



UNIVERSITI PUTRA MALAYSIA

***CROSSABILITY AND GENETIC ANALYSES OF ANDROGRAPHIS
PANICULATA NEES. POPULATIONS USING PHENOTYPIC AND
MOLECULAR MARKERS***

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FP 2012 28

CROSSABILITY AND GENETIC ANALYSES OF *ANDROGRAPHIS PANICULATA* NEES. POPULATIONS USING PHENOTYPIC AND MOLECULAR MARKERS

By

ALIREZA VALDIANI

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

May 2012

DEDICATIONS

To my dear parents and two sisters

To Babak Khorramdin

UPM &

To all those who lost their invaluable lives for a free Iran especially after June 2010

If a man has not discovered something
that he will die for, he is not fit to live.

Martin Luther King

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

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Chairman : Associate Professor Mihdzar Abdul Kadir, PhD

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Andrographis paniculata (AP) also known as the King of Bitters is a medicinal herb from the family Acanthaceae. The herb is blessed with curative effects such as anticancer, anti HIV, antiviral, anti diabetes and anti hepatitis, etc. To date AP has not been subjected to deep genetic scrutiny. The objective of this study was to evaluate the genetic mechanisms controlling the agromorphological and anticancer phytochemicals using classical and molecular methods in AP. The factors affecting low crossability as well as strategies to overcome this natural barrier were investigated in this exploration. For this

purpose, a total of seven Malaysian AP accessions from six different states including Kelantan, Negeri Sembilan, Pahang (two accessions), Perak, Selangor and Terengganu were chosen to inspect the crossability and hybrid seed production using combined molecular and morpho-phenological approaches. The effect of cross combination, style length and time of hand-pollination on crossability was reflected in the significant chi-square value using nonparametric analysis based on Kruskal-Wallis test.

For documentation of the genetic distance (GD) effect on crossability, the accessions were subjected to 55 Random Amplified Polymorphic DNA (RAPD) primers. However genetic distance based on Simple Matching method (SM) which ranged between 0.05 and 0.32 indicated the presence of a low genetic diversity among the studied accessions, but even a low GD decreased the rate of crossability.

Phylogenetic analysis was performed using three chloroplast genes consisting of Internal Transcribed Spacer Region (*ITS*), Plastid *matK* regions, *rps16* gene and *trnL-F* intron region along with phytochemical markers. The obtained results indicated that plastid genes are carrying a divergent nucleotide composition in the amplified fragments. Malaysian AP accessions are also significantly diverse ($P \leq 0.01$), in AG, NAG, and DDAG content. The novel part

of this experiment came out with amplification of *ITS* and *matK* genes in this plant.

As an important outcome, the effect of genetic distance revealed by the morphological characteristics on crossability of the accessions found to be neutral. Unlike the morphological markers, the phytochemical markers were negatively associated with crossability percentages. The GD revealed by the plastid genes based on Jukes-Cantor corrected distance, created the same results with phytochemical and RAPD markers.

In another independent experiment, twenty-four microsatellite markers were applied on the AP accessions. These markers proved the presence of a fixed heterozygosity phenomenon in the herb. This achievement was remarkable as it happens when a population experiences repeated bottlenecks in population size. Another considerable upshot of the present study was introducing the Malaysian AP accessions as a cryptic species, while according to the changes in the microsatellite repeat motifs, they were genetically different than their Indian ancestors.

The final research of this study evaluated the genetic base of the important traits using diallel analysis. The results demonstrated that both additive and non-additive gene effects contribute in controlling morphological and phytochemical traits of this species. The results also showed that most of morphological traits were under additive gene effects, in contrast, the

phytochemical traits were affected by non-additive effects. Both negative and positive heteroses was estimated for different traits at the mid-parent and better-parent levels. Perhaps such results can draw a bright horizon for breeding of the prolific varieties in the future.



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

**KEUPAYAAN KACUKAN DAN ANALISIS GENETIK POPULASI
ANDROGRAPHIS PANICULATA NEES. MENGGUNAKAN PENANDA
FENOTIP DAN MOLEKUL**

Oleh

ALIREZA VALDIANI

Mei 2012

Pengerusi : Profesor Madya Mihdzar Abdul Kadir, PhD

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Andrographis paniculata (AP) juga dikenali sebagai Raja segala pahit adalah herba perubatan dari keluarga Acanthaceae. Herba ini diberkati dengan kesan penyembuhan seperti antikanser, anti HIV, antivirus, anti diabetes dan anti hepatitis, dan sebagainya. Buat masa ini AP tidak tertakluk kepada penelitian

genetik yang mendalam. Objektif kajian ini adalah untuk menilai mekanisme genetik yang mengawal agro-morfologi dan fitokimia antikanser menggunakan kaedah klasik dan molekular dalam AP. Faktor-faktor yang mempengaruhi keupayaan kacukan rendah serta strategi untuk mengatasi halangan semula jadi ini telah dikaji dalam penyelidikan ini. Bagi tujuan ini, sebanyak tujuh AP aksesori Malaysia dari enam negeri termasuk Kelantan, Negeri Sembilan, Pahang (dua aksesori), Perak, Selangor dan Terengganu telah dipilih untuk memeriksa keupayaan kacukan dan penghasilan benih hibrid yang menggunakan gabungan pendekatan molekul dan morpho-fenolog. Kesan kacukan gabungan, panjang stil dan masa pendebungaan-tangan pada keupayaan kacukan ditunjukkan dengan nilai Chi-kuasa dua yang signifikan menggunakan analisis tak berparameter berdasarkan ujian Kruskal-Wallis.

Untuk dokumentasi kesan jarak genetik (GD) terhadap keupayaan kacukan, aksesori telah tertakluk kepada 55 random penguatan primer polimorfik DNA (RAPD). Walau bagaimanapun, jarak genetik berdasarkan kaedah pepadanan Mudah (SM) adalah antara 0.05 dan 0.32 menunjukkan kehadiran kepelbagaian genetik yang rendah di kalangan aksesori yang dikaji, walaupun GD rendah ia turut mengurangkan kadar keupayaan kacukan.

Analisis filogenetik telah dilakukan dengan menggunakan tiga gen kloroplas yang terdiri daripada Bahagian Spacer ditranskripsikan Dalam (ITS),

bagian matK Plastid, rps16 gen dan trnL-F bagian intron mengiringi dengan penanda fitokimia. Keputusan yang diperolehi menunjukkan bahawa gen plastid membawa komposisi nukleotida yang luas dalam cebisan-cebisan yang diampflikasikan. Aksesii Malaysia juga adalah bererti dari segi keluasan ($P \leq 0.01$), dari segi kandungan AG, NAG, dan DDAG. Bahagian novel eksperimen ini menghasilkan amplifikasi gen matK ITS dalam tumbuhan untuk pertama kalinya.

Sebagai hasil yang penting, kesan jarak genetik yang diturunkan oleh ciri-ciri morfologi pada keupayaan kacukan aksesii yang didapati bersikap neutral. Berbeza dengan penanda morfologi, penanda fitokimia menunjukkan korelasi negatif dengan peratusan keupayaan kacukan. GD ditunjukkan oleh gen plastid berdasarkan jarak diperbetulkan Jukes-Cantor antara kedua-dua jujukan, menunjukkan keputusan yang hampir sama dengan penanda fitokimia dan penunjuk RAPD.

Dalam satu lagi kajian bebas, 24 penanda mikrosatelit telah dikenakan ke atas aksesii AP. Penanda ini membuktikan kehadiran heterozigositi tetap dalam tumbuhan ini. Hasil ini adalah penting kerana ia berlaku apabila populasi mengalami kesesakan yang berulang kali dalam saiz populasi. Satu lagi kesudahan yang menarik kajian ini adalah memperkenalkan AP sebagai spesis kriptik semasa mengikut perubahan dalam motif mikrosatelit yang berulang,

Malaysia AP adalah berbeza dari segi genetik berbanding keturunan asalnya dari India.

Bahagian terakhir projek ini adalah menilai genetik asas sifat-sifat penting yang menggunakan analisis diallel. Hasil menunjukkan kehadiran kedua-dua kesan gen penambah dan kesan gen bukan penambah menyumbang dalam mengawal sifat-sifat morfologi dan fitokimia species ini. Hasil kajian menunjukkan bahawa kebanyakan sifat morfologi adalah di bawah kesan gen penambah manakala sifat fitokimia dipengaruhi oleh kesan gen bukan penambah. Kedua-dua heterosis negatif dan positif dicatatkan untuk sifat berbeza pada induk pertengahan dan tahap induk yang lebih baik. Keputusan analisis diallel memberi ufuk cerah untuk pembiakbakaan varieti prolifik pada masa depan.

ACKNOWLEDGEMENTS

It pleased me to take this opportunity to convey my deepest appreciation and gratitude to my supervisor Associate Professor Dr. Mihdzar Abdul Kadir for his generous help, invaluable guidance, patience and constant support throughout the completion of this thesis. I am deeply impressed by his positive energies and pure heart. I especially want to extend my heartfelt thanks and deepest gratitude to my dear co-supervisor Professor Dr. Soon-Guan Tan who was incredibly efficient, supportive and excellent in guiding me through this novel experience and his equally generous and scholarly guidance during its development. Undoubtedly, the instructions given by him led me to achieve some remarkable outcomes in the molecular experiments of my thesis. I do not remember any meeting to be held with him without fruitful technical and scientific achievements. My grateful appreciation is also due to my co-supervisor Associate Professor Datin Dr. Siti Nor Akmar Abdullah for her kind permission to work at her laboratory located in Institute of Tropical Agriculture – UPM. I would like to extend my sincere appreciation to Mr. Rahman and Mr. Nur Haizan for their support and kind cooperation in the field trials and supplementary affairs. I am certainly grateful and indebted to my dearest friend

Dr. Arash Javanmard for his valuable helps until the last minute of my study. I am indebted to Sagi for his guidance, cordial assistance for the HPLC analysis. I wish to express my warmest, sincerest thanks and profound gratitude to my colleague and friend Dr. Naghmeh Nejat. I believe her consultancy about the cross-amplification of microsatellite markers was an example of academic proficiency. I wish to express my sincere appreciation to my colleagues and close friends Dr. Vahid Omidvar and Mr. Mehdi Ebrahimi and Ms. Sonia Nikzad for their support and good wishes.

A special appreciation goes to my dear sister Dr. Dewi Hayati as I won't forget her perfect collaboration in providing SAS-DIALLEL05 software and helping me to conduct the related analysis. Mr. Talei a dear friend of mine deserves the warmest thanks due to his brotherly assistances in my statistical works.

My profound and heartiest thanks and love to my mother for her love, patience, encouragement and constant support throughout my study. My special and heartiest thanks and love towards my father for being an everlasting source of inspiration, his love, prayers, patience, continuous support and constant encouragement during the entire study period. I am forever indebted to my parents. My heartfelt thanks are extended to my sisters for their care, endless emotional support, love and assistance throughout my course of study.

Last but certainly not least, I wish to express my sincere appreciation to all those who not mentioned here that helped me to ensure the completion of my research.



I certify that an Examination Committee has met on **May 30th 2012** to conduct the final examination of **Alireza Valdiani** on his Doctor of Philosophy thesis entitled "**Crossability and Genetic Analyses of *Andrographis paniculata* Nees. Populations Using Phenotypic and Molecular Markers**" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Putra Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the degree of Doctor of Philosophy (PhD).

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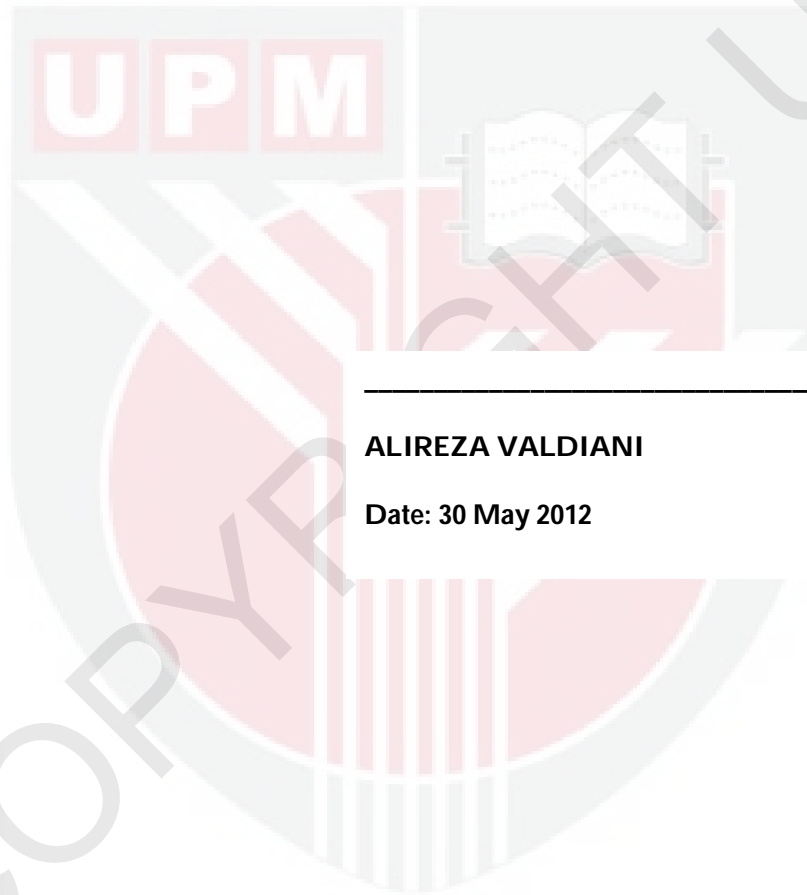
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DECLARATION

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



ALIREZA VALDIANI

Date: 30 May 2012



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