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Aspects of brown planthopper adaptation to resistant rice varieties with the *Bph3* gene

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Abstract

Despite over 30 years of deployment, varieties with the *Bph3* gene for resistance to the brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), are still effective in much of the Philippines. In the present study, we determined the effects of adaptation to one resistant variety, IR62 – assumed to possess the *Bph3* gene – on (1) resistance against a series of varieties with similar biotypical responses (presumed to contain the same major resistance genes), and (2) a differential variety with the *bph4* gene that occurs at the same chromosome position as *Bph3*. We also examined the effects of high soil nitrogen on the effectiveness of *Bph3*. Feeding, planthopper biomass, and development times were reduced in a wild BPH population when reared on IR62 compared with the susceptible standard variety TN1. However, nitrogen application increased the susceptibility of IR62. After 13 generations on IR62, BPH had adapted to the plant's resistance. Virulence of the adapted BPH against the variety 'Rathu Heenati' supports the idea that *Bph3* is present in IR62. Across similar IR varieties (IR60, IR66, IR68, IR70, IR72, and IR74), feeding, planthopper biomass, and development rates were generally higher for IR62-adapted than for non-adapted BPH; however, contrary to expectations, many of these varieties were already susceptible to wild BPH. Fitness was also higher for IR62-adapted BPH on the variety 'Babawee' indicating a close relation between *Bph3* and *bph4*. The results indicate that the conventional understanding of the genetics behind resistance in IR varieties needs to be readdressed to develop and improve deployment strategies for resistance management.

Introduction

The brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), is a major pest of rice throughout south and east Asia. Prior to the 1970s, BPH outbreaks had been largely confined to north-east Asia (Korea, Japan, China); however, with intensification of rice production during the green revolution of the 1970s and 1980s, planthoppers became a major threat to rice productivity in tropical Asia and the South Pacific (Sogawa, 1982). In response to planthopper damage at that time, the International Rice Research Institute (IRRI) produced a range of varieties with resistance to BPH (Khush, 1979; Brar et al., 2009). Resistant varieties with the *Bph1* gene derived from Mudgo were initially released in 1973, beginning with IR26, but widespread planthopper adaptation was already reported by 1975. The International Rice Research Institute responded by releasing a number of varieties (beginning with IR36 in 1976) with the *bph2* gene derived from ASD7, but again, by the early 1980s, planthopper populations had become highly adapted to these varieties (Alam & Cohen, 1998). Since 1982, a series of varieties has been released with resistance derived from PTB33 (*Bph3* and other resistance genes) and Babawee (*bph4*) (Alam & Cohen, 1998; Khush & Virk, 2005) (Table 1). Most of these are thought to possess the *Bph3* gene for resistance based on responses by laboratory BPH strains in seedbox tests (Brar et al., 2009; Jena & Kim, 2010).

Since the early 1990s, planthopper populations had been relatively low and outbreaks generally rare. However, since about 2002, outbreaks have reached an unprecedented scale and frequency throughout tropical and subtropical Asia. Hopper densities have surpassed those experienced at the height of the green revolution and major

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Table 1 Details of rice varieties evaluated for resistance against *Nilaparvata lugens* from IR62-adapted and non-adapted colonies

Variety	Origin	Year released	*Bph* gene	Extent of adoption in Philippines[1]	Predicted outcome[2]	
					Adapted	Non-adapted
IR60	IRRI Philippines	1983	*Bph3*	A preferred variety by 7% of farmers in early 1990s, declining to <1% in 2000s. Generally among the top 10 most popular varieties, especially in Mindanao	S	R
IR62	IRRI Philippines	1984	*Bph3*	Does not feature among commonly planted varieties in the Philippines	S	R
IR66	IRRI Philippines	1987	*bph4*[3]	A preferred variety by 10% of farmers in early 1990s, declining to <1% in 2000s	S	R
IR68	IRRI Philippines	1988	*Bph3*	A preferred variety by about 2% of farmers at peak adoption, declining to <1% in 2000s	S	R[4]
IR70	IRRI Philippines	1988	*Bph3*	Never exceeded 0.5% preference among farmers	S	R[4]
IR72	IRRI Philippines	1988	*Bph3*	A preferred variety by 6% of farmers at peak, <1% in 2000s	S	R[4]
IR74	IRRI Philippines	1988	*Bph3*	A preferred variety by 7.5% of farmers in 1990s, ca. 4% in 2000s; among the top six varieties for farmers in Visayas and Mindanao	S	R[4]
Rathu Heenati	Sri Lanka	Traditional	*Bph3*[5]	Not grown commercially	S	R
Taichung Native 1[6]	Taiwan	1960	None	Popular in the 1960s, this variety is now rarely grown	S	S
Babawee	Sri Lanka	Traditional	*bph4*[5]	Not grown commercially	S	R
T65-BPH25[7]	Japan	Not released	BPH25	Not grown commercially	S	S[8]
PTB33	India	Traditional	*bph2*, *Bph3*[9]	Not grown commercially	R	R

The predicted outcome of adaptation in terms of resistance against IR varieties and resistance donors is also indicated.
[1]Data for IR varieties indicate percentage of farmers from national surveys that reported a variety as commonly grown (Launio et al., 2008).
[2]Predicted susceptibility (S) or resistance (R) is based on assumed presence of *Bph3* in IR varieties and the similar positions and possibly genomic sequences of *Bph3* and *bph4* genes.
[3]As Babawee was used as a resistance donor, IR66 is suggested to contain the *bph4* gene (Khush & Virk, 2005).
[4]Varieties thought to be resistant because they are assumed to contain the *Bph3* gene, but were susceptible in this study based on planthopper fitness bioassays.
[5]*Bph3* (Rathu Heenati) and *bph4* (Babawee) have been closely mapped on the short-arm of chromosome 6 and are thought to represent the same resistance gene (Jairin et al., 2007).
[6]Cross of Dee-geo-woo-gen and Tsai-yuan-chung from 1949 (De Datta, 1981).
[7]Near-isogenic T65 line developed at Kyushu University through introgression of ADR52 with T65; ADR52 is a traditional Indian variety (Yara et al., 2010).
[8]Fujita et al. (2009).
[9]Santhanalakshmi et al. (2010).

losses have occurred in China, Thailand and Vietnam in most years (Catindig et al., 2009; Cheng, 2009). Various National Agricultural Research and Extension Systems (NARES) are again looking to host-plant resistance as a possible solution to these outbreaks (Brar et al., 2009). However, although a number of resistant varieties are currently deployed in the field, a systematic understanding of the genetics of resistance among these varieties is often lacking. Among the better understood resistance genes are *Bph1*, *bph2* and *Bph3*, which were extensively studied in the 1980s and 1990s (Sogawa & Pathak, 1970; Shigematsu et al., 1982; Velusamy & Heinrichs, 1986; Woodhead & Padgham, 1988; Stevenson et al., 1996). Breeding for introgression of these three genes probably underlies resistance in most of the current varieties deployed in Asia. This suggests that many nationally registered varieties that

are currently deemed resistant may already be ineffective against BPH. Furthermore, the popularity of IR36, IR42, and IR64, with *Bph1* and *bph2* genes, as well as a number of nationally bred lines containing the same genes (i.e., >70% of the rice area planted in Thailand and Malaysia, >80% on some islands of Indonesia, according to personal communications with NARES) will probably lead to a continuing and long-term ineffectiveness of many previously resistant varieties.

Varieties with *Bph3* have been grown in the Philippines since the early 1980s with new varieties released almost every year at least until 1988 (Khush & Virk, 2005; Launio et al., 2008) (Table 1). Initially, these releases included a series of varieties bred at IRRI. After 1988, all varieties released in the Philippines were given a Philippine Seed Board-Rice (PSB Rc) designation, irrespective of the institute that developed the variety. Some of these PSB varieties also possessed the *Bph3* gene. It is difficult to estimate the extent of planting of *Bph3*-varieties in the Philippines; surveys conducted between 1992 and 2002 indicate that about 30% of farmers preferred varieties that contained *Bph3* (although not necessarily because of the BPH resistance trait). Individual IR varieties with *Bph3* were normally favoured by 5–10% of farmers at peak adoption, but this decreased to <1% by the early 2000s (Table 1), when they were largely replaced by PSB Rc10 and PSB Rc18 (Launio et al., 2008). In screening studies at IRRI, these latter varieties were susceptible to biotype 3 (IRRI-International Rice Information System), indicating that they are not likely to contain the *Bph3* gene. In 2009, preliminary bioassays with several BPH populations from throughout the Philippines indicated that IR62, which contains *Bph3*, was still highly resistant (FG Horgan, unpubl.). This suggests that the *Bph3* gene may be more durable than were either *Bph1* or *bph2*. However, a generally low incidence of BPH in the Philippines since the 1990s and the relatively large area under susceptible varieties (including those with *Bph1* and *bph2*) suggest that the pressure of selection for virulence against *Bph3* has been low.

Planthopper colonies reared continuously on a single resistant variety have been shown to overcome resistance within 10–20 generations (Pathak & Heinrichs, 1982; Alam & Cohen, 1998; Ketipearachchi et al., 1998). The rate of adaptation differs between rice varieties, but tends to be consistent among BPH populations (Alam & Cohen, 1998). The presence or absence of undetermined resistance genes or quantitative trait loci (QTLs) may affect the rate of adaptation to different varieties that share a major resistance gene. For example, studies with IR64 and IR26, both of which contain the *Bph1* gene, suggest that IR64 has been more durable, because it also contains a major resistance QTL (Cohen et al., 1997; Alam & Cohen, 1998). Successive rearing on IR72 indicates that BPH can adapt to *Bph3* resistance in about 11 generations (Alam & Cohen, 1998), but it remains unknown as to how adaptation to this variety would affect other IR varieties with the same major resistance gene. Furthermore, recent evidence suggests that *Bph3* and *bph4* may share the same genomic sequence or resistance mechanisms: dominance or recessiveness of *bph4* depends on the genetic background of the plant, and hence, when introgressed with TN1, *bph4* behaves as a recessive gene, but when introgressed with KDML105, it behaves as a dominant gene (Jairin et al., 2007). This suggests that adaptation to *Bph3* varieties may also bestow virulence against *bph4* resistance. Understanding the nature and consequences of BPH adaptation to varieties with the *Bph3* gene could help predict requirements for resistance breeding under the current high BPH densities and improve varietal deployment strategies for sustainable BPH management.

The present study examines the hypothesis that planthopper adaptation to a variety with a major resistance gene results in the breakdown of resistance in varieties with the same gene, but this is influenced by the genetic background of each variety. We predicted that BPH reared for several generations on IR62 would have improved fitness on a series of varieties with the *Bph3* gene. However, because of the complicated pedigrees of IR varieties, we expected that some varieties may remain relatively resistant because of undetermined resistance genes and QTLs. Furthermore, the study addresses the possible shared genomic sequence of *Bph3* and *bph4*; we predicted that adaptation to *Bph3* would result in the breakdown of resistance in varieties with the *bph4* gene. We therefore examined the fitness of *Bph3*-adapted planthoppers on Babawee, a *bph4* donor variety, and compared reactions against a japonica line with the BPH25-gene, which is closely located to *Bph3* and *bph4* on the short-arm of chromosome 6 and possibly shares the same resistance mechanisms, but not the same sequence (BPH adapted to BPH25 are not virulent against *Bph3* or *bph4*; Fujita et al., 2009). We also examined the stability of resistance of IR62 under varying fertilizer (nitrogen) levels as an indication of how crop management decisions might compromise resistance durability. We predicted that IR62 would be less resistant under high-nitrogen conditions as BPH fitness is known to increase on high-nitrogen plants (Lu & Heong, 2009).

Materials and methods

Planthopper colonies

Planthoppers were collected from rice paddies in Laguna (14°10′N, 121°13′E), Philippines, during June 2009. The

Department of Laguna in South Luzon is a major irrigated rice-growing region with a high turnover of varieties (Launio et al., 2008). Planthoppers in the region are strongly virulent against *Bph1* and *bph2* (FG Horgan, unpubl.), and BPH25 (Fujita et al., 2009). Several resistant varieties with the *Bph3* gene have been grown in the region since the early 1980s, including all of the IR varieties examined in the present study. In Luzon, IR74 has been the most popular variety with *Bph3*, but has never exceeded 10% of the total rice-growing area. Since the early 2000s, IR varieties have not been widely planted in the region (Philippine Department of Agriculture, unpubl.). Evidence suggests that varieties with the *Bph3* and *bph4* genes have never exceeded 30 and 10% of the rice-growing area in Luzon respectively (Launio et al., 2008). Therefore, the Laguna BPH population was expected to have low virulence against *Bph3* and *bph4*. This was borne out in unpublished preliminary screening tests. The initial BPH population was reared on Taichung Native 1 (TN1), which is a highly susceptible rice variety. The founder population (>500 individuals) was placed in a wire mesh cage of 120 × 60 × 60 cm (h × w × l) under greenhouse conditions (temperatures ranged from 25 to 45 °C). The colony was continually fed ≥30-day-old TN1 plants. At the second generation, a group of hoppers (ca. 500) was taken from the main cage and placed on IR62 in a similar second cage. These were reared on IR62 for 13 generations (henceforth the 'adapted' or 'IR62-adapted' colony). A second colony was maintained on TN1 ('non-adapted' colony). Feeding plants for both colonies were replaced every 2 weeks.

Plant material

Seed of all varieties used in this study were acquired from the IRRI genebank. TN1 was used as a susceptible control, IR62 as a host variety for adaptation, and IR60, IR66, IR68, IR70, IR72, and IR74 were used as varieties with common biotypical responses [i.e., all were found resistant to biotypes 1–3 at IRRI (Khush & Virk, 2005)]. Rathu Heenati was included in the experiments, because it is a donor plant for *Bph3*. The highly resistant Indian variety PTB33 was used as the potential resistance donor for IR60, IR62, IR68, IR70, IR72, and IR74, and is suggested to contain the *Bph3* gene along with other resistance genes (Sidhu & Khush, 1978; Santhanalakshmi et al., 2010) (Table 1); it was used here as a resistance check. Babawee (*bph4*) was acquired from the IRRI genebank as a differential variety known to contain the *bph4* gene (Jairin et al., 2007). A japonica line (T65) introgressed with the BPH25 gene locus (henceforth T65-BPH25) was acquired from the Plant Genetics Department of Kyushu University, Japan. The *Bph3*, *bph4*, and BPH25 genes are all located on the short-arm of chromosome 6 (Jairin et al., 2007; Yara et al., 2010). Rathu Heenati, Babawee and PTB33 are traditional varieties from South Asia and are not grown in the Philippines. T65-BPH25 is not available to farmers, and the BPH25 gene has not been found in any monogenic varieties [it was identified in ADR52 together with the BPH26 gene (Yara et al., 2010)] (Table 1). Plants were seeded in saturated garden soil in the greenhouse and transplanted to #0 pots (6.5 cm diameter) at 7–10 days after sowing. Plants were watered daily and, unless indicated, were given no further nitrogen and received no chemical treatments.

Adaptation to IR62 and effects of soil nitrogen

Planthoppers from the adapted (IR62) and non-adapted (TN1) colonies were examined for their responses to IR62 under two nitrogen regimes: zero nitrogen added and nitrogen equivalent to 150 kg ha^{-1} added. Responses were compared against TN1 as a susceptible check. Three separate bioassays were conducted. The experimental unit for each bioassay consisted of a single potted plant of each variety under low and high-nitrogen and infested with adapted or non-adapted BPH. Each bioassay was replicated six times.

Bioassay 1 – Honeydew test. One gravid female was confined on each 14-day-old plant in a 5 × 5 cm plastic container (h × diameter), with a hole in the base and top through which the plant was passed. When in place, the top hole was closed using a cotton plug. A Whatman no. 1 filter paper, dipped in a solution of bromocresol green was placed at the base of the container. Bromocresol green indicates phloem-based honeydew as blue-rimmed spots, but also allows xylem-feeding to be distinguished, because this appears as white, non-rimmed spots. Following 2 h of starvation, the female planthoppers were allowed to feed for 24 h, after which the filter papers were collected and the area of each spot was measured using a digital scanner and ImageJ v. 1.48 software (National Institute of Health, Bethesda, MD, USA). Tests were conducted on a laboratory bench at 25 °C.

Bioassay 2 – Nymphal survival. Newly emerged nymphs were collected from the colonies and placed on 14-day-old rice plants of each variety and at each nitrogen level (10 nymphs per plant). The nymphs and plants were contained in 45 cm tall and 5 cm wide mylar cages and placed in a greenhouse at ambient light and temperature regimes (see above). Planthoppers were allowed to feed and develop for 15 days, after which the number of survivors and their developmental stages were recorded. The survivors were then dried and weighed.

Bioassay 3 – Population build-up. Following the honeydew tests (described above), the plastic confinement containers were removed and replaced with plastic 45 cm high and 5 cm wide mylar cages that enclosed the entire plant. The plants and gravid females were moved to a greenhouse under natural light and ambient temperatures (fluctuating between 25 and 45 °C). Plants were tended for 20 days, after which the condition of the plants and the numbers and developmental stages of the planthoppers were recorded. The planthoppers from each plant were collected, dried, and weighed, and the plants were cut at the base, dried, and weighed.

Resistance of IR varieties

Plants of IR60, IR62, IR66, IR68, IR70, IR72, and IR74 were grown in the greenhouse. These varieties are suggested to contain either the *Bph3* or *bph4* (IR66) genes (Table 1). However, there is some confusion over the gene in IR66 (Brar et al., 2009; Jena & Kim, 2010). Therefore, IR66 was included here as a variety with the same biotypical response as IR62. The differential variety Rathu Heenati was included in the experiment because of the confirmed presence of the *Bph3* gene (Jairin et al., 2007). Bioassays 1–3 were conducted on these plants as described above. The experimental unit for each bioassay consisted of a single potted plant of each variety infested by adapted or non-adapted BPH. All bioassays were replicated 10 times for each variety, with TN1 as a susceptible check.

Resistance of differential varieties with *bph4* and BPH25

Plants of Babawee, T65-BPH25, IR62, PTB33, and TN1 were grown in a greenhouse. Babawee was used as a differential variety with confirmed presence of *bph4* (Jairin et al., 2007), T65-BPH25 was used as a differential line with confirmed presence of BPH25 closely positioned on the same chromosome as *Bph3* and *bph4*, and PTB33 was used as a donor variety deemed to contain the *Bph3* and *bph2* genes as well as other resistance QTLs (Santhanalakshmi et al., 2010). Bioassays 1–3 were conducted on these plants as described above. The experimental unit for each bioassay consisted of a single potted plant of each variety infested by adapted or non-adapted BPH. All bioassays were replicated 10 times with TN1 as a susceptible check.

Statistical analysis

Some susceptible plants (TN1 and T65-BPH25) were severely affected by hopper feeding and began to show signs of hopperburn. Many of these plants had reduced biomass or died during the bioassays. Where plants were highly stressed during bioassays 3 (i.e., bioassay duration ≥15 days), planthopper fitness parameters were also affected. As a result of this, results from bioassay 3 for plants at different nitrogen levels and for differential lines are not presented (Figures 2 and 4).

Planthopper feeding-responses to rice varieties were analysed using multivariate analysis of variance (MANOVA) with 'area of phloem-spots produced' and 'area of xylem-spots produced' as dependent variables, and 'colony', 'variety', and 'nitrogen level' (where applicable) as independent factors. Planthopper population build-up and nymphal survival were each analysed as three separate tests. Planthopper number per plant, proportion of surviving nymphs per plant and total planthopper biomass per plant were analysed using a generalized linear model with colony, variety and nitrogen level (where applicable) as independent factors, and plant biomass was included as a covariate. Development was analysed using MANOVA with proportions of hoppers in each instar as dependent variables and colony, variety and nitrogen levels (where applicable) as the independent factors. Residuals were plotted following all analyses and were found to be normal and homogeneous. All statistical analyses were conducted using SPSS Version 14.0 (Carver & Nash, 2006).

Results

Adaptation to IR62 and effects of soil nitrogen

Bioassay 1. Relative honeydew areas (phloem and xylem) were affected by host-plant variety ($\lambda_{2,39} = 0.376$, $P<0.001$) and BPH colony ($\lambda_{2,39} = 0.759$, $P = 0.005$) (Figure 1). Nitrogen had no effect and there were no significant interactions ($0.896 \leq \lambda_{2,39} \leq 0.999$). Phloem-spots associated with TN1 ($F_{1,40} = 6.54$, $P = 0.014$) and the IR62-adapted colony ($F_{1,40} = 11.95$, $P = 0.001$) were larger; however, there was a near-significant variety*colony interaction ($F_{1,40} = 3.78$, $P = 0.059$) because non-adapted hoppers produced smaller phloem-spots on IR62 than on TN1 (Figure 1C and D). In contrast, xylem-spots associated with IR62 ($F_{1,40} = 64.63$, $P<0.001$) were larger than spots from TN1, irrespective of colony ($F_{1,40} = 1.95$, $P = 0.17$) (Figure 1).

Bioassay 2. TN1 plants were larger than IR62 plants ($F_{1,40} = 5.73$, $P = 0.021$), were larger where nitrogen fertilizer was added ($F_{1,40} = 85.36$, $P<0.001$), but were smaller after feeding by adapted planthoppers ($F_{1,40} = 7.92$, $P = 0.008$) causing a significant colony*variety interaction ($F_{1,40} = 4.98$, $P = 0.031$). All other interactions were non-significant (Figure 2A–D). There were no main factor effects on nymphal survival ($0.051 \leq F_{1,35} \leq 1.281$) (Figure 2E–H). There was a significant colony*nitrogen interaction ($F_{1,35} = 3.78$, $P = 0.044$) because survival of non-adapted planthoppers decreased overall with increas-

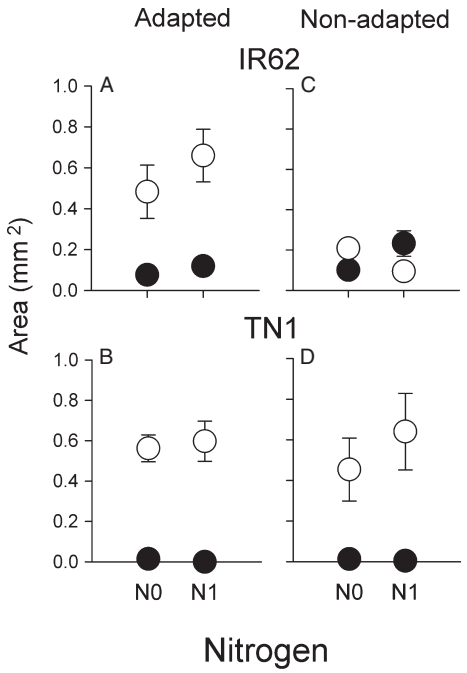

Figure 1 Mean (± SE; n = 6) honeydew production (mm^2 spot area, see Materials and methods for details) on (A, C) IR62 and (B, D) TN1 rice varieties, by (A, B) IR62-adapted and (C, D) non-adapted *Nilaparvata lugens*. Open circles = phloem; solid circles = xylem. Plants were 14 days old and grown with (N1) or without (N0) added fertilizer. Bioassays were conducted at 20 °C.

ing nitrogen (Figure 2F and H), whereas high-nitrogen levels caused an increase in survival of adapted planthoppers (Figure 2E and G). Planthopper biomass on TN1 ($F_{1,40}$ = 32.07, P<0.001) and for the IR62-adapted colony ($F_{1,40}$ = 23.40, P<0.001) was higher, and there was a significant variety*colony interaction ($F_{1,40}$ = 7.84, P = 0.008), because the biomass of non-adapted planthoppers was lower on IR62 than on TN1 (Figure 2I–L). Nitrogen level had no effect on biomass ($F_{1,40}$ = 3.06, P = 0.088), but there was a significant variety*nitrogen interaction ($F_{1,40}$ = 4.16, P = 0.048) (Figure 2I and J). Planthopper development was significantly more advanced on TN1 ($\lambda_{4,37}$ = 0.676, P = 0.005), at the higher nitrogen level ($\lambda_{4,37}$ = 0.682, P = 0.006), and for the IR62-adapted colony ($\lambda_{4,37}$ = 0.216, P<0.001) (Figure 2M–P). There were also significant variety*nitrogen ($\lambda_{4,37}$ = 0.744, P<0.024) and variety*nitrogen*colony interactions ($\lambda_{4,37}$ = 0.771, P<0.042), mainly because development was delayed for the IR62-adapted colony on high-nitrogen TN1, but increased with nitrogen addition in all other cases.

Bioassay 3. Many of the TN1 plants had symptoms of herbivore-related stress and hopperburn. Plant biomass was generally lower for TN1 ($F_{1,31}$ = 3.23, P = 0.082) causing BPH populations to decline on TN1 before the end of the experiment (Figure 2S and T). Biomass of both varieties increased with nitrogen fertilizer ($F_{1,31}$ = 8.57, P = 0.006) and was generally lower where IR62-adapted hoppers had fed ($F_{1,31}$ = 4.84, P = 0.035) (Figure 2Q–T). Based on the poor quality of the host material at the end of the bioassay, results for TN1 were eliminated from further analyses.

The numbers and biomass of BPH progeny from IR62-adapted females were higher on IR62 than those from non-adapted females; however, differences were not significant (number of planthoppers: colony: $F_{1,17}$ = 2.76, P = 0.12; nitrogen: $F_{1,17}$ = 0.231, P = 0.64; interaction: $F_{1,17}$ = 0.109, P = 0.75; total planthopper biomass: colony: $F_{1,17}$ = 0.048, P = 0.83; nitrogen: $F_{1,17}$ = 2.337, P = 0.15; interaction: $F_{1,17}$ = 0.197, P = 0.66) (Figure 2U–X). The relative proportions of life-stages on IR62 were affected by colony. However, unexpectedly, life-stages of non-adapted planthoppers were most advanced ($\lambda_{4,14}$ = 0.418, P = 0.011). Nitrogen had no apparent effect on development ($\lambda_{4,14}$ = 0.864, P = 0.70), and interactions between nitrogen levels and other factors were non-significant (Figure 2Y and Z).

Resistance of IR varieties

Bioassay 1. The relative amounts of xylem- and phloem spots produced were affected by variety ($F_{1,138}$ = 36.93, P<0.001) and hopper colony ($\lambda_{2,137}$ = 0.686, P<0.001) with no significant interaction (Figure 3A and B). The production of xylem spots was significantly higher for hoppers from the non-adapted colony ($F_{1,138}$ = 49.71, P<0.001), but was not affected by rice variety ($F_{8,149}$ = 1.098, P = 0.37) or interactions. In contrast, the production of phloem spots was higher for planthoppers from the IR62-adapted colony ($F_{1,138}$ = 36.93, P<0.001) and differed between host-plants ($F_{8,138}$ = 7.35, P<0.001). Significantly fewer phloem spots (and a smaller area) were produced when feeding on Rathu Heenati and more on TN1 compared with all other varieties (Tukey test: P<0.05) (Figure 4A and B).

Bioassay 2. Plant biomass among the nine varieties was not affected by colony-adaptation ($F_{1,149}$ = 0.093, P = 0.76) at the end of the nymphal survival experiment, but plant size differed among varieties ($F_{8,149}$ = 8.431, P<0.001). The interaction term was non-significant (Figure 3C and D). Nymphal survival was not affected by variety ($F_{8,153}$ = 1.042, P = 0.41), colony ($F_{1,153}$ = 2.625, P = 0.11), or their interaction (Figure 3E and F); variety

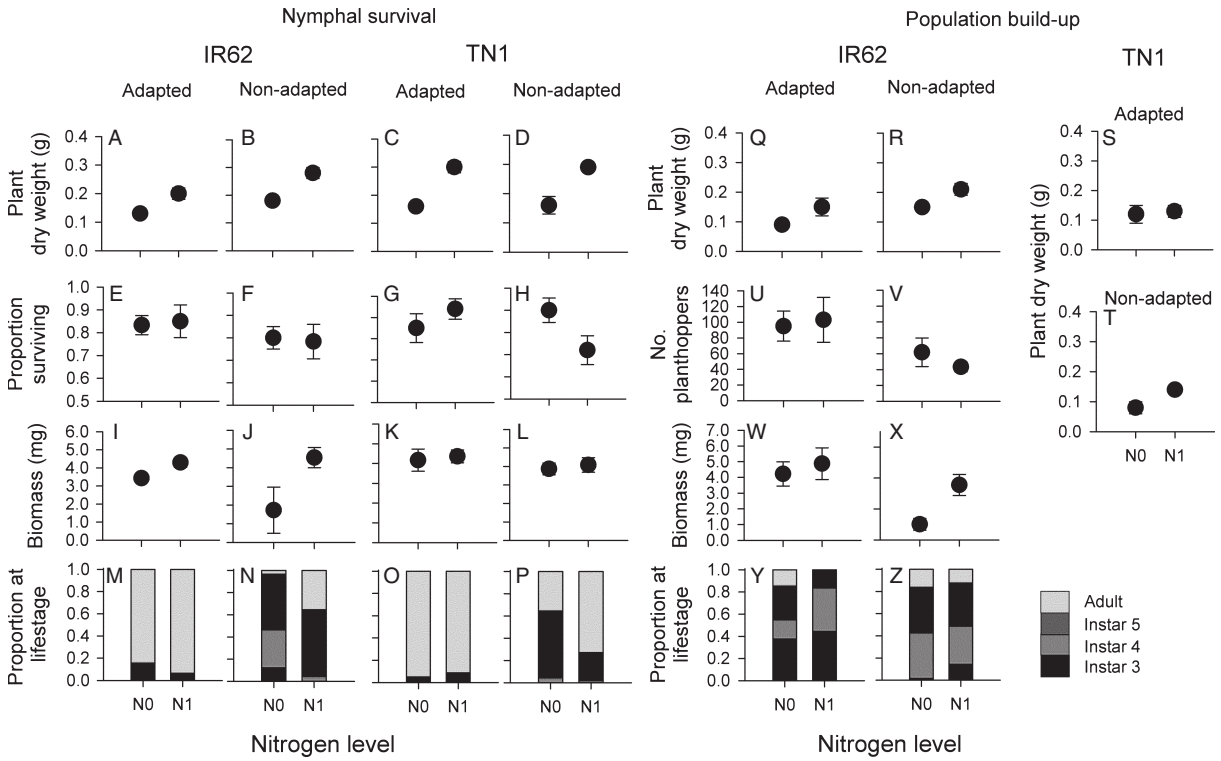

Figure 2 Results (Mean ± SE; n = 10) from (A–P) nymphal survival bioassay and (Q–Z) population build-up bioassay with adapted and non-adapted *Nilaparvata lugens* on IR62 and TN1 rice varieties. Host-plants received one of two nitrogen regimes: N0 = no nitrogen added, or N1 = 150 kg ha^{-1} nitrogen equivalent added. (A–D) Biomass of plants (g dry weight) at the end of the survival experiment (i.e., 15 days). (E–H) Proportion, (I–L) biomass (mg), and (M–P) development stages of surviving nymphs after 15 days. (Q–T) Biomass of plants (g dry weight) after attack by planthoppers. As a result of heavy feeding damage to TN1 plants (S, T), further data were not collected from bioassay 3 with TN1. (U, V) Numbers of nymphs, (W, X) population biomass (mg), and (Y, Z) development stages encountered after 20 days on plants that were infested with one gravid female. Bioassays were conducted in a greenhouse (25–45 °C).

($F_{8,151}$ = 9.232, P<0.001), but not colony ($F_{1,151}$ = 0.300, P = 0.59) affected the total planthopper biomass; however, the covariate plant biomass had a significant effect ($F_{1,151}$ = 4.068, P = 0.045). The interaction term was non-significant (Figure 3G and H). The relative proportions of planthopper life-stages among the surviving nymphs was affected by variety ($\lambda_{16,555}$ = 0.418, P<0.001), colony ($\lambda_{2,150}$ = 0.661, P<0.001) and their interaction ($\lambda_{16,555}$ = 0.520, P<0.001). Development of non-adapted nymphs on Rathu Heenati was notably delayed, and none of the nymphs advanced to second instar on this variety (Figure 3J). A significant interaction occurred because of faster development of IR62-adapted planthoppers on some varieties (IR60, IR62, IR66, and Rathu Heenati), but not on others (IR68, IR70, IR72, IR74, and TN1) compared with the non-adapted planthoppers and based on Tukey pairwise comparisons (Figure 3M and N).

Bioassay 3. Plant biomass was affected by variety ($F_{8,100}$ = 6.368, P<0.001), colony ($F_{1,100}$ = 7.966, P = 0.006), and their interaction ($F_{8,100}$ = 2.175, P = 0.036). IR62 and IR68 plants were significantly larger than all other varieties at the end of the experiment after being fed by planthoppers from both colonies (Tukey tests: P<0.05) (Figure 3K and L). Plants tended to be smaller after being fed by IR62-adapted planthoppers (Figure 3K). The numbers of planthoppers on the plants was not affected by variety ($F_{8,99}$ = 1.834, P = 0.080), colony ($F_{1,99}$ = 0.522, P = 0.47), or their interaction; however, the covariate, plant biomass, had a highly significant effect ($F_{1,99}$ = 10.883, P = 0.001) (Figure 3M and N). Variety affected total planthopper biomass ($F_{1,100}$ = 4.137, P<0.001); biomass was lowest on IR60 and highest on IR74 (Tukey tests, significant differences from all other varieties at 0.05 level). Brown planthopper biomass was not affected by colony ($F_{1,100}$ = 2.283, P = 0.13) and there was no significant interaction. Development was not affected by colony ($\lambda_{2,96}$ = 0.917, P = 0.13), but was affected by variety ($\lambda_{16,421}$ = 0.418, P<0.001) and their interaction ($\lambda_{16,421}$ = 0.569, P = 0.041). A significant

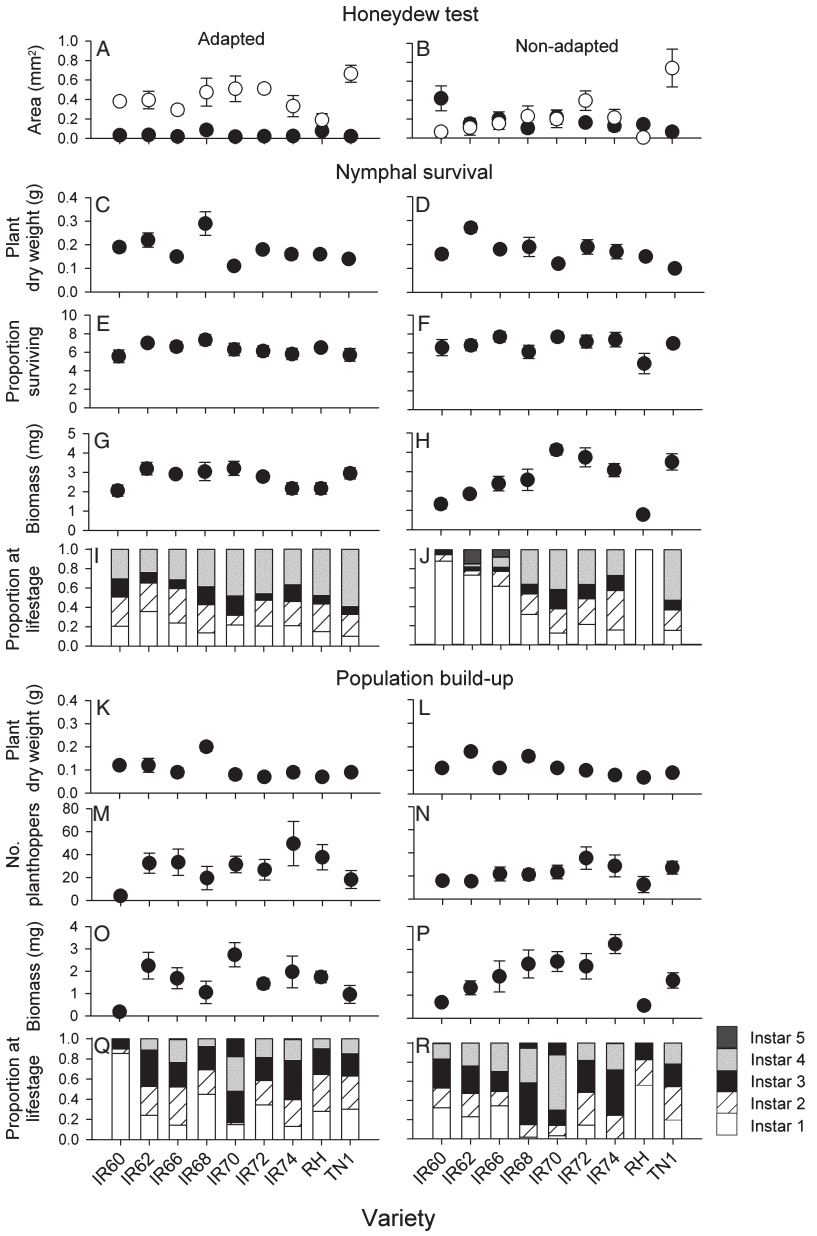

Figure 3 Mean (± SE; n = 10) fitness measures for adapted and non-adapted *Nilaparvata lugens* on IR rice varieties resistant to IRRI-biotype 3. TN1 was used as a susceptible control. (A, B) Honeydew production (mm^2 spot area, see Materials and methods for details; open circles = phloem; solid circles = xylem). (C, D) Biomass of plants (g dry weight) at the end of the survival experiment. (E, F) Proportion, (G, H) biomass (mg), and (I, J) development stages of surviving nymphs. (K, L) Biomass of plants (g dry weight) after bioassay 3. (M, N) Numbers of nymphs, (O, P) population biomass (mg), and (Q, R) development stages encountered after 20 days on plants that were infested with one gravid female. Bioassays were conducted in a greenhouse (25–45 °C).

interaction occurred because of slower development of IR62-adapted planthoppers on IR60 and faster development on Rathu Heenati compared with the non-adapted planthoppers and based on Tukey pairwise comparisons (Figure 3Q and R).

Resistance of differential varieties with *bph4* and BPH25

Bioassay 1. The relative amounts of xylem and phloem-spots produced were affected by variety ($\lambda_{8,148}$ = 0.643, P<0.001), colony ($\lambda_{2,74}$ = 0.805, P<0.001), and their interaction ($\lambda_{8,148}$ = 0.756, P = 0.007) (Figure 4A and B). The

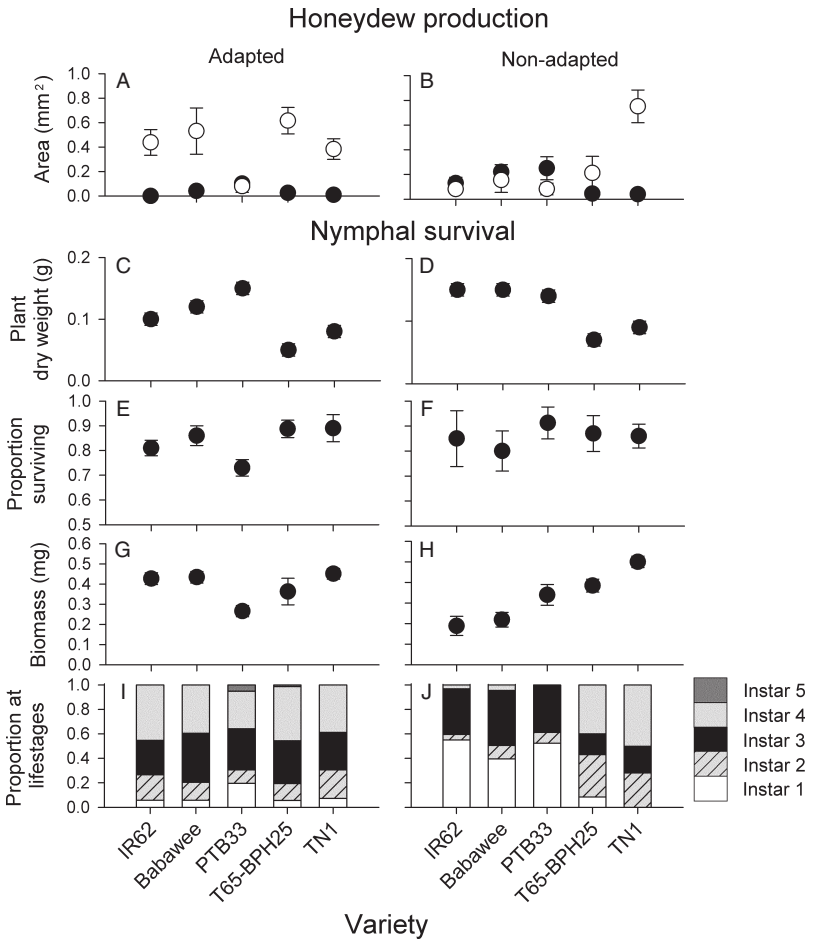

Figure 4 Mean (± SE; n = 10) fitness measures for adapted and non-adapted *Nilaparvata lugens* on differential rice varieties. TN1 was used as a susceptible control. (A, B) Honeydew production (mm^2 spot area, see Materials and methods for details; open circles = phloem; solid circles = xylem). (C, D) Biomass of plants (g dry weight) after 15 days of feeding. (E, F) Proportion, (G, H) biomass (mg), and (I, J) development stages of surviving nymphs. The corresponding bioassay 3 is not included because of high mortality of plants during the experiment. Bioassays were conducted in a greenhouse (25–45 °C).

largest xylem-spots were produced on PTB33 and the smallest on TN1 (variety, $F_{4,75} = 2.998$, $P = 0.024$; Tukey tests: $P<0.05$) and by the non-adapted colony ($F_{1,75} = 13.83$, $P<0.001$). The interaction term was non-significant. The largest phloem-spots were associated with T65-BPH25 and TN1, and the smallest with PTB33 (variety, $F_{4,75} = 8.663$, $P<0.001$; Tukey tests: $P<0.05$). Phloem-spots were larger for the IR62-adapted colony ($F_{1,75} = 10.37$, $P = 0.002$) and the interaction was significant, because phloem spot production by the IR62-adapted planthoppers was high on Babawee, T65-BPH25 and IR62, but low by non-adapted planthoppers on these plants ($F_{4,75} = 5.182$, $P = 0.001$) (Figure 4A and B).

Bioassay 2. In the nymphal survival experiment, final plant biomass was affected by variety ($F_{4,86} = 24.46$, $P<0.001$) and colony ($F_{1,86} = 6.56$, $P = 0.012$). As a result of planthopper feeding, plants of T65-BPH25 and TN1 were generally smaller (Figure 4C and D). There was also a significant interaction ($F_{4,86} = 4.87$, $P = 0.001$) because IR62 and Babawee plants were smaller when fed by IR62-adapted planthoppers than by non-adapted planthoppers. Nymphal survival was not affected by variety ($F_{4,86} = 0.374$, $P = 0.83$), colony ($F_{1,86} = 40.331$, $P = 0.57$) or their interaction (Figure 4E and F). Final planthopper biomass was affected by variety ($F_{4,86} = 10.22$, $P<0.001$) and colony ($F_{1,86} = 12.026$, $P = 0.001$). Nymphal biomass was significantly lower on IR62 and PTB33 due to poor weight-gain of non-adapted planthoppers only, thus producing a significant interaction ($F_{4,86} = 11.537$, $P<0.001$) (Figure 4G and H). The relative proportions of planthoppers at each life stage were affected by variety ($\lambda_{20,272} = 0.417$, $P<0.001$),

colony ($\lambda_{5,82} = 0.481$, $P<0.001$), and their interaction ($\lambda_{20,272} = 0.346$, $P<0.001$). Development was significantly slower for the non-adapted colony on Babawee, IR62, and PTB33 than for the IR62-adapted colony (higher proportions of first instars, few fifth instars and adults; Tukey tests: $P<0.05$) (Figure 4I and J).

Bioassay 3. There was high mortality of founder females and a low biomass of surviving plants at the end of the experiment, irrespective of colony. For this reason, the results from the population build-up experiment were not further analysed.

Discussion

Varieties with the *Bph3* gene for resistance have been grown in the Philippines for over 3 decades. Despite the rapid adaptation by field populations of BPH to previous resistance genes (*Bph1* and *bph2*), varieties with the *Bph3* gene continue to be effective against Philippine BPH populations. The present study supports the premise that the resistant variety IR62 contains the *Bph3* gene. A BPH colony reared for successive generations on IR62 adapted to the variety and had cross-virulence against Rathu Heenati, a differential variety for which the *Bph3* gene has been genetically mapped. The study confirms that the resistance of IR62 declined under high soil nitrogen, but still remained apparently more resistant than the susceptible standard TN1. A series of bioassays with both IR62-adapted and non-adapted planthoppers on a range of IR varieties supported the hypothesis that the degree of cross-virulence on varieties that possess the same resistance gene is influenced by the plant's background genetics. Among seven IR varieties that contained either the *Bph3* or *bph4* genes, resistance to a South Luzon BPH population varied considerably. Several BPH fitness measures were similar on these varieties when compared with the susceptible standard TN1. This indicates that the nature of resistance and the consequences of resistance breakdown across the varieties are not as clear-cut as in previous examples (*Bph1* and *bph2*). Finally, the study presents evidence that *Bph3* and *bph4* are similar, if not the same genes. Adaptation to *Bph3* in IR62 allowed BPH to successfully feed and reproduce on Babawee, a differential variety for which the *bph4* gene has been genetically mapped. Furthermore, the BPH25 gene, which is closely positioned to *Bph3* and *bph4*, was less effective against South Luzon BPH than previously reported.

Brown planthopper adaptation and soil nitrogen levels

The BPH has an enormous capacity to adapt to sustained biotic and abiotic stresses. In addition to overcoming rice resistance (Cohen et al., 1997; Alam & Cohen, 1998; Fujita et al., 2009; Myint et al., 2009; Seo et al., 2009), BPH populations have adapted to many chemical insecticides (Nagata & Kamimuro, 2002) and can survive under a range of climatic conditions (Wada et al., 2007, 2009). The results of the present study indicate that planthoppers reared during successive generations on IR62 had higher feeding success, were generally larger and developed more rapidly on IR62 than planthoppers that had not adapted to the plant. Similar responses have been noted in previous virulence selection studies (Pathak & Heinrichs, 1982; Alam & Cohen, 1998; Ketipearachchi et al., 1998). Many of the bioassays conducted in the present study indicate that the overall fitness of the adapted individuals was greater than non-adapted individuals even on TN1, a highly susceptible host-plant. Despite precautions taken, it is possible that a low tolerance to herbivory stress in TN1 could have affected some of the fitness measures, as planthopper responses to resistant plants are often difficult to distinguish from the effects of confining herbivores to stressed or dying plants. In particular, the results of honeydew tests were often poorly correlated with those of other bioassays. To avoid high variability, we recommend that in future comparative studies infestation densities should be kept low. Nevertheless, although variability could be reduced in some bioassays, the main effects were generally consistent across the bioassays in this study.

Fertilizers, in particular, nitrogen, improve BPH fitness on rice plants (Lu et al., 2004; Lu & Heong, 2009). Lu & Heong (2009) describe highly positive correlations between the levels of nitrogen in rice tissues and several BPH fitness parameters (nymphal survival, female weight, eggs laid, adult longevity, and egg hatchability), and negative correlations between nymphal duration and plant nitrogen content. In effect, rice susceptibility to BPH is dramatically increased under high-nitrogen conditions. Sogawa (1982) suggested that this is due to the high availability of amino acids, particularly asparagine, in the rice phloem. Asparagine stimulates BPH feeding (Sogawa, 1982). In the present study, we found that the susceptibility of IR62 also increases under high-nitrogen conditions, despite the presence of the *Bph3* gene. In particular, nymph biomass increased under high-nitrogen to levels that were on par with the susceptible standard TN1. Changes in other fitness parameters (i.e., honeydew production and nymphal development times) were less dramatic. Recent evidence suggests that the magnitude of loss of resistance under high-nitrogen conditions is dependent on plant age; young plants (as in the present study) are more heavily affected than older plants (A Peñalver, unpubl.). The effects of soil nitrogen on the efficiency and durability of resistance in rice requires further study. The possibility that resistance is compromised by high soil nitrogen suggests that the

breakdown of resistance genes may be accelerated under poor nutrient management. It also suggests that varieties with a greater capacity to absorb nitrogen and with normally higher concentrations of free asparagine or other amino acids in the phloem may be more susceptible to BPH, despite the presence of major resistance genes.

Bph3 varieties in the Philippines

Brown planthopper fitness responses to resistant plants have been used to designate populations as distinct, recognizable 'biotypes' (Claridge & den Hollander, 1980; Sogawa, 1981; Pathak & Heinrichs, 1982; Khan & Saxena, 1990; Ito et al., 1994; Takahashi et al., 1994). Laboratory-reared biotypes were used to determine resistance levels and the genetic mechanisms of resistance in IR and other varieties (Khush & Virk, 2005; Brar et al., 2009; Jena & Kim, 2010). The varieties examined here were all resistant to IRRI's biotype 1 (wild populations), biotype 2 (virulent against *Bph1*) and biotype 3 (virulent against *bph2*), thus indicating that the varieties derive their resistance from sources other than *Bph1* and *bph2*. As PTB33 or Babawee were used as resistance donors, it has been assumed that these varieties possess the *Bph3* or *bph4* genes for resistance (Brar et al., 2009; Jena & Kim, 2010). This needs to be verified using genetic markers; however, specific PTB33 and Babawee markers are not available and IR pedigrees are generally too complicated for any currently available markers to effectively determine resistance sources. As indicated in the pedigrees presented by Khush & Virk (2005), the resistance of IR varieties could be derived from several donors that were used during breeding, including the wild species *Oryza nivara*, S.D. Sharma & Shastry. Overall, this suggests that the genetics of resistance for IR varieties thought to contain *Bph3* and *bph4* genes are likely to be more complicated than previously thought and, taken together, these varieties potentially include several resistance genes and QTLs. This idea is supported by the results from the present study which indicate that, across the series, resistance based on two genes alone (*Bph3* or *bph4*) is unlikely. For example, BPH responses to IR62 and IR66 were notably different from responses to IR60 which has a more stable resistance. Nevertheless, the results of this study clearly indicate that resistance in IR62 and IR66 is largely determined by *Bph3* or a closely related gene, such as *bph4*.

The susceptibility of several IR varieties to wild-caught, non-adapted BPH was surprising. We had predicted that IR70, IR72 and IR74 would be resistant to the South Luzon BPH population. This prediction was based on observations of high resistance in IR62 and Rathu Heenati prior to commencing the present study, and because these three varieties are presumed to contain the *Bph3* gene. The susceptibility of these varieties may indicate that the *Bph3* gene is not actually present in the varieties and that resistance evaluation during breeding selection was flawed. However, the result may also indicate that the *Bph3* function in these varieties is diminished because of interactions with other genes, or that the more stable *Bph3* resistance in IR62 and Rathu Heenati is supported by other genes present in both their genomes. Exposure of all seven IR varieties in the Philippines has been relatively low. IR74 has been among the most popular of the varieties, but was never grown over large areas (Table 1), meanwhile IR68 and IR70 have never been popular among Philippine farmers. Therefore, although a local adaptation to IR74 is possible, large-scale variety-specific adaptation by BPH to IR68 and IR70 is unlikely. Responses by planthoppers to IR60 were also notably distinct; in particular, population build-up was severely retarded on this variety and xylem-feeding was significantly higher than observed in any of the other varieties. Furthermore, adaptation to IR62 did not cause an appreciable decline in the resistance of IR60 suggesting that this variety may have additional resistance that specifically affects oviposition and induces xylem-feeding. IR60 is still popular in some regions of the Philippines, but, as with IR74, never reached more than 10% of the rice grown area (Launio et al., 2008). Overall, the lack of clarity in the results of the bioassays indicates that the genetics of resistance to BPH in these IR varieties is more complex than previously suggested (Brar et al., 2009; Jena & Kim, 2010) and will need to be reviewed.

Bph3 and *bph4*

Adaptation to IR62 affected BPH responses to resistant plants containing the *bph4* gene: Babawee was resistant to non-adapted colonies, but resistance was noticeably reduced when hoppers had adapted to IR62. Similarly, resistance in IR66 – which has Babawee as a resistance donor – was reduced against IR62-adapted hoppers. This is similar to results presented by Ketipearachchi et al. (1998) where planthoppers reared on a variety with the *bph8* gene (Thai Collection 11), had increased survival on a second variety with the *Bph9* gene (Pokali). The resistance gene *Bph9* has been mapped on the long arm of chromosome 12 (Murata et al., 2000), but the location of *bph8* still remains unknown. *Bph3* and *bph4* are closely positioned on the short-arm of chromosome 6. Furthermore, *bph4* can behave as a major recessive or major dominant gene depending on the genetic background of the rice variety (Jairin et al., 2007). The *bph2* gene can similarly behave as either recessive or dominant depending on the rice variety (Athwal et al., 1971; Murai et al., 2007). This has led Jairin et al. (2007) to suggest that *Bph3* and *bph4* may represent the same gene. Our evidence supports this idea. However, in a study by Fujita et al. (2009), Babawee was moderately

resistant to Luzon BPH (34–38% adult survival), whereas Rathu Heenati was considerably more resistant (ca. 8% adult survival) to the same populations. The nature of the genetic background of these varieties may determine the discrepancies between our results and those of Fujita et al. (2009). In our study, Babawee and Rathu Heenati were equally resistant to South Luzon BPH, indicating possible major population differences between the BPH used by Fujita et al. (2009) and those used here. Associations and interactions between genes such as *Bph3*–*bph4* or *Bph9*–*bph8* needs to be further clarified. Our results corroborate the high susceptibility of lines with BPH25 in Luzon (Fujita et al., 2009). As BPH25 is also located close to *Bph3* and *bph4*, these results suggest that the similar effects of the latter two genes are not simply due to chromosome location and consequent similar resistance mechanisms.

The present study indicates that several varieties that were previously designated as resistant are now susceptible to BPH. These may share a major resistance gene, *Bph3*, with other related varieties that are still currently resistant. The study also confirms that BPH25 does not bestow resistance against Luzon BPH. This gene is one of several identified resistance genes against which BPH has already adapted (Tanaka & Matsumura, 2000; Myint et al., 2009). Resistant varieties will continue to lose their value as they are continually exposed to high BPH densities and particularly under the favourable conditions for BPH that now occur in many intensive rice production areas [with low predator/parasitoid densities because of high pesticide use, and high inputs of nitrogen fertilizers (Cheng, 2009; Lu & Heong, 2009)]. Under this high selection pressure, *Bph3*, a gene that has provided resistance for over 30 years under lower BPH densities, may now be more vulnerable to breakdown. Further efforts should be made to conserve this and other resistance genes that are still effective against BPH, and to slow the expansion of virulent BPH populations. Improved knowledge of the genetic basis for resistance among currently deployed varieties will help devise strategies to reduce gene exposure. Such strategies depend on a sound knowledge of the extent of field deployment of resistance genes before new, resistant varieties (either pyramided or monogenic) are released. Finally, a better understanding of adaptation processes and cross-virulence will help determine candidate loci to increase durability through gene pyramiding.

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