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Development of virulence to resistant rice varieties in the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae), immigrating into Japan

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Abstract
Virulence of the brown planthopper *Nilaparvata lugens* strains, which immigrated into Japan between 1997 and 1999, was examined on five rice varieties, Mudgo (carrying a resistance gene *Bph 1*), IR26 (*Bph 1*), ASD7 (*bph 2*), Norin PL10 (*Bph 3*), and Babawee (*bph 4*). Newly emerging brachypterous females of *N. lugens* were released on test rice plants at the tillering stage, and we defined the females that became heavily swollen or survived for five days as virulent. Between 45 and 87% of the females were virulent to ASD7, although such high virulence had not been detected before 1997. Between 49 and 98% of the females were virulent to Mudgo and IR26. Virulence to rice varieties carrying *Bph 1* in the *N. lugens* population has continued to become stronger since the 1988–1990 period in which changes in virulence were first found. In contrast, virulence of the *N. lugens* strains to Norin PL10 and Babawee was still at a low level, with 5 to 27% of the females being virulent. The results indicate that the resistance of *Bph 1* has probably broken down and the resistance of *bph 2* may be becoming ineffective for the *N. lugens* populations immigrating into Japan.

Key words: Resistant variety, host adaptation, rice pest, biotype, *Nilaparvata lugens*

INTRODUCTION

Insect-resistant varieties of many crops have been bred and utilized for insect pest control. Use of the resistant crop varieties has advantages in that farmers need not pay additional cost in terms of materials and labor to control the pests and the use of such crops has little deleterious effect on the environment. However, the fact that some populations of insect pests have adapted and become virulent to the previously resistant varieties presents a serious problem. Such virulence has evolved in many populations of crop pests (so-called "biotypes") (Diehl and Bush, 1984). To effectively utilize the resistant crop varieties, we should continuously monitor the virulence of pest populations to the resistant crops.

The brown planthopper (BPH), *Nilaparvata lugens* Stål, is one of the most notorious insect pests of rice in temperate and tropical Asia (Dyck and Thomas, 1979). In Southeast Asia, including the Philippines, southern Vietnam, Indonesia, and the Solomon Islands, virulence of the BPH populations has changed drastically since the 1970s when commercial rice varieties carrying the resistance genes *Bph 1* and *bph 2* were released and widely cultivated, and at present the populations are virulent to the two genes (for review see Sogawa, 1982; Gallagher et al., 1994).

The BPH occurring in East Asia, including northern Vietnam, China, Taiwan, the Korean peninsula, and Japan, is considered to be a single population with a permanent breeding area in northern Vietnam and southernmost China (Kisimoto and Sogawa, 1995). The BPH immigrating into Japan began to become virulent to *Bph 1* in 1988–1990 (Sogawa, 1992), and this change has been documented through 1996 (Tanaka, 1999b). This trend was also found in China and northern Vietnam in the same period (Yu et al., 1991; Thuat et al., 1992; Zhang et al., 1995). There were no apparent changes detected in virulence of the BPH population to other resistance genes through 1996, however (Tanaka, 1999b). In this study, we investigated virulence of the BPH immigrating into Japan between 1997 and 1999 in order to examine whether the changes in the virulence to *Bph 1* have continued and whether the virulence to other resistance genes has remained at a low level.

MATERIALS AND METHODS

Insects. We used four BPH strains for the virulence tests: (1) Isahaya-97 strain collected in Isahaya, Nagasaki Prefecture, Kyushu, Japan, on 31 July 1997; (2) Isahaya-98 collected in Isahaya on 14 August 1998; (3) Katsumoto-98 collected in Katsumoto, Iki Island, Nagasaki Prefecture, on 20 August 1998; and (4) Isahaya-99 collected in Isahaya on 21 August 1999. These BPH adults were captured from *japonica* rice varieties, Hinohikari in Isahaya and Koshihikari in Katsumoto. The BPH had been successively reared on rice seedlings of *japonica* varieties, Reiho, Shinrei, and Mochiminori, in laboratories prior to the virulence tests. These five rice varieties have no BPH-resistance genes. Rearing of BPH on seedlings of the susceptible rice varieties had no apparent effect on the virulence of BPH to resistant varieties (Tanaka, unpublished data).

For the tests of virulence to *bph 2*, we used a control strain, Nishigoshi-91, collected in Nishigoshi, Kumamoto Prefecture, Kyushu, in September 1991. This strain has exhibited low virulence to *bph 2* (Tanaka, 1999b).

Virulence tests for BPH females. Virulence of the BPH adult females was tested on four *indica* rice varieties, i.e., Mudgo (carrying *Bph 1*), IR26 (*Bph 1*), ASD7 (*bph 2*), and Babawee (*bph 4*), and a *japonica* rice line, Norin PL10 (*Bph 3*). The tests were carried out in July to October 1999 in laboratories, controlled at 25°C, 16L:8D, of National Institute of Agro-Environmental Sciences (NIAES) and Kyushu National Agricultural Experiment Station (KNAES).

The virulence of BPH is under polygenic control. This character does not, however, exhibit a continuous distribution of phenotype, but rather is a threshold character that has two distinct phenotypes, i.e., virulent and avirulent (Tanaka, 1999a). Hence, a proportion of virulent individuals in a BPH population indicates the degree of virulence of that population. Classifying virulent and avirulent BPH females was accomplished by the method of Tanaka (2000), based on survival and appearance of the abdomens of the females. The abdomens of virulent females became swollen on rice plants within a few days after their emergence, while the abdomens of avirulent females remained thin or became thinner; these two types of females could be apparently discriminated (Tanaka, 1999a). Some females had, however, medium (intermediately-swollen) abdomens, and some of them could not be easily discriminated from thin females by their abdomens. However, virulence of thin and medium females could be determined by their survival for five days (Tanaka, 2000). The heavily-swollen females should be better adapted to the rice variety.

Three seeds of test rice were sown in a 220-ml plastic cup filled with soil containing 0.07% N, 0.2% P, and 0.1% K. The rice plants were grown in greenhouses, and ca. 0.5 g of chemical fertilizer (containing 16% N, 16% P, and 16% K) was added into the cup three to four weeks after sowing. Four- to 6-week-old plants at the tillering stage were used for the tests. We cut off all the parts of the plants including the tillers and leaf blades except for a 15-cm main stem, leaving three stems in a cup. Trimming the plants in this way made it easy to observe the insects. The trimmed plants were covered with a transparent plastic cylindrical cage (5.5 cm D× 20 cm H). Ten newly emerging, up to 24-h-old, brachypterous BPH females with thin abdomens were released into the cage, and the open end of the cage was covered with gauze. The BPH were observed daily from days 2 to 5. Females whose abdomens became heavily swollen were counted and removed from the cage. We defined the females that became heavily swollen or survived for five days as virulent. We used 100 females for each test.

In the first test, the females of Isahaya-97 showed high virulence to ASD7 (see Results). To examine whether our ASD7 plants strongly exhibited the resistance trait, we conducted an additional test using the rice plants grown outdoors where the plants received more sunshine and may have grown more robustly than in the greenhouse. Furthermore, we used the Nishigoshi-91 BPH strain as a control. This additional test was conducted at NIAES in early August.

Survival and development of BPH nymphs on ASD7. To further examine the virulence of the BPH to ASD7, we investigated survival and development in the BPH nymphs of the Isahaya-97 strain on ASD7 and Reiho (control) at NIAES. We prepared test rice plants by the method just described. In this test, however, a 15-cm main stem and two 5-cm tillers of a rice plant, i.e., three stems

Table 1. Percentages of virulent females of four *Nilaparvata lugens* strains on the rice varieties carrying resistance genes[a]

Variety	Resistance gene	Laboratory[b]	*N. lugens* strain			
			Isahaya-97	Isahaya-98	Katsumoto-98	Isahaya-99
Mudgo	*Bph 1*	NIAES	51 (42)	89 (88)	78 (78)	—[c]
		KNAES	90 (90)	61 (39)	—	83 (72)
IR26	*Bph 1*	NIAES	49 (41)	92 (90)	87 (86)	—
		KNAES	98 (98)	83 (71)	—	88 (83)
ASD7	*bph 2*	NIAES	85 (85)	84 (75)	45 (41)	—
		KNAES	87 (85)	69 (26)	—	79 (59)
Norin PL10	*Bph 3*	NIAES	5 (4)	18 (14)	24 (19)	—
		KNAES	17 (1)	27 (5)	—	20 (0)
Babawee	*bph 4*	NIAES	6 (5)	10 (8)	5 (4)	—
		KNAES	15 (2)	5 (0)	—	25 (3)

[a] Figures in parentheses indicate percentage of females that became swollen within five days.
[b] Laboratory where tests were carried out. NIAES: National Institute of Agro-Environmental Sciences, KNAES: Kyushu National Agricultural Experiment Station.
[c] Absence of data indicates that the test was not carried out.

Table 2. Survival and development of *Nilaparvata lugens* nymphs of the Isahaya-97 strain on ASD7 (carrying *bph 2*) and Reiho (carrying no resistance gene)

Variety	Survival rate (%)	Nymphal period (mean±SE, days)		% Macropters		Sex ratio[a]
		Female	Male	Female	Male	
ASD7	85.3 (n=300)	14.5±0.10 (n=137)	14.0±0.11 (n=119)	0.0 (n=137)	56.3 (n=119)	0.54 (n=256)
Reiho	90.3 (n=300)	14.4±0.07 (n=141)	13.8±0.08 (n=130)	0.0 (n=141)	46.2 (n=130)	0.52 (n=271)

[a] Female/(female+male).
Between ASD7 and Reiho, there were no significant differences in survival rates ($\chi^2=3.5$, $p>0.05$), nymphal periods (ANOVA, $p>0.3$ for females and $p>0.1$ for males), % macropterous males ($\chi^2=2.6$, $p>0.1$), and sex ratios ($\chi^2=0.1$, $p>0.7$).

and six tillers in a cup, remained. Thirty newly hatched BPH nymphs were released into the plastic cage covering the test plants. Ten cages (300 nymphs) were tested for each rice variety. The surviving nymphs were transferred to another cage every six days, and the emerging adults were counted and removed from the cage.

RESULTS

Virulence of BPH females

All four BPH strains had high virulence to Mudgo and IR26 carrying *Bph 1*; more than 49% of the females were virulent (Table 1). High proportions of females of the four strains were also virulent to ASD7 (carrying *bph 2*) grown in the greenhouses (Table 1). On ASD7 grown outdoors, 74% of females of the Isahaya-97 strain were virulent (all of them became swollen), while only 13% of the Nishigoshi-91 females were virulent (10% became swollen). Thus, the ASD7 exhibited strong resistance to the Nishigoshi-91 strain. On the other hand, the four strains had low proportions of females virulent to Norin PL10 (*Bph 3*) and Babawee (*bph 4*), though values were greater than 20 in some tests (Table 1).

Survival and development of nymphs on ASD7

A high proportion of BPH nymphs developed to adults on ASD7; their survival rate was similar to that on a susceptible variety, Reiho (Table 2). The nymphal periods also showed no significant difference between the two rice varieties (Table 2). In both varieties, the sex ratio approached 0.5, and all females and about half of the males emerged in brachypterous form.

DISCUSSION

Virulence of the BPH population immigrating into Japan to rice varieties carrying *Bph 1* began to increase around 1988–1990 (Sogawa, 1992). This increase continued until at least 1996; proportions of virulent females were 39 to 66% on Mudgo and 54 to 79% on IR26 in the 1995 and 1996 BPH immigrants (Tanaka, 1999b). This study showed that the virulence to *Bph 1* still increased after 1996. In this study, the proportions of virulent females in the same BPH strains were somewhat different between the laboratories where the tests were carried out. These differences may have been due to a shift in virulence caused by dividing the BPH strain into two laboratory cultures or to differences in the robustness of test rice plants as a result of variable weather conditions, e.g., intensity and duration of sunshine and temperatures, during growth. However, trends of developing virulence to *Bph 1* were consistent. Thus, we can conclude that the BPH populations immigrating into Japan have become highly virulent to the *Bph 1*-carrying rice varieties. On the other hand, fewer than 15% of females in the BPH population were virulent to ASD7 carrying the *bph 2* gene until 1996 (Tanaka, 1999b). This study, however, revealed that the BPH population rapidly became virulent to ASD7 beginning in 1997, and the virulence remained at a high level through 1999. This is the first report that has indicated a change in virulence of BPH occurring in East Asia to a rice variety carrying *bph 2*.

In contrast, no distinct changes were observed in this study in the virulence of the BPH population to Norin PL10 (carrying *Bph 3*) or Babawee (*bph 4*). However, the proportions of virulent BPH were somewhat higher, being greater than 20% in the 1997 to 1999 immigrants, compared to those before 1997, most of which were lower than 10% in virulence (Tanaka, 1999b). It is important that we continue to carefully monitor the virulence of BPH populations to these resistance genes.

As shown above, currently the resistance of *Bph 1* gene is probably ineffective and the resistance of *bph 2* may be becoming ineffective for controlling BPH populations immigrating into Japan. Therefore, in breeding effective BPH-resistant rice varieties, we should introduce a resistance gene other than *Bph 1* and *bph 2*, including that from wild rice (e.g., Ishii et al., 1994) or multiple resistance genes into the varieties.

Migration of the East Asian BPH population has been inferred as follows (Kisimoto and Sogawa, 1995): Northward migration first occurred from the permanent breeding area, i.e., northern Vietnam and southernmost China, to southern China, then a part of the population reproducing in southern China migrated into Japan, the Korean peninsula, and temperate China. This scenario was supported by evidence that virulence to *Bph 1* has synchronously changed in BPH occurring in these regions (Sogawa, 1992). On the other hand, the BPH populations occurring in Southeast Asia became virulent to both *Bph 1* and *bph 2* in the 1970s and 1980s (Feuer, 1976; Mochida et al., 1977; Stapley et al., 1979; Medrano and Heinrichs, 1985; Sogawa et al., 1987; Huynh and Nhung, 1988). From these facts, one may suspect that the BPH immigrants in 1997–1999 tested in this study have migrated from a Southeast Asian region such as the Philippines. However, analysis of BPH migration by a computer program that monitors the low-level jet stream transporting BPH (Watanabe et al., 1988, 1990) showed that no such jet stream developed in the direction from these regions to Japan from early June through early July in the three years when the BPH immigration occurred (Matsumura, unpublished data). Consequently, the observed changes in virulence to *bph 2* may have occurred in the East Asian BPH population. We need to examine virulence to *bph 2* in BPH occurring in northern Vietnam, China, and the Korean peninsula to further confirm the BPH migration pattern.

In northern Vietnam, a rice variety known as CR203 (IR8423), which carries the *bph 2* gene, has been cultivated in some portions of paddy areas since 1984–1985 (Suzuki and Wada, 1994; Khuong, 1999). In southern China, mainly in Guandong Province, a new rice variety, Jingxian89, that carries the *bph 2* gene originated from IR36 has been cultivated since 1996, and its cultivated area reached 1,000,000 ha (Huang et al., 1999; Sogawa, personal communications). The observed changes in virulence to *bph 2* may have resulted from the evolution of the BPH population as it adapted to these rice varieties. If this is true, the composition of rice varieties cultivated in these regions including the BPH permanent-breeding and first-destination areas should have strongly affected the change in virulence of the BPH population. Analyzing the relationships between the composition of rice varieties and the BPH population structure in terms of virulence may provide helpful insight for establishing a BPH management strategy.

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