UNIVERSITI PUTRA MALAYSIA

MICROPROPAGATION OF ACACIA AURICULIFORMIS A. CUNN EX BENTH FROM DIFFERENT EXPLANT SOURCES

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FH 2000 8

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By

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Thesis Submitted in Fulfilment of the Requirement for the Degree of the Master of Science in the Faculty of Forestry Universiti Putra Malaysia

August 2000

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

MICROPROPAGATION OF *ACACIA AURICULIFORMIS* A. CUNN *EX* BENTH FROM DIFFERENT EXPLANT SOURCES

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August 2000

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Faculty: **Forestry**

Acacia auriculiformis is one of the multipurpose tree species under Leguminoceae family which has the capability to fix nitrogen from soil and rehabilitate the land. The wood properties are widely known to be suitable for pulp and paper making and can contribute as raw materials for pulp and paper industry in Malaysia. The large scale propagation of *A.auriculiformis* using seeds from open pollination will result in high variation of trees. Therefore, micropropagation can be an alternative method to mass propagate *A.auriculiformis* from selected materials (genetically improved) which can produce higher yield, uniform and high quality wood. This study sought to develop a protocol for micropropagation of *A.auriculiformis* utilizing samples from different explant sources. The explant sources were from 2 month-old *in vitro* seedlings, 5 month- old seedlings, 14 month-old trees, 6 year-old plus trees, marcotts of 6 year-old plus tree, 10 year-old plus trees, forced

flushed shoots from branch cuttings of 10 year-old trees and coppice shoots from 10 year-old trees.

This study involved the investigation on appropriate collection and sterilization techniques, appropriate media for initiation, multiplication and rooting of shoots of the explants taken from different ages of mother plants. Collection techniques by soaking samples for 24 hours in 0.1 % fungicide Benlate plus 1 % boric acid and kept in the refrigerator was the best pretreatment for sample collection from the field. Sterilization with 0.1 % mercuric chloride for 10 minutes followed with 10 % Chlorox for 5 minutes gave the highest percentage of clean and surviving explants. Incorporating 0.1 % Benlate for two weeks in initiation medium helped to reduce contamination in cultures. For shoot initiation, the results showed that low concentrations of BAP (0.1 to 0.5 mg/L or 0.44 to 2.22 μM) were sufficient for shoot initiation of juvenile and mature sources. However, explants from mature sources required a longer gestation period to start multiplying shoots. Combination of BAP with Kn showed that 0.5 mg/L (2.22 μM) BAP plus 0.1 mg/L (0.47 μM) Kn was suitable for shoots initiation of juvenile source and 0.1 mg/L (0.44 μM) BAP plus 0.1 mg/L (0.47 μM) Kn for mature sources.

Effects of different node positions showed that the percentage of contamination increased with the increase of node positions (from shoot tip) for 14 month-old and 10 year-old explant sources. Study on the effects of different basal media, strength of MS medium, gelling agents and different concentrations of sucrose

on the shoot multiplication of 5 month-old explant source resulted with the highest multiplication rate in the full strength MS medium incorporated with 30 g/L sucrose and solidified with 2 g/L Gelrite. Elongation of shoots was highest in the MS medium incorporated with 5.0 mg/L (14 μM) GA_3 , 0.02 mg/L (10.7 μM) NAA and 0.25 mg/L (1.1 μM) BAP but the shoot quality was not suitable for rooting. IBA at 2.0 mg/L (9.8 μM) and combination of 2.0 mg/L IBA (9.8 μM) with 1.0 mg/L (5.4 μM) NAA were found to be the best for *in vitro* rooting. Outplanting of *in vitro* rooted shoots in shredded coconut husk as a growing substrate gave the highest percentage of survival during acclimatization in the greenhouse. *In vivo* rooting also gave the highest percentage of survival in coconut husk. From this study, it can be concluded that there were different responses of explants *in vitro* with the different ages of mother trees.

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

MIKROPERAMBATAN *ACACIA AURICULIFORMIS* A. CUNN *EX* BENTH DARIPADA PELBAGAI SUMBER EKSPLAN

Oleh

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Acacia auriculiformis adalah salah satu spesies pokok pelbagai guna dari famili Leguminoceae yang berupaya mengikat nitrogen daripada tanah dan memulihkan sesuatu kawasan. Mutu kayunya pula diketahui sesuai untuk pembuatan palpa dan kertas, dan seterusnya boleh menyumbangkan bahan mentah kepada industri ini di Malaysia. Pengeluaran secara besar-besaran *A.auriculiformis* daripada bijibenih yang terhasil dari pendebungaan terbuka menghasilkan pokok yang bervariasi tinggi. Oleh itu mikroperambatan boleh menjadi kaedah alternatif untuk penghasilan besar-besaran *A.auriculiformis* daripada bahan terpilih (berpembaikan secara genetik) yang boleh meningkatkan pengeluaran kayu bermutu tinggi dan seragam. Kajian ini dilakukan bagi mendapatkan protokol untuk mikroperambatan *A.auriculiformis* menggunakan sampel eksplan daripada pelbagai sumber. Sumber eksplan ialah daripada anak *benih in vitro* berumur 2 bulan, anak benih berumur 5 bulan dari plot pokok terpilih, anak

pokok berumur 14 bulan, pokok terpilih berumur 6 tahun, markot daripada pokok terpilih, pokok terpilih berumur 10 tahun, pucuk terinduksi daripada keratan ranting-ranting dari pokok 10 tahun dan juga pucuk-pucuk tunasan dari tunggul pokok 10 tahun yang ditebang.

Kajian ini meliputi kaedah pengumpulan sampel dan pensterilan eksplan yang sesuai, kajian kesesuaian medium untuk inisiasi pucuk, penggandaan dan pengakaran pucuk daripada sumber eksplan pada pelbagai peringkat umur. Kaedah pengumpulan sampel dengan merendam selama 24 jam dalam 0.1 % racun kulat Benlate ditambah 1 % asid borik dan disimpan dalam peti sejuk merupakan rawatan terbaik untuk pengumpulan sampel dari lapangan. Pensterilan eksplan dengan 0.1 % merkurik klorida selama 10 minit diikuti 10 % Chlorox selama 5 minit memberikan peratus tertinggi eksplan mandiri yang bebas dari kontaminasi. Tambahan 0.1 % Benlate ke dalam medium untuk inisiasi pucuk selama 2 minggu dapat mengurangkan peratus kontaminasi dalam kultur. Hasil diperolehi menunjukkan kepekatan BAP yang rendah (0.1 – 0.5 mg/L atau 0.44 to 2.22 μM) mencukupi untuk merangsangkan inisiasi pucuk pada kedua-dua sumber eksplan; muda dan matang. Walaubagaimanapun, eksplan daripada pokok matang memerlukan tempoh gestasi yang panjang bagi memulakan penggandaan pucuk. Kombinasi BAP dengan Kinetin menunjukkan 0.5 mg/L (2.22 μM) BAP dengan 0.1 mg/L (0.47 μM) Kinetin adalah sesuai untuk inisiasi pucuk daripada sumber muda manakala 0.1 mg/L (0.44 μM) BAP dengan 0.1 mg/L (0.47 μM) Kinetin adalah sesuai untuk eksplan daripada sumber matang.

Kajian ke atas kesan kedudukan nod menunjukkan peningkatan peratus kontaminasi eksplan dengan peningkatan kedudukan nod dari hujung pucuk bagi sumber eksplan berumur 14 bulan dan 10 tahun. Kajian ke atas kesan jenis medium, kepekatan MS, jenis agar dan kepekatan sukros yang berbeza terhadap penggandaan pucuk daripada sumber eksplan berumur 5 bulan menunjukkan medium MS penuh, 2 g/L Gelrite dan 30 g/L sukros adalah paling sesuai untuk menghasilkan kadar penggandaan pucuk tertinggi. Kadar pemanjangan pucuk pula didapati tertinggi pada rawatan medium MS yang ditambah 5.0 mg/L (14 μM) GA_3 , 0.02 mg/L (10.7 μM) NAA and 0.25 mg/L (1.1 μM) BAP tetapi kualiti pucuk yang terhasil adalah tidak sesuai untuk pengakaran. IBA pada kepekatan 2.0 mg/L (9.8 μM) dan kombinasi 2.0 mg/L IBA (9.8 μM) dengan 1.0 mg/L (5.4 μM) NAA adalah paling sesuai untuk pengakaran *in vitro*. Pindah tanam anak benih yang diakar secara *in vitro* ke dalam sabut kelapa sebagai media pengakaran memberikan peratus mandiri tertinggi semasa pengikliman di rumah hijau dilakukan. Pengakaran *in vivo* juga memberikan peratus mandiri tertinggi dalam sabut kelapa. Daripada keseluruhan kajian ini, kesimpulan yang boleh dibuat ialah terdapatnya perbezaan tindakbalas bagi eksplan secara *in vitro* dengan berbezanya umur pokok induk.

ACKNOWLEDGEMENTS

In the name of Allah s.w.t The Most Gracious, The Most Merciful.

First of all, I would like to convey my appreciation to the Director General of FRIM and the Chairman of FRIM's Training Committee, Dato' Dr. Abdul Razak bin Mohd Ali and to Dr. Baskaran Krishnapillay, the Director of Forest Plantation Division for giving me the opportunity to pursue my M.Sc. in UPM. My deepest appreciation and gratitude also goes to my supervisor Associate Prof. Dr Nor Aini Ab Shukor for her advice, guidance and encouragement throughout my master's programme.

My warmest appreciation also goes to the members of my supervisory committee, Assoc. Prof. Dr. Kamis Awang, Puan Aziah Mohd Yusoff and Dr. Jamaluddin Basharuddin for their comments and suggestions that leads to the completion of this thesis.

My deepest gratitude also goes to Dr Ab Rasip Ab Ghani for his assistance on statistical analysis, all the staffs in FRIM's Tissue Culture Laboratory, Pn Fadhilah, Pn NorAsmah, Pn Syarifah, Pn Normah, Pn Rozidah, Cik Sabariah, Pn Naemah, Pn Rukiah, Pn Zamrudah, En Shahrul and En. Sharudin for their support and help, the staffs of Tree Improvement Group for assisting me during sample collection, En. Asmar Hj Hassan, FRIM's photographer, and all FRIM and UPM staffs and individuals who have helped me in one way or another throughout my study. Special thanks also goes to Norzanariah and Mohd Hallmi Ismail for their technical assistance.

Lastly, my special thanks to my parent, family and friends for their love and support throughout my study.

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° C	degree centigrade
g	gram
L	litre
mg	milligram
ml	millilitre
mm	millimetre
cm	centimetre
m	metre
mg/L	milligram per litre
g/L	gram per litre
Kg/m^3	kilogram per metre cubic
$Kcal\ kg^{-1}$	kilocalorie per kilogram
M	Molar
µM	microMolar
ppm	part per million
dbh	diameter at breast height
ANOVA	Analysis of Variance
df	degree of freedom
Ms	Mean of Square
LSD	Least Significant Different
BAP	6-Benzylamino-purine
GA_3	Gibberelic acid

2,4-D	2,4 - dichlorophenoxyaceticacid
$HgCl_2$	Mercuric chloride
NaOH	Sodium hydroxide
IAA	Indole-acetic-acid
IBA	Indole-butyric-acid
Kn	Kinetin
NAA	Naphthaleneacetic acid
MS	Murashige and Skoog medium (1962)
WPM	Lloyd and McCown Woody Plant Medium
B5	Gamborg B5 medium
2iP	N_6-isopentyladenine
pH	negative logarithm of the hydrogen concentration
w/v	weight over volume
v/v	volume over volume
min	minute
µg/ml	microgram per millilitre
$\mu molm^{-2}s^{-1}$	micromole per metre square per second
%	percentage
SDW	sterile distilled water
kPa	kilopascal (unit for pressure)
UV	ultraviolet