QTL mapping integrated with BSA-Seq analysis identifies a novel gene conferring resistance to brown planthopper from common wild rice (*Oryza rufipogon* Griff.)

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Abstract The brown planthopper (*Nilaparvata lugens* Stål, BPH) is one of the most destructive rice pests worldwide. GXU202 is a germplasm of common wild rice (*Oryza rufipogon* Griff.) with high resistance to the BPH. In this study, genetic analysis indicated that the BPH-resistant phenotype of GXU202 is controlled by a major gene. Through the combination and comparison of QTL linkage and BSA-seq analyses, a novel gene locus, *BPH41*, conferring BPH resistance was identified. This gene locus was finely mapped to a 116-kb region delimited by W4-4–3 and W1-6–3 on chromosome 4. The markers D01031 and D01045 showed high accuracy in predicting phenotypes resistant to BPH, suggesting their reliability for marker-assisted selection of *BPH41* in breeding BPH-resistant rice varieties. The present identification of *BPH41* will establish a foundation for further map-based cloning and functional characterization of the gene.

Keywords Brown planthopper · Wild rice · Resistance gene · BSA-seq · QTL analysis · Marker-assisted selection

Introduction

Rice (*Oryza sativa* L.) is one of the most important staple food crops and plays an important role in agricultural production. Brown planthopper (BPH; *Nilaparvata lugens* Stål) is a devastating pest of rice prevalent across Asia (Normile 2008). They damage rice plants by sucking sap from phloem and cause viral infections, such as grassy stunt virus and ragged stunt virus (Zheng et al. 2020). Heavy BPH infestation leads to complete drying of rice plants and the occurrence of a ‘hopper burn’, which causes severe yield losses during rice production (Backus et al. 2005). Over the long term, reducing the application of pesticides that can result in environmental pollution, high cost, pest resurgence, and stronger pathogen strain virulence (Frisvold 2019). Therefore, utilizing BPH resistance genes to develop resistant cultivars is the most promising strategy in the management of...
BPH (Bisht et al. 2019). To this end, the exploitation of genes or genetic loci conferring BPH resistance from different germplasm resources could facilitate breeding programs for resistant rice varieties. Previous studies have indicated that the genetic basis of BPH resistance is complex, in which both major and minor resistance genes with complementary or additive effects, as well as environmental interactions, are involved (Zheng et al. 2020).

To date, 40 BPH resistance genes have been identified in cultivated and wild species of rice (Akanksha et al. 2019). Among them, at least thirteen genes are clustered in two distinct regions of chromosome 4 (Hu et al. 2016). This phenomenon is consistent with the pattern of R genes, which are usually distributed on chromosomes in clusters (Mizuno et al. 2020). Specifically, six of them (Bph6, bph16, bph18, Bph27, Bph27(t) and Bph34) are distributed on the long arm of chromosome 4 (Guo et al. 2018; Hirabayashi et al. 1998; Fujita et al. 2013; Huang et al. 2013; He et al. 2013; Kumar et al. 2018); eight genes (Bph3, Bph12, Bph15, Bph17, Bph20, Bph30, Bph33, Bph35 and Bph36) and four QTLs (QBph4, QBph4.2, QBph4.3 and QBph4.4) are distributed on the short arm of chromosome 4 (Liu et al. 2015; Qiu et al. 2012; Lv et al. 2014; Sun et al. 2005; Rahman et al. 2009; Wang et al. 2018; Hu et al. 2018; Li et al. 2019; Hu et al. 2015a, b; Kamolkymolong et al. 2019; Mohanty et al. 2017).

Wild rice species have abundant genetic diversity for studying the genetic basis of resistance phenotypes to BPH (Hu et al. 2016). Previous studies have identified 24 BPH resistance genes/QTLs in 7 wild rice species with different genome types. Of which, bph20(t), bph21(t), bph22(t), bph23(t), Bph27, Bph29, BPH35 and BPH36 were derived from O. rufipogon with the AA genome (Yang et al. 2012; Hou et al. 2011; Huang et al. 2013; Wang et al. 2015; Zhang et al. 2020; Li et al. 2019); Bph34, bph39, and bph40 were derived from O. nivara with the AA genome (Kumar et al. 2018; Akanksha et al. 2019); bph11, bph16, Bph14, Bph15, and QBph4.1 were derived from O. officinalis with the CC genome (Sharma et al. 2003; Hirabayashi et al. 1998; Du et al. 2009; Lv et al. 2014; Hu et al. 2015a, b); Bph13 was derived from O. eichingeri or O. officinalis in two different studies (Liu et al. 2001; Renganayaki et al. 2002); Bph12 was derived from O. latifolia with the CCDD genome (Qiu et al. 2012); Bph20 and Bph21 were derived from O. minuta with the CCDD genome (Rahman et al. 2009); and Bph10, Bph18, and QBph4.2 were derived from O. australiensis with the EE genome (Ishii et al. 1994; Ji et al. 2016; Hu et al. 2015a, b). Therefore, identifying more BPH resistance genes/QTLs from wild rice species is of great significance in rice breeding practice.

QTL mapping is a fundamental approach for understanding the genetic inheritance of phenotypic variation. It is widely utilized in map-based cloning of candidate genes for QTLs and can provide valuable markers for marker-assisted selection (MAS) in crop breeding (Gupta et al. 2019). This conventional approach involves developing a genetic population with a segregating phenotype of target traits and requires the identification of hundreds of polymorphic markers to genotype a large number of individuals derived from the biparental cross. Thus, it is a time-consuming and laborious process and has become a bottleneck for traditional QTL mapping studies. The combination of bulked segregant analysis with whole-genome sequencing (BSA-seq) can be used to facilitate the mapping of qualitative traits that are controlled by a major gene and has been successfully applied for the rapid identification of important agronomical traits in many crop plants (Nguyen et al. 2019), such as rice, maize, wheat, barley, soybean, potato, groundnut, and sorghum (Abe et al. 2012; Klein et al. 2018; Navarro-Escalante et al. 2020; Jia et al. 2017; Song et al. 2017; Kaminski et al. 2016; Pandey et al. 2017; Han et al. 2015).

In rice, BSA-seq has been successfully applied to identify genes conferring resistance to biotic and abiotic stresses. Wu et al. (2019) mapped the genes related to partial blast resistance between 1.52 and 4.32 Mb on chromosome 6. Liang et al. (2020) identified a pi21 locus conferring basal blast resistance between 18.90 and 22.10 Mb on chromosome 4. Sun et al. (2018) identified a cold-tolerant locus between 21.84 and 23.65 Mb on chromosome 4. Tao et al. (2018) identified an aluminium toxicity tolerance gene between 4.72 and 4.87 Mb on chromosome 2. Therefore, it is also a promising approach for the identification of BPH resistance genes.

In the present study, we focused on the identification of the gene conferring BPH resistance in the common wild rice germplasm GXU202 through a combination of conventional QTL mapping and the BSA-seq approach via linkage analysis. This study aimed to (1) discover new resistance gene loci...
associated with BPH resistance using the BSA strategy; (2) validate the loci through conventional QTL mapping and perform comparisons between both methods to evaluate their reliability; (3) propose candidate genes based on genome annotation; and (4) develop reliable markers to facilitate MAS in BPH resistance breeding programs.

Materials and methods

Plant materials, mapping population, and backcrossing generation development

GXU202 (accession number) is a germplasm of common wild rice (O. rufipogon Griff.) collected from Guangxi Province, China, and maintained at Guangxi University with high resistance to BPH infestation. 9311 is an indica rice variety that is widely cultivated in southern China and that is highly susceptible to BPH. GXU202 was crossed with 9311, and the resulting F1 individuals were self-crossed twice to obtain the F2 individuals and F2:3 lines that were used as materials for screening BPH resistance lines. The obtained resistance lines were subsequently used for QTL mapping and BSA sequencing analysis. The highly resistant F3 individuals (score 0–3) were selected to be continuously backcrossed with 9311 to generate BC2F1 populations. The BC2F1 individuals were self-fertilized twice to obtain BC2F2:3 lines, which were selected for phenotypic evaluation and genotyping analysis to verify the accuracy of the mapping. All the plants were grown in an experimental paddy field (Nanning, 22.85°N, 108.26°E) located at Guangxi University, Nanning, Guangxi, China.

Evaluation of BPH resistance

BPH insects were captured from rice fields in Nanning, Guangxi Province, China, and reared on susceptible indica rice variety Taichung Native 1 (TN1) plants to produce enough nymphs for infestation in cages (50 × 50 × 100 cm) surrounded by light-transmitting fine nylon mesh. The cages and seedlings were maintained in a greenhouse under natural daylight at 26–32 °C. The standard seedbox screening test (SSST) was performed on the F2:3 families to evaluate the level of BPH resistance. Each mesh-covered tray (58 × 38 × 9 cm) filled with field mud (~5 cm deep) was planted for an average of 11 rows, including two rows of resistant (GXU202) and susceptible (9311) parental lines. Each row was planted 20–25 plants of one family. At the third-leaf stage (approximately 15 days after sowing), the seedlings were infested with second- or third-instar BPH nymphs at an average level of five insects per seedling and covered with light-transmitting fine nylon-net mesh enclosure (52 × 32 × 45 cm). When all susceptible control seedlings were wilted (scored as 9), the BPH resistance of each individual seedling was assessed according to the rating scale proposed by the International Rice Research Institute (IRRI 1988) (0, no damage; 1, very slight damage; 3, first and second leaves partially yellowing; 5 = pronounced yellowing and stunting; 7 = mostly wilting, the plant still alive; 9 = the plant completely wilted or died).

The resistance score of each F2 individual was inferred from the average resistance score of the seedlings in the corresponding F2:3 families. The test was conducted three times, and the average resistance score of each F2 line was used in subsequent analysis.

DNA extraction, bulking and whole genome resequencing

The genomic DNA of “GXU202” and “9311”, and a total of 255 F2 individuals, was extracted from the leaves collected at the tillering stage using the cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson 1980). The bulks were generated by mixing equal amounts of DNA from 30 extremely resistant (resistance score 0 ~ 4.0) F2 individuals and 30 extremely susceptible (resistance score 8.0 ~ 9.0) F2 individuals. The DNA samples of the two parents and the two bulks were subjected to whole genome resequencing using the Illumina HiSeq platform following the manufacturer’s instructions.

Data processing and analysis of variants

The raw data of each sample were trimmed for clean reads through quality control (QC) procedures. The paired reads were aligned to the reference genome of O. sativa L. ssp. indica cv. 9311 (ftp://ftpensemblgenomes.org/pub/plants/release-45/fasta/oryza_indica/dna/) using Burrows–Wheeler Aligner (BWA) software (Li and Durbin 2009). The alignments were processed by SAMTools (Li et al. 2009). The Mark
Duplicate in Picard tool (https://sourceforge.net/projects/picard/) was used to eliminate PCR duplication. Single-nucleotide polymorphism (SNP) and small InDel calling were performed by using Genome Analysis Toolkit (GATK) software (McKenna et al. 2010). Sliding window methods were used to present the SNP/InDel index of the whole genome, with a scan window size of 1 Mb and step size of 10 kb as default settings.

The association analysis of SNPs was conducted by Euclidean distance (ED) and SNP-index algorithms (Hill et al. 2013). To eliminate false-positive sites, the ∆(SNP-index) data were of the SNPs on the same chromosome fitted using the DISTANCE method (Fekih et al. 2013). ED and ∆SNP index values were plotted. The intersections between candidate regions identified using the two methods were designated candidate regions associated with BPH resistance. The association analysis of small InDels was also conducted by Euclidean distance (ED) and the InDel-index algorithm, following the same method as that of SNPs (Singh et al. 2017). Finally, the overlapping regions of the candidate regions based on SNP and InDel association analysis were designated as the final candidate intervals.

Genotyping, genetic map construction, and QTL analysis

According to the results of preliminary mapping identified through BSA-seq, we chose chromosome 4 to develop the genetic linkage map using the GXU202/9311 F2 population. A total of 310 simple sequence repeat (SSR) markers and insertion/deletion (InDel) markers distributed across chromosome 4 of the rice genome were obtained from the GRAMENE database (https://archive.gramene.org/markers/microsat/). The molecular markers were initially screened for polymorphisms between GXU202 and 9311 for QTL mapping analysis to detect the gene locus for BPH in GXU202. The sequences of the polymorphic markers are listed in Table S9.

The selected polymorphic markers were used to genotype the 255 individuals of the F2 population. QTL analysis was performed with inclusive composite interval mapping (ICIM) using the BIP (QTL mapping in the biparental populations) functionality of QTL IciMapping 4.1 (Meng et al. 2015). LOD thresholds were calculated with 1,000 permutation tests (P < 0.05) and used to declare a putative QTL.

PCRs were performed in a final volume of 10 µL with a PCR mixture including 1 µL template DNA (60 ng·µL 1), 5 µL 2×Taq Master Mix, 0.5 µL each of the forward and reverse primers (10 µM), and 3 µL ddH2O. PCR was performed in a T100™ thermal cycler (Bio–Rad, USA) with the following program: initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 30 s, annealing at 56–62 °C (for different markers) for 30 s and extension at 72 °C for 30 s; and a final extension step at 72 °C for 5 min. The amplified products were detected by agarose gel electrophoresis at a concentration of 1.5% or polyacrylamide gel electrophoresis (PAGE) at a concentration of 8%.

Results

Phenotyping of BPH resistance

In our previous evaluations, the common wild rice germplasm GXU202 with high resistance to BPH was observed under natural nursery conditions (data not shown). According to the identification criteria of BPH in the seedling bulk test, we evaluated the resistance potential of the GXU202, 9311, and F2 populations derived from the cross GXU202/9311. After infestation for 15 days, GXU202 exhibited high resistance to the BPH insects, which was scored as level 2.5 (Fig. 1a,b). In contrast, rice variety 9311 was highly susceptible to BPH, which scored 9.0 (Fig. 1a,b). These results indicated that GXU202 possesses high resistance to BPH. A total of 255 F2:3 families of GXU202×9311 were infested with BPH and scored at 15 dpi. In the F2 population, BPH resistance scores showed a continuous range from 2.5 to 9.0, with a valley between 7.0 and 7.9 in the distribution curve (Fig. 1d). Such a distribution indicates the involvement of a major gene and minor QTLs in the segregation of BPH resistance in this population.

Based on the BPH resistance phenotypic scoring, the 255 F2 individuals could be grouped as follows: 62 individuals with scores 0–4.9 were regarded as resistant groups, 128 individuals with scores 5–7.9 were moderate susceptible groups, and 65 individuals with scores 8–9 were classified as highly susceptible.
groups. The F2 populations with the phenotype of resistant, moderately susceptible, and highly susceptible presented a segregation ratio of 62:128:65, which fits well with a 1:2:1 segregation model ($\chi^2 = 0.066 < \chi^2_{0.05,2} = 5.99, P > 0.05$).

The results indicated that the inheritance of BPH resistance in the study was affected by a single Mendelian gene derived from wild rice GXU202. It was tentatively designated as BPH41. For the BSA-seq analysis, 30 extremely resistant F2 lines (resistance score 2.5–4.0) and 30 extremely susceptible F2 lines (resistance score 8.9–9.0) were selected and bulked as the R pool and S pool, respectively.

Identification of candidate regions for Bph resistance by BSA-seq analysis

16.4 GB, 17.5 GB, 12.2 GB and 17.1 GB of clean data were generated from resistant parents (GXU202, abbreviated as R01), susceptible parents (9311, abbreviated as R02), extreme resistant bulk (abbreviated as R03) and extreme susceptible bulk (abbreviated as R04), respectively (Table S1).

The average genome coverage depths of the mapped reads of the R01, R02, R03, and R04 samples were 35-, 41-, 26-, and 18-fold, respectively (Table S2). The percentages of reference bases with coverage depths above 10 were 85.34%, 88.78%, 88.1%, and 83.27% (Table S2, Fig. S1, S2). The coverage depth was evenly distributed on chromosomes (Fig. 2, Fig. S2).

In total, 2,440,152 SNPs and 512,846 small InDels were between the parental lines (R01 vs. R02), and 812,866 SNPs and 171,431 small InDels were detected between the two extreme bulks (R03 vs. R04) (Fig. S3). The SNPs and InDels were sufficiently and uniformly distributed throughout the whole genome on 12 chromosomes.

To determine the candidate regions for BPH resistance, the correlation value was calculated and plotted using the Euclidean distance (ED) algorithm and SNP/InDel-index algorithm. As expected, the plot of the ED value of SNPs and small InDels showed an obvious peak at the same interval on chromosome 4 (Fig. 2a, b). The candidate regions were detected at approximately 0.3–11.65 Mb on chromosome 4, within which the

Fig. 1 Resistance phenotypes of GXU202, 9311 and their F2 population to BPH at the seedling stage using the seedling bulk test. a Phenotypes of GXU202 and 9311 before infestation with BPH; b Phenotypes of GXU202 and 9311 after infestation with BPH; c Phenotypes of representative resistant and susceptible F2:3 lines derived from the cross GXU202/9311 after BPH infestation; d Frequency distribution of BPH resistance score in GXU202/9311 F2:3 lines population
SNP/InDel sites had higher ED values than the threshold (Fig. 2a, b; Fig. S4; Table S3; Table S5).

For the SNP/InDel-index method, the candidate regions were detected according to higher \( \Delta(SNP-/InDel\\text{-index}) \) values than the threshold (confidence interval > 99%). The detected candidate regions were mapped within the range of 4.51 Mb on chromosome 4, containing four candidate regions that cover a total length of 1.71 Mb (Figs. 2c,d, 3c,d; Table S4; Table S6).

Finally, taking overlapping targeted regions from the SNP and InDel association analysis into account, 4 tightly successive regions spanning 4.51~7.54 Mb on chromosome 4 for BPH resistance were identified. A 1.71-Mb region with 98 annotated genes was regarded as a candidate region for the \textit{BPH41} locus (Table 1).

Confirmation of the detected regions by conventional genetic mapping of chromosome 4

To validate the candidate region identified by BSA-seq, a genetic linkage map of chromosome 4 was constructed. A total of 310 SSR markers distributed across chromosome 4 were used to assess the polymorphism between the resistant parent (GXU202) and susceptible parent (9311). As a result, 23 polymorphic SSR markers evenly distributed on chromosome 4 were selected. The primer sequences for these markers are shown in Table S7. They were used to genotype the 255 individuals of the F\textsubscript{2} population for QTL analysis. Then, a genetic map of chromosome 4 was generated using QTL IciMapping 4.0 to identify BPH resistance QTL(s) based on the resistance score (Fig. 1d). The average marker interval of the genetic map was 1.50 Mb, and the maximum distance of interval is 2.49 Mb (Table S7). The order of the markers on the map is consistent with the physical order of the markers on chromosome 4 of 9311. As expected, a significant QTL peak was detected within the interval flanked by markers D01031(4.233 Mb) and D01045(5.384 Mb) (Fig. 4a). The locus had the highest LOD score of 12.70 and accounted for 24.44% of the phenotypic variation of BPH resistance, which is consistent with the results identified by...
Thus, it is considered more likely to harbour the \textbf{BPH41} locus than other regions.

**Fine mapping of BPH41**

To precisely narrow the potential region of \textbf{BPH41}, the genotyping of 1235 BC$_2$F$_2$ individuals was performed using SSR markers D01031 and D01045, resulting in the identification of 54 recombinants. Three new SSR polymorphic markers (W1-7–2, W2-2–4 and W1-3–6) were developed in the candidate region and were applied to genotype the recombinants (Fig. 4c; Table S7). All recombinants were self-pollinated to produce the BC$_2$F$_{2.3}$ lines, which were surveyed in the seedling bulk test for resistance to BPH insects. The genotypic and phenotypic analysis of the recombinants narrowed the resistance-associated region to the segment delimited by markers D01031 and W1-7–2 (Fig. 4c, d).

Furthermore, D01031 and W1-7–2, together with four additional polymorphic SSR markers (W4-4–3, W2-1–5, W1-6–3, and W2-5–1), were adopted to genotype an additional 2806 BC$_2$F$_2$ individuals, and 40 recombinants were obtained (Fig. 4d; Table S7). Based on the genotypic and phenotypic analysis of the recombinants, we finally anchored \textbf{BPH41} to the segment flanked by markers W4-4–3 and W1-6–3 on chromosome 4, cosegregating with W2-1–5 (Fig. 4d, e). The physical distance between W4-4–3 and W1-6–3 is estimated to be 115.78 kb in the 9311 genome (http://www.elabcas.cn/rice/genome_9311.html) and 116.14 kb in the Nipponbare genome (https://rapdb.dna.affrc.go.jp/tools/blast).

**Candidate genes analysis in BPH41 genomic region**

Based on the Rice Genome Annotation Project (TIGR, http://www.rice.plantbiology.msu.edu/overview.shtml), the final mapping region contained 23 predicted genes. Of these, 5 genes were expressed proteins (\textit{LOC_Os04g08630}, \textit{LOC_Os04g08650}, \textit{LOC_Os04g08660}, \textit{LOC_Os04g08690} and \textit{LOC_Os04g08749}), 3 genes were hypothetical proteins (\textit{LOC_Os04g08700}, \textit{LOC_Os04g08730} and \textit{LOC_Os04g08760}), 2 genes were transposon proteins (\textit{LOC_Os04g08710} and \textit{LOC_Os04g08720}), and 9 genes were retrotransposon proteins (\textit{LOC_Os04g08610}, \textit{LOC_Os04g08620}, \textit{LOC_Os04g08670}, \textit{LOC_Os04g08680}, \textit{LOC_Os04g08768}, \textit{LOC_Os04g08772}, \textit{LOC_Os04g08776}, \textit{LOC_Os04g08780}).
and LOC_Os04g08784). The remaining 4 proteins were predicted with assigned functions, including LOC_Os04g08600 encoding a C2H2 zinc finger protein, LOC_Os04g08640 encoding a cadmium tolerance factor, LOC_Os04g08740 encoding an ethylene receptor and LOC_Os04g08764 encoding an avr9/Cf-9 rapidly elicited protein (Table S8).

C2H2 zinc finger proteins play important roles in plant growth, development, and stress signal transduction in abiotic stress responses, such as salt, osmotic, cold, drought, oxidative and high-light stress (Han et al. 2020). Cadmium tolerance factors participate in multiple physiological processes to decrease Cd uptake and accumulation to counter Cd toxicity (Chmielewska-Bąk and Deckert 2021). Ethylene receptors participate in the regulation of ethylene in numerous growth and developmental processes, as well as responses to biotic and abiotic stress (Zhao et al. 2021). The avr9/Cf-9 rapidly elicited proteins play profound roles in R gene-mediated and ROS gene-independent early plant defence responses (De Vega et al. 2021).

Out of the 23 predicted genes, the locus LOC_Os04g08764 might be associated with biotic stress stimulus and seems to be the putative candidate gene for resistance to BPH; however, this needs further investigation.

Validation of SSR marker for BPH resistance selection

In the QTL analysis, the locus with the highest LOD score was flanked by markers D01031 and D01045 (Fig. 4a). In parallel with screening for recombinants, 541 randomly chosen BC2F2 individuals derived from the cross GXU202 × 9311 were utilized for validation of the efficacy of D01031 and D01045 to develop effective molecular markers for BPH resistance selection. This was performed by genotyping the BC2F2 population, and individuals homozygous for the resistant allele were selected to evaluate BPH resistance using their corresponding BC2F2:3 lines. For both markers, PCR amplification yielded clear bands for two parents, homozygous and heterozygous
individuals from the segregating populations (Fig. S5). As expected, both markers showed high cosegregation with the BPH reaction. There were 80 BC2F2 plants homozygous for the resistant allele among the 541 BC2F2 individuals, including 76 individuals homozygous at both sites and 4 recombinants (Table 2). In marker genotype and phenotype comparisons, when selecting individuals with BPH resistance scores of 0–4.9 as resistant, D01031 and D01045 showed only 92.31% and 89.74% accuracy, respectively (Table 3). However, if the selection boundary was broadened to a score of 0–5.9, the accuracy of D01031 and D01045 could reach 98.72% and 96.15%, respectively (Table 3).

BPH41 is located in a region clustering several BPH resistance genes

To date, 40 BPH resistance genes have been mapped to the rice genome. It has been noted that these BPH resistance genes appear to be clustered on rice chromosomes 3, 4, 6, and 12 (Hu et al. 2016). At least 8 major genes have been mapped to the adjacent or overlapping regions on the short arm of chromosome 4, including BPH3, BPH12, Bph15, Bph17, Bph20(t), Bph30, Bph33, Bph35 and Bph36 (Liu et al. 2015; Qiu et al. 2012; Lv et al. 2014; Sun et al. 2005; Rahman et al. 2009; Wang et al. 2018; Hu et al. 2018; Zhang et al. 2020; Li et al. 2019).

In the present study, Bph41 was also located on this cluster. The position of Bph41 was approximately 3.71 Mb downstream from the Bph33, and 0.42 Mb upstream from Bph12. Notably, Qbph4.4 was reported to be located in an interval ranging from 0.688 Mb to 13.07 Mb, covering the range of Bph41 (Mohanty et al. 2017) (Fig. 5). However, Bph41 was still considered a novel major gene differing from Qbph4.4 for the reasons that (I) Qbph4.4 is likely to be an indeterminate or even false QTL (Hu et al. 2018); (II) Qbph4.4 is a minor QTL, while Bph41 acts as a major gene; (III) Qbph4.4 is derived from an indica landrace in Odisha, India, while Bph41 is derived from wild sp. O. rufipogon in Guangxi, China.

**Discussion**

The integration of BSA-seq and QTL analysis identified a gene locus on chromosome 4 of GXU202 and the identification of resistance genes are important for pest control and prevention. Common wild rice GXU202 is a highly resistant germplasm to BPH, while indica cultivated rice variety 9311 exhibited obvious susceptibility (Fig. 1).

Based on a monogenic inheritance hypothesis, we combined BSA-seq and QTL analysis to identify the gene for BPH resistance in GXU202. Our study revealed a key genetic locus, designated BPH41, for BPH resistance at the seedling stage of rice. BPH41 was primarily mapped to the short arm of chromosome 4 in the interval delimited by markers D01031 and D01045 from an F2 population. In further fine mapping using the BC2F2 population, its position was finally delimited to a region flanked by markers W4-4–3 and W1-6–3, which were approximately 115.78 kb apart in the 9311 genome and 116.14 kb apart in the Nipponbare genome (Fig. 4e).

BPH41 can indeed enhance the resistance level of plants from highly susceptible (scored 9.0) to moderately resistant (scored 6.9) or more resistant and can be used for introgressing BPH41 in the background of 9311.

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**Table 2** Numbers of selected BC2F2 individuals homozygous for the resistant allele

| Genotype | No. of BC2F2 lines |
|----------|--------------------|
| D01031   | D01045             |
| B         | H                  | Total          |
| 2         | 2                  | 2              |
| H         | B                  | 0              |
| 2         | 0                  | 0              |
| B         | B                  | 76             |
|          |                    | 70             |

| Scored 0–4.9 | Scored 5.0–5.9 | Scored 0–6.9 |
|--------------|---------------|--------------|
| 0            | 0             | 0            |
| 0            | 0             | 0            |
| 5            | 1             |              |

# Genotype that is homozygous for the resistance allele; # Genotype that is heterozygous

**Table 3** The accuracy of D01031 and D01045 in genotype-and-phenotype comparisons in different criteria

| Marker | Scored 0–4.9 accuracy (%) | Scored 5.0–5.9 accuracy (%) | Scored 0–6.9 accuracy (%) |
|--------|---------------------------|-----------------------------|---------------------------|
| D01031 | 92.31                     | 98.72                       | 100.00                    |
| D01045 | 89.74                     | 96.15                       | 97.44                     |

In rice, the damage caused by BPH is a restriction factor for production. Genetic analysis of BPH resistance...
Among the genes in the cluster, BPH35 and BPH36 also originated from O. rufipogon (Zhang et al. 2020; Li et al. 2019). However, Bph41 was considered a different gene, as it is located at 1.41 Mb upstream from Bph35 or Bph36 and suggest that tightly linked loci tend to be present in clusters in particular regions of chromosomes and act in tandem (Mizuno et al. 2020).

D01031 and D01045 are valuable molecular markers in the MAS of BPH41 in breeding programs.

In the past few decades, molecular markers have played an important role in target gene location and marker-assisted selection (MAS) breeding. Identification of a tightly linked DNA marker is a prerequisite for rapidly identifying the introgressions of the desired genetic locus. In the QTL analysis results, two SSR molecular markers, D01031 and D01045, showed their potential application in breeding practice, such as marker-assisted selection and BPH-resistant locus pyramiding, for the development of rice cultivars with high BPH resistance.

It is worth noting that, compared to the high resistance of their donor parents in the F3 generation (sored 1 or 3), the resistance of the backcross progenies remained but declined. BPH41 can indeed enhance the resistance level of plants, but stronger resistance seems to be the result of the complex interaction and synergistic effect of BPH41 and other minor QTLs harboured in GXU202.

Further research perspectives

As a close ancestor of cultivated rice, wild rice species are rich sources of resistance genes against biotic and abiotic stress, such as cold, pest, pathogen, waterlogging, drought, salt, and weed. Previous genetic studies have identified 24 BPH resistance genes/QTLs in 7 wild rice species (Yang et al. 2012; Hou et al. 2011; Huang et al. 2013; Wang et al. 2015; Zhang et al. 2020; Li et al. 2019; Kumar et al. 2018; Akanksha et al. 2019; Sharma et al. 2003; Hirabayashi et al. 1998; Du et al. 2009; Lv et al. 2014; Hu et al. 2015a, b; Liu et al. 2001; Renganayaki et al. 2002; Qiu et al. 2012; Rahman et al. 2009; Ishii et al. 1994; Ji et al. 2016; Hu et al. 2015a, b). These genes have been increasingly widely utilized in breeding work to improve the BPH resistance of cultivated rice varieties. In this study, BPH41 was detected in wild rice O. rufipogon. The introgression of the fragment containing BPH41 into indica rice variety 9311 enhanced BPH resistance.

However, the population carrying the introgression fragments of wild rice species has a complex genetic background. Therefore, in further studies of BPH41, more advanced backcrossing lines, such as recombinant inbred lines (RILs) and near isogenic lines (NILs), must be constructed and applied to further fine map and clone the genes underlying BPH resistance. On the one hand, fine mapping of BPH41 with high resolution can be achieved using these rice lines. On the other hand, these advanced backcrossing lines are valuable intermediate materials in BPH resistance breeding.

The findings in this study will facilitate further exploration of the BPH41 locus, such as map-based cloning and transformation experiments of candidate genes. This study will shed light on elucidating the molecular mechanisms underlying the resistance of BPH41 and the genetic effects between BPH41 and other minor QTLs.

Authors’ contributions RL and JL designed and supervised the study. XW, YH, YZ, BD, BW, XG, YQ, YF, FL, and BQ...
performed the phenotypic data collection. XW analysed the data and drafted the manuscript. RL, JL and YZ revised and finalized the manuscript. All the authors read and approved the manuscript.

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**Declarations**

**Conflicts of interest** The authors declare that they have no conflicts of interest.

**References**

Abe A, Kosugi S, Yoshida K, Natsume S, Takagi H, Kanzaki H, Matsumura H, Yoshida K, Mitsuoka C, Tamiru M, Innan H, Cano L, Kamoun S, Terauchi R (2012) Genome sequencing reveals agronomically important loci in rice using MutMap. Nat Biotechnol 30(2):174–178. https://doi.org/10.1038/nbt.2095

Akanksha S, Jhansi Lakshmi V, Singh AK, Deepthi Y, Chiruttakumar PM, Ramdeen B, D, Sarla, N, Mangrauthia, S, K, & Ram, T. (2019) Genetics of novel brown planthopper Nilaparvata lugens (Stål) resistance genes in derived introgression lines from the interspecific cross O sativa var Swarna × O nivara. J Genetics 98:113

Backus EA, Serrano MS, Ranger CM (2005) Mechanisms of hoppersper: an overview of insect taxonomy, behavior, and physiology. Annu Rev Entomol 50:125–151. https://doi.org/10.1146/annurev.ento.49.040602.123310

Bisht DS, Bhatia V, Bhattacharya R (2019) Improving plant-resistance to insect-pests and pathogens: the new opportunities through targeted genome editing. Semin Cell Dev Biol 96:65–76. https://doi.org/10.1016/j.semcdb.2019.04.008

Chmielowska-Bańk J, Deckert J (2021) Plant recovery after metal stress-a review. Plants (basel, Switzerland) 10(3):450. https://doi.org/10.3390/plants10030450

De Vega D, Holden N, Hedley PE, Morris J, Luna E, Newton A (2021) Chitosan primes plant defence mechanisms against Botrytis cinerea, including expression of Avr9/Cf-9 rapidly elicited genes. Plant, Cell Environ 44(1):290–303. https://doi.org/10.1111/pce.13921

Du B, Zhang W, Liu B, Hu J, Wei Z, Shi Z, He R, Zhu L, Chen R, Han B, He G (2009) Identification and characterization of Bph14, a gene conferring resistance to brown planthopper in rice. Proc Natl Acad Sci USA 106(52):22163–22168. https://doi.org/10.1073/pnas.0912139106

Fekih R, Takagi H, Tamiru M, Abe A, Natsume S, Yae-gashi H, Sharma S, Sharma S, Kanzaki H, Matsumura H, Saitoh H, Mitsuoka C, Utsubo H, Uemura A, Kanzaki E, Kosugi S, Yoshida K, Cano L, Kamoun S, Terauchi R (2013) MutMap+: genetic mapping and mutant identification without crossing in rice. PLoS ONE 8(7):e68529. https://doi.org/10.1371/journal.pone.0068529

Frisvold GB (2019) How low can you go? Estimating impacts of reduced pesticide use. Pest Manag Sci 75(5):1223–1233. https://doi.org/10.1002/ps.5249

Fujita D, Kohli A, Horgan FG (2013) Rice resistance to planthoppers and leafhoppers. Crit Rev Plant Sci 32(3):162–191. https://doi.org/10.1080/07352689.2012.735986

Guo J, Xu C, Wu D, Zhao Y, Qiu Y, Wang X, Ouyang Y, Cai B, Liu X, Jing S, Shangguan X, Wang H, Ma Y, Hu L, Wu Y, Shi S, Wang W, Zhu L, Xu X, Chen R, Du B, He G (2018) Bph6 encodes an exocyst-localized protein and confers broad resistance to planthoppers in rice. Nat Genet 50(2):297–306

Gupta PK, Kulwal PL, Jaiswal V (2019) Association mapping in plants in the post-GWAS genomics era. Adv Genet 104:75–154. https://doi.org/10.1016/bs.adgen.2018.12.001

Han G, Lu C, Guo J, Qiao Z, Sui N, Qiu N, Wang B (2020) C2H2 Zinc finger proteins: master regulators of abiotic stress responses in plants. Front Plant Sci 11:115. https://doi.org/10.3389/fpls.2020.00115

Han Y, Lv P, Hou S, Li S, Ji G, Ma X, Du R, Liu G (2015) Combining next generation sequencing with bulked segregant analysis to fine map a stem moisture locus in sorghum (Sorghum bicolor L. Moench). PLoS ONE 10(5):e0127065. https://doi.org/10.1371/journal.pone.0127065

He J, Liu YQ, Liu YL, Jiang L, Wu H, Kang HY, Liu SJ, Chen LM, Liu X, Cheng XN, Wan JM (2013) High-resolution mapping of brown planthopper (BPH) resistance gene Bph27(t) in rice (Oryza sativa L.). Mol Breeding 31:549–557

Hill JT, Demarest BL, Bigsrove BW, Gorsi B, Su YC, Yost HJ (2013) MMAPR: mutation mapping analysis pipeline for pooled RNA-seq. Genome Res 23(4):687–697. https://doi.org/10.1101/gr.146936.112

Hirabayashi H, Angeles ER, Kaji R, Ogawa T, Brar DS, Khush GS (1998) Identification of brown planthopper resistance gene derived from o. officinalis using molecular markers in rice. Breed Sci,48(Suppl):82 (in Japanese)

Hou LY, Peng ST, Xing-Hua W et al. (2011) Genetic analysis and preliminary mapping of two recessive resistance genes to brown planthopper, nilaparvata lugens Sit in rice[J]. Rice Sci 18(003):238–242

Hu J, Xiao C, Cheng M, Gao G, Zhang Q, He Y (2015a) Fine mapping and pyramiding of brown planthopper resistance genes to brown planthopper, nilaparvata lugens Sit in rice[0]. Rice Sci 18(003):238–242

Hu J, Chang X, Zhao Z, Tang W, Du B, Wu W (2018) Identification and fine mapping of Bph33 a new brown planthopper resistance gene in rice (Oryza sativa L.). Rice (new York, n.y.) 11(1):55. https://doi.org/10.1186/s12284-018-0249-7

Hu J, Xiao C, He Y (2016) Recent progress on the genetics and molecular breeding of brown planthopper resistance in rice. Rice (new York, n.y.) 9(1):30. https://doi.org/10.1186/s12284-016-0099-0

Hu J, Xiao C, Cheng MX, Gao GJ, Zhang QL, He YQ (2015b) A new finely mapped Oryza australiensis-derived QTL in rice confers resistance to brown
plantthopper. Gene 561(1):132–137. https://doi.org/10.1016/j.gene.2015.02.026

Huang D, Qiu Y, Zhang Y, Huang F, Meng J, Wei S, Li R, Chen B (2013) Fine mapping and characterization of BPH27, a brown planthopper resistance gene from wild rice (Oryza rufipogon Griff.). TAG. Theoretical and applied genetics. Theoretische Und Angewandte Genetik 126(1):219–229. https://doi.org/10.1007/s00122-012-1975-7

IRRI (1988) Standard evaluation system for rice resistance to brown planthopper. International Rice Research Institute, Manila

Ishii T, Brar DS, Multani DS, Khush GS (1994) Molecular tagging of genes for brown planthopper resistance and earliness introgressed from Oryza australiensis into cultivated rice O. sativa. Genome 37(2):217–221. https://doi.org/10.1139/g94-030

Ji H, Kim SR, Kim YH, Suh JP, Park HM, Sreenivasulu N, Misra G, Kim SM, Hechanova SL, Kim H, Lee GS, Yoon UH, Kim TH, Lim H, Suh SC, Yang J, An G, Jena KK (2016) Map-based cloning and characterization of the BPH18 gene from wild rice conferring resistance to brown planthopper (BPH) insect pest. Sci Rep 6:34376

Jia Q, Wang J, Zhu J, Hua W, Shang Y, Yang J, Liang Z (2017) Toward identification of black lemma and pericarp gene BpI1 in barley combining bulked segregant analysis and specific locus amplified fragment sequencing. Front Plant Sci 8:1414. https://doi.org/10.3389/fpls.2017.01414

Kaminski KP, Korup K, Andersen MN, Sønderkær M, Andersen MS, Kirk HG et al (2016) Next generation sequencing bulk segregant analysis of potato support that differential flux into the cholesterol and stigmastanol metabolite pools is important for steroidal glycoalkaloid content. Potato Res 59:81–97

Kamolsukyeunyong W, Ruengphayak S, Chunwong P, Kusumawati L, Chaichoompu B, Gantet P (2019) Next-generation sequencing data-driven genome annotation: a rice case study. Genome Informatics 39(1):16. https://doi.org/10.5582/gi.2019.00009

Klein H, Xiao Y, Conklin PA, Govindaraju R, Kelly JA, Scanlon MJ, Whipple CJ, Bartlett M (2018) Bulked segregant analysis coupled to whole genome sequencing (BSA-Seq) for rapid gene cloning in Maize. G3 (bethesda, Md.) 8(11):3583–3592. https://doi.org/10.1534/g3.118.200499

Kuchuk K, Sarao PS, Neelam K, Amand A, Mangat GS, Brar DS, Singh K (2018) High-resolution genetic mapping of a novel brown planthopper resistance locus, Bph34 in Oryza sativa L. X Oryza nivara (Sharma & Sashtri) derived interspecific F2 population. TAG. Theoretical and applied genetics. Theoretische Und Ange wandte Genetik 131(5):1163–1171. https://doi.org/10.1007/s00122-018-3069-7

Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics (oxford, England) 25(14):1754–1760. https://doi.org/10.1093/bioinformatics/btp324

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R (2009) The sequence alignment/map format and SAMtools. Bioinformatics 25:2078–2079

Li Z, Xue Y, Zhou H, Li Y, Usman B, Jiao X, Wang X, Liu F, Qin B, Li R, Qiu Y (2019) High-resolution mapping and breeding application of a novel brown planthopper resistance gene derived from wild rice (Oryza. rufipogon Griff). Rice (new york, n.y.) 12(1):41. https://doi.org/10.1186/s12284-019-0289-7

Liang T, Chi W, Huang L, Qu M, Zhang S, Chen ZQ, Chen ZJ, Tian D, Gui Y, Chen X, Wang Z, Tang W, Chen S (2020) Bulked segregant analysis coupled with whole-genome sequencing (BSA-Seq) mapping Identifies a novel pi21 haplotype conferring basal resistance to rice blast disease. Int J Mol Sci 21(6):2162. https://doi.org/10.3390/ijms21062162

Liu GQ, Yan H, Fu Q, Qian Q, Zhang ZT, Zhai WX, Zhu LH (2001) Mapping of a new gene for brown planthopper resistance in cultivated rice introgressed from Oryza eichingeri. Chin Sci Bull 46:738–742

Liu Y, Wu H, Chen H, Liu Y, He J, Kang H, Sun Z, Pan G, Wang Q, Hu J, Zhou F, Zhou K, Zheng X, Ren Y, Chen L, Wang Y, Zhao Z, Lin Q, Wu F, Zhang X, Guo X, Cheng X, Jiang L, Wu C, Wang H, Wan J (2015) A gene cluster encoding lectin receptor kinases confers broad-spectrum and durable insect resistance in rice. Nat Biotechnol 33(3):301–305. https://doi.org/10.1038/nbt.3069

Lv W, Du B, Shangguan X, Zhao Y, Pan Y, Zhu L, He Y, He G (2014) BAC and RNA sequencing reveal the brown planthopper resistance gene BPH15 in a recombination cold spot that mediates a unique defense mechanism. BMC Genomics 15(1):674. https://doi.org/10.1186/1471-2164-15-674

McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytskyy A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA (2010) The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20(9):1297–1303

Meng L, Li H, Zhang L, Wang J (2015) QTL IciMapping: integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. The Crop Journal 3:269–283

Mizuno H, Katagiri S, Kanamori H, Mukai Y, Sasaki T, Take-moto T, Wu J (2020) Evolutionary dynamics and impacts of chromosomes regions carrying R-gene clusters in rice. Sci Rep 10(1):872. https://doi.org/10.1038/s41598-020-57729-w

Mohanty SK, Panda RS, Mohapatra SL, Nanda A, Behera L, Jena M, Sahu RK, Sahu SC, Mohapatra T (2017) Identification of novel quantitative trait loci associated with brown planthopper resistance in the rice landrace Salkathi. Euphytica 213:38. https://doi.org/10.1007/s10681-017-1835-2

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8(19):4321–4325. https://doi.org/10.1093/nar/8.19.4321

Navarro-Escalante L, Zhao C, Shukle R, Sturti J (2020) BSA-Seq discovery and functional analysis of candidate hessian fly (Mayetiola destructor) avirulence genes. Front Plant Sci 11:956. https://doi.org/10.3389/fpls.2020.00956

Nguyen KL, Grondin A, Courtiès B, Gantet P (2019) Next-generation sequencing accelerates crop gene discovery.
Trends Plant Sci 24(3):263–274. https://doi.org/10.1016/j.trendsplant.2018.11.008

Normile D (2008) Agricultural research. Reinventing rice to feed the world. Sci (new York, n.y.) 321(5887):330–333. https://doi.org/10.1126/science.321.5887.330

Pandey MK, Khan AW, Singh VK, Vishwakarma MK, Shasidhar Y, Kumar V, Garg V, Bhat RS, Chitikineni A, Janila P, Guo B, Varshney RK (2017) QTL-seq approach identified genomic regions and diagnostic markers for rust and late leaf spot resistance in groundnut (Arachis hypogaea L.). Plant Biotechnol J 15(8):927–941. https://doi.org/10.1111/pbi.12686

Qiu Y, Guo J, Jing S, Zhu L, He G (2012) Development and characterization of japonica rice lines carrying the brown planthopper-resistance genes BPH12 and BPH6. TAG. Theoretical and applied genetics. Theoretische Und Angewandte Genetik 124(3):485–494. https://doi.org/10.1007/s00122-011-1722-5

Rahman ML, Jiang W, Chu SH, Qiao Y, Ham TH, Woo MO, Lee J, Khanam MS, Chin JH, Jeung JU, Brar DS, Jena KK, Koh HJ (2009) High-resolution mapping of two rice brown planthopper resistance genes, Bph20(t) and Bph21(t), originating from Oryza minuta. TAG. Theoretical and applied genetics. Theoretische Und Angewandte Genetik 119(7):1237–1246. https://doi.org/10.1007/s00122-009-1125-z

Renganayaki K, Feitz AK, Sadasivam S, Pammi S, Harrington SE, McCouch SR, Kumar SM, Reddy AS (2002) Mapping and progress toward map-based cloning of brown planthopper biotype-4 resistance gene introgressed from Oryza officinalis into cultivated rice. O Sativa Crop Sci 42:2112–2117

Sharma PN, Ketipearchchi Y, Murata K, Torii A, Takumi S, Mori N, Nakamura C (2003) RFLP/AFLP mapping of a brown planthopper (Nilaparvata lugens Stål) resistance gene Bph1 in rice. Euphytica 129(1):109–117

Singh VK, Khan AW, Saxena RK, Sinha P, Kale SM, Parupalli S, Kumar V, Chitikineni A, Vechalapu S, Sameer Kumar CV, Sharma M, Ghanta A, Yamini KN, Muniswamy S, Varshney RK (2017) Indel-seq: a fast-forward genetics approach for identification of trait-associated putative candidate genomic regions and its application in pigeonpea (Cajanus cajan). Plant Biotechnol J 15(7):906–914. https://doi.org/10.1111/pbi.12685

Song J, Li Z, Liu Z, Guo Y, Qiu LJ (2017) Next-generation sequencing from bulked-segregant analysis accelerates the simultaneous identification of two quantitative genes in Soybean. Front Plant Sci 8:919. https://doi.org/10.3389/fpls.2017.00919

Sun L, Su C, Wang C, Zhai HQ, Wan JM (2005) Mapping of a major resistance gene to brown planthopper in the rice cultivar Rathu Heenati. Breed Sci 55:391–396

Sun J, Yang L, Wang J, Liu H, Zheng H, Xie D, Zhang M, Meng M, Jia Y, Zhao H, Zou D (2018) Identification of a cold-tolerant locus in rice (Oryza sativa L.) using bulked segregant analysis with a next-generation sequencing strategy. Rice (new York, n.y.) 11(1):24. https://doi.org/10.1186/s12284-018-0218-1

Tao Y, Niu Y, Wang Y, Chen T, Naveed SA, Zhang J, Xu J, Li Z (2018) Genome-wide association mapping of aluminum toxicity tolerance and fine mapping of a candidate gene for Nrat1 in rice. PLoS ONE 13(6):e0198589. https://doi.org/10.1371/journal.pone.0198589

Wang H, Shi S, Guo Q, Nie L, Du B, Chen R, Zhu L, He G (2018) High-resolution mapping of a gene conferring strong antibiosis to brown planthopper and developing resistant near-isogenic lines in 9311 background. Mol Breeding 38:107

Wang Y, Cao L, Zhang Y, Cao C, Liu F, Huang F, Qiu Y, Li R, Lou X (2015) Map-based cloning and characterization of BPH29, a B3 domain-containing recessive gene conferring brown planthopper resistance in rice. J Exp Bot 66(19):6035–6045. https://doi.org/10.1093/jxberr/jcv318

Wu S, Qiu J, Gao Q (2019) QTTL-BSA: a bulked segregant analysis and visualization pipeline for QTTL-seq. Interdiscip Sci Comput Life Sci 11(4):730–737. https://doi.org/10.1007/s12539-019-00344-9

Yang L, Li RB, Li YR, Huang FK, Chen YZ, Huang SS, Huang LF, Liu C, Ma ZF, Huang DH, Jiang JJ (2012) Genetic mapping of bph20(t) and bph21(t) loci conferring brown planthopper resistance to Nilaparvata lugens Stål in rice (Oryza sativa L.). Euphytica 183:161–171

Zhao H, Yin CC, Ma B, Chen SY, Zhang JS (2021) Ethylene signaling in rice and Arabidopsis: New regulators and mechanisms. J Integr Plant Biol 63(1):102–125. https://doi.org/10.1111/jipb.13028

Zhang Y, Qin G, Ma Q, Wei M, Yang X, Ma Z, Liang H, Liu C, Li Z, Liu F, Huang D, Li R (2020) Identification of major locus Bph35 resistance to brown planthopper in rice[J]. Rice Sci 27(03):237–247

Zheng X, Zhu L, He G (2020) Genetic and molecular understanding of host rice resistance and Nilaparvata lugens adaptation. Current Opinion Insect Sci 45:14–20. https://doi.org/10.1016/j.cois.2020.11.005

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