



**UNIVERSITI PUTRA MALAYSIA**

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

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*PAVONINA* (RIDL.)**

**By**

**ROSILAH BINTI AB AZIZ**

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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  $0.1 \text{ mgL}^{-1}$  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  $\alpha$ -Naphthaleneacetic acid (NAA) and Indole-butyric-acid (IBA) on root induction showed that MS medium supplemented with  $1.0 \text{ mgL}^{-1}$  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<sup>-1</sup> 2,4-D and 1.0 mg L<sup>-1</sup> 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 wildings. 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

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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  $0.1 \text{ mgL}^{-1}$  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  $1.0 \text{ mgL}^{-1}$  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  $2.0 \text{ mgL}^{-1}$  2,4-D dan  $1.0 \text{ mg L}^{-1}$  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 dipindahkan 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.



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I certify that an Examination Committee has met on ..... to conduct the final examination of Rosilah binti Ab Aziz on her degree entitle '**Micropropagation 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:

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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 or concurrently submitted for any other degree at UPM or other institutions.

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## LIST OF ABBREVIATIONS

$^{\circ}\text{C}$	degree centigrade
%	percentage
$\mu\text{M}$	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 <sub>3</sub>	Gibberellic acid



HCl	Hydrochloric acid
HgCl <sub>2</sub>	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	$\alpha$ -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 *Begonias* 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. semperflorescens*, *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

