The rice cultivar ASD7 (Oryza sativa L. ssp. indica) is resistant to the brown planthopper (BPH; Nilaparvata lugens Stål) and the green leafhopper (Nephotettix cincticeps Uhler). Here, we analyzed multiple genetic resistance to BPH and the green rice leafhopper (GRH; Nephotettix cincticeps Uhler). Using two independent F₂ populations derived from a cross between ASD7 and Taichung 65 (Oryza sativa ssp. japonica), we detected two QTLs (qBPH6 and qBPH12) for resistance to BPH and one QTL (qGRH5) for resistance to GRH. Linkage analysis in BC₁F₃ populations revealed that qBPH12 controlled resistance to BPH and co-segregated with SSR markers RM28466 and RM7376 in plants homozygous for the ASD7 allele at qBPH6. Plants homozygous for the ASD7 alleles at both QTLs showed a much faster antibiosis response to BPH than plants homozygous at only one of these QTLs. It revealed that epistatic interaction between qBPH6 and qBPH12 is the basis of resistance to BPH in ASD7. In addition, qGRH5 controlled resistance to GRH and co-segregated with SSR markers RM6082 and RM3381. qGRH5 is identical to GRH1. Thus, we clarified the genetic basis of multiple resistance of ASD7 to BPH and GRH.

Key Words: rice, multiple resistance, brown planthopper, green rice leafhopper, genetic basis, marker-assisted selection.
carrying BPH1 and BPH2 (Tanaka and Matsumura 2000). BPH does not overwinter in East Asian paddy fields, because of low temperatures, and migrates annually from northern Vietnam to East Asian countries during the rice cropping season (Otuka 2009, 2013).

GRH causes damage to susceptible rice plants in Japan (Nakasuji and Nomura 1968, Nirei and Nakazato 1975). So far, eight loci for GRH resistance derived from rice landraces and wild relatives of rice have been mapped (Fujita et al. 2013). Near-isogenic lines (NILs) and pyramided lines (PYLs) in the genetic background of the rice cultivar Taichung 65 (T65; *Oryza sativa* L. ssp. *japonica*) have been developed for all GRH resistance loci except GRH3 and qGRH9 (Fujita et al. 2010a). GRH1, GRH2, and GRH3 are no longer effective against laboratory GRH biotypes 1, 2, and 3, respectively (Hirae et al. 2007).

The rice cultivar ASD7 (*O. sativa* L. ssp. *indica*) has multiple resistance to BPH, GLH (Athwal et al. 1971), and GRH (Fujita et al. 2002). The objectives of this study were (1) to understand the genetic basis of the resistance to BPH, (2) to understand the genetic basis of the resistance to GRH, and (3) to develop NILs carrying each BPH and GRH resistance gene in the same genetic background for future breeding of insect-resistant rice cultivars.

**Materials and Methods**

**Plant materials**

F₁ hybrid seeds were obtained from a cross between resistant ASD7 and susceptible T65 (Fig. 1). The F₁ plants were self-pollinated to produce two independent F₂ populations, which were used for detection of quantitative trait loci (QTLs) conferring resistance to BPH (Fig. 1A) and GRH (Fig. 1B).

To confirm putative QTLs, the F₁ plants were backcrossed to T65 to generate BC₁F₁ plants, which were backcrossed to T65 to develop BC₂F₁ plants. Among 49 BC₂F₁ plants, 10 carrying putative QTLs were selected through marker-assisted selection (MAS) and self-pollinated. Among 156 BC₂F₂ plants, 7 heterozygous at putative qBPH12 and 20 heterozygous at putative qBPH6 were selected. Finally, four BC₂F₂ plants were chosen: 105-15, homozygous for the ASD7 allele at qBPH6 and heterozygous at qBPH12; 106-3, homozygous for the T65 allele at qBPH6 and heterozygous at qBPH12; 105-9, heterozygous at qBPH6 and homozygous for the ASD7 allele at qBPH12; and 104-45, heterozygous at qBPH6 and homozygous for the T65 allele at qBPH12. Consequently, four BC₂F₂ populations derived from the four BC₂F₂ plants were analyzed.

Preliminary near-isogenic (pre-NIL) and pyramided (pre-PYL) lines were selected from BC₂F₂ populations derived from self-pollinated progeny of BC₂F₂ plants. The pre-NIL for qBPH6 was homozygous for the ASD7 allele at qBPH6 and homozygous for the T65 allele at qBPH12; the pre-NIL for qBPH12 was homozygous for the ASD7 allele at qBPH12 and homozygous for the T65 allele at qBPH6; and the pre-PYL was homozygous for the ASD7 alleles at both qBPH6 and qBPH12.

To confirm qGRH5, mapping populations carrying qGRH5 were developed through advanced backcrossing and MAS. Among 156 BC₂F₂ plants, 3 plants heterozygous in the qGRH5 region were selected. Three BC₂F₂ populations derived from the three

![Fig. 1. Breeding scheme for mapping genes conferring resistance to BPH (A) and GRH (B) derived from the rice cultivar ASD7. The numbers of plants selected for backcrossing or self-pollination and the total plant numbers are indicated in parentheses. MAS, marker-assisted selection; pop., population.](image-url)
BC₂F₂ plants were used. A pre-NIL for qGRH5 was selected in BC₂F₂ populations derived from the self-pollinated progeny of the BC₂F₂ plants.

Insect strains
Two BPH strains were collected in Kanagawa Prefecture in 1966 (designated “1966 BPH”) and in Chikugo in 1989 (designated “1989 BPH”). The BPH strains were maintained by continuous rearing on susceptible cultivar Reiho at 25°C with 16 h light and 8 h dark. The GRH population was collected in Fukuoka Prefecture in 1991 and maintained by continuous rearing on seedlings of the susceptible japonica cultivar Nipponbare. The GRH strain was kept under the same condition.

Evaluation of BPH resistance
Strain 1989 BPH was used for F₂ analysis to overcome the resistance controlled by BPH1 (Myint et al. 2009a). Strain 1966 BPH was used for BC₂F₂ analysis to confirm potential QTLs, because it is avirulent to any of BPH resistance genes (Myint et al. 2009b). An antibiosis test for BPH resistance was conducted according to Myint et al. (2009a) with minor modifications. Each F₂ seedling was infested with 10 second-instar 1989 BPH nympha. Nymph mortality was calculated 5 days after infestation (DAI). We performed the antibiosis test in BC₂F₂ plants homozygous for T65 alleles at both QTLs: both pre-NILs and the pre-PYL. Five brachyp terous 1989 BPH females collected within 24 h after emergence were released onto a single plant 1 month after sowing. Antibiosis was then scored at 2, 3, and 5 DAI. Plants with adult BPH mortality of <40% were categorized as susceptible; plants with BPH mortality of ≥60% were categorized as resistant for mapping of the candidate QTLs. Females with a swollen abdomen were scored at 3 DAI and 5 DAI.

Evaluation of GRH resistance
An antibiosis test for GRH resistance was conducted according to Kishino and Ando (1978) with modifications. A week after sowing, 98 F₂ seedlings, 277 BC₂F₂ seedlings, and the pre-NIL carrying qGRH5 were infested with 10 first-instar nympha in test tubes. Nymph mortality was calculated at 3 DAI. For linkage analysis, plants with a nymph mortality of <30% were categorized as susceptible and those with a mortality of ≥40% were categorized as resistant.

Genotyping using SSR markers
Total DNA of each F₂ and BC₂F₂ plant was extracted from freeze-dried leaves by the potassium acetate method (Dellaporta et al. 1983). The genotypes of SSR loci were determined by PCR amplification in a PCR System-9700 (PerkinElmer, Waltham, MA, USA). The PCR reaction mixture (15 μl) contained 50 mM KCl, 10 mM Tris·HCl (pH 9.0), 1.5 mM MgCl₂, 200 μM dNTP of each, 0.2 μM primer, 1 unit of Taq polymerase (Takara, Shiga, Japan), and 25 ng of genomic DNA. The thermal cycler was programmed as follows: 5 min at 95°C; then 40 cycles of 95°C for 30 s, 53°C for 30 s, and 72°C for 30 s. The PCR products were separated in 4.5% agarose gels by electrophoresis at 250 V for 1 h in 0.5× TBE buffer. Gels were stained with ethidium bromide and photographed under ultraviolet light.

Construction of linkage maps and QTL analysis
Genetic linkage maps of the F₂ populations were constructed for BPH resistance (93 plants and 132 SSR markers) (Supplemental Fig. 1) and for GRH resistance (98 plants and 92 SSR markers) (Supplemental Fig. 2); the markers were distributed across all 12 rice chromosomes (International Rice Genome Sequencing Project 2005, McCouch et al. 2005, Temnykh et al. 2001). Mapmaker/Exp 3.0 (Lander et al. 1987) was used to determine the linkage loci and map distances. The phenotypic and genotypic data of F₂ plants were used for interval mapping with R/qtl software (Broman and Sen 2009). The critical threshold values of the logarithm of odds (LOD) scores of 3.5 for QTL detection were calculated by conducting 1000 permutation tests at an experiment-wise significance level of 0.05.

Validation of QTLs conferring resistance to BPH
To validate qBPH12 and qBPH6, four BC₂F₂ populations were analyzed for resistance to strain 1966 BPH: population 1 derived from BC₂F₂ plant 105-15 (Fig. 3A); population 2 derived from BC₂F₂ plant 106-3 (Fig. 3B); population 3 derived from BC₂F₂ plant 105-9 (Fig. 3C); and population 4 derived from BC₂F₂ plant 104-45 (Fig. 3D). The population 1 and five SSR markers RM3326, RM28466, RM7376, RM28597, and RM6396 on chromosome 12 were used for linkage analysis of qBPH12. Two SSR markers RM8120 and RM8101 on chromosome 6 were used for MAS for qBPH6.

Fig. 2. Frequency distribution of BPH nymph mortality on seedlings of an F₂ population derived from a cross between ASD7 and Taichung 65. The nymph mortality of resistant cultivar ASD7 was 85.0%, whereas the nymph mortality of susceptible cultivar Taichung 65 was 2.2%. Error bars indicate standard error.
Validation of the QTL conferring resistance to GRH

Three BC$_2$F$_3$ populations were used for linkage analysis of qGRH5 and SSR markers. Further detailed linkage analysis was conducted using SSR markers RM6082, RM3381, RM249, RM3437, and RM6024 on chromosome 5.

Results

Detection of QTLs conferring resistance to BPH

Seedlings of the parental cultivar ASD7 were highly resistant to strain 1989 BPH (85.0% nymph mortality), whereas those of the other parent, T65, were susceptible.
(4.4% nymph mortality) (Fig. 2). Nymph mortality on ASD7 × T65 F1 plants was 8.7%. Nymph mortality on 93 F2 plants showed a continuous distribution.

Two QTLs were detected for resistance to BPH that were designated qBPH6 and qBPH12 (Table 1). Interval mapping identified a LOD score peak of 5.3 ($P = 0.003$) for qBPH12 on the long arm of chromosome 12 between SSR markers RM3326 and S20103 (Table 1). qBPH12 explained 28.8% of the phenotypic variation. A LOD score peak of 3.6 ($P = 0.039$) for qBPH6 was identified on the distal short arm of chromosome 6 between SSR markers RM8120 and RM8200 (Table 1). qBPH6 explained 19.6% of the phenotypic variation.

**Validation of qBPH12 and qBPH6**

Population 1 showed discrete segregation of adult mortality as well as females with a swollen abdomen at 3 DAI (Fig. 3E, 3I, Supplemental Figs. 4, 5), with 37 plants resulting in high mortality ($\geq 60\%$) and 6 plants in low mortality ($< 40\%$); this distribution fit a 3:1 segregation ratio ($\chi^2 = 2.8$). We then conducted linkage analysis using five SSR markers near the qBPH12 locus. The 37 plants that were either homozygous or heterozygous for the ASD7 allele at SSR markers RM28466 and RM7376 gave a high level of adult BPH mortality ($\geq 60\%$), whereas plants that were homozygous for the T65 allele at these markers have low mortality ($< 40\%$). qBPH12 co-segregated with SSR markers RM28466 and RM7376 and flanked by SSR markers RM3326 and RM28597 (Fig. 4). The genetic distance between markers RM3326 to qBPH12 was 11.0 cM, and that between qBPH12 and RM28597 was 5.6 cM (Fig. 4, Supplemental Table 1).

Discrete segregation of adult BPH mortality and females with a swollen abdomen were not observed in population 2 (Fig. 3F, 3J, Supplemental Figs. 4, 5). Interval mapping in this population identified a LOD score peak of 3.6 ($P = 0.0005$) for qBPH12 on the long arm of chromosome 12 between SSR markers RM3326 and RM28766 (Supplemental Table 2).

To confirm the genetic effects of qBPH6, two SSR markers RM8120 and RM8101 were used for MAS. Two BC$_2$F$_3$ populations—population 3, derived from the BC$_2$F$_2$ plant 105-9 (Fig. 3C, 3G), and population 4, derived from the BC$_2$F$_2$ plant 104-45 (Fig. 3D, 3H)—were analyzed for resistance to strain 1966 BPH. Population 3 did not show any discrete segregation of adult BPH mortality as well as females with a swollen abdomen at 3 DAI (Fig. 3G, 3K, Supplemental Figs. 4, 5). Association between BPH resistance level and SSR markers in the region of chromosome 6 was not significant. All plants in population 4 showed very low adult BPH mortality and high percentage of females with a swollen abdomen and were categorized as susceptible (Fig. 3H, 3L, Supplemental Figs. 4, 5).

**Adult mortality of strain 1966 BPH in pre-NILs and a pre-PYL**

The resistance of the pre-NILs and a pre-PYL to BPH is shown in Fig. 5A. Adult BPH mortality on a pre-NIL carrying the ASD7 allele of qBPH12 (40.0%) was significantly lower than that on a pre-PYL carrying the ASD7 alleles of both qBPH6 and qBPH12 (85.5%) and that on ASD7 (80.0%) at 2 DAI. However, mortality on the same pre-NIL (70.0%) and pre-PYL (90.9%) did not differ significantly from that on ASD7 (86.7%) at 3 DAI. Similarly, mortality on the pre-NIL (77.5%) and the pre-PYL (96.4%) did not differ significantly from that on ASD7 (100.0%) at 5 DAI. Adult mortality on the pre-NIL carrying the ASD7 allele of qBPH6 (13.0% at 2 DAI, 13.0% at 3 DAI, and 29.0% at 5 DAI) did not differ significantly from that on the pre-NIL homozygous for T65 alleles at both QTLs (0.0% at 2 DAI, 23.0% at 3 DAI, and 30.0% at 5 DAI).

**Table 1.** Quantitative trait loci conferring resistance to the brown planthopper, Nilaparvata lugens Stål, detected by standard interval mapping in an F$_2$ population derived from a cross between ASD7 and Taichung 65

| QTL  | Chromosome | Marker interval       | Position | Peak LOD$^a$ | PEV (%)$^b$ | Additive effect$^c$ | Dominance effect$^c$ | D/A$^c$ |
|------|------------|-----------------------|----------|--------------|--------------|----------------------|----------------------|---------|
| qBPH6| 6          | RM8120–RM8200         | 1.7      | 3.6          | 19.6         | –14.7                | 10.9                 | –0.74   |
| qBPH12| 12        | RM3326–S20103         | 92.3     | 5.3          | 28.8         | –22.1                | 5.3                  | –0.24   |

$^a$ A LOD threshold of 3.5 at an experiment-wise significance level of 0.05 was used.

$^b$ Percentage of explained phenotypic variation.

$^c$ Calculated as $Z – (X + Y)/2$, where $Z$ is the trait value of heterozygous plants and $X$ and $Y$ are as above.

$^d$ Degree of dominance.
Genetic basis of multiple insect resistance in rice

Breeding Science
Vol. 65 No. 5

Genetic basis of multiple insect resistance in rice

Breeding Science 
V ol. 65 No. 5 BS

0.0% at 3 DAI, and 0.0% at 5 DAI) and that on the susceptible control T65 (4.4% at 2 DAI, 4.4% at 3 DAI, and 6.7% at 5 DAI). These data strongly suggest that \( q_{BPH6} \) contributes to a rapid antibiosis response to BPH in the presence of \( q_{BPH12} \). Females with a swollen abdomen on pre-NIL carrying ASD7 allele of \( q_{BPH6} \) were the same as T65 at 3 DAI and 5 DAI. Females with a swollen abdomen of pre-NIL carrying ASD7 allele of \( q_{BPH12} \) and pre-PYL carrying ASD7 alleles of \( q_{BPH6} \) and \( q_{BPH12} \) did not differ significantly from those on ASD7 at 3 DAI and 5 DAI (Fig. 5).

Detection of a QTL conferring resistance to GRH

ASD7 had a high level of GRH resistance (89.0% nymph mortality), whereas T65 was susceptible (1.7% nymph mortality) (Fig. 6A). The F\(_1\) plants showed a high level (83.5%) of nymph mortality. Nymph mortality on 98 F\(_2\) plants showed a continuous distribution. Interval mapping identified a LOD score peak of 24.3 (\( P = 0.004 \)) for \( q_{GRH5} \) on the short arm of chromosome 5 between SSR markers RM3322 and RM3437 (Table 2). \( q_{GRH5} \) explained 67.8% of the phenotypic variation.

Validation of \( q_{GRH5} \)

The three BC\(_2\)F\(_3\) populations derived from these plants were combined and analyzed for GRH resistance using SSR markers in the \( q_{GRH5} \) region on chromosome 5. In total, 83 plants homozygous for T65 alleles at RM6082 and RM3381 were susceptible (nymph mortality of <30%), whereas 194 plants, which were either ASD7-homozygous or -heterozygous at RM6082 and RM3381, were resistant (nymph mortality of ≥40%) (Fig. 6B). This distribution fit a 3:1 segregation ratio (\( \chi^2 = 3.6 \)), indicating that a single dominant gene controls GRH resistance in BC\(_2\)F\(_3\) populations.

\( q_{GRH5} \) genotypes unconfirmed in the BC\(_2\)F\(_3\) plants were

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**Table 2.** Quantitative trait loci conferring resistance to the green rice leafhopper, *Nephotettix cincticeps* Uhler, detected by standard interval mapping in an F\(_2\) population derived from a cross between ASD7 and Taichung 65

| QTL  | Chromosome | Marker interval | Position  | Peak LOD\( ^{a} \) | PEV (\%\( ^{b} \) | Additive effect\( ^{c} \) | Dominance effect\( ^{d} \) | D/A\( ^{e} \) |
|------|------------|----------------|-----------|-------------------|-----------------|-----------------|-----------------|---------|
| \( q_{GRH5} \) | 5          | RM3322–RM3437  | 51.5      | 24.3              | 67.8            | –38.3           | 25.6            | –0.7    |

| a A LOD threshold of 3.5 at an experiment-wise significance level of 0.05 was used.  
| b Percentage of explained phenotypic variation.  
| c Calculated as (\( X – Y \))/2, where \( X \) and \( Y \) are trait values of T65-homozygous and ASD7-homozygous plants, respectively.  
| d Calculated as \( Z – (X + Y)/2 \), where \( Z \) is the trait value of heterozygous plants and \( X \) and \( Y \) are as above.  
| e Degree of dominance.  

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Fig. 5. Adult BPH mortality and females with a swollen abdomen on preliminary near-isogenic lines and a preliminary pyramided line. Plants were used at 1 month after sowing. “T” and “A” indicate homozygous for the T65 and ASD7 alleles at marker loci. Error bars indicate standard error. Means with the same lowercase letter do not differ significantly (\( P < 0.01 \), Tukey–Kramer test). DAI, days after infestation.

Fig. 6. Adult GRH mortality and nymph mortality on preliminary near-isogenic lines and a preliminary pyramided line. Plants were used at 1 month after sowing. “T” and “A” indicate homozygous for the T65 and ASD7 alleles at marker loci. Error bars indicate standard error. Means with the same lowercase letter do not differ significantly (\( P < 0.01 \), Tukey–Kramer test). DAI, days after infestation.
confirmed using phenotypic evaluation of the BC\textsubscript{2}F\textsubscript{4} generation. \textit{qGRH5} co-segregated with RM6082 and RM3381 (Fig. 6C); no recombination was found between RM6082, RM3381, and \textit{qGRH5}. One recombination event was detected between RM249 and \textit{qGRH5}. The genetic distances were 0.2 cM between \textit{qGRH5} and RM249, 5.3 cM between RM249 and RM3437, and 5.2 cM between RM3437 and RM6024 (Fig. 6C, Supplemental Table 3).

**GRH nymph mortality in BC\textsubscript{2}F\textsubscript{3} pre-NILs**

GRH nymph mortality on the pre-NILs carrying the ASD7 allele of \textit{qGRH5} (95.0%) did not differ significantly from that on ASD7 (87.6%) at 3 DAI (Supplemental Fig. 3). Low nymph mortality (2.2%) observed on plants homozygous for the T65 allele at \textit{qGRH5} did not differ significantly from that of the susceptible control T65 (1.7%). This demonstrates that \textit{qGRH5} is the only QTL that controls the resistance of ASD7 to GRH.

**Discussion**

*Genetic basis of ASD7 resistance to BPH and GRH*

The BPH strains collected in Southeast Asia before 1976 are avirulent to Mudgo (carrying \textit{BPH1}) or ASD7 (carrying \textit{BPH2}) (Choi 1979). In Thailand in 1976, the damage score on ASD7 (0.6) was lower than that on IR32 carrying \textit{BPH2} (0.9) (Pongprasert and Weerapat 1979), suggesting the presence of other genetic factors responsible for BPH resistance in ASD7 in addition to \textit{BPH2}.

In this study, two QTLs for resistance to BPH were detected and designated \textit{qBPH12}, on chromosome 12, and \textit{qBPH6}, on chromosome 6. \textit{qBPH12} was detected as a major QTL, whereas \textit{qBPH6} improved resistance to BPH in the presence of the ASD7 allele at \textit{qBPH12} (Fig. 3E, 3F). Epistatic interaction between \textit{qBPH6} and \textit{qBPH12} was observed based on adult mortality at 2 DAI (Fig. 5A). The adult BPH mortality of a pre-PYL homozygous for the ASD7 alleles at both \textit{qBPH6} and \textit{qBPH12} was significantly
higher than that of a pre-NIL homozygous for the ASD7 allele at qBPH12 at 2 DAI. Similarly, epistatic interaction between qBPH6 and qBPH12 was slightly observed for adult mortality and females with a swollen abdomen at 3 DAI and 5 DAI (Fig. 5A, 5B). Responses to the various strains of BPH were definitely different on the pre-NIL for qBPH6 (Present study) and NIL for BPH25 (Myint et al. 2009a). The pre-NIL for qBPH6 was susceptible to all of the BPH strains (1966 BPH, 1989 BPH, and 1999 BPH) (data not shown), while the NIL for BPH25 was resistance to two of three BPH strains (1966 BPH and 1989 BPH), and was susceptible to the remaining 1999 BPH strain. Therefore, qBPH6 identified in this study was not allelic to BPH25.

One dominant QTL, qGRH5, conferring resistance to GRH was detected on chromosome 5 (Table 2). Based on the response to GRH and the location of qGRH5 and GRH1, these results indicate that qGRH5 is identical to GRH1 (Fujita et al. 2010a, Tamura et al. 1999, Yasui and Yoshimura 1999).

**Resistance loci at the genomic region of qBPH6 and qBPH12**

qBPH6 was detected on the distal end of the short arm of chromosome 6, which overlaps BPH25 (Myint et al. 2012), BPH3, BPH4 and qBPH6(t) (Jairin et al. 2007a, 2007b, 2010) and BPH20(t) (Yang et al. 2012) (Fig. 7A). qBPH12 was located on the long arm of chromosome 12, where at least 7 BPH resistance loci have been reported (Fujita et al. 2013). Among them, BPH26, derived from cultivar ADR52 (O. sativa ssp. indica), and BPH18, derived from a wild relative of rice (Oryza australiensis), have been cloned and found to be alleles of the same gene, and flanked by SNP markers DS-72B4 (22.8 Mbp) and DS-173B (22.9 Mbp) (Ji et al. 2013, Tamura et al. 2014). Another resistance gene, BPH1, has been fine-mapped (Cha et al. 2008). As this genomic region contains only BPH26, this strongly suggests that BPH1 is allelic to BPH26. On the other hand, BPH21 must be different from BPH26 because it was fine-mapped to a different location and flanked by the markers B120 (24.1 Mbp) and B122 (24.3 Mbp) (Rahman et al. 2009). Among the remaining three BPH resistance genes, the mapped position of BPH10 (Ishii et al. 1994) and BPH16 (Hisayashi et al. 2004) overlap with BPH26, but it is difficult to discriminate between these genes because of the low resolution of linkage analysis. BPH9 is located between markers G2140 (19.0 Mbp) and S2545 (21.5 Mbp) (Murata et al. 2000), which is different from the location of BPH26. In the present study, qBPH12 co-segregated with the SSR markers RM28466 (23.0 Mbp), RM7376 (23.5 Mbp) and was mapped between SSR markers RM3326 (21.8 Mbp) and RM28597 (24.7 Mbp) (Fig. 7B). In addition, the BPH26 sequences in ADR52 and ASD7 are identical (Tamura et al. 2014). These data suggested that qBPH12 is allelic to BPH26.

**Genes for resistance to insects in the future breeding programs**

NILs carrying genes that confer resistance to insects are very useful genetic resources for rice improvement. Using these NILs, we may be able to introduce these resistance genes into elite japonica cultivars by MAS and backcrossing without introducing the unfavorable traits of resistant donor cultivars such as strong photosensitivity or tall plant height. The genes related to unfavorable traits were removed during the NIL selection. In previous studies, PYLs showed significantly higher resistance to BPH or GRH than any of the NILs (Fujita et al. 2010a, Myint et al. 2009a, Qiu et al. 2012). These NILs can be used for developing pyramided lines free of any unfavorable traits. Combining different kinds of major resistance genes might contribute to the durability of host plant resistance to insect pests.

Furthermore, cultivation of multiline for specific resistance genes, which are mixtures of NILs carrying GRH or BPH resistance genes, is a useful way to reduce the damage caused by insect pests while monitoring GRH or BPH virulence in the target area of the paddy field. To prevent infection of rice blast at field, the multiline cultivars in the genetic background of elite japonica Sasanishiki and Koshikihari resistant to rice blast have been commercially cultivated for several years without any breakdown of their resistance (Ishizaki et al. 2005, Nakajima et al. 1996). The multiline for rice blast consisted of seeds mixtures of several isogenic lines for rice blast resistance genes and the combination of isogenic lines were altered according to the distribution of the races of the blast pathogens at target region. However, there is no report for multiline of insect resistance. Using by the NILs for GRH or BPH resistance genes, the multiline for insect resistance will be developed in future study. To keep stable utilization of multiline, the monitoring of insect virulence at target region is essential for selecting effective

![Fig. 7](image-url) Locations of genes and quantitative trait locus for brown planthopper resistance on (A) the distal end of the short arm of chromosome 6 and (B) the long arm of chromosome 12. Location of genes and QTL is based on physical position of flanking markers. Hatched bars represented qBPH6 and qBPH12 in this study. Black bars represented genes/QTLs in the distal short arm of chromosome 6 and the long arm of chromosome 12 cited from Fujita et al. (2013).
combination of NILs carrying resistance gene in multiline. From the viewpoint of ecological manipulation of diverse alleles at the virulence loci of the insects, multiline cultivars carrying specific genes for resistance to insect pests will be very useful.

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