

UNIVERSITI PUTRA MALAYSIA

MORPHOLOGICAL, MOLECULAR GENETIC AND HOST PLANT RELATIONSHIP STUDIES OF RICE AND WEED INFESTING POPULATIONS OF BROWN PLANTHOPPER, *NILAPARVATA LUGENS* (STAL) (HOMOPTERA: DELPHACIDAE)

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By

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Thesis Submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy in the Faculty of Science and Environmental Studies Universiti Putra Malaysia

August 2000



DEDICATION

"This thesis is dedicated to my beloved parents"



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy.

MORPHOLOGICAL, MOLECULAR GENETIC AND HOST PLANT RELATIONSHIP STUDIES OF RICE AND WEED INFESTING POPULATIONS OF BROWN PLANTHOPPER, *NILAPARVATA LUGENS* (STÅL) (HOMOPTERA : DELDPHACIDAE)

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Chairman: Professor Dr. Tan Soon Guan

Faculty: Science and Environmental Studies

A total of fifteen experiments including morphological, molecular genetic and host plant relationship studies were conducted to differentiate between two sympatric populations of brown planthopper (BPH), *Nilaparvata lugens*, one from rice (*Oryza sativa*) and the other from *Leersia hexandra*, a weed grass. The scatter plot based on seven morphometric characters indicated that *N. bakeri* was totally an isolated species. Insects with high esterase activities (usually caught off rice) and those with low esterase activities (usually caught off *L. hexandra*) showed 6-8% overlapping between the two populations of *N. lugens*. But scatter plot of the morphological characters of stridulatory organs produced distributions that were almost nonoverlapping indicating that BPH with high esterase activity usually caught off rice is different from BPH with low esterase activity usually captured from *L. hexandra*. Scanning electron micrographs showed some variations in different morphological characters between individuals from the two sympatric populations of BPH but these were not population specific.



No heterogametic mating occurred in mate choice experiments. Crosses between the two BPH populations from different host-plants showed some barriers for hybrid production. Some genetic incompatibility may exist between the two populations. After being tested for esterase activity, samples were analysed for six loci found to be polymorphic at 95% criterion namely, Mdh, Idh, Pgm, Gpi, 6Pgd and Acp. The genetic distance (average 0.182) and the existence of a diagnostic enzyme marker (GPI) between rice and *Leersia* infesting populations indicated that both populations are closely related but different species. The inheritance of GPI, IDH and MDH isozymes were studied in families generated from mating individuals of two sympatric populations of N. lugens. These isozymes were controlled by three loci, Gpi, Mdh and Idh, respectively. These loci were inherited in simple Mendelian fashions. Thirty one bands from both short and long primer RAPD were able to be tested for segregating ratios in two families of N. lugens and they were found to be inherited in simple Mendelian fashions. In the population genetic studies, two diagnostic bands, one from short primer RAPD (OPD03.7; 0.65kb) and the other from long primer RAPD (pehA#6.3; 1.00kb) were found to be present only in the Leersia infesting populations of BPH. The UPGMA cluster analyses based on both enzyme and RAPD markers showed that all the rice infesting populations of N. *lugens* clustered together as a group. On the other hand *Leersia* infesting populations of the same localities formed another distinct cluster. In host plant relationship studies, rice plants were found best suited for the establishment of the rice infesting population, and L. hexandra was a favourable host for the Leersia infesting population.



A consideration of the evidence from studies on host plant relationships, reproductive isolation, hybridization, morphometric variations, level of esterase activity, existence of diagnostic isozyme and DNA level markers, genetic distance, consensus tree and molecular variance between *N. lugens* with high esterase activity usually caught off rice and *N. lugens* with low esterase activity usually caught off rice and *N. lugens* with low esterase activity usually caught off *L. hexandra* suggested that both insect populations from Malaysia belong to closely related sibling species. This information has practical implications in formulating effective control measures against *N. lugens* which is a major pest of rice not only in Malaysia but also throughout South East Asia, South Asia and Australia.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KAJIAN MORFOLOGI, GENETIK MOLEKUL DAN PERHUBUNGAN TUMBUHAN PERUMAH BAGI POPULASI BENA PERANG, *NILAPARVATA LUGENS* (STÅL) (HOMOPTERA:DELDPHACIDAE) YANG MENJANGKITI PADI DAN LALANG

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Sejumlah lima belas eksperimen yang merangkumi kajian-kajian morfologi, genetik molekul dan perhubungan tumbuhan perumah telah dijalankan untuk membezakan dua populasi simpatrik bena perang (BPH), *Nilaparvata lugens*, daripada padi (*Oryza sativa*) dan daripada rumput lalang (*Leersia hexandra*). Plot serakan berdasarkan kepada tujuh sifat morfometrik menunjukkan bahawa *N. bakeri* adalah satu spesies yang sangat terpencil. Serangga dengan aktiviti esterase yang tinggi (biasanya pada padi) dan yang menunjukkan aktiviti estarase yang rendah (biasanya *pada L. hexandra*) didapati 6-8% bertindihan di antara dua populasi *N. lugens* tersebut. Namun begitu, plot serakan bagi sifat morfologi organ stridulatori menghasilkan taburan yang berasingan. Ini menunjukkan bahawa BPH dengan aktiviti esterase rendah yang ditangkap pada *L. hexandra*. Mikrograf elektron pengimbas menunjukkan sedikit variasi pada sifat morfologi yang berbeza antara individu-individu daripada kedua-dua populasi simpatrik BPH tetapi ia bukanlah khusus untuk



sesuatu populasi. Tiada pengawanan heretogametik berlaku dalam kajian pilihan pasangan. Kacukan antara populasi BPH dari tumbuhan perumah yang berbeza menunjukkan terdapatnya beberapa halangan untuk penghasilan hibrid. Kemungkinan terdapatnya ketidakserasian genetik antara dua populasi tersebut.

Setelah diuji dengan aktiviti esterase, sampel telah dianalisis menggunakan elektroforesis gel kanji (STAGE) bagi enam lokus yang didapati polimorfik pada kriteria 95% iaitu, Mdh, Idh, Pgm, Gpi, 6Pgd dan Acp. Jarak genetik (purata 0.182) dan kewujudan satu penanda enzim diagnostik (GPI) di antara populasi-populasi yang menjangkiti padi dan rumput lalang, L. hexandra menunjukkan kedua-dua populasi tersebut mempunyai hubungan rapat tetapi berlainan spesies. Pewarisan isozim GPI, IDH dan MDH telah dikaji dalam famili yang terhasil daripada kacukan individu-individu daripada dua populasi simpatrik bena perang, Nilaparvata lugens, dengan menggunakan elektroforesis gel kanji (STAGE). Tiga isozim ini dikawal oleh tiga lokus Gpi, Idh dan Mdh, masing-masing. Lokus tersebut diwarisi dengan menepati hukum Mendel. Tiga puluh satu jalur daripada primer pendek dan panjang RAPD telah digunakan untuk menguji kadar pengasingan pada dua famili N. lugens dan didapati diwarisi dengan menepati hukum Mendel, Dalam kajian genetik populasi, dua jalur penanda, satu daripada primer pendek RAPD (OPD03.7; 0.65kb) dan satu lagi daripada primer panjang RAPD (pehA#6.3; 1.00kb) telah dijumpai pada populasi yang menjangkiti Leersia. Analisis kelompok UPGMA yang berdasarkan kedua-dua enzim dan penanda RAPD menunjukkan kesemua populasi N. lugens yang menjangkiti padi telah dikumpulkan dalam satu kelompok. Sebaliknya populasi



yang menjangkiti *Leersia* daripada kawasan yang sama membentuk satu kelompok lain yang jelas. Dalam Kajian perhubungan tumbuhan perumah, tumbuhan padi adalah paling sesuai untuk menentukan populasi jangkitan padi dan rumput lalang, *L. hexandra* adalah perumah paling sesuai bagi populasi menjangkiti *Leersia*.

Berdasarkan pertalian tumbuhan perumah, pengasingan pembiakan, hibridisasi, variasi morfometrik, tahap aktiviti esterase, kehadiran isozim diagnostik dan penanda DNA, jarak genetik, pokok konsensi dan variasi molekul antara *N. lugens* beraktiviti esterase tinggi yang biasanya terdapat pada padi dan *N. lugens* beraktiviti esterase rendah yang didapati pada *L. hexandra* dicadangkan bahawa kedua-dua populasi BPH dari tumbuhan perumah yang berbeza dari Malaysia ini sebenarnya adalah spesies sibling yang mempunyai pertalian yang rapat. Maklumat ini mempunyai implikasi praktikal dalam merumuskan langkah-langkah kawalan berkesan ke atas *N. lugens* yang merupakan makhluk perosak utama bagi padi, bukan sahaja di Malaysia malahan di seluruh Asia Tenggara, Asia Selatan dan Australia.



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