

Food assimilated by two sympatric populations of the brown planthopper *Nilaparvata lugens* (Delphacidae) feeding on different host plants contaminates insect DNA detected by RAPD-PCR analysis

M.A. Latif^{1,6}, M.Y. Omar², S.G. Tan³, S.S. Siraj⁴, M.E. Ali⁵ and M.Y. Rafii¹

¹Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, Selangor, Malaysia

²Department of Biology, Faculty of Science, Universiti Putra Malaysia, Selangor, Malaysia

³Department of Cell and Molecular Biology, Faculty of Biotechnology and Molecular Science, Universiti Putra Malaysia, Selangor, Malaysia

⁴Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, Selangor, Malaysia

⁵Institute of Nano Electronic Engineering, Universiti Malaysia Perlis, Perlis, Malaysia

⁶Bangladesh Rice Research Institute, Bangladesh

Corresponding author: M.A. Latif

E-mail: alatif1965@yahoo.com

Genet. Mol. Res. 11 (1): 30-41 (2012)

Received June 21, 2011

Accepted September 29, 2011

Published January 9, 2012

DOI <http://dx.doi.org/10.4238/2012.January.9.4>

ABSTRACT. Contamination of insect DNA for RAPD-PCR analysis can be a problem because many primers are non-specific and DNA from parasites or gut contents may be simultaneously extracted along with that of the insect. We measured the quantity of food ingested and

assimilated by two sympatric populations of brown planthopper (BPH), *Nilaparvata lugens*, one from rice and the other from *Leersia hexandra* (Poaceae), a wetland forage grass, and we also investigated whether host plant DNA contaminates that of herbivore insects in extractions of whole insects. Ingestion and assimilation of food were reduced significantly when individuals derived from one host plant were caged on the other species. The bands, OPA3 (1.25), OPD3 (1.10), OPD3 (0.80), OPD3 (0.60), pUC/M13F (0.35), pUC/M13F (0.20), BOXAIR (0.50), peh#3 (0.50), and peh#3 (0.17) were found in both rice-infesting populations of brown planthopper and its host plant (rice). Similarly, the bands, OPA4 (1.00), OPB10 (0.70), OPD3 (0.90), OPD3 (0.80), OPD3 (0.60), pUC/M13F (0.35), pUC/M13F (0.20), and BOXAIR (0.50) were found in both *Leersia*-infesting populations of brown planthopper and the host plant. So, it is clear that the DNA bands amplified in the host plants were also found in the extracts from the insects feeding on them.

Key words: Brown planthopper; Determination; Food assimilation; DNA contamination; RAPD-PCR analysis

INTRODUCTION

The brown planthopper (BPH), *Nilaparvata lugens* (Stål), is a major pest of rice, which is widely distributed from tropical to temperate areas of Asia and Australia. This insect is a phloem-feeder and is restricted to cultivated and wild rice as their host plants. It causes “hopper burn” and complete wilting and drying of rice plants (Dyck and Thomas, 1979) and also transmits the grassy stunt and ragged stunt viral diseases (Ling, 1977). Large-scale rice crop damage caused by the pest was reported in the 1970s in several South and South East Asian countries (Dyck and Thomas, 1979).

It has been recognized that the BPH exists as a complex population, which is commonly referred to as biotypes (Sogawa, 1981). Despite the reports of possible infestation on alternative hosts and the collection of adults from a wide variety of plants, it is usually said to repeat in generations only on rice (Mochida and Okada, 1979). However, insects that feed on rice have been shown to adapt readily to a wide array of previously resistant cultivars (Pathak and Heinrichs, 1982; Claridge and Den Hollander, 1982) and this has led to speculation that *N. lugens* may form host-specific races as a stage leading to sympatric species formation (Pathak and Heinrichs, 1982).

The BPH, *N. lugens* (Stål), is generally thought to be specific to wild and cultivated rice. This pest was found to thrive on a weed grass, *Leersia hexandra*, that grows abundantly in irrigation canals near rice fields in South East Asia (Domingo et al., 1983; Sogawa et al., 1984). The individuals of this population have strong specificity for the weed host. They fail to survive when caged on rice plant (Claridge et al., 1985; Latif, 2000). Similarly, individuals of the rice infesting *N. lugens* die when confined to weed grass, *L. hexandra*. Suitability of plants as host to insects is determined by the factors that influence insect establishment on the plants. The extent of insect establishment depends on an interaction between insect responses to various plant characteristics. The main categories of behavioral and physiological responses during insect establishment on plants are feeding, metabolism of ingested food, growth, adult survival, egg production, and oviposition (Saxena, 1969).

The existence of diagnostic markers and the genetic distance between rice- and *Leersia*-infesting populations of *N. lugens* from allozymes and RAPD-PCR analyses have suggested that insects with high esterase activity (usually caught in rice) were genetically different from insects with low esterase activity (usually caught in *Leersia*) and both insects were closely related sibling species (Latif et al., 2008, 2010). The use of RAPDs for population genetics allows analysis of small amounts of DNA and the detection of a large number of polymorphisms. Nevertheless, this methodology may be limited by the difficulty to precisely replicate results because of bands associated with contaminants, and by the assumptions made in conducting the data analyses. To decrease potential methodological limitations, the DNA was extracted from legs or other parts and at least one sample per PCR set was repeated. In addition, the bands were selected from a representative insect population sample and only well-defined bands were scored (Ocampo and Wesson, 2004). Contamination is always a problem with RAPDs because the primers are non-specific. It may be a bigger problem in insect studies because DNA is often extracted from the whole organism. The present study was undertaken to determine the quantity of food ingested and assimilated by two sympatric populations of BPH as well as to investigate whether host DNA contaminates the respective insect DNA if they are extracted from whole insects. To investigate possible contamination, DNA was extracted both from insects and two types of host plants and amplified following short primer RAPD and long primer (LP)-RAPD protocols. If the same banding patterns were observed in both the insects and their respective host plants, the insect DNA could have been contaminated by host DNA.

MATERIAL AND METHODS

Quantity of food ingested and assimilated by brown planthopper, *N. lugens* of rice and *L. hexandra*

Newly emerged virgin brachypterous females of BPH from two sympatric populations, one from rice and other from weed, *L. hexandra*, were starved for 2 h and water saturated to determine the quantity of food ingested and assimilated. The experiment was laid out in complete randomized design with four treatments. Rice infesting population was caged on weed, *L. hexandra*, and rice. Conversely, *Leersia*-infesting population was caged on rice and weed, *L. hexandra*. There were eight replications for each treatment; each replication consisted of three females caged individually in parafilm sachets on three different plants of two hosts. Each female was weighed individually and enclosed singly for feeding in parafilm sachets (5 x 5 cm) on the leaf sheaths of rice plants or stems of *Leersia* plants. After 24 h, the weight of each female and its excreta were recorded separately. To assess the loss in insect body weight from catabolism, a control was established, in which the insect was denied any plant but was given access to a moist cotton swab to prevent desiccation. The amount of food ingested and assimilated by the insect was calculated as follows (Saxena and Pathak, 1977).

$$\text{Food assimilated} = W_1 \times (C_1 - C_2) / C_1 + (W_2 - W_1),$$

where W_1 is the initial weight of the insect, W_2 is the final weight of the insect, C_1 is the initial weight of the control insect, and C_2 is the final weight of the control insect. The food ingested was calculated as food assimilated plus weight of excreta.

Use of the short and long primer RAPD-PCR for the detection of contamination of BPH DNA

Sample collection for the study of DNA contamination

Twenty-five individuals each from two sympatric populations of BPH, *N. lugens*, one from rice and the other from *L. hexandra*, and the same number of young leaves from two respective host plants were collected from experimental field of UPM, Malaysia. Insect and leaves were preserved at -70°C for further use.

DNA extraction from insect

DNA from individual insect was isolated by grinding a single frozen adult with a sterile glass rod in 1.5-mL tube in 20 µL grinding buffer (0.1 M NaCl, 0.2 M sucrose, 0.1 M Tris-NaCl, pH 9.0, 0.05 M EDTA, and 0.5% SDS). The glass rod was washed with an additional 40 µL grinding buffer and the homogenate was incubated at 65°C for 40 min. After that, 10 µL 8 M potassium acetate was added and the tube was placed on ice for 40 min. The tube was spun at 14000 rpm for 20 min. The supernatant was transferred to a fresh 1.5-mL tube. One hundred microliters of chilled (-20°C) 100% ethanol was added and the DNA was allowed to precipitate at room temperature for 10 min. The tube was spun for 20 min and the ethanol was carefully removed with a pipette. The DNA pellet was washed with 100 µL chilled 70% ethanol and spun for 10 min. The DNA pellet was dried by first pouring off the ethanol and keeping the tube for 10 min at room temperature. The dried DNA pellet was suspended in 50 µL TE (Tris-EDTA, pH 8.0), and gently mixed for a few minutes. DNA was diluted with sterile distilled water to a concentration of 50 ng/µL for PCR analysis and kept in a refrigerator of -20°C.

DNA extraction from host plant

DNA from each host-plant leaf was isolated by grinding 200 mg stem with a sterile glass rod in 1.5-mL tube in 450 µL grinding buffer (0.1 M NaCl, 0.2 M sucrose, 0.1 M Tris-HCl, pH 9.0, 0.05 M EDTA and 0.5% SDS). The glass rod was washed with an additional 450 µL grinding buffer and the homogenate was incubated at 65°C for 40 min. After that, 100 µL 8 M potassium acetate was added and the tubes were placed on ice for 40 min. The tubes were spun at 14,000 rpm for 20 min. The supernatant was transferred to a fresh 1.5-mL tube. Three hundred microliters of chilled (-20°C) 100% ethanol was added and the DNA allowed to precipitate at room temperature for 10 min. Again the tubes were spun for 20 min and the ethanol was carefully removed with a pipette. The DNA pellet was washed with 300 µL chilled 70% ethanol and spun for 10 min at 14,000 rpm. The DNA pellets were dried by first pouring off the ethanol and keeping the tubes for 10 min at room temperature. The dried DNA pellets were suspended in 50 µL TE, pH 8.0, and gently mixed for a few minutes. DNA was diluted with sterile distilled water to a concentration of 50 ng/µL for PCR analysis and kept in a refrigerator at -20°C.

DNA purity and quantity

The insect and host plant DNA concentrations were measured using LKB-Ultrastep III

UV/visible spectrophotometer at absorbances of 260 and 280 nm. The DNA was considered to be pure if the ratio of OD_{260}/OD_{280} was within the range of 1.6-1.9 (Sambrook et al., 1989)

Short primer RAPD-PCR

RAPD-PCRs were carried out in 25- μ L volumes. Each reaction mixture (25 μ L) containing 10 mM Tris-HCl; 50 mM KCl; 0.1% Triton X-100; 2 mM $MgCl_2$; 0.2 M each of dATP, dGTP, dCTP, and dTTP; 5 pmol of a single primer; 80 ng of a template genomic DNA solution, and 3 U Taq DNA polymerase (Promega), was vortexed briefly to mix and centrifuged for about 5 s.

Amplification reactions were carried out in a programmable thermal cycler (GeneAmp[®], PCR system 2400, Perkin Elmer Sdn Bhd, Petaling Jaya, Malaysia) programmed as follows: pre-denaturation at 94°C for 2 min; followed by 40 cycles of denaturation at 92°C for 30 s, annealing temperature at 37°C for 30 s and primer extension at 72°C for 1 min. After the last cycle, a final extension was at 72°C for 5 min. The amplified PCR products were separated by loading 20 μ L of the reaction mixture onto 1.5% agarose gels with 1X TBE (Tris-borate-EDTA) buffer and running at 70 V for 3 h. A hundred base pair ladder (Promega) was loaded in the first lane as the marker. Gels were stained with ethidium bromide (10 μ g/ μ L) and the bands were visualised and photographed on a UV transilluminator.

LP-RAPD-PCR

LP-RAPD-PCRs were carried out in 25- μ L volumes. Each reaction mixture (25 μ L) containing 10 mM Tris-HCl; 50 mM KCl; 0.1% Triton X-100; 2-4 mM $MgCl_2$; 0.2 M each of dATP, dGTP, dCTP, and dTTP; 1.0 μ M of a single primer; 160 ng of template genomic DNA solution, and 4 U Taq DNA polymerase (Promega and Gibco, BRL) was vortexed briefly to mix and centrifuged for about 5 s.

Amplification reactions were carried out in a programmable thermal cycler (GeneAmp[®], PCR system 2400, Perkin Elmer) programmed as follows: pre-denaturation at 94°C for 3 min; followed by 40 cycles of denaturation at 94°C for 30 s, annealing temperature at 52-55°C for 30 s and a primer extension at 72°C for 2 min. After the last cycle, a final extension was at 72°C for 10 min. The amplified PCR products were separated by loading 20 μ L of the reaction mixture onto 1.5% agarose gels with 1X TBE buffer and running at 70 V for 3 h. A hundred base pair ladder (Promega) was loaded in the first lane as the marker. The gels were stained with ethidium bromide (10 μ g/ μ L) and the bands were visualised and photographed on a UV transilluminator.

Selection of short and long RAPD primers

A total of 8 primers from short and long RAPD were used in this study because these primers yielded the strongest bands and polymorphisms in two sympatric populations of BPH, one from rice and the other from *L. hexandra* and their respective host plants (Table 1).

Effect of different thermal cyclers

DNA samples were replicated thrice and amplified in two different thermocyclers: a)

Table 1. Nucleotide sequences of short and long RAPD primers used in this study.

Primer code	Sequence (5'-3')	Concentration	Taq used	Annealing temperature
Short primer RAPD				
OPA3	AGTCAGCCAC	5.19 nm	Promega	37°C
OPA4	AATCGGGCTG	5.09 nm	Promega	37°C
OPB10	CTGCTGGGAC	5.49 nm	Promega	37°C
OPD3	GTCGCCGTCA	5.74 nm	Promega	37°C
Long primer RAPD				
pUC/M13F	CGCCAGGGTTTTCCAGTCACGAC	2 mM	Promega	55°C
BOXAIR	CTACGGCAAGGCGACGCTGACG	2 mM	Promega	52°C
pehA#6	ATCGCACTTGATGCGCAGGCCGTT	4 mM	Gibco, BRL	52°C
pehA#3	CAGCAGAACCCGCGCCTGATCCAG	2 mM	Promega	52°C

GeneAmp[®], PCR system 2400, Perkin Elmer, and b) Master cycler gradient, Eppendorf, were used to observe the effects of different PCR machines on banding patterns using the same reaction conditions and PCR profile.

Detection of DNA contamination

Only the repeatable and strong bands from two sympatric populations of *N. lugens* and their respective host plants were recorded. To detect the possible contamination, DNA was extracted from two types of host plants and amplified following short primer RAPD and LP-RAPD protocols. If the same banding patterns were observed in both the insects and their respective host plants, the insect DNA could be contaminated by host DNA. The frequency of common bands both in BPH and their respective host's plant was also determined.

RESULTS

Quantity of food ingested and assimilated by brown planthopper, *N. lugens* of rice and *L. hexandra*

The quantity of food ingested and assimilated by rice- and weed-infesting individuals of *N. lugens* was significantly higher on their respective hosts (Table 2). Ingestion and assimilation of food were significantly reduced when individuals derived from one host were caged on the other host. In the case of rice-infesting populations, food ingested and assimilated per female per 24 h were numerically higher compared to the *Leersia*-infesting population, but these differences were statistically insignificant.

Table 2. Quantity of food ingested and assimilated by brown planthopper, *Nilaparvata lugens* of rice and *Leersia hexandra*.

Brown planthopper	Plant	Food ingested (mg) per female/24 h	Food assimilated (mg) per female/24 h
Rice-infesting	Rice (MR84)	21.4 ^a	1.10 ^a
	<i>L. hexandra</i>	0.4 ^b	0.05 ^b
<i>Leersia</i> -infesting	<i>L. hexandra</i>	18.3 ^a	1.02 ^a
	Rice (MR84)	0.6 ^b	0.09 ^b

Numbers followed by a common superscript lower case letter are not significantly different at 5% level by LSD.

Effect of different thermal cyclers

These studies showed that two different thermal cyclers, GeneAmp[®], PCR system 2400, Perkin Elmer, and Master cycler gradient, Eppendorf, gave similar RAPD banding patterns with the same reaction conditions and same PCR profile.

Use of the short and LP-RAPD-PCR for the detection of contamination of BPH DNA

The banding patterns of short and LP-RAPD for two sympatric populations of BPH and their respective host plants are shown in Figures 1, 2 and 3. The bands recorded from the rice- and *Leersia*-infesting population of *N. lugens* and their respective host plants using both short and LP-RAPD techniques are shown in Table 3.

A total of 34 and 24 distinct reproducible bands were recorded from the rice-infesting population of BPH and its host plant (rice plant), respectively, by PCR amplifications of four short primers RAPD. The bands, OPA3 (1.25), OPD3 (1.10), OPD3 (0.80), and OPD3 (0.60) were recorded as common in rice-infesting BPH and its host plant, rice. On the other hand, a total of 32 and 18 clear reproducible bands were recorded in *Leersia*-infesting population of BPH and its host plant, *L. hexandra*. The bands, OPA4 (1.00), OPB10 (0.70), OPD3 (0.90), OPD3 (0.80), and OPD3 (0.60) were recorded as common between the *Leersia*-infesting BPH and host plant, *L. hexandra* (Table 3).

For LP-RAPD, a total of 31 and 21 reproducible bands were recorded from rice-infesting population of BPH and its host plant (rice), respectively. The bands, pUC/M13F (0.35), pUC/M13F (0.20), BOXAIR (0.50), peh#3 (0.50), and peh#3 (0.17) were common both in rice-infesting BPH and its host plant. A total of 29 and 13 distinct bands were recorded from

Table 3. Short and long RAPD fragments (bands) recorded from two sympatric populations of brown planthopper (BPH) and their host plants.

Primers	Band (kb) recorded from rice plant	Band (kb) recorded from rice-infesting population of BPH	Band (kb) recorded from <i>Leersia</i> plant	Band (kb) recorded from <i>Leersia</i> -infesting population of BPH
Short primer RAPD fragments				
OPA3	(1.25), 1.10, 0.97, 0.80, 0.62, 0.67, 0.52, 0.35	(1.25), 1.15, 0.65, 0.60, 0.55, 0.50, 0.45, 0.35	1.05, 0.85, 0.65	1.15, 0.60, 0.50, 0.45, 0.30
OPA4	1.25, 0.92, 0.82, 0.70, 0.60	(1.00), 0.95, 0.90, 0.80, 0.72, 0.62, 0.52, 0.42, 0.40	1.10, (1.00), 0.50, 0.45	(1.00), 0.95, 0.90, 0.80, 0.72, 0.60, 0.52, 0.47, 0.42, 0.40, 0.35
OPB10	1.10, 0.90, 0.80, 0.50, 0.40	0.92, (0.70), 0.60, 0.55, 0.50, 0.42, 0.27	1.40, 0.90, (0.70), 0.45	0.92, (0.70), 0.60, 0.55, 0.37, 0.27
OPD3	1.65, 1.25, (1.10), 0.92, (0.80), (0.60)	1.60, 1.50, 1.30, 1.20, (1.10), 1.00, (0.90), (0.80), 0.70, (0.60)	1.15, 0.95, (0.90), (0.80), 0.67, (0.60), 0.42	1.60, 1.50, 1.30, 1.20, 1.00, (0.90), (0.80), 0.70, 0.65, (0.60)
Long primer RAPD fragments				
pUC/M13F	0.70, (0.35), 0.25, (0.20)	0.37, (0.35), (0.20)	0.60, (0.35), (0.20)	0.37, (0.35), (0.20)
BOXAIR	0.80, 0.57, (0.50), 0.42	1.00, 0.70, 0.60, (0.50), 0.40, 0.35, 0.30, 0.25, 0.20	0.80, (0.50), 0.42, 0.37, 0.32	1.00, 0.70, 0.60, (0.50), 0.40, 0.35, 0.30, 0.25, 0.20
peh#6	1.00, 0.75, 0.72, 0.65, 0.57, 0.42, 0.40, 0.32, 0.27	1.10, 0.85, 0.80, 0.70, 0.60, 0.37, 0.30, 0.17	0.42, 0.40, 0.27	1.20, 1.00, 0.80, 0.70, 0.50, 0.37, 0.30, 0.17
peh#3	1.10, 1.00, (0.50), 0.32, (0.17)	0.90, 0.80, 0.70, 0.60, (0.50), 0.40, 0.35, 0.30, 0.27, 0.25, (0.17)	1.12, 1.00	0.90, 0.60, (0.50), 0.40, 0.35, 0.30, 0.27, 0.25, (0.17)

Numbers within parentheses were common bands for insects and their host plant.

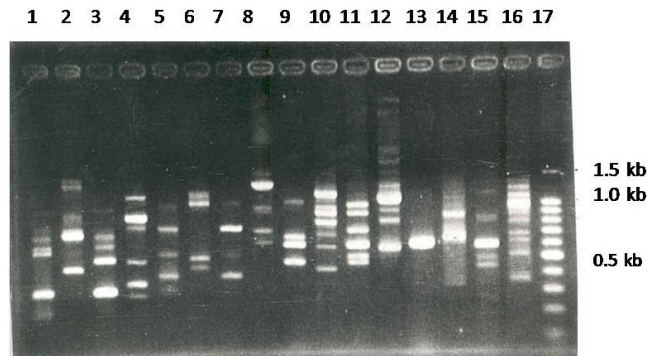


Figure 1. Short primer RAPD bands from rice and *Leersia*-infesting populations and their host plants amplified by four short primers, OPB10, OPB04, OPD03, and OPA03 (lane 1 = *Leersia* insect, lane 2 = *Leersia* plant, lane 3 = rice insect, lane 4 = rice plant, generated by OPB10; lane 5 = *Leersia* insect, lane 6 = *Leersia* plant, lane 7 = rice insect, lane 8 = rice plant, generated by OPB04; lane 9 = *Leersia* insect, lane 10 = *Leersia* plant, lane 11 = rice insect, lane 12 = rice plant, generated by OPD03; lane 13 = *Leersia* insect, lane 14 = *Leersia* plant, lane 15 = rice insect, lane 16 = rice plant, generated by OPA03; lane 17 = 100-bp DNA marker).

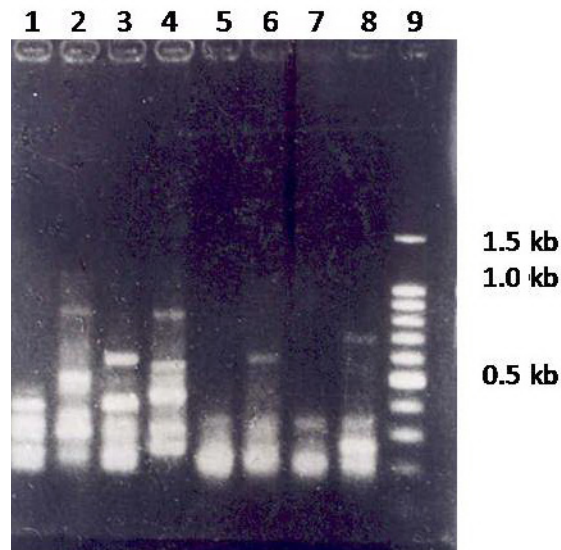


Figure 2. LP-RAPD bands from rice and *Leersia*-infesting populations and their host plants amplified by two primers, BOXAIR and pUC/M13F (lane 1 = *Leersia* insect, lane 2 = *Leersia* plant, lane 3 = rice insect, lane 4 = rice plant, from primer BOXAIR; lane 5 = *Leersia* insect, lane 6 = *Leersia* plant, lane 7 = rice insect, lane 8 = rice plant, from primer pUC/M13F; lane 9 = 100-bp DNA marker).

Leersia-infesting population of BPH and its host plant, *L. hexandra*, respectively. The bands common both in *Leersia*-infesting population of BPH and its host plant, *L. hexandra* were recorded as pUC/M13F (0.35), pUC/M13F (0.20) and BOXAIR (0.50) (Table 3).

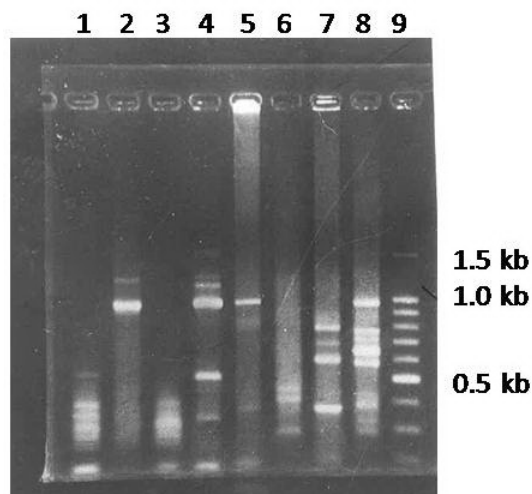


Figure 3. LP-RAPD bands from rice and *Leersia*-infesting populations and their host plants amplified by two primers, pehA#3 and pehA#6 (lane 1 = *Leersia* insect, lane 2 = *Leersia* plant, lane 3 = rice insect, lane 4 = rice plant, from primer pehA#3; lane 5 = *Leersia* insect, lane 6 = *Leersia* plant, lane 7 = rice insect, lane 8 = rice plant, from primer pehA#6; lane 9 = 100-bp DNA marker).

The percentage of frequency of common bands or marker, which existed both in two sympatric populations of BPH and their host plants, was calculated and is presented in Table 4. Irrespective of short and LP-RAPD, the frequency of common band ranged from 15 to 25% in rice-infesting population of BPH while it ranged from 62 to 100% in its host plant (rice). On the other hand, the frequency of common band ranged from 15 to 30% in *Leersia*-infesting population of BPH while it ranged from 68 to 100% in its host plant (*L. hexandra*).

Table 4. Frequency of common bands recorded from two sympatric populations of brown planthopper (BPH) and their respective host plants in short and long primers RAPD-PCR.

Band size (kb)	Frequency (%)			
	Rice-infesting BPH	Host plant (rice)	<i>Leersia</i> -infesting BPH	Host plant (<i>L. hexandra</i>)
Short primer RAPD-PCR				
OPA3 (1.25)	25	100	-	-
OPA4 (1.00)	-	-	28	100
OPB10 (0.70)	-	-	20	100
OPD3 (1.10)	21	100	-	-
OPD3 (0.90)	-	-	23	100
OPD3 (0.80)	23	100	21	100
OPD3 (0.60)	15	90	15	75
Long primer RAPD-PCR				
pUC/M13F (0.35)	12	70	15	74
pUC/M13F (0.20)	22	62	30	65
BOXAIR (0.50)	14	100	23	70
peh#3 (0.50)	25	100	-	82
peh#3 (0.17)	10	75	-	68

- = band absent.

DISCUSSION

The quantity of food ingested and assimilated by rice- and weed-infesting individuals of *N. lugens* was significantly higher on their respective host plants. Similar studies were conducted on *N. lugens* and *Sogatela furcifera* (Khan and Saxena, 1985; Velusamy, 1988; Latif, 2000). The responses of food ingested and assimilated were significantly reduced when *N. lugens* reared on resistant cultivar compared to susceptible cultivar. Similar results were recorded by Wu et al. (1986) in biotype 2 of *N. lugens* on resistant wild rice. Greater nymphal growth, adult survival and higher egg production of *N. lugens* on the susceptible cultivar, TN1, could be attributed to a higher quantity of food ingested and assimilated compared to resistant wild rice species. Other workers made similar observations with *S. furcifera* on resistant cultivars (Khan and Saxena, 1985).

A total of 9 amplicons, OPA3 (1.25), OPD3 (1.10), OPD3 (0.80), OPD3 (0.60), pUC/M13F (0.35), pUC/M13F (0.20), BOXAIR (0.50), peh#3 (0.50), and peh#3 (0.17) were common in rice-infesting populations of BPH and its host plant (rice). On the other hand, 8 bands, OPA4 (1.00), OPB10 (0.70), OPD3 (0.90), OPD3 (0.80), OPD3 (0.60), pUC/M13F (0.35), pUC/M13F (0.20), and BOXAIR (0.50) were common in *Leersia*-infesting populations of BPH and its host plant (*L. hexandra*). Therefore, the bands present in each host plant were also found in the respective insect population. This might be the contamination of DNA extracted from an insect with its gut contents. Also, contamination of host DNA might be a bigger problem in a population genetic study of two sympatric population of BPH if DNA was extracted from a whole insect. The RAPD-PCR depends on the use of random primers; it was not unexpected that bands be obtained by RAPD-PCR of the host DNA used. As with the situation in *Echinococcus* (Scott and McManus, 1994), the bands obtained with host DNA would not interfere with the parasite bands even if some were generated. However, it is extremely unlikely at the levels of the host DNA contamination likely to be present (less than 1 ng) that any host band would be generated (Zhi-Gang and Alan, 1995). Conversely, RAPD-PCR results have previously shown the resistance to high amounts of host DNA contamination (Comeau et al., 2004). Minor differences in reaction conditions can affect the probability of amplification of some products which, consequently, may seem to be absent (McClelland and Welsh, 1994). Finally, in addition to all the above, the pure complexity of the electrophoretic banding patterns can be difficult to interpret, although this is a common problem with all multiple-product techniques such as DNA fingerprinting (Lewin, 1989). However, in our study, two different thermal cyclers produced similar RAPD banding patterns with the same reaction conditions and same PCR profile. There was a potential problem worthy of note that RAPD-PCR may result in non-genetic variation as reported by several authors (Ruano et al., 1991; Latif et al., 2008). Therefore, care should be taken when analyzing the RAPD data because DNA is often extracted from the whole insect and DNA from parasites or gut contents may be simultaneously extracted.

CONCLUSIONS

Our results revealed that ingestion and assimilation of food by two sympatric populations of brown planthopper, *N. lugens*, one from rice and the other from *L. hexandra*, were reduced significantly when individuals derived from one host were caged on the other host plant. A total of 9 RAPD fragments were common in rice-infesting populations of brown planthopper and its host plant (rice), while 8 RAPD fragments were common in *Leersia*-

infesting populations of brown planthopper and its host plant (*L. hexandra*). Furthermore, the common bands between brown planthopper and their host plants indicate that DNA of either rice-infesting or *Leersia*-infesting population of brown planthopper might be contaminated with their respective host DNA.

ACKNOWLEDGMENTS

We thank the Universiti Putra Malaysia for providing research facilities and financial support to achieve these findings.

REFERENCES

- Claridge MF and Den Hollander J (1982). Virulence to rice cultivars and selection for virulence in populations of the brown planthopper *Nilaparvata lugens*. *Entom. Expert. Appl.* 32: 213-221.
- Claridge MF, Den Hollander J and Morgan JC (1985). The status of weed associated populations of the brown planthopper, *Nilaparvata lugens* (Stål)-host race or biological species. *Zool. J. Linn. Soc.* 84: 77-90.
- Comeau AM, Short S and Suttle CA (2004). The use of degenerate-primed random amplification of polymorphic DNA (DP-RAPD) for strain-typing and inferring the genetic similarity among closely related viruses. *J. Virol. Methods* 118: 95-100.
- Domingo IT, Heinrichs EA and Saxena RS (1983). Occurrences of brown planthopper on *Leersia hexandra* in the Philippines. *Intl. Rice Newsl.* 8: 17.
- Dyck VA and Thomas B (1979). The Brown Planthopper Problem. In: Brown Planthopper. Treat to rice production in Asia IRRI, Philippines, Los Banos, 3-17.
- Khan ZR and Saxena RC (1985). Behavioral and physiological responses of *Sogatella furcifera* (Homoptera: Delphacidae) to selected resistant and susceptible rice cultivars. *J. Econ. Entom.* 78: 1280-1286.
- Latif MA (2000). Morphological, Molecular Genetic and Host Plant Relationship Studies of Rice and Weed Infesting Populations of Brown Planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae). A PhD thesis Submitted in Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the Faculty of Science and Environmental Studies. Universiti Putra Malaysia, 314.
- Latif MA, Soon GT, Mohd YO and Siraj SS (2008). Evidence of sibling species in the brown planthopper complex (*Nilaparvata lugens*) detected from short and long primer random amplified polymorphic DNA fingerprints. *Biochem. Genet.* 46: 520-537.
- Latif MA, Omar MY, Tan SG, Siraj SS, et al. (2010). Interpopulation crosses, inheritance study, and genetic variability in the brown planthopper complex, *Nilaparvata lugens* (Homoptera: Delphacidae). *Biochem. Genet.* 48: 266-286.
- Lewin R (1989). Limits to DNA fingerprinting. *Science* 243: 1549-1551.
- Ling KC (1977). Rice ragged stunt disease. *Intl. Rice Res. Newsl.* 2-6.
- McClelland M and Welsh J (1994). DNA fingerprinting by arbitrarily primed PCR. *PCR Methods Appl.* 4: S59-S65.
- Mochida O and Okada T (1979). Taxonomy and Biology of *Nilaparvata lugens* (Homoptera, Delphacidae). In: Brown Planthopper; Threat to Rice Production in Asia Philippines, Los Banos, 21-44.
- Ocampo CB and Wesson DM (2004). Population dynamics of *Aedes aegypti* from a dengue hyperendemic urban setting in Colombia. *Am. J. Trop. Med. Hyg.* 71: 506-513.
- Pathak PK and Heinrichs EA (1982). Selection of biotype populations 2 and 3 of *Nilaparvata lugens* by exposure to resistant rice varieties. *Environ. Entom.* 11: 85-90.
- Ruano G, Brash DE and Kidd KK (1991). PCR: The first few cycles. *Amplifications* 7: 1-4.
- Sambrook J, Fritsch EF and Maniatis T (1989). Molecular Cloning: A Laboratory Manual. 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
- Saxena KN (1969). Patterns of insect-plants relationships determine susceptibility or resistance of different plants to an insect. *Entomol. Exp. Appl.* 12: 751-766.
- Saxena RC and Pathak MD (1977). Factor Affecting Resistant of Rice Varieties to the Brown Planthopper, *Nilaparvata lugens*. In: Proceedings of 8th Annual Conference of Pest Control Council of the Phillipines, Bacolod City, 18-20.
- Scott JC and McManus DP (1994). The random amplification of polymorphic DNA can discriminate species and strains of *Echinococcus*. *Trop. Med. Parasitol.* 45: 1-4.
- Sogawa K (1981). Biotypic variations in the brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae) at the

- IRRI, the Philippines. *Appl. Entom. Zool.* 16: 129-137.
- Sogawa K, Kilin D and Kusmayadi A (1984). A *Leersia* feeding brown planthopper (BPH) biotype in North Sumatra, Indonesia. *Intl. Rice Newsl.* 9: 20.
- Velusamy R (1988). Resistance of wild rice, *Oryza* spp., to the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae). *Crop Prot.* 7: 403-408.
- Wu JT, Heinrichs EA and Medrano FG (1986). Resistance of wild rice *Oryza* spp. to the brown planthopper *Nilaparvata lugens* (Homoptera: Delphacidae). *Environ. Entom.* 15: 648-649.
- Zhi-Gang G and Alan MJ (1995) Genetic comparison of *Neospora caninum* with *Toxoplasma* and *Sarcocystis* by random amplified polymorphic DNA-polymerase chain reaction, *Parasitol. Res.* 81: 365-370.