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Mapping of Quantitative Trait Loci of Ovicidal Response to Brown Planthopper (*Nilaparvata lugens* Stål) in Rice (*Oryza sativa* L.)

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The brown planthopper (BPH), *Nilaparvata lugens* Stål, is a serious pest of rice (*Oryza sativa* L.) in Asia. The rice ovicidal response to BPH is characterized by the formation of watery lesions which result in the death of the eggs. It is one of the factors affecting the suppression of the multiplication of BPH. To detect quantitative trait loci (QTLs) for this ovicidal response, a set of 71 rice recombinant inbred lines (F_{11}) derived from a cross between a Japonica variety Asominori with ovicidal response and an Indica variety IR24 without ovicidal response were phenotyped for grade of watery lesions (GWL) and egg mortality (EM) of BPH. GWL and EM showed a significant positive correlation ($P < 0.001$) and transgressive segregation was observed for EM. In composite interval mapping for GWL and EM with 293 RFLP marker loci, two QTLs each on the long arm of chromosome 1 (1L) and the short arm of chromosome 6 (6S) were detected for both GWL and EM. The 6S QTL explained 72.1% and 85.1% of the phenotypic variations for GWL and EM, respectively. The QTL on 1L explained 19.8% and 17.8% of the phenotypic variations for GWL and EM, respectively. Both alleles from Asominori increased GWL and EM. The Asominori allele at the 6S QTL was essential for the ovicidal response to BPH and the Asominori allele at the 1L QTL could increase the EM of BPH in the presence of the Asominori allele at the 6S QTL. It is concluded that the two RFLP loci, *R1954* linked to 6S QTL and *C112* linked to 1L QTL can be used for marker-assisted selection.

Key Words: rice (*Oryza sativa* L.), brown planthopper (BPH, *Nilaparvata lugens* Stål), ovicidal response, recombinant inbred lines (RILs), quantitative trait locus (loci) (QTL(s)), RFLP marker.

Introduction

The brown planthopper (BPH), *Nilaparvata lugens* Stål, is a serious insect pest of rice (*Oryza sativa* L.) in Asia. The insect sucks the plant sap and damages rice plants, leading to the reduction of crop vigor, plant height, number of productive tillers, filled grains and yield. Heavy infestation by BPH results in complete death of rice plants, a condition commonly known as hopperburn.

The development of detailed molecular linkage maps in rice (Causse *et al.* 1994, Harushima *et al.* 1998) has enabled to detect the quantitative trait loci (QTLs) controlling various agronomic characters. The ovicidal response to whitebacked planthopper (WBPH), *Sogatella furcifera* Horváth was characterized by the formation of watery lesions which resulted in the death of the eggs at ovipositional sites (Suzuki *et al.* 1996). The egg mortality (EM) depends on the rice developmental stages and is highest at the maximum tillering stage. Since the ovicidal response is especially prevalent in Japanese rice cultivars (Sogawa 1991), it is considered as a major factor affecting the suppression of WBPH multiplication in Japan. A total of ten QTLs for the rice ovicidal response to WBPH were detected using recombinant inbred lines (RILs) derived from a cross between a Japonica variety Asominori with ovicidal response and an Indica variety IR24 without ovicidal response (Yamasaki *et al.* 1999). The ovicidal response to BPH in Japanese rice cultivars has been recently reported by Kiyonaga *et al.* (1997), although the EM of BPH was lower than that of WBPH. The present study aims at identifying QTLs associated with the rice ovicidal response to BPH and understanding the genetic basis of the ovicidal response to BPH.

Materials and Methods

Plant materials

Recombinant inbred lines were developed by single seed descent from the progeny of an F_2 population derived from a cross between the Japonica variety Asominori and the Indica variety IR24. One hundred sixty-five F_6 lines were obtained from 227 original F_2 individual plants. From these lines, 71 were randomly selected and used for mapping. The restriction fragment length polymorphism (RFLP) map was constructed with 378 markers using the RILs of F_6, F_7 and F_{10} generations (Tsunematsu *et al.* 1996, Yamasaki *et al.* 1999). We used a framework map constructed with 293 marker loci for QTL analysis.

Brown planthopper population

The adult BPH population was collected from the paddy fields of Kyushu National Agricultural Experiment Station, Chikugo City, Fukuoka Prefecture, Japan in 1989.

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This population was maintained at Kyushu University by continuously culturing the insects on seedlings of the Japonica variety Nipponbare placed inside rearing cages. Insect cages were kept in the insectary at 25°C and under a 16 h light: 8 h dark photoperiod regime. They were allowed to mate freely in rearing boxes, and only gravid females were used for infestation.

Brown planthopper infestation

A single plant from 71 RILs (F_{11}) and three plants each from the parents and F_1 were transplanted two weeks after seeding in plastic cups and grown in a greenhouse. Eight weeks after seeding, each plant was infested with 10 to 12 BPH females for two days at 30°C and under a 16 h light: 8 h dark photoperiod regime. After removal of the insects, the plants were kept in the same conditions for three days. Experiments were conducted with two replications.

Ovicidal response to BPH

The grade of watery lesions (GWL) and the EM of BPH were scored to evaluate the plant ovicidal response to BPH. Ovipositional lesions caused by BPH were classified into three types; watery lesions, yellowish lesions and non-watery lesions. The watery lesions were defined as partial or complete watery formation in a region between two or more interveins around the ovipositional site. The GWL of the RILs ranged from 1 to 5 (1 = only non-watery lesions; 2 = many non-watery lesions and some yellowish lesions; 3 = many yellowish lesions; 4 = watery lesions (< 10 cm); and 5 = wide watery lesions (≥ 10 cm)).

Eye pigmentation of BPH was observed four days after oviposition in IR24 at 30°C. Five days after oviposition, an egg with eye spots was scored as a viable egg, whereas an egg without eye spots was considered to be dead (Fig. 1). The average GWL and EM of two replications was calculated for each RIL.

QTL analysis

The QTL analyses for GWL and EM were performed using the average values of the RILs by composite interval mapping (Zeng 1994) in QTL Cartographer v1.13 model 6 (Basten *et al.* 1998). We determined the empirical threshold levels for QTL significance by performing 1000 permutations of the data according to the method of Churchill and Doerge (1994). The genome-experimentwise thresholds for GWL and EM from the permutations under $\alpha = 0.05$ obtained a LOD value of 3.3. The loci with LOD scores over 3.3 indicated the presence of a QTL (hereafter referred to as "indicative"). To reduce type II errors, we used a LOD score of 1.5 as the threshold for confirming the presence of a QTL by permutation tests because the score was almost the same as the threshold value ($\alpha = 0.01$) in the comparisonwise critical value. The loci with LOD scores over 1.5 but under 3.3 suggested the presence of a QTL (hereafter referred to as "suggestive"). The best estimation of the QTL position was assumed to correspond to the peak significance level, and QTL support intervals were drawn for the entire region of the chromosome that exceeded the threshold value. Likelihood ratios were converted to LOD scores after division by 4.6052 (Utz and Melchinger 1996). To identify epistasis, two-way analyses of variance (two-way ANOVA) were performed for GWL and EM between all the RFLP markers in the genome based on QGene v2.26 (Nelson 1997).

Results

Frequency distribution of RILs for ovicidal response to BPH

The distribution of RILs for GWL and EM deviated significantly ($P < 0.001$) from a normal distribution (Fig. 2). However, these data were analyzed because transformations were ineffective to obtain a more normal distribution. Asominori showed a high GWL and EM, whereas IR24 showed a low GWL and EM. More than half of the RILs showed a low GWL, as in the case of IR24. Asominori and

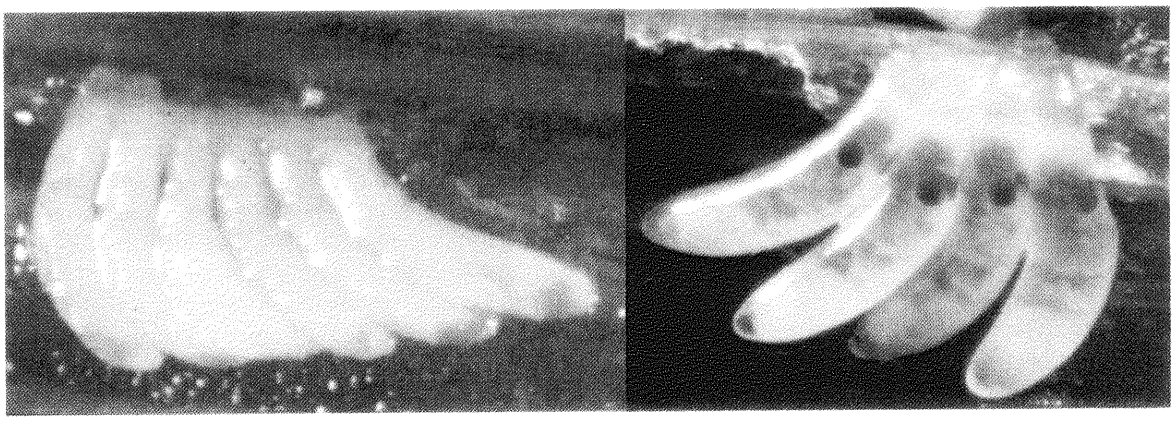

Fig. 1. Dead and viable eggs of BPH on rice five days after oviposition. a: Dead eggs without visible eye spots in a watery lesion of the Japonica variety Asominori. b: Viable eggs with clear eye spots in a non-watery lesion of the Indica variety IR24.

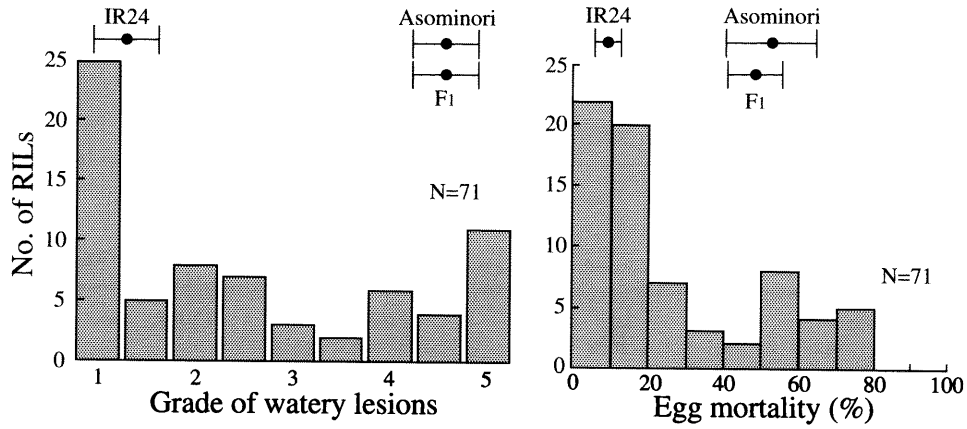

Fig. 2. Frequency distribution of GWL and EM in F_{11} RILs derived from a cross between Asominori and IR24. Points and ranges indicate the average and standard deviation, respectively.

F_1 showed a high GWL. As for EM, IR24 showed a value less than 10%, whereas Asominori showed a value higher than 40%. The F_1 showed almost the same value for EM as that of Asominori. In more than half of the RILs, the values ranged from 0 to 20%, as in the case of IR24. Three transgressive individuals for EM segregated beyond the value of Asominori ($P < 0.05$). The correlation coefficient between GWL and EM was positively significant at 0.1% level (Fig. 3).

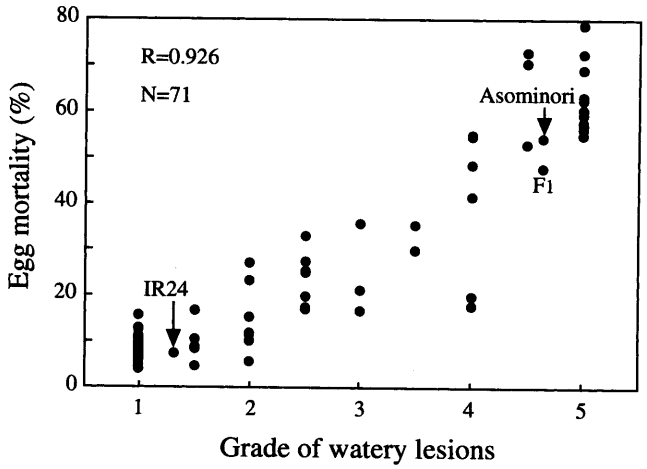

Fig. 3. Correlation between GWL and EM in F_{11} RILs derived from a cross between Asominori and IR24.

QTLs for rice ovicidal response to BPH

Two QTLs for GWL were detected on the long arm of chromosome 1 (1L) and the short arm of chromosome 6 (6S) (Table 1, Fig. 4). The 6S QTL in the interval between *XNpb165-1* and *R1954* was indicative and showed a LOD score of 18.7. The 6S QTL explained 72.1% of the phenotypic variance for GWL. The 1L QTL in the interval between *XNpb346* and *C112* was suggestive and showed a LOD score of 3.2. The 1L QTL explained 19.8% of the phenotypic variance for GWL. Similarly, the two QTLs for EM were detected in the same chromosomal regions as those of QTLs for GWL. The 6S QTL was indicative and showed a LOD score of 25.3. The 6S QTL explained 85.1% of the phenotypic variance for EM. The 1L QTL was suggestive and showed a LOD score of 3.0. The 1L QTL explained 17.8% of the phenotypic variance for EM. The Asominori alleles at the two QTLs contributed to high values of GWL and EM.

Table 1. Characteristics of QTLs associated with the ovicidal response to BPH based on composite interval mapping (QTL Cartographer, LOD ≥ 1.5) using RILs derived from a cross between Asominori and IR24

Trait	Marker interval[1)]	Chromosome[2)]	Peak LOD[3)]	Variation(%)[4)]	Effect[5)]
Grade of watery lesions	*XNpb346-C112* (0)	1L	3.2	19.8	+0.77
	XNpb165-1-R1954 (1)	6S	18.7*	72.1	+2.91
Egg mortality	*XNpb346-C112* (0)	1L	3.0	17.8	+9.18
	XNpb165-1-R1954 (1)	6S	25.3*	85.1	+46.09

* Significant at the genome-experimentwise threhold of $\alpha \leq 0.05$.

1) Underlined markers are the nearest markers to the QTLs. Figures in parenthesis indicate the genetic distance (cM) between the peak position of the LOD and the nearest marker locus.

2) S = short arm; L = long arm.

3) Log_{10} likelihood.

4) Percentage of explained phenotypic variance.

5) + sign indicates that the homozygous alleles of Asominori showed higher phenotypic effects than the homozygous alleles of IR24.

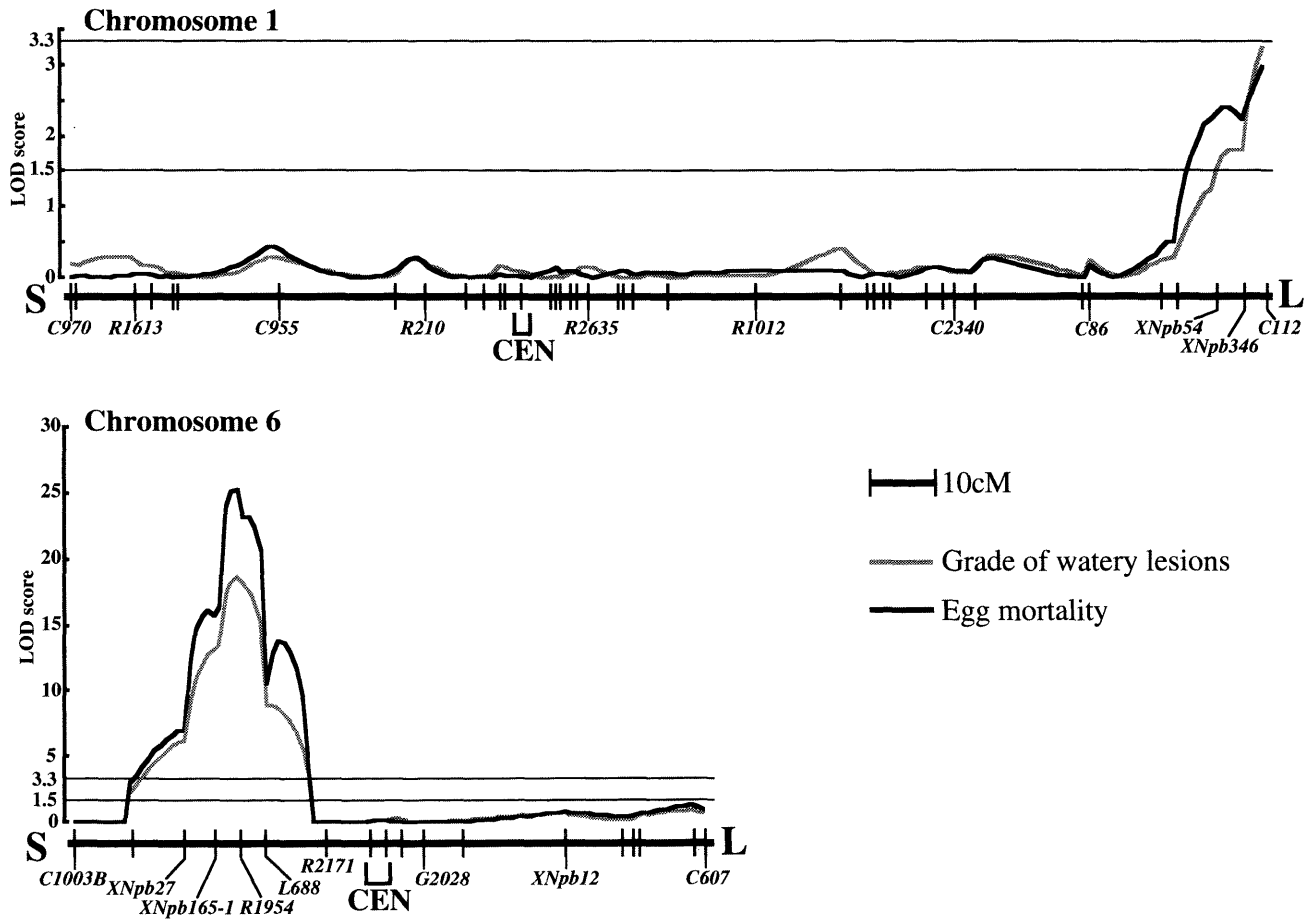

Fig. 4. QTL likelihood curves of LOD score of the ovicidal response to BPH for chromosomes 1 and 6 based on composite interval mapping. The horizontal lines indicate the level of indicative significance at LOD = 3.3 and suggestive significance at LOD = 1.5, respectively. S: short arm; L: long arm; CEN: centromere region.

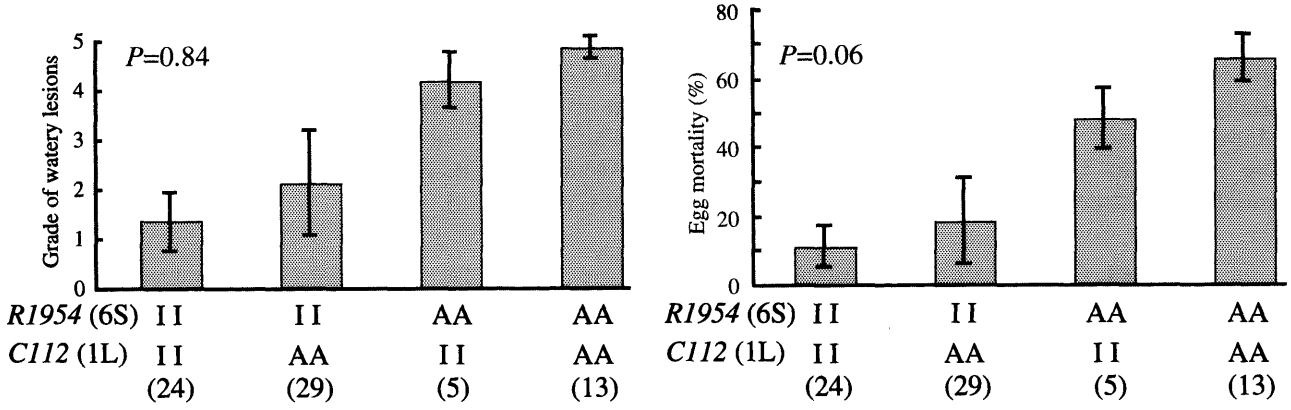

Fig. 5. Genotype class means of the QTLs on chromosomes 6 and 1 for the ovicidal response to BPH. Probability was calculated by two-way ANOVA. AA and II indicate the homozygous alleles of Asominori and IR24, respectively. Figures in parenthesis indicate the number of RILs for the genotype class. Ranges on the bars show the standard deviation.

The genotype class means for the 1L and 6S QTLs are shown in Fig. 5. The RILs having the Asominori alleles at the two QTLs exhibited the highest GWL and EM among the four classes of genotypes. It is likely that the Asominori allele at the 1L QTL increased at least EM in the presence of the Asominori allele at the 6S QTL, although two-way ANOVA failed to detect any apparent epistatic interaction ($P = 0.06$) for EM between the 1L and 6S QTLs.

Discussion

The present study clearly demonstrated that the Asominori alleles at the 1L and 6S QTLs played an important role in the rice ovicidal response to BPH. We previously reported that a total of ten QTLs for the ovicidal response to WBPH were detected using the same RILs analyzed in this study (Yamasaki *et al.* 1999). Comparison of the QTLs for the rice ovicidal response to BPH and WBPH showed that the 1L and 6S QTLs to BPH were located in the same chromosomal regions as those of the 1L and 6S QTLs to WBPH. The 6S QTL to BPH and WBPH accounted for a large part of the phenotypic variance. Therefore, it was considered that the 6S Asominori allele tightly linked to *R1954* was essential for the ovicidal response to both planthopper species. Transgressive individuals for the EM of BPH were observed in the RILs. The accumulation of the positive QTL alleles from both parents would correspond to the genetic basis of the transgressive segregation. However, the Asominori alleles at the 1L and 6S QTLs increased the EM and no QTL was observed where the IR24 allele contributed positively to the ovicidal response to BPH. It is possible that some other minor QTLs were not detected at the threshold value (LOD≥1.5) in the present QTL analysis.

Several RILs having the Asominori allele at the 6S QTL showed a high ovicidal response to BPH as well as WBPH. Since the ovicidal response is associated with a severe necrosis of the rice plant tissues, rice plants having the 6S ovicidal allele seem to be easily damaged from oviposition and physical wounds. In tropical areas, the planthopper populations can survive throughout the year if rice plants are available. We observed that the continuous recruitment of planthoppers severely reduced the number of tillers of the plants having the 6S ovicidal allele in the nursery of the International Rice Research Institute (IRRI) where planthopper populations were so small that non-ovicidal plants were not damaged. The adaptability of strong ovicidal plants may be low in the areas where planthoppers can survive throughout the year.

In temperate areas, the BPH and WBPH populations cannot survive below 10°C (Suenaga 1963) and are replaced annually by immigrants of BPH and WBPH from southern regions. The immigrants migrate from Southern part of China to Japan during the rainy season and never migrate again due to the absence of wind depressions along the frontal zone during the later seasons (Kishimoto 1976). The ovicidal response is highly expressed at the tillering stage and suppresses the multiplication of the first generation of planthopper migrants in Japan (Suzuki *et al.* 1996). Since the WBPH population reproduces only one to two generations at the tillering stage in the Western region of Japan (Kuno 1968, Hirao 1972), it is likely that the ovicidal response successfully suppresses the WBPH multiplication in the field. Thus, the rice ovicidal response may be beneficial in temperate areas. On the other hand, the BPH population builds up rapidly at the second and third generations (Kuno 1968, Hirao 1972), sometimes resulting in hopperburn during the autumn season. Since the ovicidal response attenuates after the heading stage (Suzuki *et al.* 1996), it is possible that BPH avoidance to the rice ovicidal response may contribute to the rapid multiplication of BPH.

The oviposition of the planthoppers and physical wounds lead to the formation of watery lesions on rice plants. This ovicidal response should be classified as induced resistance which is highly expressed at the tillering stage. Several genes controlling the resistance to BPH (Ishii *et al.* 1994, Hirabayashi and Ogawa 1995, 1996, Murata *et al.* 1997, 1998, Huang *et al.* 1997, Hirabayashi *et al.* 1998, 1999) and WBPH (McCouch *et al.* 1991) have been reported. These genes were identified by bulk seedling test where the susceptible seedlings wilted due to heavy damage caused by the feeding of BPH and WBPH nymphs. The present QTLs for the ovicidal response are apparently different from such resistance genes in the phenotypic expression and plant developmental stages at which they are effective. To transfer useful genes of Indica varieties without ovicidal alleles into Japonica varieties with ovicidal alleles, it is necessary that the improved lines have the Japonica alleles at ovicidal QTLs to suppress the multiplication of BPH and WBPH. The two RFLP markers detected in the present study, R1954 and C112 can be used for the identification of plants with the ovicidal response to the planthoppers.

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