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Genetic Analysis and Gene Mapping of Whitebacked Planthopper Resistance Genes from Rice Varieties

Baoxuan Nong[1,2], Biqiu Wu[3], Anlong Xu[2], Wenai He[2] and Yongfu Qiu[2]*

[1]Rice Research Institute, Guangxi Key Laboratory of Rice Genetics and Breeding, Guangxi Academy of Agricultural Science, Nanning 530007, China
[2]Agricultural College, Guangxi University, Nanning 530004, China
[3]Plant Protection Institute, Guangxi Key Laboratory of Biology for Crop Diseases and Insect Pests, Guangxi Academy of Agricultural Science, Nanning 530007, China

Baoxuan Nong and Biqiu Wu contributed equally to this work.

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Key words
Whitebacked planthopper (WBPH), *Sogatella furcifera* (Horváth), Rice, Resistance gene, Gene mapping, Honeydew excretion

ABSTRACT

The whitebacked planthopper (WBPH), also named *Sogatella furcifera* (Horváth), has become a significant threat to rice production. Identification of WBPH-resistant germplasm and genes can promote the development of resistance varieties and effectively limit pest damage. In this study, fourteen varieties of rice were surveyed for insect resistance by assessing growth rates via seedbox screening, feeding activity via measurements of honeydew excretion, and insect development by counting the number of hatched nymphs. Two resistance varieties N22 and OB677 were crossed with susceptible line 9311 to develop mapping populations, which were applied to map the resistance genes/QTLs. In the results, rice variety PTB33 showed high resistance to both brown planthopper (BPH) and WBPH, varieties N22, RBPH327, and OB677 showed moderate resistance to WBPH. Host choice test and seedling survival rates further verified the WBPH resistance of PTB33, N22, and OB677. By using two F_2 mapping populations, two WBPH resistance genes were detected in N22 and OB677. *Wbph1* was mapped on chromosome 2 of N22 in a region that harbored the markers RM13650 and RM13478. Its largest logarithm of the odds (LOD) score was 3.94, which explained a 16.6% of the phenotypic variation. *Wbph9* was mapped on chromosome 3 of OB677, where it was flanked by markers RM3513 and RM3525. It had a LOD score of 3.4, explaining a 17.2% of the phenotypic variation. Four varieties PTB33, N22, RBPH327, and OB677 showed resistance to WBPH, of which OB677 was a novel resistance germplasm; and a novel resistance gene *Wbph9* was mapped on chromosome 3. In conclusion, four WBPH resistance varieties were detected and one novel resistance gene was mapped.

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INTRODUCTION

The whitebacked planthopper (WBPH), *Sogatella furcifera* (Horváth), is a major pest that affects rice crops worldwide, especially in Asia-Pacific countries. In recent years, the frequency of WBPH outbreaks has increased markedly, resulting in a serious decline in rice yield (Hu *et al*., 2015). WBPH causes damage to rice crops through feeding, oviposition, and transmission of viruses. It feeds by directly sucking on the phloem sap, reducing crop yield and causing plants death in severe cases. In addition, WBPH, as a vector of viral diseases, can cause indirect damage. These viruses include rice grassy stunt virus (GSV) and ragged stunt virus (RSV) that cause plant dwarfism and reduce tillers (Anjaneyulu *et al*., 1986). This indirect viral-mediated damage has caused huge losses in rice production in Asia countries. For instance, more than 700,000 ha in 2011 and 500,000 ha in 2012 of rice in

* Corresponding author: yfqiu@126.com
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Abbreviations
ANOVA, Analysis of Variance; BSA, bulked segregant analysis; GSV, grassy stunt virus; InDel, Insertion/Deletion; LOD, logarithm of odds; MAS, marker-assisted selection; PCR, polymerase chain reaction; QTL, quantitative trait locus; RSV, ragged stunt virus; SRBSDV, Southern rice black-streaked dwarf virus; SSR, simple sequence repeat; WBPH, whitebacked planthopper.

Vietnam and China suffered from serious Southern rice black-streaked dwarf virus (SRBSDV) transmitted by WBPH (Zhou *et al*., 2013). It has been difficult to manage WBPH using traditional chemical applications for a long time due to its long-distance migration and quick development of resistance against pesticides. Host plant resistance is considered an efficient and environmentally friendly way to control WBPH infestation, and natural planthopper resistance genes could be exploited for developing novel resistant varieties. Therefore, the identification of rice germplasm with WBPH-resistant and the resistance genes have become an important and necessary task.

To date, 12 major WBPH resistance genes have been identified in various cultivars or wild rice species (Du *et al*., 2020). Specifically, *Wbph1* was first detected in the rice variety N22 (Sidhu *et al*., 1979); and further studies indicated that *Wbph1* co-segregated with the restriction fragment length polymorphism (RFLP) markers RG146 and RG445 in variety IR36 (McCouch *et al*., 1991). *Wbph2* was identified in variety ARC10239 and mapped to chromosome 6, where it was associated with RFLP markers RZ667, RG264, and RG64 (Angeles *et al*., 1981; Liu *et al*., 2002). *Wbph3* and *wbph4* were detected in varieties ADR52 and Podiwi A8, respectively (Hernandez and Khush, 1981). *Wbph5* was detected in variety N'Diang Marie (Wu and Khush, 1985), and *Wbph6*(*t*) was associated with RM167 on chromosome 11 (Li *et al*., 1990, 2004). *Wbph7* and *Wbph8* were derived from introgressed lines of *O. officinalis* and were mapped to the same chromosomal regions as the BPH resistance genes *Bph14* and *Bph15*, respectively (Tan *et al*., 2004). Further study indicated that *Bph14* confers resistance to both BPH and WBPH (Tan *et al*., 2004). In summary, *Wbph1*, *Wbph3*, *wbph4*, and *Wbph5* were not assigned to any chromosomes, *Wbph2* and *Wbph6* were only roughly mapped onto the chromosomes, and there were no tightly linked markers or convenient markers available for marker-assisted selection (MAS). Several quantitative trait loci (QTL) associated with WBPH resistance were also detected and mapped from variety Chunjiang 06 and Dongxiang wild species (Sogawa *et al*., 2005; Chen *et al*., 2010). At present, only very few WBPH resistance genes have been detected and finely mapped. It is reported that several cloned BPH resistance genes such as *Bph6*, *Bph14*, and *Bph3* are simultaneously resistance to BPH and WBPH (Tan *et al*., 2004; Liu *et al*., 2016; Guo *et al*., 2018). It is therefore also important to evaluate the WBPH resistance of varieties that already carry BPH resistance genes, and through which we can detect more double resistance rice germplasm. It is reported that Varieties N22, Baiganruo, Nebeshi, and Cdombo were resistant to WBPH and carried the resistance genes *Wbph1*, *Wbph2*, *Wbph3*, and *wbph4*, respectively (Sidhu *et al*., 1979; Angeles *et al*., 1981; Hernandez and Khush, 1981). Varieties Mudgo, ASD7, PTB33, Babawee, and ARC 10550 were reported to carry the resistance genes *Bph1*, *bph2*, *Bph3*, *bph4*, and *bph5*, respectively (Athwal *et al*., 1971; Lakshminarayana and Khush, 1977; Angeles *et al*., 1986; Nemoto *et al*., 1989; Khush *et al*., 1985). Despite carrying these genes, in 2006, Mudgo, ASD7, and ARC 10550 were found to be susceptible to BPH insect populations collected from rice fields in Wuhan, China (Qiu *et al*., 2011). However, it remains unclear whether the rice variety OB677 carries any planthopper resistance genes.

In this study, the purpose was to evaluate the planthoppers resistance level of collected rice varieties which carry BPH or WBPH resistance genes using the insect populations collected from rice paddy in Nanning, Guangxi; and to map the WBPH resistance genes from the detected resistance rice varieties. In detail, six rice varieties that carry BPH resistance genes and four varieties that carry WBPH resistance genes were surveyed for WBPH resistance using the seed box screening test and by assessing WBPH feeding and development. Two resistant varieties, N22 and OB677, were subject to further resistance gene mapping and genetic analyses as F_2 mapping populations. The identified planthopper resistance varieties/lines and WBPH resistance genes would greatly contribute to insect-rice breeding program.

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MATERIALS AND METHODS

Plant material and insects

Fourteen rice varieties were used in the study (Table I). In which the ten varieties N22, Baiganruo, Nebeshi, Cdombo, Mudgo, ASD7, Babawee, ARC 10550, OB677, and PTB33 were collected from the China National Rice Research Institute (CNRRI) and International Rice Research Institute (IRRI). Rice lines RBPH16 and RBPH327 were derived from the common wild rice species *O. rufipogon* Griff. and identified to be resistant to BPH and WBPH (Qiu *et al*., 2012; Yang *et al*., 2020). Varieties 9311 and TN1 were highly susceptible to BPH and WBPH. Variety PTB33 was used as the resistant control and 9311 as the susceptible control for WBPH.

WBPH and BPH insects were collected from rice fields in April 2014 in Nanning, China (22°49′ N, 108°19′ E), and reared on TN1 seedlings in a greenhouse under natural light at 26–30°C. The second to third instar nymphs and adults were used. All experiments were conducted from 2014 to 2017 in a greenhouse in Guangxi University under natural light at 26–30°C.

Table I. Varieties/lines used in the present study.

Variety	Resistance gene	Origin	Reference	Collection
N22	*Wbph1*	Indica	Sidhu *et al*., 1979	CNRRI
Baiganruo	*Wbph2*	Indica	Angeles *et al*., 1981	CNRRI
Nebeshi	*Wbph3*	Indica	Hernandez and Khush, 1981	CNRRI
Cdombo	*wbph4*	Indica	Hernandez and Khush, 1981	CNRRI
Mudgo	*Bph1*	Indica	Athwal *et al*., 1971	IRRI
ASD7	*bph2*	Indica	Lakshminarayana and Khush, 1977	IRRI
PTB33	*Bph3*	Indica	Angeles *et al*., 1986	IRRI
Babawee	*bph4*	Indica	Nemoto *et al*., 1989	IRRI
ARC10550	*bph5*	Indica	Khush *et al*., 1985	IRRI
RBPH16	*Bph27* + *Bph36*	*Oryza rufipogon* Griff.	Qiu *et al*., 2012	Local lab
RBPH327	*Bph38*	*Oryza rufipogon* Griff.	Yang *et al*., 2020	Local lab
OB677	*NA*	Indica	NA	CNRRI
9311	*None*	Indica	None	Local lab
TN1	*None*	Indica	None	Local lab

CNRRI, China National Rice Research Institute; IRRI, International Rice Research Institute; NA, no data applicable.

Evaluation of insect resistance

The seedbox screening test was performed to evaluate the resistance of 14 rice varieties to WBPH and BPH. Germinated seeds were sown in a plastic tray (48 × 39× 7 cm^3) to obtain 16 seedlings per row. A total of 10 tested rows were sown in one tray and one row of TN1 (susceptible control) was randomly sown among the tested varieties. At the second-leaf stage (approximately 7 days old), the seedlings were infested with 2nd–3rd WBPH instars at 10 insects per seedling and covered with a fine, light-transmitting mesh (44 × 34 × 44 cm^3). To evaluate BPH resistance, the same treatment was designed except that third-leaf seedlings were infested with eight nymphs per plant. When all of the control TN1 plants had died (approximately 15 days for WBPH and 9 days for BPH after infestation), each seedling was assigned a score of 0, 1, 3, 5, 7, or 9 according to the criteria for the Standard Evaluation System for Rice (IRRI, 1988; Qiu *et al*., 2010). The average resistance score of each variety was calculated from the scores of all seedlings. Both tests were performed three independent times.

To survey WBPH host choice and seedling survival rates, each plastic tray (32 × 26 × 12 cm^3) was divided into four approximately equal portions and filled with soil to a height of 8 cm. There were approximately 300 total numbers of seedlings per tray, with 70 to 90 of each variety. The two-leaf stage seedlings (approximately 7 days old) were infested with 100 female adults with swollen abdomen, and covered with a fine, light-transmitting mesh (44 × 34 × 44 cm^3). The numbers of nymphs on the seedlings were observed 10 days later, and the numbers of surviving seedlings were recorded 30 days after infestation. Three replicates were designed for the test.

Honeydew excretion quantity

To assess the feeding activity of WBPH, we quantified honeydew excreted by each insect. An average of four 7 day-old seedlings was planted uniformly in each bucket (25 cm in diameter, 15 cm in height) that was filled with soil to a height of 12 cm. Each WBPH insect with a short wing was released into a rectangular pre-weighed parafilm sachet (3.5 cm length, 3 cm width) that had been fastened onto a 35 day-old rice shoot (Pathak *et al*., 1982). Two days after infestation, each sachet containing excreted honeydew was re-weighed and the weight of the sach*et al*one subtracted to give the net weight of honeydew excreted. For the 14 varieties studied, eight plants of each variety were surveyed for honeydew excretion using three bags per plant. For F_2 plants, three bags per plant were used and the same honeydew excretion test was applied.

Development of mapping population and gene mapping

The varieties N22 and OB677 were, respectively crossed with 9311 to obtain F_1 individuals which were self-pollinated to develop two F_2 mapping populations, 9311/N22 and 9311/OB677 in 2013; and then they were used for genetic analysis and gene mapping. As the seed box screening test is approximate and subjective, it can be difficult to determine the precise resistance scores for each seedling. Furthermore, it was difficult to obtain fresh leaf

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samples for genotyping after infestation, especially when two-leaf seedlings were used and many had started to die. Therefore, the quantity of WBPH honeydew excreted after feeding on F_2 individuals was taken as the phenotypic index for resistance gene mapping.

To perform gene mapping, bulked segregant analysis was applied to detect molecular markers that were associated with WBPH resistance (Michelmore *et al.*, 1991). Specifically, two contrasting bulk DNA samples, collected from plants that produced the ten largest or least WBPH honeydew excretion weights, were used to screen the SSR or InDel marker from 12 rice chromosomes. The polymorphic markers detected between two bulk DNA samples were most possibly linked with WBPH resistance gene. Then, more polymorphic markers between parents around the linked markers were obtained and applied to analyze the genotypes of the F_2 population. After this was performed, one local genetic linkage map of the resistance gene containing region was generated by performing JoinMap 3.0 with detected genotypes (Van Ooijen and Voorrips, 2001). Finally, the resistance effect of the interested region was analyzed by MapQTL 5 (Van Ooijen, 2004). The locus with a logarithm of odds (LOD) score >3.0 was reported as one QTL and the position with the largest LOD score was taken as the target gene.

Statistical analysis

Data were analyzed using one-way ANOVA and means were compared using a least significant difference test with SPSS 13.0 (SPSS Institute Inc, Chicago, IL, USA). The seedling survival rates (%) were arcsine transformed prior to analysis.

RESULTS

Evaluation of BPH and WBPH resistance

The average WBPH resistance scores of the 14 rice varieties detected in the seedbox screening test ranged from 3.2 to 8.7 (Table II). According to the resistance evaluation criteria, varieties N22, PTB33, RBPH327, and OB677 showed high resistance to WBPH (<4.2); whereas varieties Nebeshi, Mudgo, ASD7, ARC10550, 9311, and TN1 were highly susceptible (>7.0). The other four tested varieties showed moderate resistance to WBPH. The BPH resistance scores of PTB33, Babawee, RBPH16, and RBPH327 were less than 4.0, indicating strong resistance to BPH. Variety OB677 had moderate resistance to BPH (5.2). In contrast, the resistance scores of all other nine varieties tested were >7.3 and exhibited susceptibility to BPH.

The quantity of honeydew excreted by each WBPH was measured after two days of infestation to assess its feeding activity on different rice varieties. There was a large variation in honeydew excretion (Table II). The amount of honeydew excretion was the lowest on variety PTB33 by WBPH feeding (0.2 mg). Higher amounts (>8.0 mg) of honeydew were excreted by WBPH feeding on varieties ASD7 (8.4 mg), 9311 (12.6 mg), and TN1 (9.2 mg). Less than 3.0 mg of honeydew was excreted by WBPH feeding on N22 (1.4 mg), Babawee (1.2 mg), OB677 (1.2 mg), RBPH16 (2.2 mg), and RBPH327 (2.1 mg) varieties (Table II). The number of hatched nymphs varied greatly among the plant varieties, less than 10 nymphs were observed on N22 (8), PTB33 (5), Babawee (6), and RBPH16 (9) varieties; while more than 30 nymphs were detected on Nebeshi (32), ASD7 (36), ARC10550 (32), 9311 (52), and TN1 (50) varieties (Table II). Taken in all, varieties PTB33, N22, OB677, and RBPH327 had high resistance to WBPH and showed obvious antibiotic effect on WBPH.

Table II. Evaluation of insect resistance of selected varieties of rice plants.

Variety/ Lines	WBPH resistance score	WBPH honeydew excretion (mg/2 days)	WBPH No. of nymphs	BPH resistance score
N22	4.2±0.6^{a}	1.4±0.7^{b}	8±2^{a}	7.2±0.5^{d}
Baiganruo	6.2±0.7^{b}	6.0±2.2^{cd}	14±3^{ab}	8.2±0.6^{e}
Nebeshi	7.1±1.1^{c}	6.2±1.6^{cd}	32±10^{c}	7.3±1.3^{d}
Cdombo	6.6±0.9^{bc}	4.6±1.8^{c}	12±5^{ab}	7.6±1.2^{d}
Mudgo	7.9±1.2^{cd}	4.8±2.3^{c}	28±9^{c}	7.9±0.9^{de}
ASD7	8.1±0.5^{cd}	8.4±2.2^{d}	36±8^{c}	8.2±0.7^{e}
PTB33	3.2±0.6 ^{a}	0.2±0.03^{a}	5±2^{a}	2.7±0.4^{a}
Babawee	4.6±0.7^{ab}	1.2±0.7^{b}	6±2^{a}	3.6±0.7^{ab}
ARC10550	8.1±0.8^{cd}	6.8±2.1^{cd}	32±9^{c}	8.3±0.4^{e}
RBPH16	5.2±0.9^{ab}	2.2±0.8^{b}	9±2^{a}	3.4±0.6^{ab}
RBPH327	4.0±0.6^{a}	2.1±0.6^{b}	16±6^{ab}	3.5±0.8^{ab}
OB677	3.8±0.8^{a}	1.2±0.4^{b}	11±4^{ab}	5.2±0.7^{c}
9311	8.5±0.7^{d}	12.6±3.2^{e}	52±11^{d}	8.7±0.3^{f}
TN1	8.7±0.3^{d}	9.2±2.8^{d}	50±7^{d}	8.9±0.4^{f}

Different lowercase letters in the same column indicate significantly different according to the LSD test (P <0.05).

Verifying the resistance of varieties N22 and OB677

Previous study indicated that variety RBPH327 showed high resistance to both WBPH and BPH, and one same chromosome region controls the resistance (Yang *et al.*, 2020). Variety PTB33 also confers resistance to WBPH and BPH simultaneously and usually is taken as

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control. Here, varieties N22 and OB677 were detected to be resistance to WBPH and considered for further resistance gene mapping. Therefore, insect host choice and seedling survival rates were surveyed to verify the resistance of N22 and OB677 to WBPH. Variety PTB33 was taken as a resistance control (Fig. 1A). As there had too much individuals and the nymphs was small, it was hard to exactly count the number. But obvious more WBPH nymphs were observed on the susceptible plants at 10 days after infestation (Fig. 1B). There were 9311 plants (susceptible control) that were almost dead at 30 days after infestation and its average survival rate was 2.3%; while the survival rates of N22, OB677, and PTB33 (resistant control) were 83.6%, 74.7%, and 98.2%, respectively (Fig. 1C, D). These results indicated that PTB33 showed high resistance to WBPH; whereas N22 and OB677 were moderately resistance to these insects.

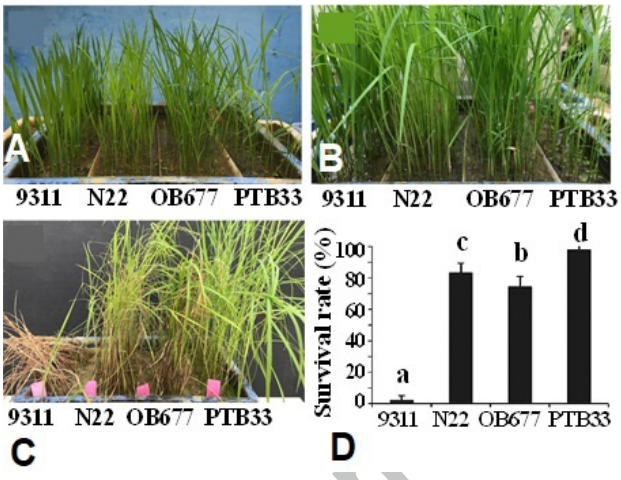

Fig. 1. Detection of WBPH host choice and seedling survival rate. (A) Before infestation with WBPH, (B) at 10 days and (C) 30 days after infestation. (D) Seedling survival rate of the four plant varieties.
Note: Different lowercase letters in Fig. 1d indicate significantly different according to the LSD test (P <0.05).

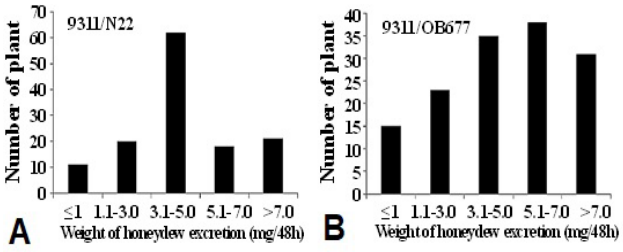

Fig. 2. Distribution of honeydew excretion weight of each WBPH per 48 h after feeding on the plants of F_2 mapping population (A) 9311/N22 and (B) 9311/OB677.

Distribution of honeydew excretion by WBPH feeding on the F_2 plants

The weight of honeydew excreted varied from 0.3 mg to 13.1 mg in WBPH feeding on the F_2 rice populations of 9311/N22 containing 132 individuals, and most weights were clustered in the region of 3.1–5.0 mg (Fig. 2A). The weight of honeydew excreted also varied in the F_2 population of 9311/OB677 having 142 individuals and ranged from 0.4 mg to 11.3 mg. No significant peaks of specific regions were observed in the mapping population (Fig. 2B). The results suggested that there may be one or more QTLs controlling the segregation of WBPH resistance in the F_2 population.

Table III. Mapping of WBPH resistance genes.

Marker	Position (cM)	LOD Score	PEV (%)[1]	Addi-tive[2]	Domi-nant
Chromosome 2					
2M021	0	1.53	6.7	-1.7	-2.0
RM530	7.4	2.71	11.6	-2.7	-1.9
RM13650	13.5	3.84	16.2	-3.2	-2.4
Wbph1	14.5	3.94	16.6	-3.3	-2.4
RM13478	14.6	3.92	16.5	-3.3	-2.4
2M15.039	21.1	2.33	10.2	-2.3	-2.7
Chromosome 3					
RM231	0	1.7	10.1	-0.4	-0.4
RM7395	4.8	2.4	12.8	-0.5	-0.2
RM3513	6.2	3.0	15.2	-0.5	-0.3
Wbph9	6.4	3.4	17.2	-0.5	-0.3
RM3525	6.6	3.3	16.4	-0.5	-0.3
3E51292	11.6	1.2	7.2	-0.3	-0.3

[1]The percentage of total phenotypic variance (PEV %) was contributed by the locus. [2]Additive means additive effect of the associated marker indicated from 9311; dominant means dominant effect of the associated marker indicated from 9311.

Identification of the WBPH resistance gene in rice varieties N22 and OB677

A total of 216 and 197 polymorphic markers were respectively detected between N22 and 9311 or OB677 and 9311 after checked by more than 1120 makers. Subsequently, two chromosomal regions were detected to be polymorphic in the two bulk DNA samples that were checked by the polymorphic markers. One region was located on the long arm of chromosome 2 of N22, and the other was on chromosome 3 of OB677 (Table III). The two local genetic linkage groups identified were 21.1 cM and 11.6 cM for N22 and OB677, respectively. The marker orders were in accordance with the genome of variety

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Nipponbare (Kawahara *et al*., 2013). Interval mapping with MapQTL 5 was then used to analyze the genotype and phenotype of the F_2 individuals. Subsequently, one major QTL with the largest LOD score of 3.94 was detected between RM13650 and RM13478, which explained a 16.6% of the phenotypic variation in the 9311/N22 F_2 mapping population. This was designated *Wbph1* as the gene had been previously identified in N22 (Sidhu *et al*., 1979). Another QTL was harbored with markers RM3513 and RM3525, which had a LOD score of 3.4, and explained a 17.2% of the phenotypic variation in the 9311/OB677 F_2 mapping population. The detected QTL was designated *Wbph9* according to the rules for nomenclature (Yamazaki *et al*., 2010), as no WBPH resistance gene in this region had been previously reported.

DISCUSSION

Over the years, researchers have been prioritizing detecting resistance varieties and lines, contributing to this research effort, but few have been used in resistance breeding projects, or for detecting resistance genes. With the increasing risks posed by GSV and RSV infection, it has recently become essential and urgent to identify WBPH resistant germplasm and genes or QTLs to drive pest control research. In this study, we have identified several WBPH-resistant varieties that have been verified with insects collected from Nanning, Guangxi province, and can be used for resistant rice breeding programs.

A total of eight major WBPH resistance genes have been identified from rice varieties or wild rice species to date. Interestingly, *Wbph7*(*t*) and *Wbph8*(*t*) were suggested to be the same loci as *Bph14* and *Bph15*, respectively (Tan *et al*., 2004), indicating resistance to both WBPH and BPH. Many additional varieties or lines have also been shown to be resistant to both WBPH and BPH (Cao *et al*., 1993; Huang *et al*., 2012). Several cloned BPH resistance genes (*Bph3*, *Bph6*) have also been proven to confer resistance to both these planthoppers, suggesting there could share mechanisms of resistance in some of these varieties. Interestingly, variety N22 was susceptible to BPH, and OB677 was moderately resistant to BPH, but both were moderately resistant to WBPH (Table II). These results suggested that insect populations probably could change the resistance level conferred by the resistance genes.

In the present study, we mapped two resistance genes/QTLs, *Wbph1* and *Wbph9*. *Wbph1* derived from N22 was mapped to the long arm of chromosome 2 that harbored markers RM13650 and RM13478. A WBPH resistance QTL that was associated with increased seedling survival rate was identified in the Dongxiang wild species, on the short arm of chromosome 2, flanked by markers RM1285 and RM555 derived (Chen *et al*., 2010). We also showed that *Wbph9* was on chromosome 3, flanked by markers RM3513 and RM3525. *Wbph7*(*t*) was also mapped to chromosome 3, in a region that harbored markers R1925 and G1318 (Tan *et al*., 2004). However, these regions were downstream of *Wbph9*, according to the marker locations. Sogawa *et al*. (2005) detected several QTLs on chromosomes 2 and 3 that were associated with immigrant density of WBPH or seedling mortality. However, the probable physical positions of these QTLs were different from that of *Wbph1* and *Wbph9* described in our study. Taken together, we believe that both *Wbph1* and *Wbph9* are novel and unique, and do not correspond to existing genes or QTLs.

Several complementary methods have been used to evaluate WBPH resistance, including the seedbox screening test, honeydew excretion weight, and hatched nymph numbers to identify genes and QTLs associated with resistance. For example, Sogawa *et al*. (2005) detected five QTLs associated with honeydew excretion weight on chromosomes 2, 3, and 4. Chen *et al*. (2010) detected three QTLs on chromosomes 2, 5, and 9 from the Dongxiang wild rice species that were associated with seedling survival rate. These discoveries suggest that one gene or QTL can confers multiple traits, or multiple resistant genes or QTLs could regulate a single trait. In the present study, *Wbph1* and *Wbph9* were respectively identified from N22 and OB677 using the honeydew excretion weight method. It is possible that both genes could also confer resistance when assessed based on other phenotypes, including seedling survival rate or insect development. Indeed, the influence conferred by *Wbph1* and *Wbph9* on seedling survival rates of N22 (83.6%) and OB677 (74.7%) were partially verified (Fig. 1) and the numbers of hatched insects on N22 (8) and OB677 (11) were also influenced by the presence of these genes (Table II).

The scores of LOD and PEV reflect the genetic effects conferred by the genes/QTLs in one mapping population. In the present study, the LOD scores of *Wbph1* and *Wbph9* were 3.94 and 3.4, respectively, and the respective PEV were 16.6% and 17.2%, which are generally considered to be low (Table III). Similar results were reported by Chen *et al*., who showed that the PEV of three QTLs ranged from 6.5% to 8.0%. However, Sogawa *et al*. (2005) suggested the PEV of three QTLs *qIMG*-4, *qHND*-4, and *qEGN*-4 were 78.4%, 71.7%, and 58.7%, respectively. To explore the reasons for the diversity of these PEVs, improved evaluation methods are required, to obtain more objective phenotypic scores. Larger mapping populations for gene mapping studies may also improve the PEV. Finally, the detected genes or QTLs may account for multiple traits and not all these genetically dependent traits may have

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been identified using the existing methods described in the present study.

CONCLUSION

The rice varieties PTB33, N22, RBPH327, and OB677 showed resistance to WBPH, of which OB677 was a novel resistance variety. Gene mapping indicated that *Wbph1* was mapped on chromosome 2 of N22 in a region harbored with the markers RM13650 and RM13478; and a novel resistance gene *Wbph9* was mapped on chromosome 3 of OB677, where it was flanked by the markers RM3513 and RM3525. Further fine mapping of the resistance genes may contribute to cloning of the genes and allow their usage in breeding insect resistant varieties.

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Statement of conflicts of interest

The authors have declared no conflict of interest.

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