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PROPAGATION OF SEALING WAX PALM , CYRTOSTACHYS RENDA BLUME, USING IN VITRO TECHNIQUES

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By

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Thesis submitted in fulfilment of the requirements for the degree of Master of Science in the Faculty of Food Science and Biotechnology, Universiti Pertanian Malaysia.

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LIST OF ABBREVIATIONS

ADH	Alcohol dehydrogenase
BAP	6-benzylaminopurine
2,4-D	2,4-dichlorophenoxyacetic acid
DNA	Deoxyribonucleic acid
DTT	Dithiothreithol
EST	Esterase
EDTA	Ethylenediaminetetracetic acid
GA3	Gibberellic acid
G6PDH	Glucose-6-phosphate dehydrogenase
GOT	Glutamate-oxaloacetate transaminase
IAA	Indole-3-acetic acid
IBA	Indole-butyric acid
IDH	Isocitrate dehydrogenase
2iP	2-isopentenyl adenine or 6-(λ,λ-dimethylallylamino)purine
mM	Millimolar
μM	Micromolar
μ	micron
M	Molar
MS	Murashige and Skoog medium formulation (1962)
MMS	Modified Murashige and Skoog medium formulation
MTT	(3-(4,5-Dimethylthiazolyl-2)-2,5 diphenyl tetrazolium bromide)

m.w.	Molecular weight
NAA	∝-naphthaleneacetic acid
NAD	ß-nicotinamide adenine dinucleotide
NADP	ß-NAD phosphate
OPM	Medium for oil palm
ppm	Parts per million
PRX	Peroxidase
PMS	Phenazine methosulphate
PGI	Phosphoglucose isomerase
PGM	Phosphoglucomutase
PVP	Polyvinylpyrrolidone
w/w	Weight/weight
WPM	Woody Plant Medium
Y3	Eeuwens Medium Formulation

Abstract of the thesis presented to the Senate of Universiti Pertanian Malaysia in fulfilment of the requirements for the Degree of Master of Science.

PROPAGATION OF SEALING WAX PALM, *CYRTOSTACHYS RENDA* BLUME, USING *IN VITRO* TECHNIQUES

BY

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Chairperson: Associate Professor Dr. Hasanah Mohd. Ghazali
Faculty: Food Science and Biotechnology

A series of experiments on the culture of vegetative tissues and embryos of *Cyrtostachys renda* Blume were conducted to explore the possibility of *in vitro* culture as an alternative system of propagation. Isozyme typing of the vegetative tissues used as explants was also carried out.

Unemerged leaf tissue of about 1 cm with petiole attached and probably including shoot (L1), was used as explant. The preceding two 1-cm leaf sections before L1 were also used as explants. However, the youngest tissue (L1) used was least-proned to browning and survived in culture whereas the preceding two sections died in culture.

Three different culture media were tested, namely modified Murashige and Skoog (MMS), oil palm medium (OPM) and Woody Plant Medium (WPM). MMS was found to be suitable

for the explant of *C. renda* whereas OPM and WPM were found to be unsuitable.

2,4-dichlorophenoxyacetic acid (2,4-D) at 750 μM was lethal to the explant. Several responses were also observed after 270 days of culture. Development of shoots, "rootlike" structures and calli occurred in medium containing 250 μM 2,4-D with 600 μM NAA. Proliferation of shoot and expansion of explant were obtained in medium containing 1000 μM NAA. "Rootlike" structures developed and the explant expanded in medium containing 200 μM NAA.

Effects of anti-oxidants incorporated into the media, namely polyvinylpyrrolidone (PVP) and dithiothreithol (DTT) were also investigated. Explants survived longer (120 days) in medium containing 500 mgl^{-1} PVP compared to medium containing 250 mgl^{-1} PVP (60 days) in all combinations of 2,4-D and NAA studied. DTT at 100 mgl^{-1} had no significant effect in reducing browning and survival of the explants in all combinations of auxins studied.

Two types of anti-oxidants were used in pre-treatment of explants namely, (i) 10 mgl^{-1} ascorbic acid, (ii) 5 mgl^{-1} citric acid and 10 mgl^{-1} ascorbic acid. Both

anti-oxidants were in 1/2 strength MS inorganic salts with 2% sucrose. Data obtained showed that both anti-oxidants prevented browning after excision of explants and during culture.

In the study on *in vitro* culture of zygotic embryos, MMS medium was used. Development of only shoot was obtained in treatment containing 5 μM NAA and 4 μM BAP. Calli which later developed into projections were formed in treatment with 200 μM 2,4-D.

In isozyme typing of both *Cyrtostachys renda* and *Metroxylon sagu*, activities for alcohol dehydrogenase, isocitrate dehydrogenase and 6-phosphogluconate dehydrogenase were detected. Peroxidase activity was observed at the cathodal and anodal portions in both species. Phosphoglucomutase activity was present in *M. sagu* but not in *C. renda*.

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PERAMBATAN PINANG RAJA,
***CYRTOSTACHYS RENDA* BLUME,**
DENGAN MENGGUNAKAN TEKNIK-TEKNIK *IN VITRO*

OLEH

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Satu siri eksperimen kultur tisu vegetatif dan embrio *Cyrtostachys renda* Blume telah dilakukan untuk mengkaji kaedah kultur *in vitro* sebagai satu sistem pembiakan alternatif. "Typing" isozim tisu vegetatif yang digunakan sebagai eksplan telah juga dilakukan.

Daun yang belum muncul, kira-kira 1 cm panjang berserta petiol dan kemungkinan termasuk pucuk (L1) telah digunakan sebagai eksplan. Dua keratan tisu daun berikutnya, setiap satu berukuran kira-kira 1 cm panjang telah juga digunakan sebagai eksplan. Walaupun begitu tisu yang termuda (L1), paling kurang mengalami pemerangan dan hidup di dalam kultur, sementara kedua-dua keratan berikutnya mati di dalam kultur.

Tiga media berlainan komposisi telah diuji iaitu media Murashige dan Skoog yang diubahsuai (MMS), medium kelapa sawit (OPM) dan Medium Woody Plant (WPM). MMS didapati sesuai untuk eksplan *C. renda* tetapi OPM dan WPM didapati tidak sesuai.

Asid 2,4-diklorofenoksiasetik (2,4-D) pada kepekatan 750 μM telah menyebabkan eksplan mati. Beberapa respon telah diperhatikan selepas 270 hari kultur. Perkembangan pucuk, struktur menyerupai akar dan kalus wujud dalam kombinasi medium yang mengandungi 250 μM 2,4-D dan 600 μM NAA. Proliferasi pucuk dan pengembangan eksplan didapati dalam medium mengandungi 1000 μM NAA. Struktur menyerupai "akar" berkembang dan eksplan mengembang dalam medium mengandugi 200 μM NAA.

Kesan anti-oksidan yang dimasukkan kedalam medium iaitu polivinilpirrolidone (PVP) dan dithiothreithol (DTT) juga telah dikaji. Eksplan didapati hidup lebih lama (120 hari) di dalam medium yang mengandungi 500 mgl^{-1} PVP berbanding dengan medium mengandungi 250 mgl^{-1} (60 hari) pada semua kombinasi auksin yang dikaji. DTT pada kepekatan

100 mgl^{-1} tidak berkesan mengurangkan pemerangan pada semua kombinasi auksin yang dikaji.

Dua jenis anti-oksidan telah digunakan dalam pra-perlakuan keatas eksplan iaitu, (i) 10 mgl^{-1} asid askorbik dan, (ii) 5 mgl^{-1} asid sitrik dan 10 mgl^{-1} asid askorbik. Kedua-dua anti-oksidan telah dilarutkan ke dalam 1/2 MS garam inorganik dengan 2% sukros. Kedua-dua jenis anti-oksidan mencegah pemerangan selepas pemotongan eksplan dan semasa pengkulturan.

Dalam kajian kultur embrio zigotik, medium MMS telah digunakan. Perkembangan pucuk hanya didapati dalam rawatan 5 μM NAA dan 4 μM BAP. Kalus yang seterusnya berkembang kepada juluran-juluran, terbentuk dalam rawatan 200 μM 2,4-D.

Dalam "typing" isozim kedua-dua *Cyrtosatchys renda* dan *Metroxylon sagu*, aktiviti enzim-enzim alkohol dehidrogenase, isositrat dehidrogenase dan 6-fosfoglukonat dehidrogenase didapati. Aktiviti peroksidase dicerap pada bahagian katod dan anod dalam kedua-dua spesis. Aktiviti fosfoglukomutase hadir dalam *M. sagu* tetapi tiada dalam *C. renda*.

CHAPTER I

INTRODUCTION

Cyrtostachys renda Blume or sealing wax palm is classified under the subfamily Arecoideae, tribe Areceae and subtribe Cyrtostachydinae. The species is synonymous to *C. lakka* and is well distributed and cultivated in Peninsular Malaysia, South Thailand, Sumatra and Borneo (Uhl and Dranfield, 1987). In the wild, *C. renda* is confined to peat swamp forests, usually near coastal areas. It is a commercially important ornamental plant, used in landscape as borders for avenues, in clumps or as potted plants.

The palm is mainly propagated through seeds. However, seeds are normally limited because they are seasonal and germination is generally low. The common method for propagating this species is through the use of suckers which can be separated from mature mother plants. However, growth of the suckers are slow. Therefore, it is important to develop a tissue culture method to propagate this species in order to provide clonal planting materials.

In propagating a plant species through tissue culture, establishment of aseptic culture, multiplication of propagula and preparation for reestablishment of plants in soil need to be addressed. Factors such as type of explant, growth requirements, combination of hormones or growth regulators in respect of types and levels and culture environments have to be identified. Procedures on species in the same family or one closest in respect of taxonomy could be used as guidelines. However, plants under the same taxon may not have common or same physiological characteristics enabling it to respond similarly *in vitro*. *In vitro* culture of zygotic embryos could provide some information which would be useful in *in vitro* culture of vegetative tissues. *In vitro* culture of vegetative tissues and embryos of *C. renda* have not been previously reported.

In studying the genetic variations in plants, isozymes analysis has been used extensively. To support the *in vitro* experiments on vegetative tissues of *C. renda*, similar isozymes analysis of the explants used was, as well relevant.

Thus the objectives of the study are:

1. to develop a protocol for *in vitro* propagation of *C. renda* which involves selection of suitable vegetative tissues as explants and establishment of the culture.
2. to carry out a preliminary study on embryo culture of *C.renda* which can provide useful information for normal growth and development in *in vitro* culture, and
3. to compare isozymes in selected explants of *C. renda* and *Metroxylon sagu*.

CHAPTER II

LITERATURE REVIEW

Taxonomy and morphology of palms

The Palmae (Arecaceae) comprises of six subfamilies namely Coryphoideae, Calamoideae, Nypoideae, Ceroxyloideae, Arecoideae and Phytelephantoideae. Basically, palms can be described and identified by their morphological characters in terms of habit, stem, armature, leaf, inflorescence, fruit and seed (Uhl and Dransfield, 1987).

The vegetative body of a palm can be solitary (monopodial) as in oil palm and coconut or in cluster (sympodial) as in sago and sealing wax palm (Uhl and Dransfield, 1987). In most palms the stems are not terminated by a flower or an inflorescence, and described as "pleonanthic" behaviour. The shoots of hapaxanthic palms are terminated or suppressed after or during inflorescences are produced such as in *Metroxylon sagu*. There are no palms which are herbaceous and develop bulbs or corms.

Palms are monocotyledons which lack cambium and the wood consists of primary tissues, which originate from the growing tip. The anatomy of palms have been extensively described by Tomlinson (1961 and 1990).

Uhl and Dransfield (1987) described the morphology, anatomy, relationship and distributions of species within the Palmae family. There are also other literatures on the taxonomy of palms such as by Corner (1966) and Whitmore (1973).

Economic importance of palms

Oil palm (*Elaeis guineensis*, Arecoideae), coconut (*Cocos nucifera*, Arecoideae) and date palm (*Phoenix dactylifera*, Coryphoideae) are important as sources of nutrition and are of high economic value. Coconut and oil palms are sources of edible oil. Palm oil is one of the ingredients in margarine, cooking oil, ice-cream, baked goods and mayonnaise and is used in the manufacture of soap, candles and detergents (Reynolds, 1982). Residue from extracted kernel (palm kernel cake) is a good source of carbohydrate. Copra (dried coconut endosperm) is used in making desserts and sweets. Liquid coconut endosperm or

milk produces drinks and gelatinous endosperm in Makapuno variety is a delicacy. Date is an important dietary staple and consumed fresh or dried.

Sago palm (*Metroxylon sagu*, Calamoideae) is important in the production of sago starch, sugar, toddy and heart of palm salad (Reynolds, 1982). In addition, Krishnapillay (1986) reviewed the economic uses of sago palm such as for the purpose of human food, industrial starches in paper and textile manufacturing, animal feed and production of glucose, alcohol and dextrine. Other palms, for example peach palm or pejibaye (*Bactris gasipaes* H.B.K.) is a source for hearts of palm and fruits (Litz et al., 1985).

Palms such as *Howeia forsteriana* Becc. (Arecoideae) and *Chamaedorea costaricana* Oerst. (Ceroxyloideae) are ornamentals of commercial value (Reynolds, 1982). Rattan, (*Calamus manan*, Calamoideae) is useful in making furniture.

Cyrtostachys renda Blume - the sealing wax palm (morphological and other characters)

The habits of *C. renda* is either solitary or clustered, pleonanthic and monoecious (Uhl and Dransfield,

1987). The stem is erect. The leaves are pinnate and the sheaths are tubular, forming red-orange crownshaft. Tomlinson (1961) found that the leaves of *Cyrtostachys* could be distinguished from those of other arecoid palms by the sinous epidermal cell walls and fibrous hypodermis.

The inflorescence is protandrous, infrafoliar, highly branched to 3 orders and the peduncle is usually short. The staminate flowers consist of 3 sepals , 9-15 stamens and the pistillate flowers have 3 sepals and 3 petals. The gynoecium is unilocular and the ovule is pendulous from the apex of the locules. The fruit of *C. renda* has one seed which is ellipsoidal and black in colour. The fruit has smooth epicarp, thin, oily, fibrous mesocarp and thin endocarp. The seed is globose or ellipsoidal, apically attached and the hilum is orbicular. The endosperm of the seed is homogeneous. The embryo is basal. The palm has adjacent-ligular germination. The chromosome number of this species is n=16.

Conventional methods of propagation in palms

The conventional methods of propagation in the palms mentioned earlier have many limitations. Propagation of oil