Adaptation of the brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), to BPH resistant rice cultivars carrying *bph8* or *Bph9*

Yasaratna Ketipearachchi,¹ Chukichi Kaneda¹, ², ³ and Chiharu Nakamura¹, ², *

¹Division of Biological Resources, The Graduate School of Science and Technology, and ²Laboratory of Plant Genetics, Department of Biological and Environmental Science, Faculty of Agriculture, Kobe University, 1 Rokkodai-cho, Nada-ku, Kobe 657–8501, Japan

(Received 24 December 1997; Accepted 12 June 1998)

**Abstract**

Colonies of the brown planthopper (BPH), *Nilaparvata lugens* (Stål), virulent against two resistant rice cultivars carrying BPH resistance genes, either *bph8* or *Bph9*, were selected under laboratory conditions. Two BPH resistant indica cultivars, Thai Col. 11 (a *bph8* carrier) and Pokkali (a *Bph9* carrier), were used as selection hosts on which the wild-type population of BPH was continuously cultured. The virulence or adaptation was monitored on the resistant hosts during the selection process based on the following five parameters; survival rate, wing dimorphism, oviposition, egg hatch and egg hatchability. Selection appeared to be achieved at the 9th–15th generations depending on the parameters and the selection hosts. The colony selected on Thai Col. 11 showed a high level of virulence not only against the selection host but also against another *bph8* carrier, cv. Thai Col. 5. The colony selected on Pokkali also showed virulence on the selection host but this virulence was unstable and not effective against another *Bph9* carrier, cv. Balamawee. Our results suggested the presence of virulent BPH individuals (forerunners) in the wild population that could effectively be selected on resistant rice hosts with specific major resistance genes. The genetic system of BPH virulence and rice resistance is discussed.

**Key words:** Biotype, brown planthopper (*Nilaparvata lugens*), host plant resistance, rice (*Oryza sativa*), virulence

**INTRODUCTION**

The brown planthopper (BPH), *Nilaparvata lugens* (Stål), is one of the most destructive pests of rice throughout the rice-growing tropical, subtropical and temperate areas in Asia and Oceania. BPH causes severe damage on rice plants either directly by phloem feeding or indirectly by transmitting viral diseases (Rivera et al., 1966; Ling et al., 1978; Sogawa and Cheng, 1979). Periodic BPH outbreaks were recorded in temperate rice-growing countries such as Korea and Japan (Mochida and Okada, 1979), but BPH was a minor pest in the past in its endemic habitats. BPH became a key pest in tropical Asia in the 1970s when the rice agroecosystem changed drastically after the adoption of high-yielding cultivars under heavy use of agrochemicals, especially nitrogenous fertilizers and insecticides. The severity of the damage and the frequency of the outbreaks along with the hazardous effects of insecticides prompted researchers to look for sources of natural resistance to achieve BPH control (Sogawa, 1982).

The International Rice Research Institute (IRRI) in the Philippines released the world’s first BPH resistant cultivar, IR26, for commercial cultivation in November 1973 (IRRI, 1976). This cultivar, harboring a dominant resistance gene, *Bph1*, was effective against the BPH population in the Philippines. IR26 soon be-
came popular and was extensively cultivated in many rice-growing countries in tropical Asia. It was, however, realized by the end of 1975 that this cultivar had become susceptible to the BPH populations in the Philippines, Indonesia, Vietnam, Sri Lanka and the Solomon Islands (Stapley et al., 1979). Later, cultivars bred to carry a recessive gene, bph2, also succumbed to the virulent BPH population (Panda and Khush, 1995). Thus far, 10 major BPH resistance genes have been discovered in the germplasm collections of rice (Panda and Khush, 1995; Murata et al., 1997). A large number of cultivars with monogenic resistance from Bph1, bph2, Bph3 or bph4 have been bred and used for cultivation in the last 25 years. But, rice cultivars having single major resistance genes have had to be replaced one after the other within several cycles of cultivation due to the occurrence of new biotypes (Rombach and Gallagher, 1994). The incidence of virulent BPH biotypes therefore has continued to be a major problem in rice production.

BPH biotypes differ in their ability to infest and feed on rice cultivars with specific major BPH resistance genes (Panda and Khush, 1995). Many reports have indicated the presence of different BPH biotypes in sympatric as well as allopatric BPH populations (Pathak and Khush, 1979; Sogawa, 1992; Takahashi et al., 1994). Some BPH biotypes have shown simultaneous virulence or adaptation to several BPH resistance genes (Cheng, 1975; Sogawa, 1982; Panda and Khush, 1995). It was also suggested that the wild-type BPH populations have the potential to accumulate virulence against different BPH resistance genes upon culturing for generations on resistant rice cultivars (Pathak and Heinrichs, 1982; Rombach and Gallagher, 1994; Nemoto and Yokoo, 1994).

Three sympatric biotypes were selected from Southeast Asian BPH populations and designated as biotype 1, biotype 2 and biotype 3 (IRRI, 1976; Pathak and Heinrichs, 1982; Panda and Khush, 1995). Biotype 1 has the ability to infest only rice cultivars which lack genes for BPH resistance, while biotype 2 can feed on Bph1 carriers in addition to cultivars susceptible to biotype 1. Biotype 3 can feed on bph2 carriers and cultivars susceptible to biotype 1. However, the mechanisms of biotype occurrence and the shift in the natural BPH populations have yet to be clarified. We attempted to select for BPH colonies which are virulent against rice hosts carrying two major resistance genes, either a recessive gene bph8 or a dominant gene Bph9. Changes in the virulence or adaptation of BPH colonies were monitored on susceptible and resistant hosts throughout the selection process based on survival rate, wing dimorphism, oviposition and egg hatching.

MATERIALS AND METHODS

BPH culture. A wild-type BPH population was maintained in a growth chamber under LD 15-9 h and day-night temperatures of 30-25°C on a susceptible japonica cultivar, 'Nipponbare.' This wild-type population was a descendant of a mixture of wild-type BPH populations obtained from Kyushu National Agricultural Experiment Station and Hyogo Prefectural Agricultural Research Center. For maintenance of the BPH population, rice plants were replaced every three-weeks. Two-month-old plants of two resistant indica cultivars, 'Thai Collection 11' (hereafter abbreviated as Thai Col. 11, a bph8 carrier) and 'Pokkali' (a Bph9 carrier) were used for the challenge infestation. They were individually grown in 8 cm diameter pots placed under the same conditions as for the maintenance of BPH. A susceptible indica cultivar, 'IR24', was used as the control. The plants were covered with culture cages (8.3 cm in diameter and 33 cm in height) and random samples of 200 first instar nymphs were infested. Initial infestation was made using nymphs of the wild-type BPH and the number of surviving BPH was counted at the 23rd day after infestation. In the subsequent generations, infestation was made using nymphs hatched from the same host cultivars (selection hosts). The colony that was selected on Thai Col. 11 was designated as TC colony, while that selected on Pokkali was designated as PO colony.

Virulence test. The virulence or adaptation of BPH to the selection hosts was monitored during the selection process based on survival (number and percentage of surviving BPH), wing dimorphism (number of brachypterous
and macropterous forms among surviving adults), oviposition (number of eggs laid per plant), egg hatch (number of eggs hatched per plant) and percent hatchability of eggs. The wild-type BPH colony and two independent BPH colonies selected on the resistant hosts, i.e. TC and PO colonies, were used for these tests. Four other indica cultivars were used as additional hosts, including two resistant cultivars, 'Thai Col. 5' (a carrier of bph8) and 'Balama-wee' (a carrier of Bph9), and two susceptible cultivars, IR24 and 'Taichun Native 1' (TN1).

Changes in the survival rate (percent survival) were monitored during the selection process by infesting first instar nymphs onto 3-week-old plants (10 nymphs per plant) inside glass test tubes (29 × 3 cm), incubated under LD 15–9 h and day-night temperature of 30–25°C (test 1). Moist cotton wads were placed at the bottom of tubes and the tops were covered with muslin cloth for ventilation. The rates of survival and wing dimorphism were also studied using BPH infested on 2-month-old selection hosts grown in culture cages (8.3 cm in diameter and 33 cm in height) (test 2). Changes in oviposition and egg hatch were monitored in the following manner. Three-week-old plants grown in trays (22.5 × 30 cm) under the randomized complete block design with 5 replications (5 plants per cultivar) were infested for 3 days with BPH adults (3 couples per plant) inside ventilated transparent plastic cages (26 × 33.5 × 33.5 cm). Plants were removed from the tray on the 5th day from the date of infestation and placed individually in glass test tubes (20 × 2 cm) with wet cotton wads around the roots, and the tops were covered with muslin cloth. The number and percentage of emerging nymphs from each plant was monitored for 3 weeks. The plants were then dissected to count the number of unhatched eggs. The total number of unhatched eggs plus the number of nymphs emerging from each plant was considered as the total oviposition. ANOVA was performed by MSTAT (Freed and Eisensmith, 1989) after square root transformation. Mean separation was made by t test.

RESULTS

Survival rate

The survival rate (percent survival) of BPH at the 23rd day after infestation of the first instar nymphs of the wild-type BPH was 73% on IR24, 47% on Thai Col. 11 and 45% on Pokkali (Table 1). On Thai Col. 11 and Pokkali, 34% and 31% of the surviving BPH were underdeveloped, respectively. The relative percentage of the brachypterous form among adults was larger than that of the macropterous form on susceptible hosts in both sexes. Conversely, higher frequencies of the macropterous form were observed on resistant hosts.

The wild-type BPH and randomly selected nymphs of their descendants on Thai Col. 11 and Pokkali were continuously cultured on the two resistant host cultivars. During the selection process, the rate of survival was monitored in test 1. Figure 1 shows the changes in the percentage survival of BPH of the wild-type, TC and PO colonies on Thai Col. 11 and Pokkali. The wild-type colony showed a considerable fluctuation in survival rate. As compared with

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Female B</th>
<th>Male B</th>
<th>Underdeveloped BPH</th>
<th>Total % survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR24</td>
<td>48 ± 2</td>
<td>7 ± 2</td>
<td>34 ± 2 11 ± 2</td>
<td>0</td>
</tr>
<tr>
<td>Thai. Col. 11</td>
<td>15 ± 2</td>
<td>26 ± 3</td>
<td>4 ± 1 21 ± 1</td>
<td>34 ± 3</td>
</tr>
<tr>
<td>Pokkali</td>
<td>13 ± 1</td>
<td>22 ± 3</td>
<td>9 ± 3 24 ± 4</td>
<td>31 ± 1</td>
</tr>
</tbody>
</table>

Numbers of surviving BPH were counted at 23 days after infestation of 200 first instar nymphs onto 2-month-old plants of a susceptible cultivar IR24 and two resistant cultivars, Thai Col. 11 and Pokkali. Figures are relative percentages of B (brachypterous) and M (macropterous) forms and underdeveloped BPH among the survivors.
the wild-type, however, both TC and PO colonies showed higher survival rates, respectively, on Thai Col. 11 and Pokkali after some generations. The difference between the wild type and the selected colonies became significant at the 10th generation in the TC colony and at the 14th generation in the PO colony. The survival rate and wing dimorphism among adults were further studied at the 12th-26th generations of the selection process in test 2 (Fig. 2). The survival rate of both TC and PO colonies on the resistant hosts did not increase as compared with the results shown in Table 1. This might be ascribed to differences in the experimental conditions, particularly different developmental stages of the host plants (3-week-old in test 1 and 2-month-old in test 2). Male adults often emerged earlier than females and many died by the time of data collection. Therefore, only data obtained on female adults are shown for wing dimorphism. A brachypterous form was prevalent in the TC colony on Thai Col. 11 throughout the
generations studied. In contrast, the relative frequency of the brachypterous form in the PO colony on Pokkali showed a notable fluctuation depending on the generation.

**Oviposition**

Figure 3 shows the changes in oviposition by the wild-type and TC colonies on Thai Col. 11 and the wild-type and PO colonies on Pokkali during the 1st to the 18th generation. The experiment was done under competitive infestation (simultaneous infestation to the two susceptible and four resistant cultivars randomly grown in the cage). A significantly higher oviposition by the TC colony than the wild-type colony on Thai Col. 11 was observed at the 12th generation, while the difference with the wild-type became significant at the 9th generation in the PO colony. The results of oviposition by these selected colonies on the two susceptible and four resistant cultivars at the 12th generation are shown in Fig. 4. Both colonies showed

![Graph showing changes in oviposition by TC and PO colonies on Thai Col. 11 and Pokkali during generations 1 to 18.](image)

**Fig. 3.** Changes in oviposition (number of eggs deposited per plant) by wild-type, TC and PO colonies. (A) ■, wild-type on Thai Col. 11; ●, TC colony on Thai Col. 11; (B) ×, wild-type on Pokkali; ▲, PO colony on Pokkali. Data represent means of five replicates taken after infestation of newly emerged adults on 3-week-old plants (3 couples per plant). The coefficient of variation was 22%. * indicates differences at the 1% level of significance.

![Graph showing oviposition by BPH of wild-type, TC, and PO colonies on susceptible and resistant cultivars.](image)

**Fig. 4.** Oviposition by BPH of the wild-type, TC and PO colonies on the susceptible and resistant cultivars. Data represent means of five replicates taken after infestation of newly emerged adults of the 12th generation on 3-week-old plants (3 couples per plant). The coefficient of variation was 27%. Bars denoted by different letters indicate differences at the 1% level of significance.
much higher oviposition than the wild-type BPH on all cultivars tested except for the PO colony on Balamawee. The TC colony showed the highest oviposition on both Thai Col. 11 and Thai Col. 5. The PO colony showed the highest oviposition on Pokkali but the lowest on Balamawee.

Under the experimental conditions employed here, the wild-type colony could feed only on the susceptible hosts, thus its effective infestation density could have been 3 times higher than that of the PO and TC colonies. The inhibition of feeding due to the high density of resistant plants might explain why the wild-type colony showed significantly low oviposition even on susceptible hosts. The observed high oviposition by the PO and TC colonies on the selection hosts, however, clearly demonstrated their adaptation.

Egg hatch

Changes in egg hatch during the selection process were quite similar to those in oviposition in both TC and PO colonies with regard to susceptible and resistant selection hosts. The rates of egg hatch obtained using the selected BPH colonies on the susceptible and resistant cultivars at the 12th generation are shown in Fig. 5. Significant correlations were observed between oviposition and egg hatch in the wild-type BPH (r=0.90), TC colony (r=0.98) and PO colony (r=0.94). Both TC and PO colonies showed higher rates of egg hatch than the wild-type BPH for all the cultivars tested except Balamawee. The TC colony showed the highest rate of egg hatch for both Thai Col. 11 and Thai Col. 5, while the PO colony showed no apparent virulence against Balamawee in terms of egg hatch.

Percent egg hatchability was calculated from the data for oviposition and egg hatch. Figure 6 shows the changes in hatchability of eggs deposited by the wild-type and TC colonies on Thai Col. 11 and the wild-type and PO colonies on Pokkali during the 1st to the 18th generation. The TC colony showed a continuous increase in egg hatchability on the selection host. The difference between the wild-type colony and the TC colony became significant at the 12th generation. A similar trend was observed in the PO colony on Pokkali, but a significantly higher egg hatchability than that observed for the wild-type occurred only at the 15th generation.

DISCUSSION

Continuous culturing of the wild-type BPH on resistant rice cultivars that possess either a recessive gene bph8 or a dominant gene Bph9 resulted in the selection of BPH colonies that show virulence against the respective selection hosts. The gradual but significant increases in
Virulent BPH Colonies against Rice BPH Resistance Genes

Fig. 6. Changes in percent hatchability of eggs deposited by BPH of wild-type, TC and PO colonies on the resistant cultivars. (A) ■, wild-type on Thai Col. 11; ○, TC colony on Thai Col. 11; ×, wild-type on Pokkali; ▲, PO colony on Pokkali. Data represent means of five replicates taken after infestation of the newly emerged adults on 3-week-old plants (3 couples per plant). The coefficient of variation was 12%. * indicates differences at the 5% level of significance.

the rates of survival, oviposition and egg hatch by the BPH colonies on the selection hosts indicated their adaptation. The different parameters tested, however, depicted different time requirements for adaptation to the selection hosts. Significant virulence of TC and PO colonies against the selection hosts was judged, respectively, at the 10th and the 14th generation based on the rate of survival. Virulence became significant at the 9th–15th generation on the bases of oviposition and egg hatch. A considerable fluctuation, however, was observed in wing dimorphism in the PO colony, indicating its unstable virulence against Pokkali. Pathak and Heinrichs (1982) demonstrated the selection of virulent BPH (biotypes 2 and 3) against two resistant hosts (with Bph1 or bph2) in about 8 generations on the basis of BPH survival and the bulked seedling tests. Although the time required for selection in our study appears to be longer than that of others (Pathak and Heinrichs, 1982; Sogawa, 1982; Nemoto and Yokoo, 1994), the difference can easily be ascribed to factors such as selection intensity, BPH genotypes, BPH population size and resistance genes of selection hosts. It has been suggested that the natural population of BPH includes some proportions of pre-existing biotypes which, upon selection, become forerunners of new biotypes (IRRI, 1976; Pathak and Heinrichs, 1982). The observed rate of BPH survival on the resistant hosts during the initial stage of the selection supports this hypothesis. It is, thus, suggested that the adaptation to specific major resistance genes provides selectable advantages for such forerunners to increase their relative frequency in the BPH population under selection.

An important question related to the effectiveness of selection is whether BPH virulence and/or its specificity is controlled by single major genes or by polygenes. The work by Cheng and Chang (1979) proposing a gene-for-gene relationship could not be repeated by others. The results of Sogawa (1981) were inconclusive; he found a possible major gene for one biotype but polygenic inheritance for another. den Hollander and Pathak (1981) demonstrated the polygenic basis of BPH virulence. We showed that the TC colony selected on Thai Col. 11 possesses virulence not only against the selection host but also against the non-selection host with the same resistance gene, bph8. The observed virulence of the TC colony against two cultivars carrying an identical major resistance gene suggests the specificity of its virulence against bph8. Although the genetic basis of virulence or adaptation of the selected BPH colonies remains to be elucidated, the effectiveness of the selection for BPH virulence against a given major resistance gene observed in our study suggests that the number of selectable
major genes involved in determining BPH virulence is rather small.

Another important question is that of the mechanism of rice resistance to BPH. Among 10 resistance genes so far described, six are reported to be dominant and four are recessive (Murata et al., 1997). Resistance mechanisms controlled by dominant genes and recessive genes are likely to be different. In relation to this, the observed increases in oviposition and egg hatch, especially egg hatchability, in the two selected BPH colonies are noteworthy. The observation seems to indicate the presence of some common virulence mechanism that is effective against rice cultivars carrying major dominant genes and recessive genes. It has been shown that leaf sheath tissues surrounding eggs of the whitebacked planthopper cause physiological egg mortality in resistant rice plants, suggesting the presence of an ovicidal substance(s) (e.g. benzyl benzoate) (Seino et al., 1996; Suzuki et al., 1996; Seino and Suzuki, 1997). Recently we have observed significant growth inhibition of an Indian meal-moth, Plodia interpunctella (Lepidoptera: Phycidae), after infestation of this storage insect pest on rice grain-powder prepared from the two BPH resistant cultivars (Naemullah et al., unpublished). The result suggests that grains of these BPH resistant rice cultivars contain toxic substance(s) effective against insect pests other than BPH. The relationship between the growth inhibition of the storage pest and BPH resistance genes should be elucidated. The effect of both dominant and recessive resistance genes on the development of BPH eggs deposited in leaf sheath tissues of the resistant hosts must be further studied.

The PO colony did not show virulence against Balamawee, which was reported to be another Bph9 carrier (Nemoto et al., 1989). Balamawee showed the highest level of resistance against all the BPH colonies studied. This result suggests that Balamawee may carry additional resistance gene(s). Along with the two major BPH colonies described in this study, several subcolonies including ones that show simultaneous virulence to bph8 and Bph9 have been developed by altering the selection hosts (Ketipearachchi and Nakamura, 1998). These colonies may provide useful experimental means for further characterization of BPH biotypes.

ACKNOWLEDGEMENTS

Y. Ketipearachchi is a recipient of the scholarship from the Ministry of Education, Science, Sports and Culture of Japan (Monbusho). Contribution No. 103 from the Laboratory of Plant Genetics, Kobe University.

REFERENCES


Freed, R. D. and S. P. Eisensmith (1989) MSTAT, MSTAT-C version 1.3. Crop and Soil Sciences Department, Michigan State University, East Lansing, MI, USA.


Virulent BPH Colonies against Rice BPH Resistance Genes


