Rice Science, 2004, 11(3): 86－90
http://www.ricescience.info

Mapping of a New Gene *Wbph6*(t) Resistant to the Whitebacked Planthopper, *Sogatella furcifera*, in Rice

LI Xi-ming[1, 2], ZHAI Hu-qu[1, 2], WAN Jian-min[1], MA Liang-yong[2], ZHUANG Jie-yun[2], LIU Guang-jie[2], YANG Chang-deng[2]
([1]*Nanjing Agricultural University, Nanjing 210095, China*; [2]*State Key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou 310006, China*)

Abstract: A rice population consisting of 90 TN1/Guiyigu F_3 lines was employed to analyze the linkage between DNA markers and a new gene *Wbph6*(t) conferring resistance to whitebacked planthopper, *Sogatella furcifera*. By using the mapping approach of bulked extremes and recessive class, *Wbph6*(t) was mapped onto the short arm of chromosome 11 with a genetic distance of 21.2 cM to SSLP marker RM167.

Key words: gene mapping; restriction fragment length polymorphism; simple sequence length polymorphism; *Sogatella furcifera*; resistance to pest insect; rice

The whitebacked planthopper (WBPH), *Sogatella furcifera* Horváth, is one of the most destructive insects of rice in the rice-growing area of China and South-East Asia. Growing and development of resistant varieties is the most economic and effective way to control any target insect pest. Identification and utilization of new genes conferring WBPH resistance has been one of the important objectives of rice breeding. So far, five WBPH resistance genes have been identified and named, including four dominant genes *Wbph1*, *Wbph2*, *Wbph3* and *Wbph5*, and one recessive gene *wbph4* [1-5]. Genetic analyses we had made since 1984, suggested that a single dominant gene controlled the resistance to WBPH in rice landraces Guiyigu, Biangu, Daqigu and Biangu from Yunnan, and this gene was non-allelic to the five WPBH resistant genes reported previously. This new gene was tentatively designated as *Wbph6*(t) [6, 7]. DNA-marker based gene mapping of *Wbph6*(t) was conducted in this study.

MATERIALS AND METHODS

Rice plant materials

The mapping population used was an F_3 population of Taichung Native 1 (TN1)/Guiyigu. The female parent TN1 is a susceptible variety commonly employed as the susceptible control. The male parent Guiyigu carrying *Wbph6*(t) is a rice landrace from Yunnan Province[7]. TN1 and Guiyigu were crossed at the Experimental Farm of China National Rice Research Institute (CNRRI), Fuyang in the autumn of 1998. Both the parents and F_1 plants were planted in the paddy fields of Lingshui, Hainan Province during the winter of 1998 and spring of 1999. The F_2 plants were grown at CNRRI farm in the summer of 1999. Seeds harvested from each F_2 plants were divided into two parts, one for evaluation of insect resistance and the other for DNA extraction.

Insect bio-assay

The F_3 populations and the parents were evaluated for resistance to WBPH in June to July 2000, using the standard bulked screening test[8] developed by the International Rice Research Institute (IRRI). The pre-germinated rice seeds were sown in a plastic tray (60 cm×45 cm×10 cm). Each F_3 line was sown in a single row of 25 plants in triplicate fashion. Three rows of each parent were sown in each tray. Unhealthy and weak seedlings were removed at 2-leaf stage. Each plant was inoculated with 7－8 WBPH nymphs at 1^{st}－2^{nd} instars. To prevent the escape of WBPH nymphs, the trays were covered with a nylon net (12 m×1 m×1.2 cm). Reaction of the F_3 lines was scored when the mortality of the susceptible control, TN1 seedlings, reached 95%. Test insects were maintained in a CNRRI greenhouse for 5-6 generations.

RFLP and SSLP analysis

The parental lines were planted during 1999. Rice leaves were collected from a single plant at the maximum tillering stage. The F_3 population was planted in 2000. Based on the results of resistance evaluation to WBPH, 90 F_3 lines with similar resistance scores across three replications were selected. At 5－6 leaves stage, leaves from 20 plants of each line were collected, their

Received: 12 September 2003; Accepted: 24 December 2003 .
Corresponding author: Zhai Hu-qu (liximing@mail.hz.zj.cn)

genotype referring to the genotype of F_2 plants. Total DNA was extracted using the method modified by Lu and Zheng [9]. Two types of molecular markers, restriction fragment length polymorphism (RFLP) and simple sequence length polymorphism (SSLP) were employed.

RFLP analysis was initiated as described by McCouch et al.[10], but probe labeling, hybridization and signal detection were performed using the ECL direct nucleic acid labeling and detection systems (Amersham Pharmacia Biotech). DNA samples were digested with *Bam*H I, *Eco*R I, *Eco*R V and *Hin*d III restriction enzymes. One hundred and thirty one probes detecting high polymorphism within indica rice sub-species in our previous experiments were selected for a survey of parental polymorphism. By mixing DNAs from 10 susceptible and 10 resistant lines, respectively, two extremes i. e. R and S DNA pools were constructed and assayed with polymorphic probes. All RFLP probes were from Tanksley's laboratory, Cornell University, USA.

SSLP was surveyed using MAPPAIR™ SSR primers (Research Genetics Inc). The PCR protocol of the manufacturer was followed. Template DNA was pre-denatured at 94℃ for 1 min followed by 30 cycles of 94℃ for 30 s, 57℃ for 45 s, 72℃ for 1 min, and a final 5 min incubation at 72℃. Two sets of R and S DNA pools were designed from 10 resistant and 10 susceptible lines, by mixing DNAs of five lines from each pool. The DNA pools and the parental DNAs were diluted 20 times with 1×TE (pH 8.0) and used for SSLP analysis. Positive primers detected, were used for population analysis. The amplified products were resolved by electrophoresis on a 2.5% Metaphor® agarose gel (BMA) and 100 bp DNA Ladder Plus (MBI) was used as the length marker. After electrophoresis and staining with EB, the products were visualized using GDS 8000 gel analysis system (UVP). Bands were scored as 1 for homozygote from resistant parent Guiyigu, 2 for TN1, and 3 for heterozygote.

Data analysis

Co-segregation between the resistance gene and DNA markers was analyzed with the method of bulked extreme and recessive class [11]. Linkage analysis was undertaken using MAPMAKER/EXP3.0 [12,13], and genetic distances were measured in centiMorgan (cM) derived using the Kosambi function.

RESULTS

Screening for WBPH resistance in parents and F_3 lines

Average mortalities of the susceptible parent TN1 and the resistance parent Guiyigu were 97.2% and 18.3%, respectively. Among the F_3 lines screened, 90 lines displayed an identical response to WBPH in overall three replications. Average seedling mortality of F_3 lines ranged as 12 – 100%, forming a continuous distribution with several peaks (Fig. 1). The average seedling mortality of most F_3 lines was lower than the value of susceptible TN1 and higher than the value of resistant Guiyigu, indicating that Guiyigu might harbor several resistant genes.

Detection of the positive markers

Among 131 RFLP probes and 150 SSLP primers used for parental survey, 54 probes and 45 primers were able to detect polymorphism between TN1 and Guiyigu. However, only marker RM167 located on rice chromosome 11 was shown to be positive. Two susceptible pools had a single DNA fragment from the susceptible parent, while two resistant pools bands from both parents (Fig. 2).

Mapping of the resistance gene *Wbph6*(t)

DNA preparations of the 20 individuals for constructing DNA pools were assayed using marker RM167. Of the 10 susceptible individuals, 6 TN1 homozygotes, 4 heterozygotes and no Guiyigu

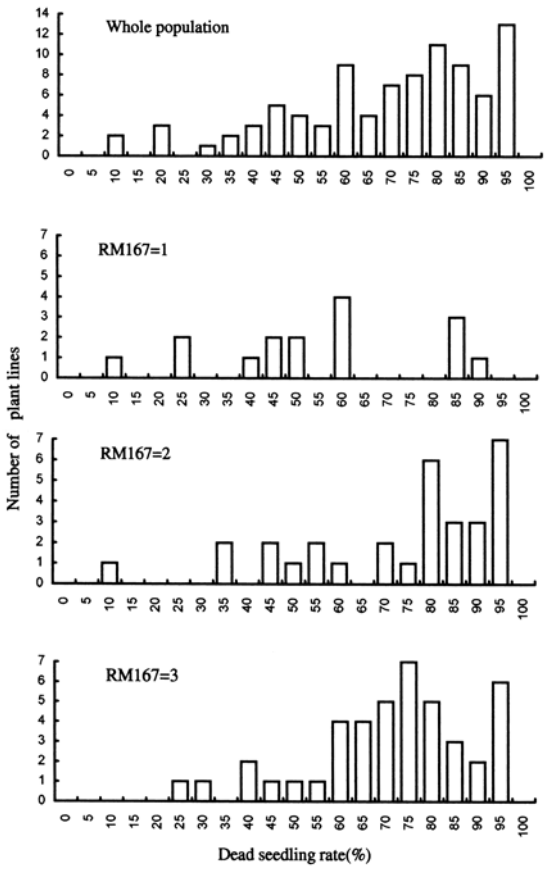

Fig. 1. Resistance segregation in the TN1/Guiyigu F_3 population scored as the dead seedling percentage of F_3 lines infested with 1 – 2 instar of *S. furcifera*.

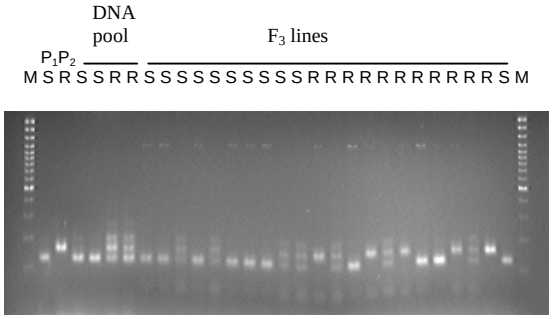

Fig. 2. Cosegregation between SSLP marker RM167 and the resistance to whitebacked planthopper in the TN1/Guiyigu population.

M, 100 bp DNA ladder; P_1, TN1; P_2, Guiyigu; S, Susceptible lines; R, Resistant lines.

homozygotes, were detected. It was estimated that the recombination ratio between RM167 and the resistance gene was 20±0.8%, or 21.2 cM. Of the 10 resistant individuals, 5 Guiyigu homozygotes, 3 heterozygotes and 2 TN1 homozygotes, were screened. Obviously, the recombination of resistance gene and RM167 could not explain the status of TN1 homozygote in the resistance individuals pool. Other resistance genes might also be operated for the genetic control of WBPH resistance in the population.

Other individuals of F_3 lines were also analyzed using RM167. According to the banding patterns of RM167, the whole F_3 population was categorized into three sub-populations (Fig. 1). Of the 16 lines when RM167 was scored as 1 (the Guyigu homozygote), the percentages of dead seedlings in 12 lines were less than 65% and 4 lines higher than 85%. If the F_3 population was classified into two groups based on the peaks and values of the whole population at 65%, then recombination between the resistance gene and RM167 occurred in 4 individuals. If all of the recombination were from double crossing-over, the recombination ratio should be 25.0 ± 0.6%. On the other hand, if all of the recombination were from single crossing-over, the ratio should be 12.5 ± 0.3%. In the sub-population of RM167=2 (the TN1 homozygote), the recombination ratio between resistance gene and RM167 was 14.5–29.0%. These results were similar to those obtained using the bulked susceptible extremes, explaining that RM167 was linked to a gene conferring resistance to

Table 1. Genotypes at three marker loci of ten susceptible.

Marker	Paternal genotype (Guiyigu)	Heterozygote	Maternal genotype (TN1)
RM167	0	4	6
RM287	1	4	4
RM21	1	5	4

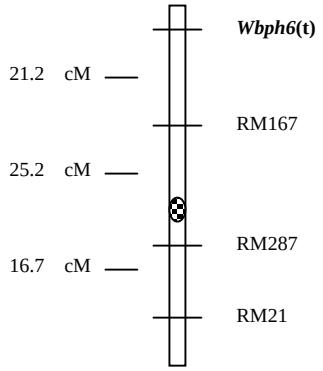

Fig. 3. Location of *Wbph6*(t) on chromosome 11 of rice.

The chromosome is drawn as a blank bar with dotted portion referring to centromere.

WBPH and the resistant allele was derived from Guiyigu. As the resistant allele of WBPH resistance gene *Wbph6*(t), we designated, was also from Guiyigu and this work was the first mapping study for WBPH resistance genes in Guiyigu, the same designation *Wbph6*(t) was given to the gene mapped here.

In the sub-population of RM167=3 (the heterozygote), most of the F_3 lines were moderately resistant to WBPH. If RM167 is linked to *Wbph6*(t), a small proportion of lines in this sub-population would have either homozygotes at *Wbph6*(t) loci due to single crossing-over and the majority would have inter-mediate resistance due to segregation within the lines. The phenotypic performance in the sub-population of RM167=3 was in support of the genetic linkage between RM167 and *Wbph6*(t).

All the F_3 lines were assayed with RM287 and RM21, which were linked to RM167. Results indicated that linkages between the markers and *Wbph6*(t) were weaker than that between RM167 and *Wbph6*(t) (Table 1). In the SSLP map reported [14], RM167 was located on the short arm of chromosome 11 and RM287 and RM21 on the lower positions, suggesting that *Wbph6*(t) was located at a position upper than RM167 locus on the short arm of rice chromosome 11 (Fig. 3).

DISCUSSION

In the present investigation, we utilized a TN1/Guiyigu $F_{2:3}$ population to map the new WBPH resistance gene *Wbph6*(t) in rice landrace Guiyigu from Yunan. Results obtained from recessive susceptible extremes and sub-population analysis based on positive makers suggested that *Wbph6*(t) was located on the short arm of rice chromosome 11 with a genetic distance of 21.2 cM to RM167. Since no polymorphism between TN1 and Guiyigu was detected for other RFLP and

SSLP markers on the short arm of chromosome 11 used in the parental survey, further work is still required to verify the *Wbph6*(t) location.

So far, no considerable progress has been made on the mapping of genes conferring resistance to WBPH in rice, except that Liu et al. [16] tagged resistant gene *Wbph2* in rice variety ARC10329 to RFLP markers on chromosome 6 of rice, but the chromosomal locations of other WBPH genes remained still unclear. Using an F_2 population from a cross between IR36 and its *Wbph1* isoline IR58034-1, McCouch found that the resistant gene *Wbph1* was co-segregated with RFLP markers RG146 and RG445. Since no more polymorphic markers were detected between the parents and RG146 and RG445 were multi-copy markers, chromosomal location of *Wbph1* was not clarified in their study [15]. Until now, there have been no reports on the mapping of WBPH-resistant genes on rice chromosome 11. However, Kadirvel et al. conducted a comparison on the differences in dry weight of rice leaves and roots of IR64/Azucena DH population at seedling stage under the condition with and without of insect infestation. A QTL conditioning dry weight difference was located between RG167 and RG103 on chromosome 11, which is in an interval just below the interval harboring *Wbph6*(t). It is worthwhile to determine whether a cluster of WBPH resistance genes presents in this region.

Mapping of the resistant genes to the brown planthopper (BPH), *Nilaparvata lugens*, has made remarkable advancements. BPH resistance genes *Bph1*, *bph2*, *Bph3*, *bph4* and *Bph10*(t) were mapped on chromosome 3, 4, 10 and 12 of rice, respectively [15,19-26]. QTLs linked to BPH resistance were also detected on rice chromosome 1, 2, 3, 4 and 8[18]. Nevertheless, none of the studies have mapped BPH resistance genes on chromosome 11. Although the present study is not able to tag *Wbph6*(t) with tightly linked markers, however, mapping of the new gene on chromosome 11 is highly valuable for understanding the genetic basis of host resistance to rice planthopper and for marker-assisted selection of WBPH resistance.

As employed in the present study, phenotyping on F_3 lines have been generally applied to solve the problem of un-replicability of F_2 plants. However, the use of F_3 population in gene mapping studies would reduce dominant sensitivity due to its segregation, and the genetic effects were generally underestimated. Owing to the use of F_3 lines for the evaluation of WBPH resistance and the small sample size, it is unlikely to detect genes with minor contribution to WBPH resistance in TN1/Guyigu population. We are currently constructing a recombinant inbred line population of Guiyigu/TN1, and the population will be used to validate the results we have obtained and to fine map *Wbph6*(t).

ACKNOWLEDGEMENT

This study was supported by National Natural Science Foundation of China (30270818).

REFERENCES

1 Angeles E R, Khush G S, Heinrichs E A. New genes for resistance to whitebacked planthopper in rice. *Crop Sci*, 1981, **21**: 47–50.

2 Wu C F, Khush G S. A new dominant gene for resistance to whitebacked planthopper in rice. *Crop Sci*, 1985, **25**(3): 505–509.

3 Hernandez J E, Khush G S. Genetics of resistance to whitebacked planthopper in some rice varieties. *Oryza*, 1981, **18**: 44–50.

4 Sidhu G S, Khush G S, Medrano F G. A dominant gene in rice for resistance to whitebacked planthopper and its relationship to other plant characteristics. *Euphytica*, 1979, **28**: 227–232.

5 Nair R V, Masajo E M, Khush G S. Genetic analysis of resistance to whitebacked planthopper in twenty-one varieties of rice *Oryza sativa* L. *Theor Appl Genet*, 1982, **61**: 19–22.

6 Li X M, Min S K, Xiong Z M, Hu G W. Screening of resistant resource and genetical analysis of resistance to whitebacked planthopper (WBPH) *Sogatella furcifera* (Horváth) in rice varieties (*Oryza sativa* L.). *Acta Genet Sin*, 1987, **14**(6): 413–418. (in Chinese with English abstract)

7 Li X M, Min S K, Xiong Z M, Hu G W. Genetics analysis of resistance to whitebacked planthopper *Sogatella furcifera* (Horváth) in four rice varieties (*Oryza sativa*) of Yunnan Province. *Chinese J Rice Sci*, 1990, **4**(3): 113–116. (in Chinese with English abstract)

8 IRRI. Inheritance of resistance to whitebacked planthopper. *In*: IRRI Annual Report for 1978. Manila: IRRI, 1979. 71–75.

9 Lu Y J, Zheng K L. A simple method for isolation of rice DNA. *Chinese J Rice Sci*, 1992, **6**(1): 47–48. (in Chinese with English abstract)

10 McCouch S R, Kochert G, Yu Z H, Wang Z Y, Khush G S, Coffman W R, Tanksley S D. Molecular mapping of rice chromosomes. *Theor Appl Genet*, 1988, **76**: 815–829 .

11 Zhang Q F, Shen B Z, Dai X K, Mei M H, Saghai Maroof M A, Li Z B. Using bulked extremes and recessive class to map genes for photoperiod-sensitive genic male sterility in rice. *Proc Natl Acad Sci USA*, 1994, **91**: 8675–8679.

12 Lander E, Green P, Abrahamson J, Barlow A, Daley M, Lincoln S, Newburg L. MAPMAKER: an interactive computer package for constructing primary genetic maps of experimental and natural populations. *Genomics*, 1987, **1**: 174-181.

13 Lander E S, Bostein D. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*, 1989, **121**:185–199.

14 Temnykh S, Park W D, Ayres N M, Cartinhour S, Hauck N, Lipovich L, Cho Y G, Ishii T, McCouch S R. Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). *Theor Appl Genet*, 2000, **100**: 697–712.

15 McCouch S R, Khush G S, Tanksley S D. Tagging genes for disease and insect resistance via linkage to RFLP markers. *In*: Rice Genetics Ⅱ. Manila: IRRI, 1991. 443–449.

16 Liu Z Y, Liu G J, Sogawa K, Zheng K L. Tagging the gene *Wbph2* in ARC 10239 resistant to the whitebacked planthopper *Sogatella furcifera* by using RFLP markers. *CRRN*, 2001, **9**(2): 10.

17 Kadirvel R, Maheswaran M, Gunathilagaraj K. Molecular mapping of quantitative trait loci (QTL) associated with whitebacked planthopper in rice. *IRRN*, 1999, **24**(3): 12–13.

18 Alam S N, Cohen M B. Detection and analysis of QTLs for resistance to brown planthopper, *Nilaparvata lugens*, in a doubled-haploid rice population. *Theor Appl Genet*, 1998, **97**: 1370–1379.

19 Ikeda R, Kaneda C. Trisomic analysis of the gene *Bph-1* for resistance to brown planthopper *Nilaparvata lugens* Stål, in rice. *Jpn J Breeding*, 1983, **33**:40–44.

20 Ikeda R, Kaneda C. Genetic analysis of resistance to brown planthopper *Nilaparvata lugens* Stål, in rice. *Jpn J Breeding*, 1981, **31**: 279–285.

21 Hirabayashi H, Ogawa T. RFLP mapping of *Bph-1* (brown planthopper resistance gene) in rice. *Breeding Sci*, 1995, **45**: 369–371.

22 Jeon Y H, Ahn S N, Choi H C, Hahn T R, Moon H P. Identification of a RAPD marker linked to a brown planthopper resistance gene in rice. *Euphytica*, 1999, **107**: 23–28.

23 Ishii T, Brar D S, Multani D S, Khush G S. Molecular tagging of gene for brown planthopper resistance and earliness introgressed from *Oryza australiensis* into cultivated rice, *O. sativa*. *Genome*, 1994, **37**: 217–221.

24 Murata K, Fujiwara M, Kaneda C, Takumi S, Mori N, Nakamura C. RFLP mapping of a brown planthopper (*Nilaparvata lugens* Stål.) resistance gene *bph2* of indica rice introgressed into a japonica breeding line “Norin-PL4”. *Genes Genet Syst*, 1998, **73**: 359–364.

25 Huang N, Parco A, Mew T, Magpantay G, McCouch S, Guiderdoni E, Xu J C, Subudhi P, Angeles E R, Khush G S. RFLP mapping of isozymes, RAPDs and QTLs for grain shape, brown planthopper resistance in doubled-haploid rice population. *Mol Breeding*, 1997, **3**: 105–113.

26 Huang Z, He G, Shu L, Li X, Zhang Q. Identification and mapping of two brown planthopper resistance gene in rice. *Theor Appl Genet*, 2001, **102**: 929–934.

27 Li J X, Yu S B, Xu C G, Tan Y F, Gao Y J, Li X H, Zhang Q F. Analyzing quantitative trait loci for yield using a vegetatively replicated F_2 population from a cross between the parents of an elite rice hybrid. *Theor Appl Genet*, 2000, **101**: 248–254.