UNIVERSITI PUTRA MALAYSIA

MICROPROPAGATION OF ACACIA CRASSICARPA A. CUNN. EX BENTH

GRIFFIN AKENG

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By

GRIFFIN AKENG

Thesis submitted in Fulfilment of the Requirements for the
Degree of Master of Science in the Faculty of Forestry
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Dedication

To my father,
mother,
sisters
And
Cecilia

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

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Chairman: Associate Professor Nor Aini Ab Shukor, Ph.D.

Faculty: Forestry

Micropropagation through tissue culture technique offers an alternative to vegetative propagation to mass propagate selected trees for large-scale forest plantation. Therefore, this study aimed to develop a protocol for the micropropagation of *A. crassicarpa*. It involved the determination of an appropriate sterilisation technique for seeds, suitable explants to be used, appropriate plant growth regulators and medium for culture initiation and culture maintenance.

Rinsing with commercial clorox (15%) for at least 15 minutes was found to be effective to reduce contamination rate to as low as 10%. Nodal stem segment and leaf obtained from 2 month-old aseptically germinated seedlings were

used as explants in this study. Nodal stem segment was found to be the most appropriate explant for shoot formation when cultured on a MS medium supplemented with BAP. The highest mean number of shoots (5) and the longest mean shoot elongation (8 mm) occurred on a medium supplemented with 0.5 mg/L BAP. The longest mean shoot length (8 mm) and the highest mean number of explant obtained per culture (7) were obtained on medium without any plant growth regulator. When cultured on a medium supplemented with 2,4-D, nodal stem segment explant developed roots and callus after 14 days in culture incubation. The highest mean number of roots (8.3 ≅8) and the longest mean root length (12.0 ≅12mm) were obtained from the medium supplemented with 10.0 and 2.0 mg/L 2,4-D respectively while the highest intensity of callus (+++) was obtained from a medium supplemented with higher concentrations of 2,4-D (6.0, 8.0 and 10.0 mg/L). Leaf explants on the other hand, failed to develop shoot when cultured on a medium supplemented with BAP where they were swollen and eventually died. However, they produced roots and callus when cultured on a medium supplemented with 2,4-D. The highest mean number of roots (20.6 ≅21) and the longest mean root length (10.4 ≅10mm) were obtained from the medium supplemented with 10.0 and 2.0 mg/L 2,4-D respectively while the highest intensity of callus (+++) was produced on a medium supplemented with 8.0 and 10.0 mg/L 2,4-D. The calli produced were compact, watery and white in colour.

The shoots were then transferred onto the MS medium containing 1.0 and 2.0 mg/L BAP to stimulate shoot multiplication. The highest multiplication rate (4.2) was obtained from the second subculture on a medium supplemented with 2.0 mg/L BAP. A comparative study showed that IBA performed better than NAA where the former produced 100% rooting and having an average of 7 roots per culture as compared to the latter, which only produced 70% rooting and having an average of 2 roots per culture. Shoots obtained from the fourth subculture were found to produce higher percentage of rooting (60%) than those obtained from the initial culture (21%) but having lower mean root number (1.9 ≅2) and shorter mean root length (19.7 mm) compared to those obtained from the initial culture (an average of 2.3 ≅2 roots per culture and mean root length of 22.0 mm). Survival rate of plantlets was higher (100%) when transferred into the autoclaved mixture of soil, sand and peat (3:3:1) than those transplanted in an unautoclaved soil mixture (6.6%). Survival percentages of the plantlets in the culture room and green house condition were 85% and 100% respectively.

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

PEMBIAKAN MIKROPERAMBATAN *ACACIA CRASSICARPA* A. CUNN. EX BENTH

Oleh

GRIFFIN AKENG

Mei 2000

Pengerusi : Profesor Madya Nor Aini Ab Shukor, Ph.D.

Fakulti : Perhutanan

Pembiakan mikroperambatan melalui teknik kultur tisu memberikan satu alternatif kepada pembiakan tampang untuk menghasilkan stok tanaman daripada pokok terpilih untuk tujuan penubuhan ladang hutan secara besar-besaran. Dengan itu, kajian ini bertujuan untuk menghasilkan protokol untuk teknik mikroperambatan untuk *A. crassicarpa*. Ia melibatkan penentuan teknik pensterilan yang sesuai untuk biji benih, jenis eksplan yang sesuai, hormon penggalak pertumbuhan dan media untuk permulaan dan penyimpanan kultur yang sesuai.

Membasuh biji benih menggunakan larutan klorox (15%) untuk tempoh sekurang-kurangnya 15 minit didapati sangat berkesan untuk

mengurangkan kadar kontaminasi sehingga 10%. Segmen nodal batang dan daun yang diperolehi daripada anak benih berumur 2 bulan digunakan sebagai eksplan dalam kajian ini. Eksplan segmen nodal batang didapati lebih sesuai untuk penghasilan pucuk apabila dikulturkan di atas medium yang dibekalkan dengan BAP. Pembentukan pucuk yang terbaik dari segi purata bilangan pucuk tertinggi (4.5 ≅5) dan pemanjangan pucuk terpanjang (8 mm) diperolehi daripada media yang dibekalkan dengan kepekatan 0.5 mg/L BAP manakala purata pucuk terpanjang (8 mm) dan purata bilangan eksplan terbanyak (7) dihasilkan di dalam media tanpa hormon penggalak. Apabila dikulturkan di atas media yang dibekalkan dengan 2,4-D, eksplan segmen nodal batang telah menghasilkan akar dan kalus. Purata bilangan akar tertinggi diperolehi daripada segmen nodal batang (8.3 ≅3) yang dikulturkan di atas media yang dibekalkan dengan 10.0 mg/L 2,4-D manakala purata akar terpanjang (12.0 mm) diperolehi daripada media yang dibekalkan dengan 2.0 mg/L 2,4-D. Intensiti kalus tertinggi (+++) diperolehi daripada media yang dibekalkan dengan kepekatan 2,4-D yang tinggi (6.0, 8.0 dan 10.0 mg/L). Bagi eksplan daun pula, ia hanya membengkak dan mati apabila dikulturkan di atas media yang dibekalkan dengan BAP. Apabila dikulturkan di atas media yang dibekalkan dengan 2,4-D eksplan daun akan menghasilkan akar dan kalus. Bilangan purata akar tertinggi (20.6 ≅21) diperolehi daripada media yang dibekalkan dengan 10.0 mg/L 2,4-D dan purata akar terpanjang (10.3 ≅10mm) diperolehi daripada media yang

dibekalkan dengan 2.0 mg/L 2,4-D. Intensiti kalus tertinggi (+++) diperolehi daripada media yang dibekalkan dengan 8.0 dan 10.0 mg/L 2,4-D. Kalus yang diperolehi adalah padat, cair dan berwarna putih.

Penggandaan pucuk dapat dirangsangkan dengan memindahkan pucuk ke media MS yang mengandungi 1.0 dan 2.0 mg/L BAP. Purata bilangan pucuk terbanyak (4.2) diperolehi daripada subkultur keempat pada media yang dibekalkan dengan 2.0 mg/L BAP. Kajian keupayaan pengakaran menunjukkan bahawa IBA adalah lebih baik dari NAA. Peratus penghasilan akar untuk pucuk yang dibekalkan dengan IBA adalah 100% berbanding dengan NAA yang hanya menghasilkan 70% pengakaran. Purata bilangan akar daripada media yang dibekalkan dengan IBA adalah lebih tinggi (7) berbanding dengan NAA (2). Kajian perbandingan keupayaan mengakar menunjukkan pucuk yang diperolehi dari subkultur keempat lebih mudah berakar (60%) berbanding pucuk yang diperolehi daripada kultur permulaan (21%). Peratus kemandirian anak pokok *in vitro* selepas diaklimitasikan ke dalam campuran tanah steril, pasir dan tanah gambut (3:3:1) adalah 100% berbanding 6.6% di dalam campuran tanah tidak disterilkan. Aklimitasi anak pokok di bilik kultur dan rumah hijau menghasilkan 85% dan 100% kadar kemandiran masing-masing.

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LIST OF ABBREVIATIONS

ANOVA	Analyses of variance
a.s.l.	Above sea level
BAP	Benzyaminopurine
B5	Gamborg *et al.* medium
dbh	Diameter at breast height
df	Degree of freedom
$HgCl_2$	Mercuric chloride
h	hour
IAA	Indole acetic acid
IBA	Indole butyric acid
Kg	Kilogram
M	Molar
mg/L	Milligram per liter
MSO	Murashige & Skoog's medium without hormone
NAA	Naphthaleneacetic Acid
NaOH	Sodium Hydroxide
ss	Sum of square
pH	Negative logarithm of the hydrogen concentration
TDZ	Thidiazuron
WPM	Woody Plant Medium
Z	Zeatin
µm	Micromolar
$\mu molm^{-2}s^{-1}$	Micromol per meter cube per second
%	percentage

TERMINOLOGY

Acclimatisation	Show change in the physiology of an organism/plant, as a result of its exposure to a changed environment
Adventitious	Development of shoots or roots from unusual origin on a leaf or stem tissue other than the axils or apex, often dependent on close physical or temporal association with organised or semi-organised tissues or cells.
Asepsis	Cleanliness of the materials/explants from any harmful contaminants/bacteria
Autotropic	(of an organism/plant) independent of outside sources of organic substances for provision of its own organic constituents, which it can manufacture from inorganic material
Auxins	Plant hormone naturally synthesised (Indole-3-Acetic Acid-IAA) in the apex and transported downward the stem. Also occurred in synthetic form (Naphthalene Acetic Acid-NAA and Indole-3-butyric Acid-IBA), auxins influence cell elongation, cell division, induction of primary vascular tissue, adventitious root formation, senescence, fruit growth, out growth of axillary buds and sex expression.
Axillary shoots	Shoot buds formed at the juncture of the leaf and the stem.
Callus	Actively growing relatively undifferentiated tissue, devoid of macroscopic organised structure, normally produced in higher plants in response to wounding or infection but often formed *in vitro* during the artificial culture of plant tissue.
Cytokinin	A class of growth regulators chemically and functionally hormone zeatin, cytokinins stimulate cell division, cell and/or shoot differentiation, lateral bud break etc.

Dediferrentiation	A process whereby specialised, non-dividing cells begin to proliferate by mitotic division presumed to involve regression to a differentiated state.
Explant	The tissue taken from a plant or seed and transferred to a culture medium to establish a tissue cultures system or regenerates a plant.
Growth	An irreversible increase in volume or mass associated with the development, it usually involves cell division, expansion, differentiation and morphogenesis.
Heterotrophic	An organism that requires a supply of a carbon compound as a source of energy and for growth such organisms usually cannot fix carbon dioxide in the light
Induction	Determination and/or initiation of a plant structure, organ or process *in vitro* as the results of a specific stimulus.
Inflorescence	Flowering shoot
Initial culture	A culture started from cells, tissues or organ taken directly from organisms
In vitro	A sterile artificial environment typically in glass vessels, in which cultured cells, tissue, organs or whole plants may reside.
In vivo	Literally "in life" applied to any process occurring in a living whole organism
Juvenile	A phase in the sexual cycle of a plant characterised by differences in appearance from the adult and which lacks the ability to respond to flower inducing stimuli
Micropropagation	Rapid vegetative propagation of a plant *via* small pieces of tissue and usually beyond that obtained in nature. The process includes many steps-stock plant cares, explant selection and sterilisation, media manipulation to obtain proliferation, rooting, acclimation, and growing on of liners