



UNIVERSITI PUTRA MALAYSIA

**GENETIC VARIATION, HYBRIDIZATION, *In-Vitro* GERMINATION, AND
MORPHOMETRIC STUDIES OF WILD ORCHIDS (*Aerides* spp.)**

SIVANASWARI CHALAPARMAL

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By

SIVANASWARI CHALAPARMAL

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

March 2015

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

GENETIC VARIATION, HYBRIDIZATION, *In-Vitro* GERMINATION AND MORPHOMETRIC STUDIES OF WILD ORCHIDS (*Aerides* spp.)

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March 2015

Chairman : Associate Professor Thohirah Lee Abdullah. PhD
Faculty : Agriculture

Aerides odorata, *Aerides odorata* var. Yellow, *Aerides quinquevulnera* var. Calyana, *Aerides flabellata* and *Aerides houlletiana* are five wild and endangered orchid species with immense floricultural traits. Comparative study using Polymerase Chain Reaction (PCR) based marker, Inter Simple Sequence Repeat (ISSR), were performed to access the genetic variation among these wild orchids. Among 15 ISSR primers tested, 14 primers showed reproducible bands. 71 bands out of 75 bands were polymorphic. *Aerides odorata* and *Aerides odorata* var. Yellow with *Aerides flabellata* and *Aerides houlletiana* were closest genetically. While, *Aerides quinquevulnera* var. Calyana was found to be most genetically diverse as compared to the other four species. Physical clustering of the loci present in the species were identified. These new polymorphic ISSR loci clearly delineate genetic variation and will aid in investigating the extent of gene flow taking place between species of *Aerides*. The intergeneric hybrids were synthesized by performing reciprocal crosses between species studied. The crosses performed were *Aeride sodorata* × *Aerides quinquevulnera* var. Calyana, *Aerides odorata* × *Aerides flabellata*, *Aerides odorata* var. Yellow × *Aerides quinquevulnera* var. Calyana and their reciprocals. *Aerides odorata* × *Aerides quinquevulnera* var. Calyana and its reciprocal cross were the most compatible cross.

Seed morphometry and scanning electron microscopic studies of four *Aerides* species and four hybridized seeds were presented. All the presently investigated taxa were different in their seed size, shape and colour. Seed colour ranges from pale yellow to yellow and brown. Variation between maximum and minimum in the length/width ratio of seed were discussed. Data on the seed volume showed that higher seed volume is the result of both greater length and width. Special attention was paid to study the morphology of parent and hybridized seeds and different stages of protocorms produced *in vitro*. Seed types were recognized according to the classification of Dressler. Gathering of these data is to provide genetic information for development of breeding strategies and conservation of endangered as well as economically important orchid species strategies. Successfully hybridized seeds of the hybridization study were subjected into *in-vitro* study to

determine the effects of kinetin and 6-Benzylaminopurine (BAP) (0.5, 1.0, 1.5, 2.0 mg/L) to their germination.

In vitro germination response of the both immature hybrid embryos of *Aerides odorata* × *Aerides quinquevulnera* var. *Calyana* and *Aerides quinquevulnera* var. *Calyana* × *Aerides odorata* were found to be best on Murashige and Skoog medium supplemented with kinetin 1.5 mg/L and BAP 1.5 mg/L respectively. These two plant growth regulators with 1.5mg/L concentration level also resulted in earliest growth and shortest time for shoot and root development of both hybrids.



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**VARIASI GENETIK, HIBRIDISASI, PERCAMBAHAN *In-Vitro* DAN
PENGAJIAN MORPHOMETRIK ORKID LIAR (*Aerides* spp.)**

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Pengerusi : Profesor Madya Thohirah Lee Abdullah. PhD
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Aerides odorata, *Aerides odorata* var. Kuning, *Aerides quinquevulnera* var. Calyana, *Aerides flabellata* dan *Aerides houlletiana* adalah lima spesies orkid liar dan terancam yang mempunyai ciri-ciri yang sangat penting dalam bidang florikultur. Kajian perbandingan menggunakan penanda ISSR berasaskan PCR, telah dijalankan untuk mengakses variasi genetik antara orkid liar. Antara 15 ISSR primer diuji, 14 primer menunjukkan jalur yang jelas. 71 jalur daripada 75 jalur adalah polimorfik. *Aerides odorata* dan *Aerides odorata* var. Kuning bersama *Aerides flabellata* dan *Aerides houlletiana* adalah rapat dari segi genetik. Walaubagaimanapun, *Aerides quinquevulnera* var. Calyana didapati paling jauh genetiknya berbanding dengan empat spesies lain. Kelompok lokus fizikal hadir dalam spesies telah dikenalpasti. Lokus ISSR polimorfik tersebut jelas menggambarkan variasi genetik dan akan membantu dalam menyiasat sejauh mana aliran gen yang berlaku di antara spesies *Aerides*. Kacukan di antara genus disintesis dengan melaksanakan kacukan dua hala di antara mereka. Kacukan-kacukkan yang dibuat adalah *Aerides odorata* × *Aerides quinquevulnera* var. Calyana, *Aerides odorata* × *Aerides flabellata*, *Aerides odorata* var. Kuning × *Aerides quinquevulnera* var. Calyana dan juga kacukan bersilang. *Aerides odorata* × *Aerides quinquevulnera* var. Calyana dan kacukan bersilang adalah kacukkan yang paling serasi.

Morphometrik benih dan pengimbasan kajian elektron mikroskopik pada empat *Aerides* spesies dan empat biji benih hibridisasi telah dibentangkan. Semua taksa yang kini disiasat adalah berbeza dalam bentuk, saiz benih, dan juga warna. Pelbagai warna benih didapati dari kuning pucat, kuning dan coklat. Perbezaan maksimum dan minimum dalam nisbah panjang/lebar benih telah dibincangkan. Data kepada jumlah benih menunjukkan bahawa kelantangan benih yang lebih tinggi hasil daripada kedua-dua panjang dan lebar yang lebih tinggi. Perhatian khusus telah dibuat untuk mengkaji morfologi induk dan benih hibridisasi, dan juga pelbagai peringkat protokom yang dihasilkan '*in-vitro*'. Kajian molekul menyiasat tahap perubahan genetik antara spesies induk dan jarak genetik *Aerides quinquevulnera* var. Calyana × *Aerides odorata* sebagai hibrid. Ini adalah kerana

hibrid ini telah memberi peratusan percambahan optimum berbanding *Aerides odorata* × *Aerides quinquevulnera* var. *Calyana*. Pengumpulan data ini adalah untuk memberikan maklumat genetik untuk pembangunan strategi pembiakan dan pemuliharaan terancam serta strategi spesies orkid penting dari segi ekonomi. Benih daripada kedua-dua kacukkan yang berjaya didedahkan di atas pembiakan 'in-vitro' untuk mengkaji kesan kinetin dan BAP (0.5, 1.0, 1.5, 2.0 mg/L) untuk percambahan.

Sebagai tindakbalas percambahan 'in-vitro', kedua-dua embrio hibrid *Aerides odorata* × *Aerides quinquevulnera* var. *Calyana* dan *Aerides quinquevulnera* var. *Calyana* × *Aerides odorata* didapati terbaik di dalam media Murashige dan Skoog yang ditambah dengan kinetin 1.5 mg/L dan BAP 1.5 mg/L.



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LIST OF ABBREVIATIONS

1 st	first
2ip	2-isopentenyladenine
AF	<i>Aerides flabellata</i>
AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
AO	<i>Aerides odorata</i>
AOY	<i>Aerides odorata</i> var. Yellow
AQ	<i>Aerides quinquevulnera</i> var. Calyana
BAP	6-Benzylaminopurine
Bp	Base pair
CRD	Completely Randomized Design
dH ₂ O	Distilled water
DNA	Deoxyribonucleic acid
GDP	Gross National Product
HMS	Half Murashige and Skoog
hr	Hour
ISSR	Inter simple sequence repeat
ITS	Internal transcribed spacers
L	Length
LM	Light microscope
MARDI	Malaysian Agriculture Research and Development Institute
MS	Murashige and Skoog
PCR	Polimerase Chain Reaction
PGR	Plant growth regulator
RAPD	Randomly Amplified Polymorphic Deoxyribonucleic acid
SEM	Scanning electron microscope
W	Width

CHAPTER 1

INTRODUCTION

Plant enthusiast and scientists alike has always been fascinated by the beauty and mystery of orchids. *Orchids exhibit an incredible range of diversity in size, shape and colour of their flowers.* Majority of the cultivated orchids are native of tropical countries. Over the years, orchid has evolved from a hobbyist's market into highly commercial market. Large-scale cultivation of orchid cut flowers and potted orchids is now the trend. Orchids play an important part in Malaysia's economy. Malaysia's orchid trade estimated at some RM80 – RM100 million a year. Malaysian orchids are sent off and sold daily in places as far away as London, New York, and Tokyo. The Malaysia Plan looks to orchids as a growth market. The performance of the floriculture industry has gradually improved to the point of producing 126 million stalks in 2005, and looking to produce 147 million stalks by 2010 (Whyte, 2007).

Malaysian forests are home of more than 2500 species in Sabah and Sarawak, and a further 850 species in Peninsular Malaysia. *Aerides odorata*, *Aerides odorata* var. Yellow, *Aerides flabellata*, *Aerides quinquevulnera* var. Calyana and *Aerides houlletiana* are five rare and endangered vandaceous orchids with immense floricultural traits. All of these orchids have an attractive fragrance. They are wild, vastly distributed in Malaysia and have not been domesticated. Endemic orchids of the region are facing the grim possibility of extinction from intense biotic pressures such as forest fires, indiscriminate wild collection and illegal trade by the local people. Hence, conservation and sustainable utilization assume greater importance to save the dwindling orchid wealth especially the rare and endangered ones. Collecting information on the level and pattern of genetic variation of wild orchids is the first step to facilitate their conservation. Knowledge about genetic variation is considered the baseline for conservation. Molecular markers are powerful tools to measure genetic relatedness and evolution which could lead the selection of parents for hybridization of orchids. Hybridization introduces new dimension in floriculture industry with constant production of better breeds. Synthesis of better hybrid orchids will certainly reduce the threatening pressure on their wild parents.

The orchid seeds are non-endospermic hence, they could not provide nutrient for seed germination. They require mycorrhizal association for their germination. Knudson (1922) discovered that orchid seeds can be germinated on a simple sugar containing medium. Subsequently, he proposed a new nutrient solution for the germination of orchid seeds in 1946. However, alteration of nutrients in the medium and addition of the plant growth regulators needed to obtain maximum germination rate and also optimum growth. Since then many species have been successfully raised in-vitro from seeds especially the rare ones which has difficulties in growth in natural condition.

Developmental studies of orchid embryo are scarce. Barthlott and Ziegler (1981) have recognized 20 different seed types and Viji *et al.* (1992) have reported seed characteristics in 53 species of orchids including their taxonomic significance. There are very few reports on orchid seed morphometry. Only few taxa of orchids have been investigated so far (Vijet *al.*, 1992; Rani *et al.*, 1993; Augustine *et al.*, 2001). Therefore, documentation of the seed information is vital for many uses.

Hence, the objectives of these studies are:

1. To quantify genetic variation between the five *Aerides* species in the study.
2. To produce primary hybrids from the crosses of *A.odorata* (AO) x *A. quinquevulnera* var. Calyana (AQ), *A.odorata* (AO) x *A.flabellata* (AF), *A.odorata* var. Yellow (AOY) x *A. quinquevulnera* var. Calyana (AQ) and their reciprocals.
3. To determine the effect of Kinetin and BAP and its concentration levels on seed germination percentage (%) of *A.odorata* x *A. quinquevulnera* var. Calyana and its reciprocal cross.
4. To illustrate the seed morphometric characterization of the parents and their offsprings of several *Aerides* species.
5. To describe early growth (embryogenesis) of hybrids, *A.odorata* x *A. quinquevulnera* var. Calyana and *A. quinquevulnera* var. Calyana x *A.odorata*.

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