



UNIVERSITI PUTRA MALAYSIA

***BIOCHEMICAL CHANGES IN DIFFERENT STAGES OF
DENDROBIUM ORCHID FLOWERS AND IN VIVO FLOWERING
VIA EXOGENOUS CYTOKININ APPLICATION***

NISHA NAMBIAR

FBSB 2013 25



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**DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA**

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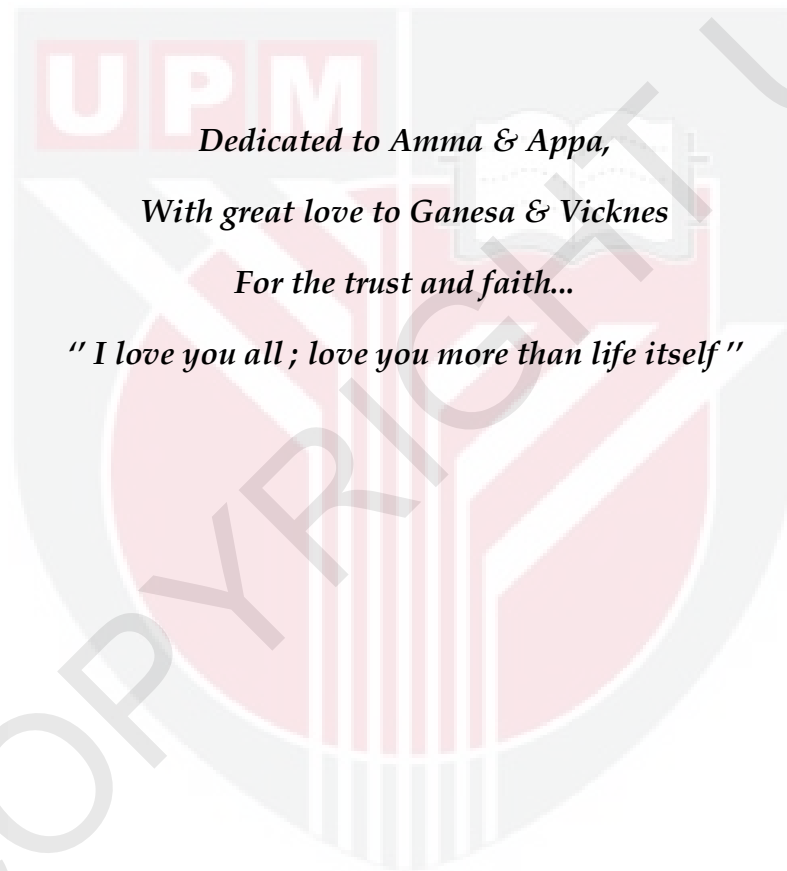
**Thesis submitted to the School of Graduate Studies, Universiti Putra
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Philosophy**

April 2013

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Dedicated to Amma & Appa,

With great love to Ganesa & Vicknes

For the trust and faith...

" I love you all ; love you more than life itself "

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the Degree of Doctor of Philosophy

**BIOCHEMICAL CHANGES IN DIFFERENT STAGES OF
DENDROBIUM ORCHID FLOWERS AND *IN VIVO* FLOWERING VIA
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NISHA NAMBIAR

April 2013

Chairman: Professor Maziah Mahmood, PhD

Faculty: Biotechnology and Biomolecular Sciences

Orchids capture the interests of many because of their large diversity, large number of species and varied forms and structures of its flowers. The focus of the present study will be on investigating the biochemical changes that occur in the flowers of four commercially important *Dendrobium* orchids (*D. Angel White*, *D. Dragon Eye*, *D. Aredang Green* and *D. Aredang Blue*) at four different developmental stages (buds, half-opened flower, fully bloomed flower and wilted flower). *Dendrobium* orchids are tropical orchids suitable to be grown under hot and humid environment and are also commercially important as they are mostly used as cut flower. This study would be important towards the understanding of biochemical changes which can occur in a flower prior to flowering and throughout the developmental process of a flower and this information

will be useful in the manipulation of flowering induction and the flower longevity. Results from the biochemical characteristics of the selected *Dendrobium* flowers revealed that protein content increased from the bud stage up to the full bloom stage but reduced thereafter during the wilting stage. Soluble protein content ranged from 9.01 to 12.51 mg/g FW and the total non-soluble protein content was recorded to be ranging from 4.18 to 9.29 mg/g FW. In this study, the relationship between carbohydrate and glucose content throughout the development of flowers from the bud stage to the wilting stage was investigated as well. The total carbohydrate and glucose content was observed to be the highest in half-opened flowers of all four *Dendrobium* orchid hybrids. The anthocyanin analysis of the *Dendrobium* flowers showed that the total anthocyanin content was the highest in *D. Dragon Eye* (deep purple flower) with 27.06 nmol/g FW and the lowest in *D. Angel White* flowers (white coloured) with only 0.59 nmol/g FW. The results obtained from the various enzyme activities in *Dendrobium* orchid flowers showed the possible role played by antioxidative enzymes during flowering of orchids can be applied in linking both flowering and senescence process because the enzymes showed different activity level in all the hybrids as their activities were the highest in flowers at wilting stage, showing the essential role played by these enzymes in regulating the senescence process in *Dendrobium* flowers. In this study also, the role of BAP application in promoting earlier flowering of a selected *Dendrobium* orchid (*D. Angel White*) was

investigated. The first inflorescence stalk was formed on day 53 in the plants exposed to 200, 250 or 300 mg/L of BAP followed by nine days later for plants sprayed with 150 mg/L of BAP. Plants that were not sprayed with BAP (control) developed approximately 13 leaves per plant, whereas those sprayed with 200 mg/L BAP produced the highest number of leaves followed by plants treated with 150 mg/L of BAP by producing 40.9% and 23.6% more leaves than the control plants. The highest number of flowers per inflorescence (14) was recorded for the plants treated with 200 mg/L BAP followed by plants treated with 150 mg/L BAP which showed 31% less number of flowers than the plants treated with 200 mg/L of BAP. This finding can further be exploited to increase flowering and to promote earlier flowering of other orchids. The quality of flowers obtained were determined based on the total protein content and reducing sugar content in the flowers where the total protein and sugar content increased as the concentrations of BAP applied increased ranging from 24.3 to 61.2 $\mu\text{g/g}$ FW and 1175 to 1220 $\mu\text{g/g}$ FW, respectively. This showed that the quality of the flowers was not affected by the BAP treatment. Furthermore, BAP treatment on *D. Angel White* at all concentrations also improved the chlorophyll content of its leaves. This study indicates a great potential for speeding up orchid flowering whereby in future, the application of plant growth regulators to plants would be able to stimulate synchronous flowering of orchids which can help *Dendrobium* orchid types in maintaining a steady position in the floriculture industry.

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

**PERUBAHAN BIOKIMIA DALAM PELBAGAI PERINGKAT
BUNGA ORKID *DENDROBIUM* DAN PEMBUNGAAN *IN VIVO*
MELALUI KAEDAH APLIKASI SITOKININ**

Oleh

NISHA NAMBIAR

April 2013

Pengerusi: Professor Maziah Mahmood, PhD

Fakulti: Bioteknologi dan Sains Biomolekul

Tanaman orkid berjaya menarik perhatian orang ramai disebabkan oleh kepelbagaian diversiti, jumlah spesies yang besar dan juga kerana variasi dari segi bentuk dan struktur bunga tersebut. Fokus utama kajian ini adalah untuk mengenalpasti perubahan biokimia yang berlaku di dalam bunga orkid jenis *Dendrobium* di dalam empat peringkat perkembangan. Empat jenis orkid *Dendrobium* yang dipilih untuk digunakan dalam kajian ini adalah *D. Angel White*, *D. Dragon Eye*, *D. Aredang Blue* dan *D. Aredang Green*. Orkid jenis *Dendrobium* dipilih dalam kajian ini kerana orkid ini sesuai dikomersilkan di Malaysia dan banyak digunakan dalam industri keratan bunga kerana orkid *Dendrobium* merupakan orkid jenis tropika yang sesuai ditanam di bawah cuaca yang panas. Kajian ini penting kerana ia akan memberi maklumat yang lebih mendalam tentang

perubahan biokimia yang berlaku di dalam bunga sepanjang perkembangan proses pembungaannya. Maklumat ini seterusnya boleh digunakan untuk membolehkan manipulasi proses penghasilan bunga dan proses meningkatkan tempoh tahan bunga tersebut. Keputusan kajian biokimia menunjukkan bahawa kandungan protein dalam bunga meningkat dari peringkat putik bunga ke peringkat bunga mekar tetapi berkurangan di peringkat bunga layu. Kandungan protein larut didapati berada dalam lingkungan 9.01 to 12.51 mg/g berat basah manakala kandungan protein tak larut pula dalam julat 4.18 to 9.29 mg/g berat basah. Dalam kajian ini, hubungan di antara kandungan karbohidrat dan glukosa dengan perkembangan bunga *Dendrobium* turut dikaji. Bagi kesemua hibrid orkid yang digunakan, kandungan karbohidrat dan glukosa didapati paling tinggi di dalam bunga separa-buka. Analisa kandungan antosianin dalam bunga orkid *Dendrobium* pula menunjukkan kandungan pigmen yang paling tinggi dalam *D. Dragon Eye* (bunga berwarna ungu tua) dengan amaun 27.06 nmol/g berat basah dan paling kurang dalam *D. Angel White* (bunga putih) dengan amaun hanya sebanyak 0.59 nmol/g berat basah. Keputusan yang diperolehi menunjukkan aktiviti enzim tertinggi dalam bunga pada peringkat layu di mana ini menunjukkan kepentingan enzim ini dalam proses senesens bunga orkid *Dendrobium*. Dalam kajian ini juga, kesan beberapa kepekatan BAP dalam pencetusan pembungaan dalam orkid jenis *D. Angel White* telah dijalankan. Pembentukan infloresens bunga terawal telah

direkodkan pada hari ke 53 di dalam pokok orkid yang didedahkan kepada BAP pada kepekatan 200, 250 dan 300 mg/L BAP, diikuti dengan sembilan hari kemudian bagi pokok orkid yang didedahkan kepada BAP pada kepekatan 150 mg/L BAP. Pokok yang tidak diberi rawatan BAP menghasilkan 13 daun, manakala pokok yang diberi rawatan 200 mg/L BAP menghasilkan bilangan daun tertinggi diikuti dengan pokok yang diberi rawatan BAP sebanyak 150 mg/L BAP dengan menghasilkan 40.9% dan 23.6% lebih banyak daun daripada pokok kawalan. Bilangan bunga paling tinggi (14) dihasilkan oleh pokok yang diberi rawatan 200 mg/L BAP diikuti dengan pokok yang diberi 150 mg/L BAP yang menunjukkan 31% lebih rendah bilangan bunga jika dibandingkan dengan pokok yang didedahkan kepada 200 mg/L BAP. Penemuan ini berguna untuk meningkatkan produktiviti dari segi pembungaan dan juga bagi mempercepat proses ini dalam orkid jenis lain. Kualiti bunga yang dihasilkan turut dinilai melalui kandungan protein dan glukosa di dalam bunga di mana keputusan menunjukkan kandungan kedua-dua komponen ini meningkat di dalam bunga iaitu dari 24.3 to 61.2 $\mu\text{g} / \text{g}$ berat basah dan 1175 to 1220 $\mu\text{g} / \text{g}$ berat basah, masing-masing selaras dengan peningkatan kepekatan BAP yang digunakan. Ini menunjukkan bahawa kualiti bunga orkid ini tidak terjejas oleh sebab rawatan BAP. Malah, BAP pada semua kepekatan turut meningkatkan kandungan klorofil di dalam daun *D. Angel White*. Hasil kajian ini mempunyai potensi untuk mempercepatkan proses pembungaan dalam orkid

Dendrobium di mana di masa-masa akan datang, penggunaan pengawalatur pertumbuhan yang lain mampu menghasilkan proses pembungaan yang stabil dan serentak di mana ini boleh membantu dalam memajukan industri florikultur negara.



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~ *THANK YOU* ~

I certify that a Thesis Examination Committee has met on 29th April 2013 to conduct the final examination of Nisha Nambiar on her thesis entitled “Biochemical changes in different stages of *Dendrobium* orchid flowers and *in vivo* flowering via exogenous cytokinin application” 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 degree of Doctor of Philosophy.

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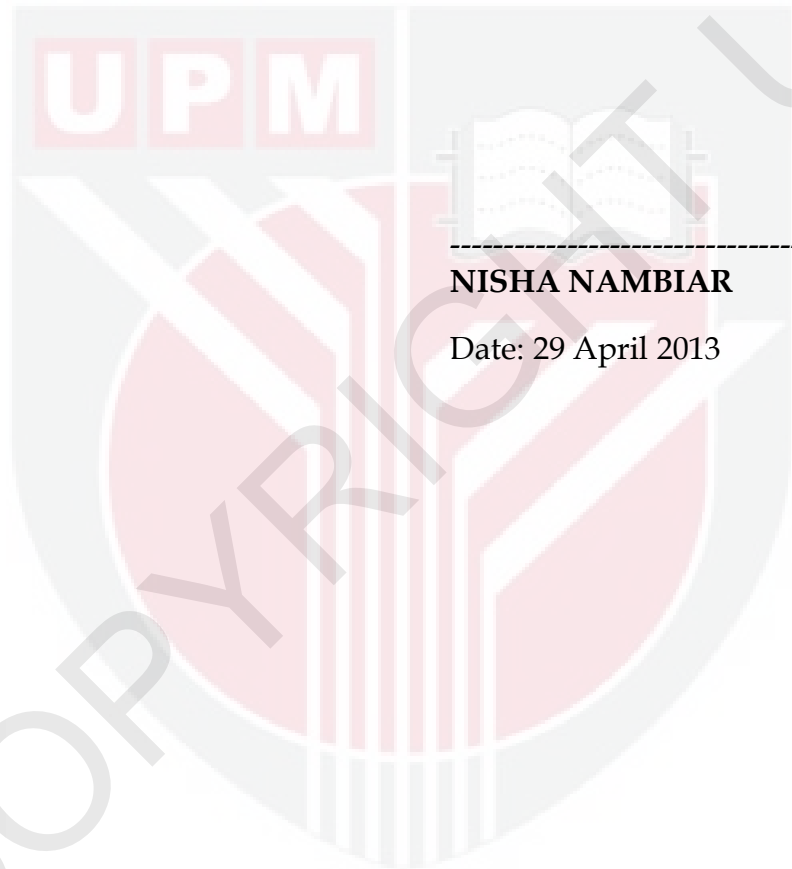
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DECLARATION

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



NISHA NAMBIAR

Date: 29 April 2013

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