recognized as one of the most economic and effective measures in controlling SBPH and RSV.

The *indica* cultivar 'Kasalath', expressing strong antixenosis and antibiosis to SBPH, was screened for resistance to SBPH out of more than 100 rice accessions in a bulk seedling test. In this study, we tested a mapping population of 98 backcross inbred lines (BILs), derived from a backcross of 'Nipponbare' (*japonica*)/'Kasalath' (*indica*)//'Nipponbare', for resistance to SBPH and detected several QTLs for SBPH resistance. The objectives of this study were to map the QTLs for resistance to SBPH in 'Kasalath' and to identify molecular markers linked to these loci to be used in marker-assisted selection programmes for breeding rice varieties resistant to SBPH and RSV.

Materials and Methods

Plant materials: Ninty-eight BILs, provided by Yano at Rice Genome Research Program, National Institute of Agrobiological Resources, Tsukuba, Japan, were developed from a backcross of 'Nipponbare' (*japonica*)/'Kasalath' (*indica*)//'Nipponbare' by single seed descent method. 'Kasalath' is resistant to SBPH, while 'Nipponbare' is susceptible. 'Rathu Heenati' and 'Wuyujing 3' were used as the resistant and the susceptible controls, respectively.

Insect population: The SBPH population used for infestation was originally collected from a rice field in Nanjing, China, and was maintained on barley in a greenhouse for four generations before transferred to 'Wuyujing 3' rice in the greenhouse of Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China. The SBPH population was confirmed to be non-viruliferous by dot-immunobinding assay and PCR detection (Qin et al. 1994).

Modified seedbox screening test: A modified seedbox screening test was applied to evaluate resistance of parental and control varieties, as well as the 98 BILs, as described previously (Duan et al. 2007). Twenty-five germinated seeds were sown in a row of 20 cm length with a spacing of 4.0 cm between rows in a plastic box. Four rows of 'Kasalath', 'Nipponbare', 'Rathu Heenati' and 'Wuyujing 3' each were randomly planted in the same box.

Table 1: Evaluation criteria of reactions to SBPH in rice seedling stage

Damage	Scale	Resistance reaction
No visible damage	0	I
Very slightly damage	1	HR
Partial yellowing of the first and the second leaves	3	R
Pronounced yellowing, and some seedlings slight stunning	5	MR
Seedlings showing signs of wilting and severe stunning	7	S
Seedlings dead	9	HS

SBPH, small brown planthopper; I, immune; HR, highly resistant; R, resistant; MR, moderately resistant; S, susceptible; HS, highly susceptible.

To evaluate SBPH resistance in the BIL population, about 60 uniform germinated seeds of each BIL, parental and control varieties were sown in a plastic pot of 8 cm diameter with a hole in the base. Generally, 28 pots, together with one pot of each parent and control variety, were placed in a 65 (length) × 44 (width) × 14 (height) cm plastic seedbox. All the seeds evaluated were incubated at 26 ± 2°C with sunlight. About 2 cm depth of water was maintained in the seedbox.

At the 1.5- to 2.0-leaf stage, the seedlings were infested with second to third instar SBPH nymphs at 15 insects per seedling. Scoring all entries in each seedbox was conducted when 'Wuyujing 3' seedlings were about 90% dead after 14 ± 1 day infestation according to the standard evaluation systems (IRRI, 1988). The scale of each entry was then calculated based on the weighted average of the seedlings tested (Table 1).

Antixenosis test: Fifteen germinated seeds for each entry were grown in a row in a 65 × 44 × 14 cm plastic seedbox at 26 ± 2°C. At the 1.5- to 2.0-leaf stage, seedlings were transferred into cages covered with nylon net and infested with second to third instar SBPH nymphs at a rate of five insects per seedling. The number of insects was counted on each seedling at 8:00 AM and 4:00 PM, and the insects were then dispersed in order to distribute them evenly among seedlings after counting every day (Nemoto et al. 1994). The average number of insects on each entry was calculated and regarded as the value of antixenosis test after 5-day investigation.

Antibiosis test: Five germinated seeds for each entry with four replicates were grown in a glass with 6 cm in diameter and 15 cm high at 26 ± 2°C. At the 1.5- to 2.0-leaf stage, seedlings were infested with 1- to 2-instar SBPH nymphs at a rate of 20 insects per glass. At 10 days after infestation, the survival percentage of insects on each variety was calculated and regarded as the value of antibiosis test.

QTL analysis: A linkage map constructed from the BIL population was used for mapping QTLs associated with resistance to SBPH. The linkage map comprised of 245 restriction fragment length polymorphism (RFLP) markers, covering 1179.5 cM of the rice genome with an average marker interval of 4.8 cM. QTL analysis of SBPH resistance was performed using composite interval mapping implemented in Windows QTL Cartographer 2.5 software (Basten et al. 2002). A log of odds (LOD) threshold of 2.5 was used to claim the presence of putative QTL. The percentage of variation explained by a QTL for traits and the additive effect were also estimated by the software.

Results

Evaluation for SBPH resistance by modified seedbox screening test

The resistance scales of 'Kasalath' and 'Nipponbare' indicated that 'Kasalath' was resistant whereas 'Nipponbare' was susceptible to SBPH. 'Rathu Heenati' was highly resistant whereas 'Wuyujing3' was highly susceptible to SBPH (Table 2).

Table 2: Reaction of parental and control varieties to SBPH in modified seedbox test

Variety	Number of seedlings tested	Resistance scale (mean ± SE)	Resistance reaction
'Kasalath'	75	2.0 ± 0.27	R
'Nipponbare'	75	7.0 ± 0.39	S
'Rathu Heenati'	75	0.8 ± 0.16	HR
'Wuyujing 3'	75	8.4 ± 0.31	HS

SBPH, small brown planthopper; R, resistant; S, susceptible; HR, highly resistant; HS, highly susceptible.

In the modified seedbox screening test, 98 'Nipponbare'/'Kasalath'//'Nipponbare' BILs showed transgressive segregation for resistance to SBPH and resistance scores exhibited a continuous distribution with a range from 1.5 to 9.0, indicating a polygenic control of the resistance to SBPH in this population (Fig. 1a).

QTL analysis of SBPH resistance

Three QTLs for SBPH resistance, designated *Qsbph3b*, *Qsbph11d* and *Qsbph11e*, were mapped on chromosomes 3 and 11 by composite interval mapping with LOD scores of 3.14, 2.95 and 4.12. These QTLs explained 13.8%, 12.6% and 23.5% of the phenotypic variance in this population, respectively (Fig. 2). As indicated by the additive effect, resistance alleles at *Qsbph3c*, *Qsbph8* and *Qsbph11f* came from 'Kasalath', 'Nipponbare' and 'Kasalath', respectively (Table 3).

Antixenosis response against SBPH and QTL mapping

'Kasalath' confers strong antixenosis against SBPH. The antixenosis values of 'Kasalath' and 'Nipponbare' were 2.0 and 8.0, respectively, which were significantly different. Continuous and transgressive segregation was also observed in this population with a range of insect number from 1.0 to 10. The normal distribution of antixenosis values indicated that minor genes controlled antixenosis resistance to SBPH (Fig. 1b).

A total of three QTLs, *Qsbph3c*, *Qsbph8* and *Qsbph11f*, conferring SBPH antixenosis were detected on chromosomes 3, 8 and 11 in the region of R2170–C1135, C390–R1943 and S2260–G257 with LOD scores of 3.19, 2.58 and 3.36, respectively. These QTLs explained 36.4% of the total phenotypic variance observed in this BIL population (Fig. 2 and Table 3).

Antibiosis test and QTL detection

The antibiosis values of the parental varieties 'Kasalath' and 'Nipponbare' were 51% and 75%, respectively, indicating that 'Kasalath' expressed relatively strong antibiosis to SBPH feeding. The continuous distribution of the rate of survival of nymphs with a range from 45 % to 83 % in the BIL population showed that minor genes governed antibiosis resistance (Fig. 1c). A total of two QTLs, designated *Qsbph2* and *Qsbph11g*, conferring antibiosis to SBPH were mapped on chromosomes 3 and 11, with LOD scores of 3.23 and 3.52, accounting for 13.8% and 14.7% of the phenotypic variance in the BIL population, respectively (Table 3 and Fig. 2).

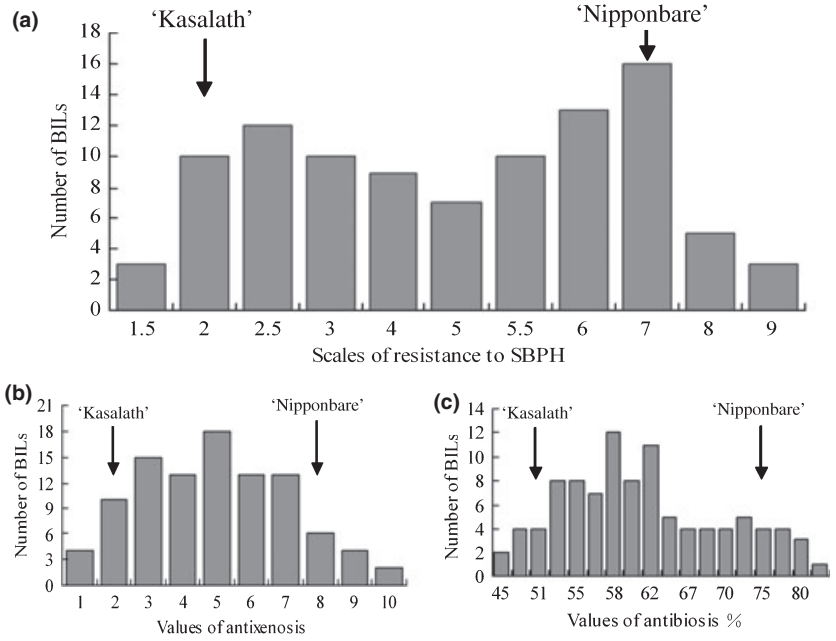

Fig. 1: Frequency distribution of SBPH resistance in 'Nipponbare'/'Kasalath'//'Nipponbare' BIL population. (a) Resistance scales in the modified seedbox screening test. The scales of parental varieties 'Nipponbare' and 'Kasalath' were 7.0 and 2.0, respectively. (b) Distribution of antixenosis values. The antixenosis values of 'Kasalath' and 'Nipponbare' were 2.0 and 8.0, respectively. (c) Distribution of antibiosis values. The antibiosis values of 'Kasalath' and 'Nipponbare' were 51% and 75%, respectively

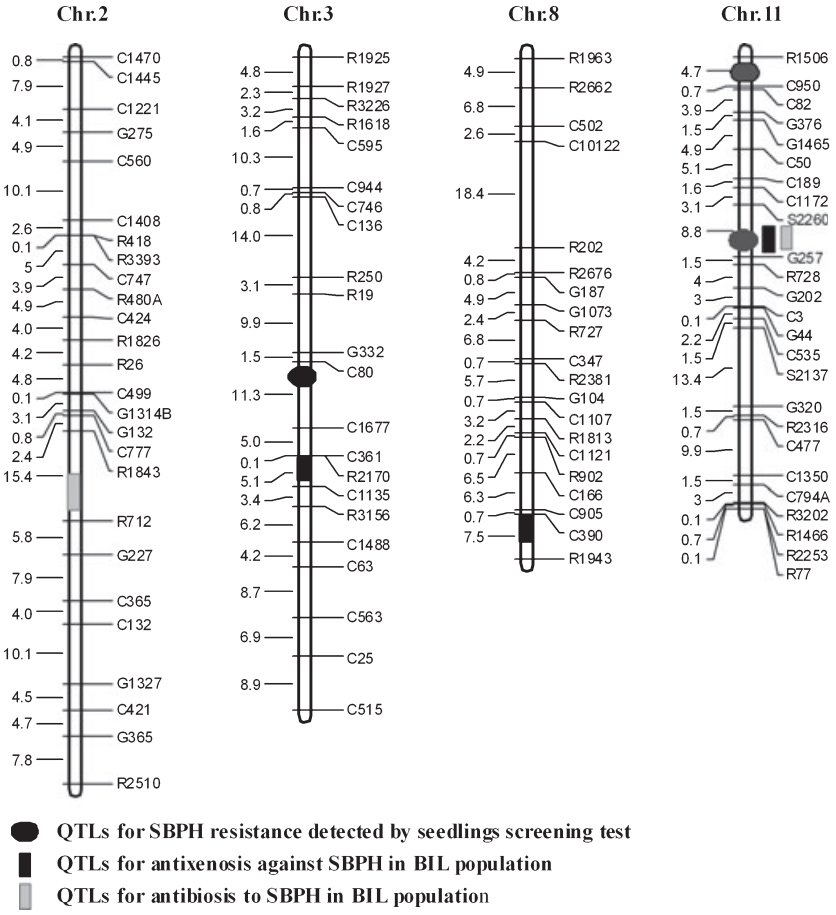

Fig. 2: Chromosome location of QTLs for resistance to small brown planthopper in a backcross inbred line (BIL) population

Discussion

QTL analysis for different resistance phenotypes will reveal the genetic mechanism of resistance and discover the stable resistant locus, and then provide valuable information for germplasm improvement and crop breeding for resistance. In the present study, we used three phenotypic systems to measure resistance to SBPH in 'Nipponbare'/'Kasalath'// 'Nipponbare' BIL population. Three QTLs for SBPH resistance were located on chromosomes 3 and 11 by the modified seedbox screening test, accounting for 49.9% of the total

Table 3: QTLs for SBPH resistance detected in 'Nipponbare'/'Kasalath'//'Nipponbare' BIL population by three phenotypic screening methods

Phenotyping methods	QTL	Marker interval	Chromosome	LOD score	Variance explained (%)	Additive effect
Seedbox screening test with modification	*Qsbph3b*	C80–C1677	3	3.14	13.8	−1.086
	Qsbph11d	R1506–C950	11	2.95	12.6	0.941
	Qsbph11e	S2260–G257	11	4.12	23.5	−2.361
Antixenosis test	*Qsbph3c*	R2170–C1135	3	3.19	12.5	−1.856
	Qsbph8	C390–R1943	8	2.58	10.3	1.282
	Qsbph11f	S2260–G257	11	3.36	13.6	−1.771
Antibiosis test	*Qsbph2*	R1843–R712	2	3.23	13.8	−1.059
	Qsbph11g	S2260–G257	11	3.52	14.7	−2.278

QTL, quantitative trait loci; SBPH, small brown planthopper; BIL, backcross inbred line; LOD, log of odds.

phenotypic variance. In addition, three QTLs associated with antixenosis and two QTLs for antibiosis were identified, which explained 36.4% and 28.5% of the total phenotypic variance, respectively. It was suggested that antixenosis and antibiosis contributed to protection against SBPH feeding in 'Kasalath'. *Qsbph11e*, *Qsbph11f* and *Qsbph11g* were detected in the region between S2260 and G257 on chromosome 11, together with resistance to SBPH originating from 'Kasalath', which indicates that the locus is stable and significant in conferring resistance in 'Kasalath'.

The phenotype scale of the seedbox screening test is an accumulative effect of antixenosis, antibiosis and tolerance. *Qsbph11e* mapped on chromosome 11 was associated with antixenosis and antibiosis, and likely tolerance. Seedbox test is a rapid method for large-scale screening of resistance to the pest, whereas antixenosis and antibiosis tests reveal the mechanism of resistance (Heinrichs et al. 1985), which is especially valuable in the assessment of SBPH resistance.

The entries with strong antixenosis against viruliferous SBPH may markedly decrease the chance of sucking and feeding. For instance, the accessions releasing volatile repulsive chemicals can repulse this planthopper's settlement and probing and thus reduce the chance of transmitting RSV. Furthermore, even if the entries with antixenosis are likely to increase tentative probing by the planthopper, the chance of transmission of RSV can be still reduced because successful communication of RSV needs more than 30 min of successive sucking (Kisimoto 1967). The antibiosis-resistant cultivars can lead to abnormal growth and development of the pest, thereby decreasing feeding, whereas tolerance usually does not affect feeding of the pest. Therefore, an understanding of the mechanism of resistance will be very useful to develop resistant varieties with high levels of antixenosis and/or antibiosis to SBPH.

The *indica* cultivar 'Kasalath' from Assam, India, one of the origin areas of rice, is also resistant to some other pests such as brown planthopper (*Nilaparvata lugens* Stål) and RSV (Su et al. 2002, Sun et al. 2006), indicating that this variety is desirable for pest resistance breeding. A major-effect QTL for RSV resistance was mapped in the region between S2260 and G257 on chromosome 11 (Ding et al. 2005, Sun et al. 2006), the same region harbouring QTL for SBPH resistance detected in this study (Fig. 3). This indicates a close correlation between SBPH and RSV resistance in 'Kasalath'. Therefore, it is speculated that the gene for resistance to SBPH is likely to be tightly linked to the gene for RSV resistance in 'Kasalath', which coincides with a previous study that showed a close relation between SBPH and RSV resistance in many rice accessions (Lin et al. 2000). Rice stripe disease caused by RSV will be controlled effectively once damage by SBPH, the vector

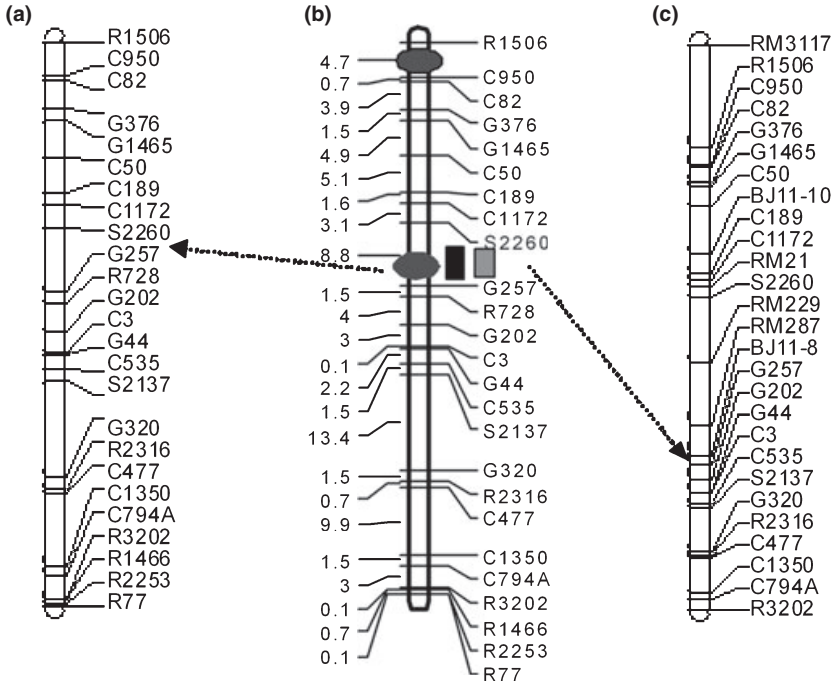

Fig. 3: Comparison of QTLs for resistance to SBPH and RSV on chromosome 11 detected in 'Nipponbare'/'Kasalath'//'Nipponbare' BIL population. (a) A stable QTL for resistance to RSV located in the region between S2260 and G257 (Ding et al. 2005). (b) *Qsbph11e*, *Qsbph11f* and *Qsbph11g* were located in the region between S2260 and G257 in the present study. (c) A locus for RSV resistance was located in the region between BJ118 and G257 (Sun et al. 2006)

of RSV, is reduced markedly or avoided. Undoubtedly, study on SBPH resistance is important in controlling RSV and SBPH.

Qsbph3c associated with antixenosis detected in this study and the QTL for whitebacked planthopper resistance mapped by Yamasaki et al. (1999) in IR24/Asominori RIL were both in the vicinity of marker C1135 on chromosome 3. In addition, both *Qsbph2* for SBPH antibiosis in the present study and the QTL against BPH (Su et al. 2002) were located in the region between R1843 and R712 on chromosome 2. Based on the evidence discussed above, it is suggested that this region harbours genes for resistance to piercing sucking insects, which can be useful for the development of varieties resistant to multiple insects and/or diseases by marker-assisted selection.

Acknowledgements

We thank Yano of Rice Genome Research Program, National Institute of Agrobiological Resources, Tsukuba, Japan, for kindly providing the BIL population, Shi-Xian Zhang of the Institute of Plant Protection, Beijing, China, for providing SBPH insects and Shuang Zhao of the Department of Agronomy, Jilin Agricultural University, Changchun, China, for technical assistance in this study. This project was supported by the National 973 Program (2004CB117204) and the National Key Research Program of 10th Five-Year-Plan of China (2004BA525B02-04).

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