

Mapping of Brown Planthopper Resistance Gene Introgressed from *Oryza nivara* into Cultivated Rice, *O. sativa*

Min-Tze Wu^{1*}, Charng-Pei LI¹, Jen-Ren CHEN¹, Shou-Horng Huang²
Hsin-Mei Ku³

¹Biotechnology Division, Taiwan Agricultural Research Institute, Taichung 413, Taiwan

²Department of Plant Protection, Chiayi Agricultural Experiment Station, Taiwan Agricultural Research Institute, Chiayi 600, Taiwan

³Department of Agronomy, National Chung Hsing University, Taichung 402, Taiwan

*Corresponding author: wu@tari.gov.tw

Abstract

Brown planthopper (BPH) is one of the most destructive insect pests of rice in Asia. Wild rice species have been shown to be valuable sources for breeding resistant cultivars. In this study, SSR mapping of BPH resistance was conducted using an F₁₀ family derived from a cross between an introgression line, '852T034', from *Oryza nivara* (Accession number 102165), and a susceptible *japonica* variety, 'Tainung 71'. Bulked analysis was used to evaluate the resistance of F_{10:11} families. A BPH resistant gene contributed by 852T034 was mapped between two flanking SSR markers, RM16655 and RM3317, on the short arm of chromosome 4. This is the first time a BPH resistance gene derived from the wild species *O. nivara* was mapped. The tightly linked SSR markers will be facilitated marker-assisted breeding and provide the basis for map-based cloning of BPH resistant gene.

Key words: Brown planthopper, *Oryza nivara*, Wild rice, Genetic mapping, Marker-assisted breeding

INTRODUCTION

The brown planthopper (BPH), *Nilaparvata lugens* (Stal.), is one of the most destructive phloem-sap-sucking insect pests of rice (*O. sativa* L.) and can cause significant yield losses. Heavy infestations cause complete drying and plant death known as hopper burn. Not only the BPH feeds on the rice plant directly but also transmits rice grassy stunt virus and ragged stunt virus that cause severe disease (Khush and Brar, 1991). The application of BPH resistance genes has been recognized as the most economic, effective measure for BPH management and the most environmental friendly. Several resistance loci associated with BPH resistance have been reported and reviewed. Among these, *Bph1*, *bph2*, *Bph3* and *bph4* have been used extensively in rice breeding programs (Chen et al. 2006). In recent years, rice cultivars carrying *Bph1* or *bph2*, however, have lost their ability against BPH in Taiwan and the situation is getting worst probably related to global climate change.

Wild species of *Oryza* are potential source of new genes for resistance to BPH. Since few useful genes from wild germplasm accessions have been explored, there is still great potential for exploring novel genes (Xiao et al. 1998). In previous studies, eleven out of 21 BPH resistance loci derived from wild species have been reported in rice (Jena et al. 2006; Rahman et al. 2009). They are *Bph10* on the short arm of chromosome 12 from *O. australiensis* (Ishii et al. 1994), *Bph11(t)* and *bph12(t)* on the long arm of chromosome 3 and chromosome 4 from *O. officinalis* (Hirabayashi et al. 1998), *Bph12(t)* on the short arm of chromosome 4 from *O. latifolia* (Yang et al. 2002), *Bph13(t)* on the long arm of chromosome 2 from *O. eichingeri* (Liu et al. 2001), *Bph20(t)* and *Bph21(t)* on the short arm of chromosome 4 and the long arm of chromosome 12 from *O. minuta* (Rahman et al. 2009). Numerous studies have identified QTLs associated with BPH resistance on the adjacent chromosome regions near *Bph1* and *bph2* (Sharma et al. 2004). At least, six of these studies identified a major BPH resistance QTL located on the

short arm of chromosome 4 using both inter- and intra-species cross (Hunag et al. 2001; Yang et al. 2002, 2004; Huang et al. 2001; Sun et al. 2005; Rahman et al. 2009). In interspecific crosses, it was noticed that the wild accessions were the one always contributing the resistant allele at BPH resistance locus. a

Oryza nivara is an annual wild rice species and is possibly one of the direct ancestor of *O. sativa*. An introgression line '852T034' derived from a cross between *O. nivara* and *japonica* type Tainung 67 was selected and tested its resistance to BPH. The objectives of this study were to identify SSR markers closely linked to the BPH genes in order to be facilitated marker-assisted selection (MAS) in Taiwan rice breeding program and to map the major resistance genes on rice chromosome for further gene cloning.

Materials and Methods

Plant materials

The introgression line, 852T034, derived from an interspecific cross between Tainung 67 and a wild species *O. nivara* (Acc. No. 102165) show consistent resistance to BPH. 852T034 and Tainung 71 (a mega variety susceptible to BPH) were used as parents to develop a F₁₀ population consisting 199 progeny for genetic analysis. The F_{10:11} from each F₁₀ plants were bioassay for BPH resistance. To test SSR markers linked to BPH resistance, a F₂ population derived from a cross between 852T034 and 871948, an *O. officinalis* susceptible introgression line, was generated for genetic analysis and F_{2:3} families for BPH bioassay.

BPH bioassay

A pure BPH population was developed and maintained from single colony of BPH biotype 1 in the greenhouse facility of Chiayi Agricultural Experiment Station of TARI. The bioassay was performed with bulk analysis and seedbox screening technique. Seedlings at three-leaf stage were infested with second- and

third-instar nymphs density of 2-3 nymphs per seedling. The reaction against the BPH was scored following the guidelines of Standard Evaluation System for Rice (IRRI, 2002). Taichung Native 1 susceptible to all biotype of BPH was used as the susceptible check and Mudgo (*Bph1*) and H105 (*bph2*) were used as the resistant checks.

DNA preparation and SSR analysis

Plant DNA was extracted from the leaves of rice plants using Wizard Genomic DNA extraction kit (Promega, United State). SSR analysis was performed according to the procedure of McCouch et al. (1997) with minor modification. The original source and motifs for all SSR markers used in this study could be found in the Gramene database (<http://www.gramene.org/>), McCouch et al. (2002) and IRGSP (2005). Amplification reactions were carried out in a 20 µl containing 50 ng DNA, 50 µM of each primer, 10x PCR buffer [100 mM Tris (pH 8.3), 500 mM KCl, 15 mM MgCl₂], 250 µM of each dNTP and 0.6 U of *Taq* polymerase (Protech Inc., Taiwan). PCR was performed in a PTC-200 Thermocycler (MJ Research Inc., United State) programmed as 5 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 55°C and 1.5 min at 72°C, with a final extension of 5 min at 72°C. Amplified PCR products were detected by electrophoresis in 3% SFR agarose gels (Amersco Inc., United State) staining with ethidium bromide.

Mapping QTL for BPH resistance

Linkage groups and the order of markers were determined using MAPMAKER/EXP 3.0 (Lander et al. 1987). The Kosambi mapping function was selected to correct the recombination frequency to genetic distances (cM). Marker regression and interval QTL mapping were carried out using Map Manager QTX 0.30 (Manly et al. 2001) with a likelihood ratio statistic (LRS) threshold 20 and a probability level of 0.01 for declaring the presence of putative QTLs.

RESULTS

BPH resistance evaluation

The BPH resistance phenotypes of the $F_{10:11}$ were evaluated when the 90% seedlings of the susceptible check 'TN1' were killed by BPH after infestation. The results showed the average severity scores of the parents '852T034' and 'Tainung 71' were 3.7 and 7.7, respectively. '852T034' showed the highest of BPH resistance. The severity scores of the 199 $F_{10:11}$ infested with BPH displayed a continuous distribution, ranging from 3 to 9, with two apparent peaks around 3 and 7 (Fig. 1).

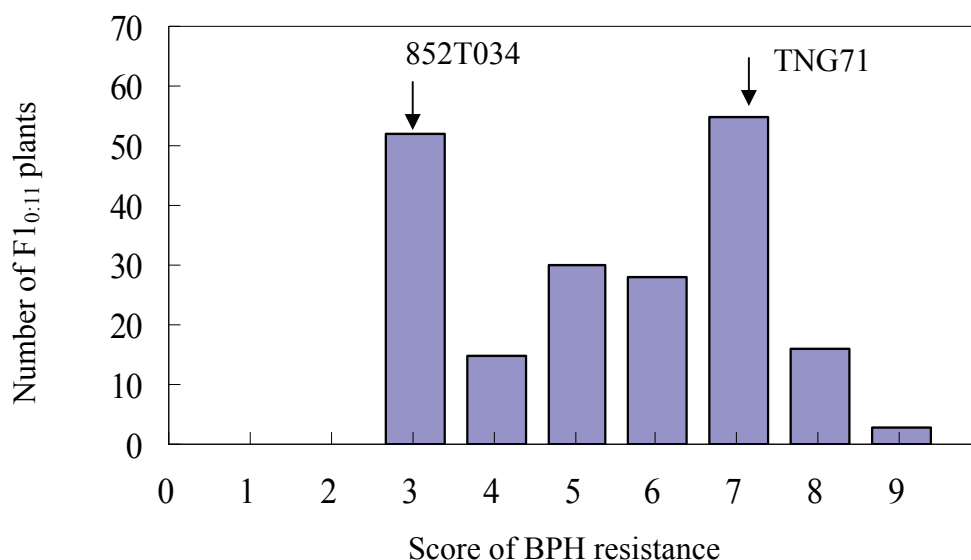


Fig 1. The frequency distribution of BPH resistance scores of $F_{10:11}$ lines derived from 852T034 \times TNG71 in 2009 first crop.

Identification of SSR markers linked with the BPH resistance gene

To identify SSR markers tightly linked to BPH resistance, a total of 157 SSR markers, selected from 12 chromosomes with an interval of ~15 cM were tested. A linkage map was constructed by SSR genotyping 199 F_{10} individuals, and a major QTL for BPH resistance was identified. The BPH resistance locus detected with a LRS 136 was located between RM16655 and RM3317 on the short arm of

chromosome 4 (Fig. 2) and explained 50% of the phenotypic variance of BPH resistance in this population and the resistance was contributed by ‘852T034’.

Line	BPH	Chromosome 4						
		RM3536	RM16554	RM16563	RM16655	RM3317	RM7113	RM3308
TNG71	7.7	A	A	A	A	A	A	A
852T034	3.7	B	B	B	B	B	B	B
R006	4.3	B	B	B	B	B	B	B
R008	9.0	A	A	A	A	A	B	B
R010	7.7	H	A	A	A	A	B	A
R011	3.7	A	B	B	B	B	A	A
R012	9.0	A	A	A	A	A	B	H
R019	8.3	H	A	A	A	A	A	A
R022	5.0	B	B	B	B	B	A	B
R029	7.7	H	A	A	A	A	B	B
R032	3.0	B	B	B	B	B	B	A
R033	6.3	H	A	A	A	A	B	B
R036	6.3	A	A	A	A	A	B	B
R047	7.0	A	A	A	A	A	A	A
R074	5.7	H	H	A	H	H	B	B
R099	5.0	B	B	B	B	B	A	B
R118	4.3	B	B	B	B	B	A	A

Fig 2. Phenotype (BPH resistance) and graphical genotype of randomly selected F₁₀ individuals. A: Tainung 71 allele, B: 852T034 allele, H: heterozygous

MAS test for the BPH resistance gene linked to SSR markers

The SSR markers RM16655 and RM3317 were further validated as R- or S-associated DNA markers by genotyping the F₂ progenies with different genetic background of Tainung 71. One hundred and forty-two F₂ seedlings from a cross between 852T034 and a susceptible *O. officinalis* introgression line 871948 were genotyped and F_{2:3} families were used for BPH bioassay. 71% and 79% homozygous allele of 852T034 at RM16655 and RM3317 loci in the F₂

population showed resistance scores ≤ 5 , respectively. More than 90% F₂ individuals detecting homozygous allele of 852T034 showed different degrees of increasing BPH resistance (Table 1). The results indicated linked SSR markers have great potential used in marker-assisted selection.

Table. Distribution of BPH resistance scores by genotypes of markers RM16655 and RM3317 in F₃ of 852T034 x 871948

Marker	Genotype ^z	Number of lines in BPH resistance score class						Total	Mean score
		RS \leq 5	(%)	5<RS<7	(%)	RS \geq 7	(%)		
RM16655	1/1	14	26	14	26	26	48	54	6.74
	1/2	15	50	7	23	8	27	30	5.57
	2/2	41	71	13	22	4	7	58	4.72
RM3317	1/1	14	25	16	28	27	47	57	6.74
	1/2	23	53	12	28	8	19	43	5.35
	2/2	33	79	6	14	3	7	42	4.55

^a 1/1 denotes the homozygous genotype of '871948', 1/2 denotes the genotype of the heterozygous, 2/2 denotes the homozygous genotype of '852T034'; RS, Resistance Score.

DISCUSSION

BPH is a major biotic stress in rice production in most Asian countries. In Taiwan, BPH resistance genes such as *Bph1* and *bph2* were introduced into *japonica* cultivars by conventional breeding methods. However, because of changes in BPH biotype and infestation patterns, varieties with the *Bph1* gene for resistance have become susceptible. It is also reported that biotype changes occurred because of the immigration of new biotypes of BPH from China, Vietnam and Philippines by summer wind since BPH never overwinters in Taiwan (Cheng and Huang, 2004). Therefore, identification of a new source of BPH resistance genes followed by introducing into *japonica* cultivars is an important objective in breeding programs.

Wild rice species are important resources of disease and insect resistance in crop genetic improvement (Tanksley and McCouch, 1996). Several wild species, including *O. latifolia*, *O. minuta*, *O. nivara*, *O. officinalis* and *O. punctata*, possess resistance to various biotypes of BPH (Renganayaki et al. 2002; Yang et al, 2004). In this study, a BPH resistance gene explained 50% phenotypic variations in the F2 population was mapped on the short arm of chromosome 4 and the resistance was contributed by '852T034' allele derived from *O. nivara* . This location is very close to BPH loci of *Qbp2*, *Bph12(t)*, *Bph15*, *Bph17* and *Bph20(t)* (Hunag et al. 2001; Yang et al. 2002, 2004; Huang et al. 2001; Sun et al. 2005; Rahman et al. 2009). It is very interesting to notice that BPH resistance genes derived from different wild rice species were mapped on the same region of chromosome 4. Disease resistance genes of plants were reported often clustering in the same chromosome region. It remains to be investigated whether the distribution of insect pests resistance genes is similar to that of disease resistance genes.

BPH populations can quickly overcome single resistance gene under natural conditions. New resistance genes are always needed for rice improvement and breeding against BPH. Therefore, the resistance genes of '852T034' can be a useful BPH resistance donor for a new resource of BPH resistance. The transfer of BPH resistance genes from wild species to different variety backgrounds can be greatly facilitated by using MAS (Tanksley and Nelson. 1996; Moncada et al. 2001). There has been great progress in the development of MAS for BPH in recent years. SSR and CAPS markers linked to *Bph1*, *bph2* and *Bph3* were used widely in Japan, Korean and Thailand MAS programs (Sharma et al. 2004; Cha et al. 2008; Jairin et al. 2009). But relatively few varieties or lines have been reported to be successfully developed by this method. One of the problems lies in the crossovers between markers and genes caused the segregation of the resistance in the population and deviated selection.

In this study, we conducted SSR mapping of wild species *O. nivara* and identified the SSR markers closely linked to BPH resistance locus, and verified by the

efficiency of MAS. Both RM16655 and RM3317 showed over 90% accuracy predicting BPH resistance in the F₂ generation derived from 852T034 x 871948. Thus RM16655 and RM3317 could be applied to the MAS of the trait of BPH resistance in rice breeding programs.

REFERENCES

- Chen, J. W., L. Wang, X. F. Pang, and Q. H. Pan. 2006. Genetic analysis and fine mapping of a rice brown planthopper (*Nilaparvata lugens* Stal) resistance gene *bph19(t)*. *Mol. Gen. Genomics* 275: 321-329.
- Cheng, C. H., and S. H. Huang, 2004 Population fluctuations and forecasting of the white-backed planthopper. *Plant Prot. Bull.* 46: 315-332.
- Huang, Z., G. He, L. Shu, X. Li, and Q. Zhang. 2001. Identification and mapping of two brown planthopper resistance genes in rice. *Theor. Appl. Genet.* 102:929–934.
- Ishii, T., D.S. Brar, D.S. Multani, and G.S. Khush. 1994. Molecular tagging of genes for brown planthopper resistance and earliness introgressed from *Oryza australiensis* into cultivated rice, *O. sativa*. *Genome* 37:217–221.
- Jairin J., S. Teangdeerith, P. Leelagud, J. Kothcharerk, K. Phengrat, M. Yi, A. Vanavichit and T. Toojinda. 2009. Development of rice introgression lines with brown plant hopper resistance and KDML105 grain quality characteristics through marker-assisted selection. *Field Crops Res.* 110:263-271.
- Jena K. K., J. U. Jeung, J. H. Lee, H. C. Choi and D. S. Brar. 2006. High-resolution mapping of a new brown planthopper (BPH) resistance gene, *Bph18(t)*, and marker-assisted selection for BPH
- Khush, G.S. and D.S. Brar. 1991. Genetics of resistance to insects in crop plants. *Adv. Agron.* 45:223-274
- Liu G.Q., H.H. Yan, Q. Fu, Q. Qian, Z.T. Zhang, W.X. Zhai and L.H. Zhu. 2001. Mapping of a new gene for brown planthopper resistance in cultivated rice introgressed from *Oryza eichingeri*. *Chin. Sci. Bull.* 46:1459-1462.
- Manly, KF, R. H. Jr. Cudmore, and J. M. Meer. 2001. Map Manager QTX, cross-platform software for genetic mapping. *Mammalian Genome* 12:930-932.

- Moncada P., C. P. Martinez, J. Borrero, M. Chatel, J. H. Gauch, E. Guimaraes, J. Tohme, and S. R. McCouch. 2001. Quantitative trait loci for yield and yield components in an *Oryza sativa* × *Oryza rufipogon* BC₂F₂ population evaluated in an upland environment. *Theor. Appl. Genet.* 102: 41-52.
- Rahman M.L., W. Jiang, S.H. Chu, Y. Qiao, T. H. Ham, M. O. Woo, J. Lee, M. S. Khanam, J. H. Chin, J. U. Jeung, D. R. Brar, K. K. Jena, and H.J. Koh. 2009. High-resolution mapping of two rice brown planthopper resistant genes, *Bph20(t)* and *Bph21(t)* from *Oryza minuta*. *Theor. Appl. Genet.* 11 Aug. [Epub]
- Renganayaki, K., A. K. Fritz, S. Sadasivam, S. Pammi, S. E. Harington, S. R. McCouch, S. M. Kumar and A. S. Reddy. 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.
- Sun L.H., C.M. Wang, C.C. Su, Y.Q. Liu, H.Q. Zhai and J.M. Wan. 2006. Mapping and marker-assisted selection of a brown planthopper resistance gene *bph2* in rice (*Oryza sativa* L.). *Acta Genetica Sinica*, 2006, 33 (8) : 717–723.
- Tanksley, S. D. and J. C. Nelson. 1996. Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTL from unadapted germplasm into elite breeding lines. *Theor. Appl. Genet.* 92 : 191-203.
- Tanksley S. D. and S. R. McCouch 1996 Seed Banks and Molecular Maps: Unlocking Genetic Potential from the Wild. *Science* 277:1063 – 1066.
- Xiao J, Li J, S. Grandillo, A. Sang-Nag, L. Yuan, S.D, Tanksley and S.R. McCouch. (1998) Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon*. *Genetics* 150: 899–909.
- Yang, H., X. Ren, Q. Weng, L. Zhu, and G. He. 2002. Detection and analysis of QTL for resistance to brown planthopper, *Nilaparvata lugens* (Stal), in rice (*Oryza sativa* L.), using backcross inbred lines. *Hereditas* 136:39–43.
- Yang, H., A. You, Z. Yang, F. Zhang, R. He, L. Zhu and G. He. 2004. High-resolution genetic mapping at *Bph15* locus for brown planthopper resistance in rice (*Oryza sativa* L.), using backcross inbred lines. *Theor. Appl. Genet.* 110:182-191.