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Virulence of long-term laboratory populations of the brown planthopper, *Nilaparvata lugens* (Stål), and whitebacked planthopper, *Sogatella furcifera* (Horváth) (Homoptera: Delphacidae), on rice differential varieties

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Abstract
The virulence of laboratory strains of the brown planthopper (BPH), *Nilaparvata lugens* (Stål), and the whitebacked planthopper (WBPH), *Sogatella furcifera* (Horváth), collected in Japan between 1966 and 2005, was evaluated using rice differential varieties carrying different planthopper resistance genes. The BPH strain collected in 1966 was avirulent to all the rice varieties tested. In contrast, the 1989, 1999 and 2005 strains were virulent to Mudgo, which carries *Bph1*. The 1999 and 2005 strains were virulent to ASD7 (*bph2*). Thus, the virulence status of the laboratory BPH strains was the same as in previous reports. The 1989, 1999, and 2005 WBPH strains were virulent to N22 (*Wbph1*), Mudgo, ASD7, Babawee (*bph4*) and Chin Saba (*bph8*); the 1999 and 2005 WBPH strains were also virulent to ARC10239 (*Wbph2*). Although the virulence status of WBPH in Japan has not previously been studied, the present results suggest that the effectiveness of the *Wbph1* resistance gene broke down before 1989, while that of *Wbph2* broke down between 1989 and 1999. The present study showed that long-term mass rearing in the laboratory has not affected virulence status. Thus, these strains will be useful to analyze resistance genes against BPH and WBPH.

Key words: Virulence; rice differential varieties; biotype; *Nilaparvata lugens*; *Sogatella furcifera*

INTRODUCTION

The brown planthopper (BPH), *Nilaparvata lugens* (Stål), and the whitebacked planthopper (WBPH), *Sogatella furcifera* (Horváth), are widely distributed throughout Asia and are two major insect pests of rice. Many rice varieties with resistance to these planthoppers have been developed and released to farmers for commercial cultivation; however, the situation became alarming when the resistance of these new varieties diminished because of the apparent selection for virulent biotypes of the pests (Khush, 1979). Laboratory studies showed that exposure of BPH to resistant varieties of rice resulted in an increased preference of the insect for the resistant variety in successive generations (Kaneda and Kisimoto, 1979). Monitoring of field populations revealed that BPH migrating to Japan showed increased virulence against the resistance gene *Bph1* around 1988–1990 (Sogawa, 1992a, b). Virulence against *bph2* in migrating BPH populations was first identified in Japan in 1997 (Tanaka, 1999; Tanaka and Matsumura, 2000), and virulence has remained at a high level through 1999. In contrast to BPH, there is little information on changes in virulence to resistance genes in WBPH.

The National Agricultural Research Center for Kyushu Okinawa Region (KONARC), Japan, has maintained BPH and WBPH laboratory strains collected in Japan at different times, 1966, 1989, 1999 and 2005. As described above, the virulence status of BPH populations immigrating into Japan has changed in recent years (Sogawa, 1992a, b; Tanaka, 1999; Tanaka and Matsumura, 2000). Therefore, the laboratory strains may have a different viru-

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lence status to the current natural populations, depending on when the collection was made; however, it is not known whether the virulence status of the laboratory strains has remained unchanged following mass rearing over many generations. If the strains have retained their original virulence status, they will be invaluable for plant breeding studies, in particular for analysis of known resistance genes or for mapping newly identified resistance genes. Thus, we screened the virulence characteristics of laboratory strains of BPH and WBPH using rice differential varieties carrying different resistance genes.

MATERIALS AND METHODS

Plant materials. Twelve rice differential varieties with different resistance genes to BPH and WBPH were used: N22 (which carries the *Wbph1* gene), ARC10239 (*Wbph2*), ADR52 (*Wbph3*), Podiwi-A8 (*wbph4*), N'Daing Marie (*Wbph5*), Manggar (*Wbph5*), Mudgo (*Bph1*), ASD7 (*bph2*), Rathu Heenati (*Bph3*, *Bph17*), Babawee (*bph4*), Chin Saba (*bph8*), Balamawee (*Bph9*) and Taichung 65 (T65) (no resistance genes). ADR52 and Podiwi-A8 have resistance genes (*Wbph3* and *wbph4*, respectively) against WBPH (Hernandez and Khush, 1981), but are also resistant to BPH (Sonoda et al., 2003).

Insect strains. Four BPH populations were used: (1) Hatano-66 (collected in Hatano, Kanagawa Pref. in 1966); (2) Chikugo-89 (collected in Chikugo, Fukuoka Pref. in 1989); (3) Isahaya-99 (collected in Isahaya, Nagasaki Pref. in 1999); and (4) Nishigoshi-05 (collected in Koshi, Kumamoto Pref. in 2005). Three WBPH populations were used: (1) Chikugo-89 (collected in Chikugo, Fukuoka Pref. in 1989); (2) Nishigoshi-99 (collected in Koshi, Kumamoto Pref. in 1999); and (3) Nishigoshi-05 (collected in Koshi, Kumamoto Pref. in 2005). These populations were separately maintained as seven laboratory strains by continuous rearing on rice variety 'Reiho' with no resistance gene at 25±1°C under 16 h light/8 h dark conditions at KONARC, Japan.

Virulence test. Seeds of the test variety were sown individually in plastic cups (220 ml) with soil. One-month-old seedlings were trimmed to 15 cm height, and each trimmed plant was covered with a transparent plastic cylindrical cage (5 cm D×25 cm H). Five brachypterous (short-wing form) BPH females or five macropterous (long-wing form) WBPH females within 24 h after emergence were released into the cage and the open end was covered with a nylon cloth. The number of surviving insects and the shape of their abdomen were monitored 5 days after infestation (DAI). We classified females with heavily swollen abdomens or that survived for five days as virulent. This system of classification is identical to that used by Tanaka (2000) for identifying virulent and avirulent females. Eight independent replicates of the experiment were performed. All tests were conducted in May, 2006.

Statistical analysis. Data were analyzed using ANOVA and treatment means were subjected to multiple comparisons using the Tukey-Kramer multiple comparison test (SAS Institute Inc., 2003). The rate of surviving insects (%) was arcsine transformed prior to analysis.

RESULTS AND DISCUSSION

Only a small proportion of Hatano-66 BPH females survived on rice differential varieties that carried a resistance gene(s) (range 0.0 to 7.5%) and the surviving females did not all show a swollen abdomen (Tables 1 and 2). Thus, the Hatano-66 BPH strain was avirulent to the varieties tested, except for T65 that does not carry a resistance gene. In contrast, high survival rates were found for Chikugo-89, Isahaya-99 and Nishigoshi-05 on the Mudgo variety (Table 1) and most females displayed swollen abdomens (Table 2). The Isahaya-99 and Nishigoshi-05 strains were also virulent on the ASD7 variety and showed high rates of survival and high proportions of insects with swollen abdomens (Tables 1 and 2). These results generally agreed well with those obtained by previous studies (Sogawa, 1992a, b; Tanaka, 1999; Tanaka and Matsumura, 2000). There were no other distinct changes in the virulence of the 1999 (Tanaka and Matsumura, 2000) and 2005 BPH migrants (Tables 1 and 2), suggesting that the virulent status of the migrant population in Japan in 2005, which caused severe damage to rice crops (Watanabe et al., 2007), did not differ from that of 1999.

Tanaka and Matsumura (2000) reported that the rate of virulent BPH infestation on the Babawee variety was greater for the 1997 to 1999 migrants

Table 1. Survival rates (%, mean±SE) of *Nilaparvata lugens* females on rice differential varieties carrying resistance genes

Variety	Resistance gene	*Nilaparvata lugens* strains[a,b]			
		Hatano-66	Chikugo-89	Isahaya-99	Nishigoshi-05
Mudgo	*Bph1*	7.5±3.6 b	85.0±3.2 a	95.0±3.2 a	90.0±5.3 a
ASD7	*bph2*	5.0±3.2 b	5.0±3.2 b	80.0±5.3 a	87.5±5.2 a
Rathu Heenati	*Bph3*, *Bph17*	2.5±2.5 b	2.5±2.5 b	5.0±3.2 b	2.5±2.5 b
Babawee	*bph4*	2.5±2.5 b	2.5±2.5 b	17.5±4.5 b	20.0±5.3 b
Chin Saba	*bph8*	7.5±3.6 b	10.0±3.7 b	7.5±3.6 b	12.5±5.2 b
Balamawee	*Bph9*	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b
ADR52	*Wbph3*	5.0±3.2 b	5.0±3.2 b	5.0±3.2 b	5.0±3.2 b
Podiwi-A8	*wbph4*	2.5±2.5 b	2.5±2.5 b	7.5±3.6 b	2.5±2.5 b
Taichung 65	no resistance gene	90.0±3.7 a	82.5±5.9 a	90.0±5.3 a	90.0±3.7 a

[a] Hatano-66, Chikugo-89, Isahaya-99, and Nishigoshi-05 strains were collected in 1966, 1989, 1999, and 2005, respectively.
[b] Means followed by the same letter are not significantly different at $p<0.01$, by the Tukey-Kramer multiple comparison test.

Table 2. Proportion (%, mean±SE) of *Nilaparvata lugens* females with swollen abdomens five days after infestation

Variety	Resistance gene	*Nilaparvata lugens* strains[a]			
		Hatano-66	Chikugo-89	Isahaya-99	Nishigoshi-05
Mudgo	*Bph1*	0.0±0.0	80.0±0.0	92.5±3.7	87.5±5.3
ASD7	*bph2*	0.0±0.0	0.0±0.0	77.5±5.9	85.0±5.0
Rathu Heenati	*Bph3*, *Bph17*	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Babawee	*bph4*	0.0±0.0	0.0±0.0	5.0±3.3	7.5±3.7
Chin Saba	*bph8*	0.0±0.0	0.0±0.0	7.5±3.7	7.5±3.7
Balamawee	*Bph9*	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
ADR52	*Wbph3*	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Podiwi-A8	*wbph4*	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Taichung 65	no resistance gene	90.0±3.7	82.5±5.9	90.0±5.3	90.0±3.7

[a] Hatano-66, Chikugo-89, Isahaya-99, and Nishigoshi-05 strains were collected in 1966, 1989, 1999, and 2005, respectively.

than pre-1997. Here, we found that the resistance level of the Babawee variety to the Nishigoshi-05 strains (Tables 1 and 2) was similar to that in 1999 BPH immigrants (Tanaka and Matsumura, 2000). Therefore, it will be important to continuously monitor the virulence of BPH populations to this variety. The five varieties with different resistance genes to BPH and WBPH, i.e. Rathu Heenati, Chin Saba, Balamawee, ADR52 and Podiwi-A8, were highly resistant to all BPH strains tested (Tables 1 and 2).

The Isahaya-99 BPH strain used in this study is the offspring of the Isahaya-99 strain tested in Tanaka and Matsumura (2000). The virulence status of this strain tested in 2006 (Tables 1 and 2) was similar to those tested in 1999 (Tanaka and Matsumura, 2000). This suggests that the long-term mass rearing of the insects in the laboratory (at least 7 years) did not change the virulence status of BPH.

In the case of WBPH, no information on the virulence status of the immigrant populations into Japan has been available; however, if the long-term mass rearing of WBPH in the laboratory did not change the virulence status as well as BPH, we discussed the change of virulent status of WBPH using strains previously collected and maintained.

The survival rates of Chikugo-89, Nishigoshi-99 and Nishigoshi-05 WBPH females on ADR52, Podiwi-A8, N'Daing Marie, Manggar, Rathu Heenati and Balamawee varieties were low (range 0 to 30%) (Table 3). Similarly, the proportions of females with swollen abdomens within 5DAI were comparatively low (Table 4). In contrast, for strains

Table 3. Survival rates (%, mean±SE) of *Sogatella furcifera* females on rice differential varieties carrying resistance genes

Variety	Resistance gene	*Sogatella furcifera* strains[a,b]		
		Chikugo-89	Nishigoshi-99	Nishigoshi-05
N22	*Wbph1*	87.5±3.6 a	85.0±5.0 ab	84.3±6.9 a
ARC10239	*Wbph2*	15.0±5.0 bc	67.5±8.4 b	85.0±6.3 a
ADR52	*Wbph3*	0.0±0.0 c	17.5±5.9 c	17.5±7.0 b
Podiwi-A8	*wbph4*	10.0±5.3 bc	12.5±5.3 c	15.0±6.3 b
N'Daing Marie	*Wbph5*	30.0±7.5 b	22.5±5.9 c	22.5±7.0 b
Manggar	*Wbph5*	17.5±8.0 bc	22.5±9.6 c	30.0±7.5 b
Mudgo	*Bph1*	80.0±5.3 a	90.0±3.8 ab	95.0±3.3 a
ASD7	*bph2*	75.0±5.0 a	85.0±6.3 ab	95.0±3.3 a
Rathu Heenati	*Bph3*, *Bph17*	10.0±3.8 bc	7.5±3.6 c	10.0±3.8 b
Babawee	*bph4*	92.5±5.3 a	95.0±3.3 ab	100.0±0.0 a
Chin Saba	*bph8*	95.0±3.3 a	97.5±2.5 a	97.5±2.5 a
Balamawee	*Bph9*	5.0±5.0 c	7.5±3.6 c	15.0±5.0 b
Taichung 65	no resistance gene	94.4±5.3 a	97.5±2.5 a	97.5±2.5 a

[a] Chikugo-89, Nishigoshi-99, and Nishigoshi-05 strains were collected in 1989, 1999, and 2005, respectively.
[b] Means followed by the same letter are not significantly different at $p<0.01$, by the Tukey-Kramer multiple comparison test.

Table 4. Proportion (%, mean±SE) of *Sogatella furcifera* females with swollen abdomens five days after infestation

Variety	Resistance gene	*Sogatella furcifera* strains[a]		
		Chikugo-89	Nishigoshi-99	Nishigoshi-05
N22	*Wbph1*	85.0±3.3	77.5±5.9	77.1±6.3
ARC10239	*Wbph2*	0.0±0.0	60.0±6.5	75.0±5.0
ADR52	*Wbph3*	0.0±0.0	0.0±0.0	0.0±0.0
Podiwi-A8	*wbph4*	0.0±0.0	0.0±0.0	0.0±0.0
N'Daing Marie	*Wbph5*	0.0±0.0	0.0±0.0	0.0±0.0
Manggar	*Wbph5*	0.0±0.0	0.0±0.0	0.0±0.0
Mudgo	*Bph1*	80.0±5.3	87.5±3.7	92.5±3.7
ASD7	*bph2*	70.0±3.7	82.5±5.9	85.0±5.0
Rathu Heenati	*Bph3*, *Bph17*	0.0±0.0	0.0±0.0	0.0±0.0
Babawee	*bph4*	90.0±5.3	90.0±3.8	95.0±3.3
Chin Saba	*bph8*	92.5±3.7	97.5±2.5	92.5±3.7
Balamawee	*Bph9*	0.0±0.0	0.0±0.0	0.0±0.0
Taichung 65	no resistance gene	93.0±5.3	90.0±5.3	90.0±5.3

[a] Chikugo-89, Nishigoshi-99, and Nishigoshi-05 strains were collected in 1989, 1999, and 2005, respectively.

collected between 1989 and 2005, very high proportions of WBPH females survived on N22, Mudgo, ASD7, Babawee and Chin Saba (range 75 to 100%), at rates similar to that on the T65 variety (Table 3). Almost all females on these varieties had swollen abdomens within 5DAI (Table 4). A relatively small proportion of Chikugo-89 females survived on ARC10239 (15.0%) and did not show swollen abdomens, whereas high proportions of Nishigoshi-99 and Nishigoshi-05 females survived and had swollen abdomens on this variety (Tables 3 and 4). These results suggest that the effectiveness of the resistance of variety N22, which carries the *Wbph1* gene, broke down before 1989 and that resistance in ARC10239 (*Wbph2*) broke down between 1989 and 1999. No distinct changes were observed in the virulence of 1999 and 2005 WBPH migrants.

This study revealed that long-term laboratory strains of BPH and WBPH had a different status of

virulence to resistant varieties. Thus, these strains will be useful to analyze resistance genes against BPH and WBPH. Recent molecular mapping suggests that highly resistant rice varieties carry multiple genes for resistance to BPH (Huang et al., 2001; Sonoda et al., 2003; Sun et al., 2005). In the present study, we found that ADR52, Podiwi-A8, Rathu Heenati and Balamawee showed high resistance to all BPH and WBPH strains, suggesting that these varieties may carry multiple resistance genes. Previous studies concluded that BPH resistance in ADR52 and Podiwi-A8 varieties were controlled by quantitative trait loci (Sonoda et al., 2003), while resistance in Rathu Heenati was likely to be controlled by two major genes (*Bph3*, *Bph17*) and two minor genes (Sun et al., 2005; Jairin et al., 2007). These multiple genes help maintain their durable resistance to different BPH and WBPH populations. To effectively utilize resistant rice varieties, it will be necessary to continuously monitor virulent status of rice planthoppers migrating to Japan. Moreover, sharing information among Asian countries on the virulence characteristics of rice planthoppers with regard to resistant rice varieties will be a powerful strategy for rice planthopper management in Asia.

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