



UNIVERSITI PUTRA MALAYSIA

**REPRODUCTIVE SYSTEM AND GENETIC DIVERSITY OF HEMPEDU
BUMI (*ANDROGRAPHIS PANICULATA*) GERMPLASM IN
PENINSULAR MALAYSIA**

**CHIA SOK HUA
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**Reproductive System and Genetic Diversity of
Hempedu Bumi (*Andrographis paniculata*)
Germplasm in Peninsular Malaysia**

By

CHIA SOK HUA

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirement for the Degree of Master
Science**

October 2009



DEDICATION

I would like to dedicate my thesis to:

My beloved parents,

Chia Peng Hwi

Eng King Luang

for giving me constant encouragement, sacrifices and supports

and

My beloved husband,

Hoong Seng Soi

who has been great source of motivation and inspiration



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Master of Science

**Reproductive System and Genetic Diversity of Hempedu Bumi
(*Andrographis paniculata*) Germplasm in Peninsular Malaysia**

By

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October 2009

Chairman: Associate Professor Mohd Said Saad, PhD

Faculty: Agriculture

Andrographis paniculata is an erect annual plant belongs to the family of Acanthaceae. This species have been reported to possess medicinal properties such as antiviral, antithrombotic, anticancer, anti-inflammatory and many others. In spite of its significant medicinal importance, fundamental aspects regarding reproductive biology, mating system and genetic diversity of *A. paniculata* has been poorly investigated. The objectives of this study are to (a) determine the reproductive biology which includes the occurrence of stigma receptivity, anther dehiscence and pollen viability (b) determine the occurrence of naturally self and/or cross pollination and (c) elucidate the genetic diversity of *A. paniculata* germplasm in Peninsular Malaysia using molecular marker.



Reproductive biology of *A. paniculata* was studied by investigating floral development, stigmatic receptivity, anther dehiscence and pollen viability. Two pollination treatments namely bagged and unbagged were imposed on seven accessions to study the mating system through self-compatibility and inbreeding depression. A total of 80 accessions of *A. paniculata* representing six different states, namely Perak, Selangor, Negeri Sembilan, Kelantan, Terengganu and Pahang were subjected to Random Amplified Polymorphic DNA (RAPD) assay using 10 primers to elucidate genetic diversity of this plant in Peninsular Malaysia.

Results showed that floral development of *A. paniculata* can be categorised into five stages based upon flower buds length associated with timing of anther dehiscence and stigma receptivity. The anthers started to dehisce when flower buds reached the size of 11.5 mm (Stage III) and stigma became fully receptive at 12 mm (Stage IV). Both events occurred almost simultaneously when corolla still remained close. In addition, stigma of *A. paniculata* was closely attached to anthers from stage II until flower opening. Thereby, the mode of mating system of *A. paniculata* can be defined as pollination in flower bud before anthesis, also known as preanthesis cleistogamy. This mechanism of mating system greatly promotes the occurrence of autonomous self-pollination.

Fruit and seed set of bagged and unbagged flowers were not significantly different ($P > 0.05$), indicating the species is self-compatible. The fitness of progeny derived from seeds of bagged flowers was similar as compared to

the unbagged flowers in all three life stages (germination, seedlings and fruiting plants). Results have shown absence of inbreeding depression in *A. paniculata*, further supporting the finding of self-compatibility that it is a naturally self-pollinated species.

Jaccard's similarity coefficients based on RAPD were ranged between 0.81 to 1.00, indicating the presence of high similarity and thereby low genetic diversity among the accessions. Cluster analysis had grouped the accessions into two major groups and five subgroups. The AMOVA revealed significant ($P < 0.01$) genetic differences within the states (65.62%) and among states within the regions (26.26%).

It can be concluded that *A. paniculata* is a naturally and predominantly self-pollinated species. The *A. paniculata* germplasm in Peninsular Malaysia is low in genetic diversity. Therefore, hybridization using conventional crossing can be carried out to produce new segregating genotypes. Future breeding program on this plant should use the approach of self-pollinated species. Apart from that, germplasm collection from other countries particularly its origin, South India and Sri Lanka would be beneficial to obtain more diverse genetic composition in this species.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**Sistem Pembiakan dan Keperlbagaian Genetik Hempedu Bumi
(*Andrographis paniculata*) Germplasma di Semenanjung Malaysia**

Oleh

CHIA SOK HUA

Oktober 2009

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Andrographis paniculata merupakan tumbuhan semusim yang tegak daripada famili Acanthaceae. Spesis ini telah dilaporkan memiliki keupayaan perubatan seperti antivirus, antitrombosis, antikanser, anti-inflamatori dan banyak lagi. Meskipun dengan kepentingan signifikannya dalam perubatan, aspek asas berkaitan biologi pembiakan, sistem pengawanan dan kepelbagaian genetikanya kurang dikaji. Objektif kajian ini adalah untuk (a) mengenalpasti biologi pembiakan termasuk perlakuan kematangan stigma dan anter dan juga keburnasan debunga (b) mengenalpasti perlakuan pendebungaan sendiri dan/atau pendebungaan kacukan secara semulajadi dan (c) menjelaskan genetik kepelbagaian germplasma *A. paniculata* di Semenanjung Malaysia dengan menggunakan penanda molekular.

Biologi pembiakan *A. paniculata* telah dikaji dengan menyiasat perkembangan bunga, kematangan anter dan stigma dan juga keburnasan debunga. Dua rawatan pendebungaan iaitu beg and tanpa beg telah diberi

ke atas tujuh aksesori untuk mengkaji sistem pengawanan melalui keserasian sendiri dan kemelesetan penginbredan. Sebanyak 80 aksesori *A. paniculata* mewakili enam negeri, iaitu Perak, Selangor, Negeri Sembilan, Kelantan, Terengganu dan Pahang telah dikenakan esei DNA Polimorfik Teramplifikasi Rawak (RAPD) menggunakan 10 primer untuk menjelaskan kepelbagaian genetik pokok ini di Semenanjung Malaysia.

Keputusan menunjukkan bahawa perkembangan bunga *A. paniculata* dapat dikategorikan kepada lima peringkat berdasarkan kepada kepanjangan kudup bunga dan dihubungkan dengan waktu kematangan anter dan stigma. Anter mula menjadi matang apabila saiz kudup bunga mencapai 11.5 mm (Peringkat III) dan stigma menjadi matang keseluruhannya pada 12 mm (Peringkat IV). Kedua-dua acara berlaku hampir serentak ketika korola masih tutup. Tambahan pula, stigma *A. paniculata* didapati rapat mendekati dengan anter dari peringkat II sehingga pembukaan bunga. Justeru itu, sistem pengawanan ini boleh diklasifikasikan sebagai pendebungaan dalam kudup bunga sebelum antesis, juga dikenali sebagai preantesis kleistogami. Mekanisma sistem pengawanan ini sangat menggalakkan pendebungaan sendiri secara berotonomi.

Penghasilan buah dan biji benih antara bunga yang dibeg dan tanpa dibeg menunjukkan tiada perbezaan yang bererti ($P > 0.05$), mengindikasikan spesies ini adalah berkeserasian sendiri. Kecergasan progeni daripada biji benih bunga yang dibeg adalah sama berbanding dengan biji benih bunga yang tidak dibeg dalam ketiga-tiga peringkat hidup (percambahan, anak

benih dan pokok berbuah). Keputusan telah menunjukkan ketiadaan kemelesetan penginbredan dalam *A. paniculata*, menyokong dengan penemuan keserasian sendiri bahawa ia adalah spesies yang menjalankan pendebungaan sendiri secara semulajadi.

Koefisien persamaan Jaccard's berdasarkan kepada RAPD berada dalam lingkungan 0.81 kepada 1.00, mengindikasikan kehadiran persamaan yang tinggi dan justeru itu genetik kepelbagaian yang rendah di antara akses. Analisis kluster telah mengelompokkan akses tersebut kepada dua kumpulan utama dan lima subkumpulan. Keputusan AMOVA mendedahkan perbezaan genetik yang bererti ($P < 0.01$) di dalam negeri (65.62%) dan antara negeri dalam rantau (26.26%).

Kesimpulannya, *A. paniculata* merupakan spesies yang menjalankan pendebungaan sendiri secara semulajadi dan berkeutamaan. Lantaran itu, hibridikasi menggunakan kacukan tradisional dapat dijalankan untuk menghasilkan genotip baru yang bersegregasi. Program pembiakbakaan pada masa depan atas pokok ini perlu menggunakan pendekatan spesies pendebungaan sendiri. Selain daripada itu, koleksi germplasma *A. paniculata* dari negara lain terutama tempat asalnya, India Selatan dan Sri Lanka akan mendatangkan kebaikan dalam memperolehi komposisi genetik yang berkepelbagaian dalam spesies ini.

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I certify that a Thesis Examination Committee has met on 29 October 2009 to conduct the final examination of Chia Sok Hua on her thesis entitled "Reproductive System and Genetic Diversity of Hempedu Bumi (*Andrographis paniculata*) Germplasm in Peninsular Malaysia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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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.

CHIA SOK HUA

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LIST OF ABBREVIATIONS/NOTATIONS

Acc	: Accession
AFLP	: Amplified Fragment Length Polymorphism
AMOVA	: Analysis of Molecular Variance
bp	: Base pair
CD4	: Cluster of differentiation 4
CTAB	: Cetyl trimethylammonium bromide
CV	: Coefficient of Variation
DNA	: Deoxyribonucleic acid
dNTP	: Deoxynucleotide triphosphate
EDTA	: Ethylenediamine tetra-acetic acid
HIV	: Human Immunodeficiency Virus
ISSR	: Inter Simple Sequence Repeats
MgCl ₂	: Magnesium chloride
M	: Molar
mM	: Milimolar
ng	: Nanogram
OD	: Optical Density
PCR	: Polymerase Chain Reaction
PIC	: Polymorphism Information Content
PROC	: Procedure (SAS command)
PVP	: Polyvinylpyrrolidone
RAPD	: Random Amplified Polymorphic DNA
RFLP	: Restriction Fragment Length Polymorphism

RNA	: Ribonucleic Acid
rpm	: Rounds per minute
SAS	: Statistical Analysis Software
SE	: Standard Error
SNP	: Single Nucleotide Polymorphism
SSR	: Simple Sequence Repeats
Taq	: <i>Thermus aquaticus</i>
TE	: Tris EDTA
UV	: Ultraviolet
w/v	: Weight per volume
μg	: Microgram
μl	: Microliter

CHAPTER 1

INTRODUCTION

Andrographis paniculata is an annual and erect plant belongs to the family Acanthaceae. It is believed to originate from South India and Sri Lanka (Hooker, 1885; Bhat and Nanavati, 1978). *Andrographis paniculata* is widely found and cultivated in tropical and subtropical Asia, South-east Asia and India (Chang and But, 1986; Kapoor, 2001).

Since time immemorial, it has been widely used traditionally to treat liver disorder and numerous ailments (Chadha, 1985). In Malaysia, the plant is known as 'Hempedu Bumi' or 'Akar Cerita' and has long been used to treat hypertension, tonsillitis, flu and chest pains (Muhammad and Mustafa, 1994). Clinical studies have reported that *A. paniculata* possess various medicinal properties which include antiviral (Calabrese et al., 2000), antithrombotic (Thisoda et al., 2006), anticancer (Jebril et al., 2003; Rajagopal et al., 2003), hepatoprotective (Upadhyay et al., 2001) and anti-inflammatory (Gabrielian et al., 2002; Liu et al., 2007).

Despite its significant medicinal importance, fundamental aspects regarding reproductive biology, mating system and genetic diversity of *A. paniculata*

