



UNIVERSITI PUTRA MALAYSIA

**MICROPROPAGATION OF ENDANGERED SLIPPER ORCHID,
PAPHIOPEDILUM ROTHSCILDIANUM (RCHB. F.) STEIN**

NG CHYUAM YIH

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By

NG CHYUAM YIH

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
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June 2011

Abstract of thesis presented to Senate of Universiti Putra Malaysia in fulfillment of the requirements for the Degree of Master of Science

**MICROPROPAGATION OF ENDANGERED SLIPPER ORCHID,
PAPHIOPEDILUM ROTHSCHILDIANUM (RCHB. F.) STEIN**

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June 2011

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Faculty: Biotechnology and Biomolecular Sciences

Paphiopedilum rothschildianum, a highly endangered slipper orchid species endemic to Mount Kinabalu, Sabah, Malaysia, is strictly protected under Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). A study was carried out to establish *in vitro* culture system and to develop efficient propagation strategies to prevent this species from being extinct. The effect of casein hydrolysate, peptone and tryptone peptone at concentrations of 0.5, 1.0 and 2.0 g/L on multiple shoots induction of *P. rothschildianum* were studied. Additions of 1.0 g/L peptone into PGR-free ½ MS has resulted in the formation of 2.9 shoots per nodal stem explants; whereas, 2.8 shoots were produced from a single shoot after 16 week of culture on PGR-free ½ MS supplemented with 2.0 g/L tryptone-peptone. The study was also conducted to investigate the effects of cytokinins, organic additives and combination of cytokinin and organic additives on multiple shoot formation of *P. rothschildianum*. The additions of 4 µM BAP together with 2 g/L peptone and 60 g/L potato homogenate in ½ MS had markedly

increased the number of multiple shoot formed with an average of 8.8 shoots per nodal stem explant after 12 weeks of culture. The effects of other organic additives such as banana homogenate, potato homogenate, tomato homogenate and coconut water were also evaluated on the *in vitro* growth and development of *P. rothschildianum* plantlets. Among these organic additives, addition of potato homogenate had effectively promoted the formation of new shoots (3.1 shoots per plantlet) and leaves (1.8 leaves per plantlet); and addition of tomato homogenate has shown to promote roots formation (6.2 roots per explant) in *P. rothschildianum* plantlets. On the other hand, the presence of coconut water was inhibitory to the proliferation of *P. rothschildianum* plantlets. Well developed plantlets with four to five leaves and roots were acclimatized and transferred to a glasshouse with 90% survival rate. Propagation of *P. rothschildianum* through formation of protocorm-like body (PLB) was also established. Secondary PLBs were successfully induced from primary PLBs which obtained from stem derived-callus, after 8 weeks of culture on $\frac{1}{2}$ MS supplemented with 4 μ M kinetin with an average of 4.1 PLBs formed per explant. Subsequent subculture of the secondary PLBs onto PGR-free $\frac{1}{2}$ MS containing 60 g/L banana homogenate for an additional 8 weeks has resulted in the three fold increased in the number of new PLBs (tertiary PLB) formation. Approximately 70% of these newly formed PLBs were regenerated into complete plantlets after 8 weeks of culture on PGR-free $\frac{1}{2}$ MS containing 20% (v/v) coconut water. The outcomes of this study were suitable for *in vitro* conservation of this highly endangered species and could also be applied for large-scale production of this fabulous slipper orchid toward meeting the increasing demand of ornamental industry.

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

**PROPAGASI MIKRO BAGI 'ORKID SELIPAR' TERANCAM,
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Paphiopedilum rothschildianum, sejenis 'Orkid Selipar' yang berasal dari Gunung Kinabalu, Sabah, Malaysia, adalah species orkid yang amat terancam dan dibawah perlindungan ketat 'Convention on International Trade in Endangered Species of Wild Fauna and Flora' (CITES). Bagi mengelakkan kepupusan *P. rothschildianum*, kajian telah dijalankan untuk membangunkan sistem kultur 'in vitro' dan strategi propagasi efisien bagi *P. rothschildianum*. Kesan daripada kasein hidrolisat, pepton dan tripton-pepton pada kepekatan 0.5, 1.0 dan 2.0 g/L terhadap pembentukan tunas berganda dari *P. rothschildianum* telah dikaji. Penambahan 1.0 g/L pepton ke dalam ½ MS bebas hormon telah menghasilkan 2.9 tunas pada eksplan batang, sedangkan sebanyak 2.8 tunas dapat dihasilkan dari anak pokok selepas kultur selama 16 minggu diatas ½ MS bebas hormon yang mengandungi 2.0 g/L tripton-pepton. Kajian juga telah dijalankan untuk menentukan keberkesanan dan peranan sitokinin dan aditif organik dalam pembentukan tunas berganda terhadap *P. rothschildianum*. Penambahan 4 µM BAP bersama-sama dengan 2 g/L pepton dan 60 g/L homogenat kentang telah meningkatkan jumlah pembentukan tunas berganda dengan signifikan, dan sebanyak 8.8 tunas telah dihasilkan pada eksplan batang selepas dikultur

selama 12 minggu. Kesan daripada pelbagai jenis aditif organik kompleks (homogenat pisang, homogenat kentang, homogenat tomato dan air kelapa) terhadap pertumbuhan dan perkembangan anak pokok *P. rothschildianum* juga dikaji. Kajian menunjukkan bahawa penambahan homogenat kentang ke dalam media kultur telah mengaruh pembentukan tunas baru (3.1 tunas) dan daun (1.8 daun) pada anak pokok *P. rothschildianum*; sedangkan penambahan homogenat tomato ke dalam media kultur telah mengaruh pembentukan akar pada anak pokok *P. rothschildianum* (6.2 akar). Dibalikinya, penambahan air kelapa telah merencat pembiakan anak pokok *P. rothshildianum*. Anak pokok yang berkembang dengan baik dan mempunyai empat hingga lima daun pucuk dan akar telah diaklimatisasi dan dipindahkan ke rumah kaca dengan kadar hidup sebanyak 90%. Propagasi *P. rothschildianum* melalui pembentukan 'protocorm-like body' (PLB) juga telah dibangunkan. Selepas 8 minggu berkultur diatas $\frac{1}{2}$ MS mengandungi 4 μ M kinetin, PLB primer yang berasal dari kalus telah berjaya membentuk 4.1 PLB sekunder. Subkultur PLB sekunder selanjutnya diatas $\frac{1}{2}$ MS bebas hormon yang mengandungi 60 g/L homogenat pisang selama 8 minggu telah mengakibatkan peningkatan sebanyak tiga kali ganda dalam pembentukan PLB baru dari PLB sekunder. 70% dari PLB yang baru terbentuk ini telah berkembang dan manghasilkan anak pokok selepas 8 minggu dikultur diatas $\frac{1}{2}$ MS bebas hormon yang mengandungi 20% (v/v) air kelapa. Hasil dari kajian ini adalah sesuai untuk digunakan dalam pemuliharaan *in vitro* spesies teracam ini dan juga boleh digunakan untuk pengeluaran secara besar-besaran 'Orkid Selipar' ini demi memenuhi permintaan industri pokok hias yang kian meningkat.

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I certify that a Thesis Examination Committee has met on **21 June 2011** to conduct the final examination of Ng Chyuam Yih on his thesis entitled “Micropropagation of an Endangered Slipper Orchid, *Paphiopedilum rothschildianum* (Rchb. f.) Stein in accordance with the Universities and University College 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, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

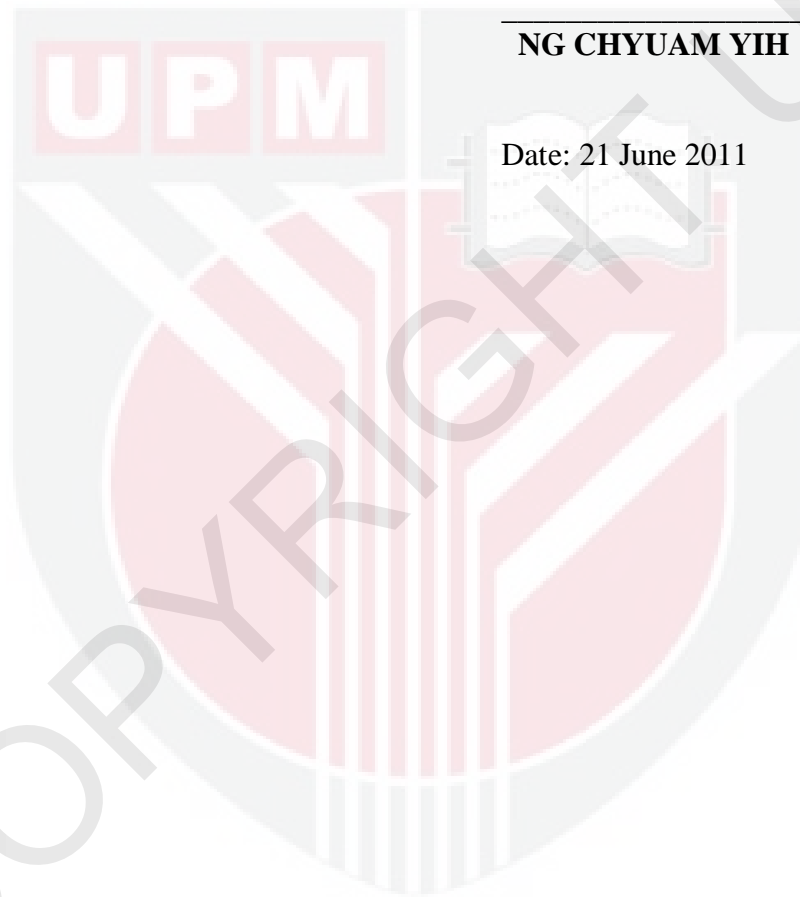


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