

**STUDY ON THE EFFECTS OF EXOGENOUS HORMONE AND
HAIRY ROOTS INDUCTION IN BOESENBERGIA ROTUNDA
FOR COMPOUNDS PRODUCTION**

SUZILAWATI ABDULLAH SANI

**FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR**

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AND HAIRY ROOTS INDUCTION IN BOESENBERGIA
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Name of Candidate: SUZILAWATI BINTI ABDULLAH SANI
(I.C/Passport No: 770421-10-5240)

Registration/Matric No: SGF090004

Name of Degree: MASTER OF BIOTECHNOLOGY

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ABSTRACT

Boesenbergia rotunda is a medicinal plant belongs to the family of Zingiberaceae which contains major compounds that exhibits pharmaceutical activities. In this study, the methods of plant growth regulators and hairy roots transformation for *in-vitro* root culture induction in *B. rotunda* were established. Regenerated roots morphology and compounds accumulation in *in-vitro* roots and hairy roots induced were also analyzed. Media containing either NAA alone or in combinations with BAP at different concentrations (0, 0.5, 1.0, 2.0 mg/ mL and 0, 0.5, 1.0, 1.5, 2.0 mg/ mL of NAA and BAP, respectively) were used to initiate *in-vitro* roots. The highest number of roots was 16.5 ± 3.4 per explant, when 0.5 mg/ mL NAA was used. For hairy roots transformation, *B. rotunda* apical meristem was infected using *A. rhizogenes* strain A4. The effects of infection times (20 mins and 1 hr), co-cultivation periods (1, 2, 3 days) and acetosyringone (AS) on the transformation were investigated. Based on PCR, amplification of *rolC* gene analysis, infection times of 20 mins to 1 hr followed by 2 days of co-cultivation period in the presence of 10 μ M AS in the cultivation media resulted in 33.3% of transformation efficiency. The profile and content of compounds in *in-vitro* and hairy roots were evaluated using HPLC analysis. HPLC peaks of alpinetin, pinocembrin, cardamonin, pinostrobin and panduratin A were identified and quantified. *In-vitro* roots induced from plant growth regulators exhibited higher bioactive compounds content as compared to controls and hairy roots induced via transformation. Supplementation of 0.5 mg/ mL NAA in MS media showed higher accumulation of alpinetin, pinocembrin, cardamonin and panduratin A, while combination of BAP and NAA with similar concentration (0.5 mg/ mL) showed higher pinostrobin content as compared to control. It is also found that exogenous PGR, enhanced the production of cardamonin contents as compared to natural rhizome.

ABSTRAK

Boesenbergia rotunda adalah tumbuhan tradisional Malaysia yang mengandungi bioaktif komposisi yang boleh dijadikan bahan campuran di dalam farmasi perubatan. Dalam kajian ini, pelbagai situasi untuk mengkultur *B. rotunda* akar bagi penghasilan bioaktif komposisi telah diselidik. Kesan terhadap penghasilan bioaktif komposisi ini telah dikaji dengan menggunakan kaedah akar induksi melalui penggunaan hormon secara luaran dan 'hairy root' transformasi. Bilangan akar yang terhasil dan kandungan bioaktif komposisi telah dinilai bagi setiap kaedah yang digunakan. Pertumbuhan akar *B. rotunda* telah diuji kesannya melalui penggunaan NAA sahaja atau pelbagai kepekatan kombinasi BAP dan NAA. Kepekatan 0, 0.5, 1.0, 2.0 mg/ mL dan 0, 0.5, 1.0, 1.5, 2.0 mg/ mL bagi NAA dan BAP telah digunakan untuk induksi pertumbuhan akar. Bilangan akar tertinggi (16.5 ± 3.4 per explant) telah diperolehi apabila explant dikultur di dalam media mengandungi 0.5 mg/ mL NAA sahaja. Sebaliknya dalam kaedah 'hairy root' transformasi, bacteria *A. rhizogenes* jenis A4 telah digunakan. Kesan masa jangkitan yang berbeza (20 minit and 1 jam), dan kala ko-kultivasi (1, 2, 3 hari) termasuk kehadiran acetosyringone terhadap transformasi telah dinilai. Berdasarkan keputusan positif terhadap amplifikasi *rolC* gene dalam PCR analisis, telah mendedahkan, untuk tumbuhan species ini, jangkitan 20 minit hingga 1 jam diikuti oleh dua hari kala ko-kultivasi dengan kehadiran 10 μ M AS di dalam kultur media diperlukan untuk menghasilkan 33.3% kecekapan transformasi. Butiran bioactive dan kandungannya dalam kedua-dua kaedah telah dinilai melalui HPLC analisis. Puncak alpinetin, pinocembrin, cardamonin, pinostrobin dan panduratin A dikenalpasti dan dinilai. Penggunaan hormon luaran mempamerkan kandungan bioaktif komposisi yang

tinggi berbanding *in-vitro* normal akar dan 'hairy root' transformasi akar. Penggunaan 0.5 mg/ mL NAA di dalam MS kultur media menunjukkan kandungan alpinetin, pinocembrin, cardamonin dan panduratin A yang tinggi, manakala, kandungan pinostrobin di dapati tinggi apabila kombinasi BAP dan NAA pada kepekatan sama (0.5 mg/ mL) digunakan di dalam kultur media. Kaedah penggunaan hormon secara luaran didapati menambahbaik kandungan cardamonin berbanding dengan rhizome semula jadi.

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ABBREVIATIONS

μ	Micro
μl	Microliter
°C	Degree Celcius
%	Percent
ANOVA	Analysis of Variance
AS	Acetosyringone
BAP	6-Benzylaminopurine
bp	base pair
cm	centimetre
CTAB	Cetyltrimethyammonium bromide
dH ₂ O	distilled water
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
EtOH	Ethanol
<i>et al.</i>	et alia
EtBr	Ethidium bromide
FWD	Forward
g	Gram
g/L	gram per liter
HCl	Hydrochloric acid
L	Liter
LB	Luria-Bertani media
M	Molar

mg	milligram
MgCl ₂	Magnesium chloride
Mins	Minutes
ml	Mililiter
mM	MiliMolar
MS	Murashige and Skoog
MSO	MS media without plant growth regulator
NAA	α -naphthalene acetic acid
N ₂	Nitrogen gas
NaOH	Sodium hydroxide
NaCl	Sodium chloride
OD	Optical density
PBIU	Plant Biotechnology Incubator Unit
PCR	Polymerase Chain Reaction
PGH	Plant Growth Hormone
PGR	Plant Growth Regulator
RAM	Root apical meristem
RNase	Ribonuclease
REV	Reverse
Ri-plasmid	Root-inducing plasmid
rpm	Rotation per minute
SD	Standard Deviation
spp	Subspecies
SAM	Shoot apical meristem
TAE	Tris acetate EDTA
T-DNA	Transferred DNA

TE	Tris EDTA
TL-DNA	T-DNA left region
TR-DNA	T-DNA right region
U	Unit
UV	Ultraviolet
V	Volt
<i>vir</i>	Virulence
v/v	Volume over volume
w/v	Weight over volume