UNIVERSITI PUTRA MALAYSIA

MICROPROPAGATION AND HEAVY METAL CONTENT OF *BEGONIA PAVONINA* (RIDL.)

ROSILAH BINTI AB AZIZ

IB 2006 14

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By

ROSILAH BINTI AB AZIZ

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

October 2006

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

MICROPROPAGATION AND HEAVY METAL CONTENT OF *BEGONIA PAVONINA* (RIDL.)

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October 2006

Chairman : Faridah Qamaruz Zaman, PhD

Institute : Bioscience

This study were conducted to develop a micropropagation protocol for *Begonia pavonina* (Ridl.) and to analyze the heavy metals content in tissue culture derived and wild plants. Various media treatments were tested for shoot multiplication and root induction of *B. pavonina*. Results obtained showed that Murashige and Skoog (1962) basal medium (MS) supplemented with 0.1 mgL^{-1} 6-Benzylamino-purine (BAP) produced higher mean number of shoots (7.60) and high mean length of shoots (2.20 cm). Studies on the effects of α-Naphthaleneacetic acid (NAA) and Indole-butyric-acid (IBA) on root induction showed that MS medium supplemented with 1.0 mgL^{-1} NAA gave the highest mean number of roots per shoot (14.47). Root formation can be seen as early as two weeks of incubation. The survival rate of this tissue cultured derived plants in the greenhouse after three months were about 80%. An efficient protocol for somatic embryogenesis from leaf explant of *B. pavonina*

was also developed. Of the media tested, MS supplemented with 2.0 mgL^{-1} 2,4-D and 1.0 mg L^{-1} BAP was found to be the most effective for the production of somatic embryos. Somatic embryos were successfully developed into plantlets when single embryos were transferred onto MS hormone-free medium. Plantlets were successfully transplanted in FRIM's nursery.

Heavy metals content in three different parts (leaves, stems and roots) of tissue cultured plants (*in vitro*) and wildings were analyzed using Inductively Coupled Plasma (ICP). The analyses showed that the level of heavy metal tested for both tissue cultured and wildings of *B. pavonina* were below the maximum permissible limits. Results also indicated that accumulation of heavy metal was higher in the roots, (both in wildings and tissue cultured plantlets) compared to stems and leaves. Zinc (Zn), Iron (Fe) and Manganese (Mn) were highly accumulated in tissue culture derived plantlets, while the content of Cadmium (Cd), Copper (Cu) and Lead (Pb) in tissue culture derived plantlets were lower compared to those of wildlings. Mercury (Hg) and Arsenic (As) were not detected in both tissue culture derived plantlets and wildings of *B. pavonina*.

Histological analysis were done using a standard protocol by Malaysian Palm Oil Board (MPOB). The histological analysis of the embryogenic callus tissue of the *B. pavonina* explants showed that they were made up of small meristematic cells with dense cytoplasm, with small vacuoles and were very rich in soluble protein (which were stained blue with Naphthelene Blue Black). Three types of callus were

identified, however, only Type 3 callus produced globular embryos after eight weeks of culture, and subsequently to the heart-shaped structures. From this study, it can be concluded that micropropagation *B. pavonina* can be accomplished via organogenesis and somatic embryogenesis. Analysis of heavy metals content in tissue culture derived plantlets and wildling were below the maximum permissible limits, hence proven safe to be eaten.

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

PERAMBATAN MIKRO DAN KANDUNGAN LOGAM BERAT BAGI *BEGONIA PAVONINA* (RIDL.)

Oleh

ROSILAH BINTI AB AZIZ

Oktober 2006

Pengerusi : Faridah Qamaruz Zaman, PhD

Institut : Biosains

Kajian ini dijalankan untuk membangunkan teknik perambatan mikro bagi *Begonia pavonina* (Ridl.) dan menganalisa kandungan logam berat di dalam anak didik hasilan kultur tisu dan anak liar. Pelbagai rawatan media telah diuji untuk menghasilkan penggandaan pucuk dan pertumbuhan akar bagi *B. pavonina*. Keputusan yang diperolehi menunjukkan bahawa media Murashige and Skoog (1962) (MS) yang ditambah dengan 0.1 mgL^{-1} BAP menghasilkan purata bilangan pucuk yang tertinggi (7.60) dan ketinggian pucuk yang tertinggi (2.20 cm). Kajian ke atas kesan NAA dan IBA di dalam meransangkan pertumbuhan akar menunjukkan media MS yang ditambah dengan 1.0 mgL^{-1} NAA memberikan purata bilangan akar yang tertinggi (14.47) bagi setiap pucuk. Pembentukan akar berlaku seawal dua minggu selepas dikulturkan di dalam media pengakaran.. Kadar kemandirian anak didik hasilan kultur tisu di tapak semaian adalah 80% selepas tiga

bulan. Protokol untuk embriogenesis somatik dari daun pokok *B. pavonina* telah berjaya dibangunkan. Keputusan menunjukkan kombinasi media MS yang ditambah dengan 2.0 mgL^{-1} 2,4-D dan 1.0 mg L^{-1} BAP adalah yang paling sesuai dan efektif dalam penghasilan embriogenik somatik. Sel embriogenik ini berjaya membentuk plantlet apabila individu embrio dipindahkan ke media MS tanpa hormon. Plantlet berjaya dpindahkan ke tapak semaian FRIM.

Analisis kandungan logam berat di dalam tiga bahagian tisu yang berbeza (daun, batang dan akar) bagi anak didik dan anak liar dilakukan dengan menggunakan alat Inductively Coupled Plasma (ICP). Keputusan menunjukkan kandungan logam-logam berat yang diuji di dalam anak didik dan anak liar *B. pavonina* adalah di bawah aras maksimum yang dibenarkan. Keputusan menunjukkan bahagian akar anak didik mengumpul logam berat yang lebih tinggi dan anak liar berbanding di bahagian daun dan batang. Zink (Zn), Ferum (Fe) dan Manganese (Mn) menunjukkan pengumpulan yang tertinggi di dalam anak didik, manakala kandungan Cadmium (Cd), Kuprum (Cu) dan Plumbum (Pb) di dalam anak didik adalah kurang berbanding kandungan di dalam anak liar. Kandungan Raksa (Hg) dan Arsenik (As) tidak dikesan di dalam kedua-dua anak didik dan anak liar *B. pavonina*.

Analisis histology dijalankan mengikut protokol yang telah dibangunkan oleh Lembaga Kelapa Sawit Malaysia (MPOB). Analisis histologi yang dijalankan ke atas tisu kalus embriogenik eksplan *B. pavonina* menunjukkan bahawa tisu kalus itu mengandungi sel-sel meristemik, sitoplasma dan sel-sel vakuol yang kecil dan kandungan protein yang banyak (apabila ditandakan dengan Naphthelene Blue Black). Teknik ini dapat mengenalpasti tiga jenis kalus telah dapat dikenalpasti tetapi hanya kalus Jenis 3 menghasilkan embrio berbentuk bulat selepas lapan minggu dikulturkan, dan seterusnya membentuk struktur berbentuk hati 'heart'. Dari keseluruhan kajian ini, dapat disimpulkan bahawa perambatan mikro bagi B. pavonina dapat dibangunkan melalui teknik organogenesis dan embriogenik somatic. Analisis kandungan logam berat bagi anak didik dan anak liar menunjukkan ia di bawah aras maksimum yang dibenarkan, yakni dibuktikan ia selamat untuk dimakan.

ACKNOWLEDGEMENTS

In the name of Allah S.W.T The Most Gracious, The Most Merciful

I would like to convey my appreciation to the Director General of FRIM, Dato' Dr Abdul Razak bin Mohd Ali and the Chairman of FRIM Training Committee (JKL), Dato' Dr Abdul Latif bin Mahmood, to Dr Baskaran Krishnapillay (Senior Director of Forest Biotechnology) and Dr Marzalina Mansor (Director of Forest Biotechnology Programme) for giving me the opportunity to pursue my MSc. in UPM.

My deepest appreciation and gratitude also goes to my supervisor Dr Faridah Qamaruz Zaman, the members of the supervisory committee, Dr Kodiswaran Kandasamy (FRIM) and Dr Parameswari Namasivayam (UPM) for their support, kindness, comments and suggestions that leads to the completion of this thesis.

My appreciation goes to Dr Anuar Rahim and Dr Osmanu for their guidance and assistance on statistical analysis, Dr Wan Rasidah (Soil Lab, FRIM) Dr Siti Zawiyah, Dr Che Fauziah, and Pn Esah for their assistance on heavy metal analysis. To all the staff in Tissue Culture Lab Pn Hasnida, Pn Sharifah, Pn Normah, Kak Sabariah, Rohani, Pn Naemah, Pn Rozidah, Pn Zamrudah, Pn Rukiah, Syafidah, Pn Thila and Shidah for their support. Not forgotten to my

friends Datie, Liza, Sun, Brian and individuals who directly or indirectly helped me throughout my study.

This thesis is especially dedicated to my mother and father for their love and courage throughout my study. To my sisters and brothers and to my fiancé (Saiful), thank you very much for the support.

I certify that an Examination Committee has met on ..... to conduct the final examination of Rosilah binti Ab Aziz on her degree entitle 'M**icropropagation techniques and comparative analysis of heavy metal content in *Begonia pavonina* (Ridl)**' in accordance with Universiti Putra Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Nor' Aini Mohd Fadzillah, PhD
Associate Professor,
Faculty of Science,
Universiti Putra Malaysia
(Chairman)

Nor'Aini Ab Shukor, PhD
Professor
Faculty of Forestry
Universiti Putra Malaysia
(Internal Examiner)

Saleh Kadzimin, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Chan Lai Keng, PhD
Associate Professor
Centre of Biological Science Studies
Universiti Sains Malaysia
(External Examiner)

_____
HASANAH MOHD GHAZALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

Faridah Qamaruz Zaman, PhD
Lecturer
Institute of Bioscience
Universiti Putra Malaysia
(Chairperson)

Kodi Isparan Kandasamy, PhD
Senior Scientist
Forest Research Institute of Malaysia
(Member)

Parameswari A/P Namasivayam
Lecturer
Faculty of Biotechnology and Biomolecular Science
Universiti Putra Malaysia
(Member)

_____
AINI IDERIS, PhD
Professor//Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 8 MARCH 2007

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.

ROSILAH BINTI AB AZIZ

Date:

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LIST OF ABBREVIATIONS

^{0}C	degree centrigrade
%	percentage
µM	microMolar
2iP	2-isopentyl adenine
2,4-D	2,4-Dichlorophenoxyacetic acid
ABA	Abscissic acid
ANOVA	Analysis of variance
As	Arsenic
BAP	6-Benzylamino-purine
B5	Gamborg B5 media (1968)
cm	centimeter
Co	Cobalt
Cr	Chromium
Cu	Copper
df	degree of freedom
EC	Embryogenic cells
Fe	Ferum
FRIM	Forest Research Institute of Malaysia
g	gram
g/L	gram per litre
GA_3	Gibberellic acid

HCl	Hydrochloric acid
$HgCl_2$	Mercuric chloride
Hg	Mercury
IBA	Indole-butyric-acid
ICP	Inductively Coupled Plasma
KC	Knudson C media (1946)
Kin	Kinetin
kPa	kilopascal (unit of pressure)
L	litre
LAF	Laminar air flow cabinet
LSD	least significant different
M	Molar
mg	milligram
mg/L	milligram per litre
ml	millilitre
mm	millimeter
Mn	Manganese
MPOB	Malaysian Palm Oil Board
Ms	mean of square
MS	Murashige and Skoog media (1962)
NAA	α-Naphthaleneacetic acid
NaOH	Sodium hydroxide

NBB	Naphthol blue black
NEC	non-embryogenic cells
Ni	Nickel
Pb	Lead
pH	negative logarithm of the hydrogen concentration
ppm	part per million
SDW	sterile distilled water
Spp	species
TDZ	Thidiazuron
UV	ultraviolet
V	vacuoles
v/v	volume over volume
w/v	weight over volume
Zn	Zinc

CHAPTER 1

INTRODUCTION

Begonia belongs to the family of Begoniaceae and is widely distributed throughout the tropical and subtropical regions, from America to Asia. Over 10,000 kinds of hybrids and cultivars are available in this family (Kiew, 1991). To date, more than 1,500 species have been named with more new species waiting to be discovered (Kiew, 2005).

Since it is an important ornamental plant throughout the world, commercial large-scale propagation of these valuable *Begonia*s are considered necessary to meet the ever increasing demand (Kiew and Kee, 2002). Over 200 species have been introduced by commercial growers. Among some of the commercially important species are *B. sempenflorences, B. tuberhybrida, B. elatior, B. cheimanta* and *B. socotrana* (Takayama, 1990). *Begonia pavonina* is a plant species endemic to Malaysia, and is known to be found only in the Cameron Highlands (Peninsular Malaysia) (Kiew, 1991). Although *in vitro* propagation of several *Begonia* species has been well documented, but no report is available on the *in vitro* propagation of *B. pavonina*.

Plants are considered to be poisonous which when eaten, can have a bad effect on the normal health of man and domestic animals. According to Marion and Anthony (1984), the amount that has been eaten to produce an adverse effect on health can

range from a relatively small to a much greater quantity, and/or consumed over a prolong period. Plants needed micronutrients like Zn, Mn, Pb, Cu and Co for metabolic activity, respiration and transpiration. However, if the concentration exceeds the standard level, this may cause toxicity to plants. Therefore, it is unsafe for human and animal consumption (Wild, 1993).

Justification and objectives

Begonia pavonina is an endemic plant to Cameron Highlands, Malaysia. Though, development of micropropagation protocol is an important aspect in the conservation of *B. pavonina*. Production of plants through tissue culture technique can ensure the sufficient supply of this species so that the natural habitat of this species can remain undisturbed. This is especially important to safeguard the sustainability of resources from natural forest. The micropropagation strategy used in *B. pavonina* can be a model study in propagating and conserving other endemic species of *Begonia* in Peninsular Malaysia such as *Begonia rajah* (Johor), *Begonia abdullahpieei* (Perak), *Begonia sibthorpiodes* (Kedah), *Begonia phoeniogramma* (Selangor), *Begonia isopteroidea* (Pahang), *Begonia jiewhoei* (Kelantan), *Begonia alpine* (Pahang), *Begonia koksunii* (Perak) and *Begonia tigrina* (Kelantan).

Begonia spp. is believed to have anti-inflammatory properties and were used in cooking by the indigenous people in Malaysia (Zainon A.S., pers. comm.). Some *Begonia* spp. have also been used as diuretics, purgatives or emetics. Several