UNIVERSITI PUTRA MALAYSIA

GENETIC VARIATION OF *HELOPELTIS THEIVORA* WATERHOUSE AND ITS HOST PLANT *CAMELLIA SINENSIS* L.

SITI NOOR HAJJAR BT MD LATIP

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

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Bismillahirrahmanirrahim

I dedicated this thesis to my beloved parents, Haji Md. Latip b. Haji Mahmood and Hajjah Siti Sariah Soleh for their support, patient and endless love.

For my husband and our little princess Rabia'tul Adawiyah bt Zainal Abidin for being so patient.

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

GENETIC VARIATION OF *HELOPELTIS THEIVORA* WATERHOUSE AND ITS HOST PLANT *CAMELLIA SINENSIS* L.

By

SITI NOOR HAJJAR BT MD LATIP

September 2006

Chairman: **Associate Professor Rita Muhamad, PhD**

Faculty : **Agriculture**

Helopeltis theivora is known as a pest of cocoa and tea in Malaysia. Insecticides have been used for the control of the mirids since cocoa and tea are grown widely in this country. Several mechanisms of insecticide resistance have been proposed and metabolic detoxification was shown to play a major role in insecticide resistance. Although *H. theivora* is recognized as a leaf-destroying pest of tea, little is known about the genetic background of the mirid, *H. theivora* and its host plant, tea. This lack of information has lead to the loss of genetic variability and allelic differences of tea clones planted in Malaysia. Little molecular work has been done in *H. theivora* but some information can be obtained for tea.

In a study on the evaluation of the levels infestations of *H. theivora* on different varieties of tea, the results showed that the tea varieties could be categorized into three different groups namely resistant, intermediate and susceptible. For tea, RAPD and RAMs markers were used to study the genetic relationships among the resistant and susceptible

varieties found in Bukit Cheeding and Sungai Palas. Both the dendrograms based on the RAMs and RAPD markers, respectively grouped the tea varieties into two clusters with the intermediate varieties grouping with the resistant (first cluster) or susceptible (second cluster) varieties. The genetic differences are based on their geographical distributions and partially based on their resistance towards damage by the insect (*H. theivora*).

Two DNA markers were used to identify the genetic variation of *H. theivora*, namely random amplified polymorphic DNA (RAPD) and random amplified microsatellites (RAMs). For RAPD and RAMs markers, after screening 20 arbitary primers, 8 primers were identified as being useful for generating RAPD markers and 5 primers for RAMs markers. A dendrogram obtained through the use of these DNA level markers showed that the first cluster pooled both the populations of *H. theivora* from BOH estate at Bukit Cheeding and Sungai Palas together while the population of *H. theivora* from Tanah Rata clustered by itself. This showed that the populations of *H. theivora* were not clustered together based on their geographical distributions. This clustering pattern could be due to *H. theivora* (eggs and nymphs) being present on the planting materials from Bukit Cheeding and planted at Sungai Palas since both the plantations are owned by Boh.

Eighteen microsatellite primer pairs were designed for *H. theivora*. Out of these, six were found to be polymorphic (CT2, CT4, CT9, CT15, CT17 and CT18). This study showed that the RAMs technique is suitable and efficient for isolating single locus DNA microsatellites markers for *H. theivora*.

Enzyme studies using inhibitors, polyacrylamide gel electrophoresis (PAGE) and gluthatione-s-transferase (GST) analysis using a spectrophotometer were used to identify the metabolic enzymes involved in the development of resistance in *H. theivora*. The results showed that of the 25 enzymes screened for 8 enzymes were detectable in *H. theivora*. The dendrogram resulting from the cluster analysis based on isoenzyme data grouped the Bukit Cheeding and the Tanah Rata populations together while the Sungai Palas population clustered by itself. For the Bukit Cheeding and Tanah Rata populations, insecticides were not frequently sprayed when compared with the Sungai Palas population. Esterases and oxidases were enzymes observed to play roles in the insecticide resistance mechanism in *H. theivora*. Meanwhile, for GST analysis the presence of low activity of GST was detected in *H. theivora*. This results show that the resistance of *H. theivora* towards insecticides appeared not to be due to an increased detoxification by gluthatione s-transferase.

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

VARIASI GENETIK *HELOPELTIS THEIVORA* DAN PERUMAHNYA *CAMELLIA SINENSIS* L.

Oleh

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H. theivora dikenali sebagai perosak koko dan teh di Malaysia. Racun serangga telah digunakan untuk mengawal *H. theivora* sejak koko dan teh ditanam secara meluas di negara ini. Beberapa mekanisme rentan kepada racun serangga telah dicadangkan dan metabolisme detoksifikasi memainkan peranan penting dalam kerentanan racun serangga. Walaupun *H. theivora* telah dikenali sebagai perosak daun teh, hanya sedikit latarbelakang genetik *H. theivora* dan perumahnya, teh diketahui. Ini menyebabkan kekurangan maklumat mengenai kepelbagaian genetik dan perbezaan alel dalam klon teh yang ditanam di Malaysia. Hanya sedikit kajian genetik yang telah dibuat mengenai *H. theivora* tetapi beberapa informasi boleh didapati bagi teh.

Dalam kajian untuk menilai kerentanan varieti teh terhadap *H. theivora*, keputusan menunjukkan varieti teh boleh dikategorikan kepada tiga kumpulan iaitu rentan, pertengahan dan mudah diserang *H. theivora*. Untuk kajian bagi teh, penanda RAMs dan RAPD diuji untuk melihat perbandingan genetik diantara varieti di Bukit Cheeding dan

Sungai Palas. Kedua-dua dendrogram ini menunjukkan penanda RAMs dan RAPD membahagikan varieti teh kepada dua kluster iaitu varieti pertengahan dan rentan dalam kluster pertama dan pertengahan masing-masing dan mudah diserang dalam kluster kedua. Perbezaan genetik bagi teh adalah berdasarkan kedudukan geografi dan sebahagian berdasarkan pada kerentanan terhadap kerosakan oleh serangga (*H. theivora*).

Dua penanda DNA yang telah digunakan untuk mengenalpasti variasi genetik *H. theivora* iaitu *random amplified polymorphic DNA* (RAPD) dan *random amplified microsatellites* (RAMs). Bagi penanda untuk RAMs dan RAPD, daripada 20 primer yang diuji, lapan primer untuk RAPD dan lima primer untuk RAMs telah dikenalpasti sesuai untuk *H. theivora*. Kluster yang pertama mengumpulkan populasi *H. theivora* dari estet Boh di Bukit Cheeding dan Sungai Palas bersama manakala populasi dari Tanah Rata di kluster yang kedua. Berdasarkan keputusan daripada penanda RAMs dan RAPD didapati walaupun populasi *H. theivora* dari kawasan yang sama (Cameron Highlands) tetapi ia tidak berada dalam kluster yang sama. Ini disebabkan kemungkinan telur dan nimfa *H. theivora* yang terdapat pada pokok teh di Banting dibawa dan ditanam di Sungai Palas kerana kedua-dua estet ini dimilki oleh Boh.

18 pasangan primer mikrosatelit telah direka untuk *H. theivora*. Daripada jumlah ini, 6 primer mikrosatelit yang direka didapati polimorfik (CT2, CT4, CT9, CT15, CT17 dan CT18). Kajian ini menunjukkan teknik RAM sesuai dan efisien untuk memencilkan DNA mikrosatelit untuk *H. theivora*.

Kajian isoenzim menggunakan elektroforesis gel poliakrilamida (PAGE) dan analisis gluthatione-s-transferase (GST) menggunakan spektrofotometer dijalankan untuk mengenalpasti metabolik pada enzim yang terlibat dalam perkembangan kerentanan *H. theivora*. Keputusan menunjukkan daripada 25 enzim yang diuji, 8 enzim yang dikenalpasti di dalam *H. theivora*. Dendrogram menunjukkan analisis kluster mengumpulkan populasi Bukit Cheeding dan Tanah Rata bersama manakala populasi Sungai Palas di dalam kluster yang tersendiri. Populasi Bukit Cheeding dan Tanah Rata kurang disembur dengan racun serangga dibandingkan dengan populasi Sungai Palas. Enzim esterase dan oksidasi didapati memainkan peranan penting dalam mekanisme rentan kepada racun perosak dalam *H. theivora*. Manakala bagi analisis GST, kehadiran aktiviti GST yang rendah dikesan pada *H. theivora.* Keputusan ini menunjukkan resistan *H. theivora* terhadap racun serangga tiada kaitan dengan peningkatan detoksifikasi *gluthatione-s-tranferase.*

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I certify that an Examination Committee has met on 20 September 2006 to conduct the final examination of Siti Noor Hajjar Md Latip on her Doctor of Philosophy thesis entitled "Genetic Variation of *Helopeltis theivora* and its Host Plant *Camellia sinensis* L." in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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SITI NOOR HAJJAR BT. MD. LATIP

Date: 2 NOVEMBER 2006

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α	alpha
ATP	adenosine triphosphate
β	beta
bp	base pairs
dH_2O	distilled water
ddH_2O	double distilled water
dNTP	deoxyribonucleotide
GST	gluthatione-s-transferase
Kb	kilobase
λ	lambda
LB	Luria-Bertani
mM	milimolar
ng	nanogram
nmol	nanomole
PCR	polymerase chain reaction
RAPD	randomly amplified polymorphic DNA
RAMs	randomly amplified microsatellites
rpm	revolutions per minute
µg	microgram
µl	microliter
µM	micromolar

UPGMA	unweighted pair-group method with arithmetic mean
V	volts
w/v	weight/volume
xg	centrifugal force
OP	Organophosphates

CHAPTER 1

INTRODUCTION

The mirid, *Helopeltis theivora* Waterhouse (Hemiptera: Miridae) or the mosquito bug is known to be a pest of tea (Cranham, 1966; Wilson, 1999) and cocoa (Entwistle, 1972). The insect feeds on the young tea shoots, which after one feeding lesion could cause dieback of the shoots. Severe damage on young tea plants could cause stunting of plant growth. Tea production could virtually stop ("blackout") (Sidhu and Saikia, 1999). Insecticides have been used for the control of the mirids since cocoa is grown widely in this country. In insect pest management, insecticides continue to be one of the main features.

The widespread used of insecticides has been shown to cause the development of resistance in many insects (Madharan and Abraham, 1983). Dzolkhifli *et al*., (1998) reported the development of resistance in the Sungai Tekam, Pahang and Serdang populations of *H. theivora* to γ-HCH, deltamethrin and cypermethrin. Ismail (2002) reported that the Sungai Tekam population showed increasing resistance to chlorpyrifos, γ-HCH, deltamethrin and cypermethrin when compared to the Serdang and Banting populations.