

## Forward the Design Breeding of Resistance to Planthoppers in Rice

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**Key Words:** Rice, brown planthopper (BPH), resistance gene, nearly-isogenic line (NIL), pest management

### Abstract

The incorporation of host plant resistance to planthoppers into elite rice cultivars and the sustainability of pest management using resistant cultivars are important for a stable food supply in most rice production areas. The brown planthopper (BPH), *Nilaparvata lugens* Stål., is one of the most serious and destructive rice pests that can be found throughout rice growing areas in Asia. Twenty one major BPH resistance genes have been identified in several Indica cultivars and wild relatives. Recent molecular mapping of genes which are resistant to the planthoppers suggested that highly resistant cultivars/accessions often carried multiple genes for resistance. It suggests that gene pyramiding which combines more than two resistance genes derived from same/different donor(s) will delay the occurrence of virulent biotypes. Here, I describe the identification of BPH resistance genes and the development of nearly-isogenic lines for each resistance gene in order to improve rice cultivars through molecular genetic approach. Genetic enhancement of rice exotic germplasm is future target for breeding elite cultivars with resistance to the BPH. Marker-assisted selection, gene pyramiding, and map-based approach for gene isolation were discussed. We also discussed the monitoring of the genetic constitution of the BPH population; those probably involve several virulent types to the specific resistance gene(s), for sustainable pest management using resistant cultivars against the BPH.

### Introduction

The planthopper species damage the plant epidermises and parenchyma with their stylets and suck the plant sap from the phloem. Among the phloem-feeding insects, the brown planthopper (BPH), *Nilaparvata lugens* Stål., is the most serious insect pest of rice (*Oryza sativa* L.) throughout Asia. The populations migrate from China to Japan during the rainy season every year (Kisimoto 1976). Though the migrant populations are not large, the progenies sometimes break out. The insect sucks out the plant sap and causes damage to rice plants such as reduction of crop vigor, plant height, productive tillers, perfect grains, and yield. In extreme cases, a heavy infestation of BPH results in complete necrosis of the rice plants, a condition commonly known as ‘hopper burn’. It influences yield loss and also causes poor grain quality. The BPH is also a vector of the grassy stunt virus and ragged stunt virus, which seriously decrease rice production. Several kinds of resistant cultivars and accessions of rice against the planthoppers have

been reported (Heinrich et al 1985). Host plant resistance to insects has been classified into three mechanistic types: antibiosis, antixenosis and tolerance (Painter 1951). However, distinguishing between these mechanisms against the planthopper species have been difficult with bulk seedling tests (Athwal et al 1971). Kishino and Ando (1978) established a simple method for evaluating antibiosis to the green rice leafhopper (GRH), and the survival ratio of the GRH nymphs was examined on the tested cultivars. Using this evaluation method, genetic analyses of resistance to the GRH have been carried out, and at least six loci for the GRH resistance have been identified with the aid of DNA markers. The simple sequence repeat (SSR) marker loci are widely distributed throughout the genome and can be easily analyzed using a polymerase chain reaction (PCR). The SSR markers have been used extensively to map agronomically important loci in rice, such as disease and insect resistance. It has opened the door to further revelations regarding the mechanisms of host plant resistance to the insect pests through molecular mapping and the cloning of genes which will confer resistance to the phelom-sucking insects such as BPH and GRH. The relationship between rice and the GRH is a model case of plant-insect interaction. The knowledge obtained from a series of molecular cloning is expected to reveal the sucking resistance and system of host-plant resistance breakdown.

The objectives of the present study are to understand the genetic basis for a resistance to BPH found in rice cultivars and accessions of wild rice, as well as facilitate the use of germplasm for future rice improvements. First, a quantitative trait loci (QTL) analysis for resistance to the insect pests was conducted using an initial mapping population derived from the cross between a susceptible cultivar and a resistant accession. Subsequently, new loci for the resistance to insect pests were mapped onto a molecular linkage map using a nearly isogenic population, which was developed by continuous backcrossing and marker-assisted selection (MAS) of the targeted QTL region. Finally, we discuss the necessity of monitoring genetic constitutions of the insect populations, which probably involve several virulent types to the specific resistance gene(s), for sustainable pest management using resistant cultivars against the insect pests.

## **Materials and Methods**

### **Plant materials**

Nine rice cultivars with different levels of resistance to the BPH: ADR52, PodiwiA-8, Mudgo, ASD7, Rathu Heenati, Babawee, Chin Saba, Balamawee, Taichung 65 (T65) (no resistance gene) were used. Nearly-isogenic lines derived from the BPH resistant cultivar ADR52 were used.

### **Insect strains**

Three BPH strains were evaluated: (1) Hatano-66 strain collected from Hatano, Kanagawa Prefecture, Japan in 1966, (2) Chikugo-89 strain collected from Chikugo, Fukuoka Prefecture, Japan in 1989, and (3) Isahaya-99 strain collected from Isahaya, Nagasaki Prefecture, Japan in 1999. They are maintained separately by continuously rearing on susceptible variety 'Reihou' at  $25\pm 1^{\circ}\text{C}$  under 16 h light and 8 h dark condition in the National Agricultural Research Center for Kyushu Okinawa Region (KONARC). Chikugo-89 strain was used to map the QTLs involved in ADR52. The BPH strains collected in 1989 and 1999 in Japan have different virulence characteristics to rice differential cultivars (Myint et al., 2009). Chikugo-89 strain was virulent to

Mudgo carrying *Bph1* but avirulent to ASD7 carrying *bph2*. The BPH strain collected in 1999 was virulent to Mudgo carrying *Bph1* and ASD7 carrying *bph2*.

In 2006, ten populations of BPH were collected in East Asia. Four populations were collected from the Red river delta (Vietnam-HT, Vietnam-HP) in Northern Vietnam and Mekong river delta (Vietnam-TGL, Vietnam-TGH) in Southern Vietnam. Three populations were collected from the Philippines; Northern and Central Luzon islands (Philippines-NE-B, Philippines-AN) and Mindanao island (Philippines-CG). Each population from Japan (Japan-KG), China (China-FJF), and Taiwan (Taiwan-CH) were collected. These populations were maintained by continuous rearing on susceptible cultivars 'Reiho' at 25±1°C under 16h light and 8h dark conditions in the laboratory of Pest Management System, National Agricultural Research Center for Kyushu-Okinawa Region, Kumamoto, Japan. Thus, these BPH strain was used for monitoring the current status of virulence in BPH strains against the resistance gene, *bph20(t)* and *Bph21(t)*.

### **Evaluation of the resistance to BPH**

The one-month old plants were trimmed and covered with a transparent plastic cylindrical cage (5.5 cm D x 20cm H). The antibiosis test were carried out using the method described by Tanaka (2000). Five brachypterous females within 24h after emergence were released to a single rice plant at one month after sowing. The score was observed from 3 to 5 DAI. The adult mortality as well as the shape of abdomen were examined. Females that formed a heavily swollen abdomen or survived for five days were defined as virulent, and females died within five days as avirulent. Plants with adult mortality found to be less than 30% were categorized as susceptible and those with adult mortality found to be greater than 70% were categorized as resistant. The experiment was carried out with 8 replications.

### **Statistical analysis**

The data was analyzed using two-way ANOVA. *bph20(t)*-NIL, *Bph21(t)*-NIL and *bph20(t)/Bph21(t)*-PYL were developed through continuous backcrossing with T65 and MAS. These NILs and PYL were evaluated for BPH resistance at the seedling stage and T65 was used as a susceptible control. The resistance level was compared by the adult mortality and by females of a swollen abdomen at 5 DAI. The experiment was carried out with seven replications. Data were analyzed using one-way ANOVA and treatment means were pair-wise compared using the Tukey-Kramer test (SAS Institute Inc., 2003). The adult mortality (%) and females with a swollen abdomen (%) was arcsine transformed prior to analysis.

## **Results and Discussion**

### **QTL analyses of highly resistant cultivars to BPH and developing the NILs**

The advent of detailed molecular linkage maps in rice has made it possible to detect the quantitative trait loci (QTLs) which control agronomic characters such as biotic and abiotic stresses. In screening germplasm resistance to the BPH under antibiotic tests, four Indica cultivars, ADR52, Podiwi A8, ASD7 and Balamawee, were selected as highly resistant. QTL analyses for antibiosis to BPH were conducted using F2 populations derived from a cross between a susceptible Japonica cultivar and resistant Indica cultivars. The study has assured future mapping of the BPH-resistance

gene using nearly isogenic populations developed through marker-assisted selection (MAS). In the case of ADR52, a total of three QTLs controlling antibiosis to the BPH were detected on chromosomes 5, 6 and 12. Nearly isogenic lines (NILs) and pyramided lines (PYLs) for respective QTLs were developed through continuous backcrossing and MAS. The newly identified resistance genes on chromosomes 6 and 12 were tentatively designated as *bph20(t)* and *Bph21(t)*, respectively.

### **Development of NILs and pyramided lines PYLs for the BPH resistance genes**

Since highly resistant cultivars often carried multiple genes for resistance to the BPH, a nearly isogenic population was necessary to map the BPH resistance gene, precisely. The MAS for BPH resistance genes with advanced backcrossing with the recurrent parent can facilitate transferring the resistance to the BPH from the resistant cultivars and wild relatives. The NILs and PYLs derived from the resistant germplasm are useful not only for the improvement of BPH resistance in rice improvement but also for monitoring BPH virulence to the specific resistance gene.

### **Understanding the mechanisms of a breakdown of the resistance gene**

Virulent insect pests, the so-called new biotypes, often appear after the release of modern improved varieties of rice that carry a single major gene for resistance to the insect pests. These pests represent a serious threat to rice paddies, because they have acquired virulence to the specific resistance gene, which will have subsequently lost its effectiveness in insect pest management. For example, the BPH population migrating into Japan began to become virulent to the *Bph1* (*Brown planthopper resistance 1*) in the late 1980s (Sogawa 1992) and has become highly virulent for rice cultivars carrying both *Bph1* and *bph2* since the late 1990s (Tanaka and Matsumura 2000). The virulent biotypes of the BPH were experimentally identified by continuous rearing of the BPH on resistance lines, each carrying a single major gene for BPH resistance (Ketipearachchi et al 1998). By a similar methodology, virulent biotypes against each of the three resistance genes *Grh1*, *Grh2* and *Grh3* were isolated (Hirae et al, 2007). It suggests that natural strains of GRH are likely to feed on rice plants having a single major gene for the resistance. In contrast, virulent biotypes against the PYL carrying both *Grh2* and *Grh4* did not occur experimentally (Hirae et al, 2007). In line with these findings, we have demonstrated that, although the nymph mortality of *Grh4*-NIL showed susceptibility to the GRH, the PYL carrying *Grh2* and *Grh4* showed higher nymph mortality than *Grh2*-NIL. Additionally, both *Grh2* and *Grh4* have been essential to express resistance to the green leafhopper (GLH), which is closely related to the GRH and a major vector of Tungro, a destructive viral disease found in tropical rice fields in Asia (Yasui and Yoshimura 1999). The PYLs carrying *Grh2* and *Grh4* may thus have an important role in expressing durable resistance to the rice leafhoppers. It suggests that gene pyramiding that combines multiple resistance genes with different mechanistic types will suppress the dominance of virulent biotypes in the insect population. The PYLs carrying these resistant genes may suppress the dominance of virulent biotypes and show durable resistance to the GRH. To study the durability of resistance to insect pests, the development of PYLs carrying multiple resistance genes is essential using MAS and advanced backcrossing with a recurrent parent.

### **Virulence of the Asian BPH strains against rice differential cultivars**

Matsumura demonstrated that the adult survival rate and the ratio of virulent females of the 10 Asian BPH strains on 6 differential cultivars and T65 are a susceptible check (**Proceedings: this issue**). Based on the resistance spectrum, the Asian BPH strains seem to be classified into three groups: the first group virulent to Mudgo and ASD7 but avirulent to the other 4 differential cultivars, the second group involving quite high percentage of the BPH individuals virulent to Babawee and ADR52 in addition to Mudgo and ASD7, the third group partially virulent to Babawee in addition to Mudgo and ASD7. The first group involved BPH strains collected from Japan, China, Taiwan and two strains of Northern Vietnam. The second group consisted of the two BPH strains collected from Southern Vietnam. The third group consisted of three strains from the Philippines, one which was collected from Mindanao Island and involved about half of BPH individuals virulent to Mudgo and ASD7. We concluded that the cultivars Rathu Heenati and Balamawee are still keeping a broad spectrum of resistance against the Asian BPH strains.

### Monitoring the BPH virulence using rice NILs

Adult survival rates and development of the proportion of BPH females with swollen abdomen are shown in Table 1 and Table 2, respectively. Small proportions of Hatano-66 and Chikugo-89 females survived on NILs (*bph20(t)*-NIL and *Bph21(t)*-NIL), PYL (*bph20(t)*, *Bph21(t)*-PYL), and ADR52 (resistant check) ranging from 0 to 20.0% and all the females that survived did not show a swollen abdomen. In contrast, high proportions of Isahaya-99 females survived on the *bph20(t)*-NIL (87.5%) and on *Bph21(t)*-NIL (100.0%). Eighty percent of Isahaya-99 females showed swollen abdomens on the two NILs. However, adult survivorship in Isahaya-99 strain remained small on the PYL and ADR52 (12.5 and 20.0%, respectively), and all the surviving females did not show swollen abdomens. These results indicate that Hatano-66 and Chikugo-89 strains were avirulent to *bph20(t)*-NIL, *Bph21(t)*-NIL and their PYL. However, Isahaya-99 strains was virulent to *bph20(t)*-NIL, *Bph21(t)*-NIL but avirulent to the PYL.

**Table 1.** Adult survival rates (%) of *Nilaparvata lugens* strains on NILs and their PYL of rice

Line	Resistance gene	<i>Nilaparvata lugens</i> strains <sup>a, b</sup>		
		Hatano-66	Chikugo-89	Isahaya-99
<i>bph20(t)</i> -NIL	<i>bph20(t)</i>	20.0±6.5 b	5.0±3.3 b	87.5±3.7 a
<i>Bph21(t)</i> -NIL	<i>Bph21(t)</i>	2.5±2.5 c	2.5±2.5 b	100.0±0.0 a
<i>bph20(t)</i> , <i>Bph21(t)</i> -PYL	<i>bph20(t)</i> , <i>Bph21(t)</i>	0.0±0.0 c	2.5±2.5 b	20.0±3.8 b
ADR52 (R. check)	<i>bph20(t)</i> , <i>Bph21(t)</i>	0.0±0.0 c	2.5±2.5 b	12.5±3.6 b
T65 (S. check)	no resistance gene	82.5±2.5 a	85.0±5.0 a	95.0±3.3 a

<sup>a</sup> Hatano-66, Chikugo-89, Isahaya-99 strains were collected in 1966, 1989 and 1999, respectively.

<sup>b</sup> Means (mean ± S.E.) followed by the same letter are not significantly different at  $P < 0.01$ , by the Tukey-Kramer multiple comparison test.

**Table 2.** The proportion (%) of *Nilaparvata lugens* females whose abdomen became swollen on NILs and their PYL of rice

Line	Resistance gene	<i>Nilaparvata lugens</i> strains <sup>a, b</sup>		
		Hatano-66	Chikugo-89	Isahaya-99
<i>bph20</i> (t)-NIL	<i>bph20</i> (t)	0.0±0.0 b	0.0±0.0 b	80.0±3.8 a
<i>Bph21</i> (t)-NIL	<i>Bph21</i> (t)	0.0±0.0 b	0.0±0.0 b	80.0±5.3 a
<i>bph20</i> (t), <i>Bph21</i> (t)-PYL	<i>bph20</i> (t), <i>Bph21</i> (t)	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b
ADR52 (R. check)	<i>bph20</i> (t), <i>Bph21</i> (t)	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b
T65 (S. check)	no resistance gene	80.0±3.8 a	83.0±5.9 a	90.0±3.8 a

<sup>a</sup> Hatano-66, Chikugo-89, Isahaya-99 strains were collected in 1966, 1989 and 1999, respectively.

<sup>b</sup> Means (mean ± S.E.) followed by the same letter are not significantly different at  $P < 0.01$ , by the Tukey-Kramer multiple comparison test.

Tables 3 and 4 showed that the adult survival rate and the ratio of the virulent females of the 10 Asian BPH strains on the NILs and the PYL carrying the BPH resistance genes are derived from ADR52. Based on the resistance spectrum to NILs and the PYL for *bph20*(t) and *Bph21*(t), the Asian BPH strains seem to be classified into four groups: the first group avirulent to all the tested lines, the second group virulent to the *Bph21*(t)-NIL but avirulent to the *bph20*(t)-NIL and the *bph20*(t)+*Bph21*(t)-PYL, the third group virulent to both the *bph20*(t)-NIL and the *Bph21*(t)-NIL but avirulent to the *bph20*(t)+*Bph21*(t)-PYL, and the fourth group virulent to all tested lines. The first group is the Mindanao strain, which could not adapt to any lines. The second group consisted of BPH strains from China, and Taiwan. The third group consisted of BPH strains from Japan, Northern Vietnam and two Luzon strains from the Philippines. The discrimination between the second and the third groups is still ambiguous because of differentiation among the BPH strains from China, Taiwan and Japan as well as Northern Vietnam which have never been identified. The fourth group consisted of BPH strains from Southern Vietnam, those were most virulent and half of the adult females had swollen abdomens on the PYL within 5 days. The results indicate that both of the BPH resistance genes, *bph20*(t) and *Bph21*(t), are necessary to express broad-spectrum resistance against the East Asian BPH strains. The PYL, however, had lost their resistance against the Southern Vietnam strains of BPH. Monitoring the virulence of BPH strains using the NILs and the PYLs will open the door for utilization of the BPH resistant cultivars and sustainable pest management in the Asian rice field.

Table 3. Survival rates (means  $\pm$  S.E.) of Asian *Nilaparvata lugens* strains on rice nearly isogenic lines and pyramided line

Variety	Resistance gene	Asian <i>N. lugens</i> strain*									
		Philippines AN-06	Philippines NE-B-06	Philippines CG-06	Taiwan CH-06	China FJF-06	Japan KG-06	Vietnam HT-06	Vietnam HP-06	Vietnam TGL-06	Vietnam TGH-06
<i>bph20</i> (t)-NIL	<i>bph20</i> (t)	25.7 $\pm$ 5.3 b	85.7 $\pm$ 3.4 a	60.0 $\pm$ 5.7 b	31.4 $\pm$ 8.9 b	31.9 $\pm$ 4.8 b	85.7 $\pm$ 3.4 a	94.3 $\pm$ 5.3 a	60.0 $\pm$ 4.0 b	74.3 $\pm$ 5.3 ab	88.6 $\pm$ 6.8 a
<i>Bph21</i> (t)-NIL	<i>Bph21</i> (t)	37.1 $\pm$ 2.6 b	80.0 $\pm$ 5.7 a	91.4 $\pm$ 3.7 a	85.7 $\pm$ 3.4 a	80.0 $\pm$ 4.0 a	88.6 $\pm$ 5.5 a	91.4 $\pm$ 5.5 a	94.3 $\pm$ 3.4 a	94.3 $\pm$ 3.4 a	91.4 $\pm$ 3.7 a
<i>bph20</i> (t)+ <i>Bph21</i> (t)-PYL	<i>bph20</i> (t), <i>Bph21</i> (t)	28.6 $\pm$ 7.9 b	17.1 $\pm$ 6.3 b	25.7 $\pm$ 6.7 c	28.6 $\pm$ 5.5 b	20.0 $\pm$ 4.0 b	17.1 $\pm$ 6.3 b	28.6 $\pm$ 3.7 b	25.7 $\pm$ 5.3 c	71.4 $\pm$ 6.8 b	65.7 $\pm$ 3.4 b
Taichung 65	no resistance gene	91.4 $\pm$ 5.5 a	91.4 $\pm$ 5.5 a	94.3 $\pm$ 3.4 a	97.1 $\pm$ 2.6 a	94.3 $\pm$ 3.4 a	91.4 $\pm$ 3.7 a	97.1 $\pm$ 2.6 a	94.3 $\pm$ 3.4 a	91.4 $\pm$ 5.5 ab	91.4 $\pm$ 3.7 a

\* Means followed by the same letters are not significantly different at  $P < 0.01$ , by the Tukey-Kramer multiple comparison test.

Table 4. The proportion (%) of *Nilaparvata lugens* females with swollen abdomen at five days after infestation

Variety	Resistance gene	Asian <i>N. lugens</i> strain*									
		Philippines AN-06	Philippines NE-B-06	Philippines CG-06	Taiwan CH-06	China FJF-06	Japan KG-06	Vietnam HT-06	Vietnam HP-06	Vietnam TGL-06	Vietnam TGH-06
<i>bph20</i> (t)-NIL	<i>bph20</i> (t)-NIL	0.0 $\pm$ 0.0	37.1 $\pm$ 4.8	34.3 $\pm$ 5.3	5.0 $\pm$ 0.0	6.0 $\pm$ 0.0	62.9 $\pm$ 2.6	82.9 $\pm$ 6.3	51.4 $\pm$ 5.5	68.6 $\pm$ 3.7	71.4 $\pm$ 3.7
<i>Bph21</i> (t)-NIL	<i>Bph21</i> (t)-NIL	11.4 $\pm$ 3.7	51.4 $\pm$ 3.7	77.1 $\pm$ 6.3	77.1 $\pm$ 2.6	80.0 $\pm$ 4.0	85.7 $\pm$ 5.3	71.4 $\pm$ 3.7	77.1 $\pm$ 6.3	88.6 $\pm$ 3.7	85.7 $\pm$ 3.4
<i>bph20</i> (t)+ <i>Bph21</i> (t)-PYL	<i>bph20</i> (t), <i>Bph21</i> (t)	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	48.6 $\pm$ 5.5	51.4 $\pm$ 5.5
Taichung 65	no resistance gene	88.6 $\pm$ 5.5	91.4 $\pm$ 5.5	94.3 $\pm$ 3.4	97.1 $\pm$ 2.6	94.3 $\pm$ 3.4	85.7 $\pm$ 5.3	97.1 $\pm$ 2.6	88.6 $\pm$ 5.5	80.0 $\pm$ 5.7	85.7 $\pm$ 3.4

\* The data were given as mean  $\pm$  S. E.

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