



UNIVERSITI PUTRA MALAYSIA

**MICROPROPAGATION OF SENTANG
(AZADIRACHTA EXCELSA (JACK) JACOBS)**

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**MICROPROPAGATION OF SENTANG
(*AZADIRACHTA EXCELSA* (JACK) JACOBS)**

By

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Dedicated to my father, mother

sisters and brothers

for their love and support



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LIST OF ABBREVIATION

AgNO ₃	= Argentum Nitrate
BAP	= Benzyl-aminopurine
B5	= Gamborg B5
°C	= degree centigrade
cm	= Centimetre
dbh	= Diameter breast height
df	= Degree of freedom
DNMRT	= Duncan New Multiple Range Test
FE-EDTA	= Ferreous ethylenediaminetetraacetic acid
GA ₃	= Gibberelic acid
h	= Hour
HgCl ₂	= Mercuric chloride
IAA	= Indole-acetic acid
IBA	= Indole-butyric acid
IPA	= Indole-propionic acid
K	= Kinetin
kg	= Kilogramme
M	= Molar
m	= Metre
mm	= Millimetre
mg l ⁻¹	= Milligramme per litre
min	= Minute
nm	= Nanometre
NAA	= Naphthaleneacetic acid
ss	= Sum of square
pH	= Negative logarithm of the hydrogen concentration
TDZ	= Thidiazuron



vpm	=	Volume per million
WPM	=	Woody plant medium
Zea	=	Trans-zeatin
2IP	=	N ₆ -isopentyladenine
2,4-D	=	2,4-dichlorophenoxyacetic acid
μm	=	Micromolar
μg/ml	=	Microgramme per millilitre
μmolm ⁻² s ⁻¹	=	Micromol per metre cube per second
μw/cm ²	=	Microwatt per cubic centimetre
%	=	Percentage

TERMINOLOGY

Adventitious- Developing from unusual points of origin, such as shoots or roots arising from a leaf or stem tissues other than the axils or apex, often dependent on close physical or temporal association with organized or semiorganized tissues or cells

Autotrophic- Self-sufficient for growth condition, self-reliant

Auxins- A class of growth regulators, chemically and functionally related to the natural Indole-acetic acid (IAA). Auxins stimulate new cell division, cell enlargement, the formation of shoot apices or buds, the induction of somatic embryogenesis, and may promote rooting

Axillary bud- Shoot buds formed at the juncture of the leaf and the stem (the axil)

Bud- An undeveloped shoot covered with protecting scales, consists of a very short shoot axis and primordia of leaves or floral parts

Callus - Actively growing relatively undifferentiated tissue, devoid of macroscopic organized structure, normally produced in higher plants in response to wounding or infection but often formed *in vitro* during the artificial culture of plant tissue

Culture medium -A mixture of organic and inorganic nutrients used for the cultivation of cells

Cytokinin- A class of growth regulators chemically and functionally related to the natural hormone zeatin, cytokinins stimulate cell division, cell and/or shoot differentiation, lateral bud break etc

Dedifferentiation- A process whereby specialized, nondividing cells begin to proliferate by mitotic division presumed to involve regression to an differentiated state

Development- Qualitative change undergone by organism via differentiation and growth from its beginning to maturation

Differentiation- The process of biochemical and structural changes by which cells become specialized in form and function

Explant- The tissue taken from a plant or seed and transferred to a culture medium to establish a tissue culture system or regenerate a plant



Ethylene- A gaseous plant hormone involved in fruit maturation, abscission and senescence. It is produced by certain tissue cultures

Growth- An irreversible increase in volume or mass associated with the development, it usually involves cell division, expansion, differentiation and morphogenesis

Heterotrophic - An organism which 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

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 characterized by differences in appearance from the adult and which lacks the ability to respond to flower inducing stimuli

Meristemoid- Meristem-like cells located in areas of a plant or culture other than the meristem, e.g. a center of cell division activity within a callus

Micropropagation- Rapid vegetative propagation of a plant *via* small pieces of tissue and usually beyond that obtained in nature

Morphogenesis - The development of form or structure

Mutation- A change in the genetic material or a cell that is heritable

Organogenesis- Initiation of an organ or the production of a plantlet *in vitro* through the sequential usually non-synchronized initiation of root and shoot structures connected by vascular system

Plantlet- A tiny plant with a distinct root and shoot system formed *via* tissue culture either by embryogenesis or organogenesis

Primary culture -A culture started from cells, tissues or organ taken directly from organisms

Regeneration - Laboratory techniques for forming a new plant or organ from cultured cells



Shoot tip culture- Culture of a structure consisting of the shoot apical meristem plus one to several primordial leaves

Somaclonal variation- Variation which occurs in cultures of cell and tissues that may be either genetic or epigenetic

Subculture- The transfer or subculture of cells, with or without dilution, from one culture vessel to another containing fresh culture medium

Tissue culture- A general term used to describe the development of tissue in culture under sterile conditions

Totipotency- The ability of a somatic cell *in vitro* to regenerate a whole organism either via organogenesis or embryogenesis

Vegetative propagation- Somatic nonsexual propagation of plant parts without fertilization

Vitrification- A physiological disorder associated with specific changes in the appearance of induced organ *in vitro* where leaves become translucent, appearing glassy or water logged and their needles adhered to each other



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**MICROPROPAGATION OF SENTANG
(AZADIRACHTA EXCELSA (JACK) JACOBS)**

By

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The large-scale propagation of sentang (*A. excelsa* (Jack) Jacobs) through seeds is impractical as the seeds are recalcitrant and they lose viability in a short period. Therefore this study sought to develop a protocol for the micropropagation of sentang. It involved the determination of an appropriate sterilization technique, a suitable explant to be used, appropriate medium and plant growth regulators for shoot formation, multiplication and rooting, as well as estimating the rate of multiplication. Means of solving problems of defoliation during rooting and shoot elongation were also developed. A comparative study on the rootability of shoots excised from the initial culture and final subculture was also conducted.



Nodal stem segment, petiole nodal segment, shoot tip and young leaves from 7 month-old seedling were explants tested in this study. Shoot tip was found to be the best explant producing the highest percentage of shoot formation (93.3%). A concentration of 20-25% commercial clorox applied for 40 minutes was the best sterilization method for shoot tip explants that yielded 100% aseptic cultures. Shoot formation in terms of percentage of explants with shoot, shoot elongation, number and length of axillary shoot and number of explants obtained per culture was found to be the most prolific when 2.0 mg l^{-1} BAP was added to either B5, MS or WPM medium. The most optimum combination of medium and cytokinin was that of MS medium with 0.5 mg l^{-1} BAP which produced a multiplication rate of 2 within 53 days.

Following this, short shoots were then transferred into the MS medium containing 1.0 mg l^{-1} BAP and 0.24% gelrite to stimulate their elongation. In the rooting study, 2.0 mg l^{-1} NAA was found to be the best concentration of auxin in terms of percentage of adventitious root formation, as well as their number and length. Defoliation could be overcome during the rooting phase when the shoots were transferred into MS hormone free medium but with an addition of 0.24% gelrite. A comparative study on the rootability of shoots excised from the initial and fourth subcultures showed that shoots from the latter performed better in terms of the number and length of adventitious roots produced. Survival percentage of 65.4% was achieved after two weeks of transplanting in the nursery.

