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TOWARDS THE DEVELOPMENT OF A PROTOCOL FOR CRYOPRESERVATION OF EMBRYOS OF RAMBUTAN (NEPHELIUM LAPPACEUM L.)

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TOWARDS THE DEVELOPMENT OF A PROTOCOL FOR CRYOPRESERVATION OF EMBRYOS OF RAMBUTAN (*NEPHELIUM LAPPACEUM* L.)

BY

HIEW YEE HOOI

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DEDICATED

to my parents

for their understanding
and encouragement which have been a
constant source of inspiration
for me throughout this study

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LIST OF ABBREVIATIONS

The following abbreviations were used in the text:

NAA	α-Napthalene acetic acid
BAP	Benzylaminopurine
2iP	2-isopentyl adenine
LSD	Least Significant Difference
DNMRT	Duncan's New Multiple Range Test
RH	Relative Humidity
EtOH	Ethyl Alcohol
w/w	weight by weight
v/v	volume by volume
min	minutes
^{o}C	Degree centigrade
mg	miligram
g	gram
l	litre
ul	microlitre
g/l	grams per litre
mg/l	miligrams per litre
MS	Murashige and Skoog Medium formulation
%	percentage
DMSO	Dimethylsulfoxide
rpm	revolution per minute
GA_3	Gibberellic acid

Abstract of the thesis presented to the Senate of Universiti Pertanian Malaysia as fulfilment of the requirements for the degree of Master of Science

TOWARDS THE DEVELOPMENT OF A PROTOCOL FOR CRYOPRESERVATION OF EMBRYOS OF RAMBUTAN (Nephelium lappaceum L.)

by

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Supervisor : Professor Chin Hoong Fong

Faculty : Agriculture

Key Words : Nephelium lappaceum, excised embryos, cryopreservation.

Long term storage of recalcitrant seeds by conventional storage methods thus far have been unsuccessful. In this study, the excised embryos from seeds of Rambutan (Nephelium lappaceum), a recalcitrant tropical fruit species has been attempted for long term storage through cryopre

Stage of fruit maturity was found to be one of the important factor for the survival of embryos *in vitro*. The embryos excised from mature unripe fruits were selected as the most appropriate for use in this study.

Minimizing variability in fresh weight and moisture content was found to be essential in this study. Selection of uniform sized embryos, measuring 3.0^2-4.0^2mm in surface area per side of the cubical blocks of cotyledonary tissue that enclosed the embryos; ensured uniform fresh weight and moisture content.

A modified Murashige and Skoog medium containing 1.0 mg/l each of NAA and Kinetin, 2 g/l of activated charcoal and 170 mg/l of $NaH_2PO_4.H_2O$ was found to be suitable medium for the development and growth of the excised embryos of rambutan.

Critical moisture limit of rambutan embryos was found to be around 10-15%. Attempts at direct storage of excised embryos in liquid nitrogen after desiccation to near critical moisture limit was found to be unsuccessful.

Embryos cryoprotected with 10%DMSO+10%Glycerol for 6 hours followed by partial desiccation to moisture contents of 29-33% and slow freezing to -40^{o}C at approximately 1^{o}C/min before plunging into liquid nitrogen survived cryopreservation and gave approximately 40% viability. However, the surviving embryos were abnormal in growth with either root or shoot development only.

Improvement in growth and development of cryopreserved embryos was attempted by supplementing the initially established medium with additional growth promoting substances namely, GA_3, glutamine, arginine, asparagine and adenine sulphate. However, the development of the surviving embryos in the enriched medium was not different from the initial medium in which abnormal growth of plantlets was observed.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia bagi memenuhi syarat-syarat untuk memperolehi Ijazah Master Sains.

TERHADAP PERKEMBANGAN SATU PROTOKOL BAGI PENYIMPANAN KRIO EMBRIO RAMBUTAN (Nephelium lappaceum L.)

oleh

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Setakat ini, penyimpanan biji benih tempoh panjang melalui kaedah penyimpanan biasa, belum menemui kejayaan. Dalam kajian ini, embrio yang telah diasingkan dari biji benih rambutan (Nephelium lappaceum), sejenis spesis rekalsitran, telah dikaji sebagai bahagian tumbuhan untuk penyimpanan tempoh panjang melalui kaedah kriogenik.

Peringkat kematangan buah didapati sebagai satu faktor penting demi pengekalan keadaan terus hidup embryo secara in vitro. Embrio dari biji benih dalam buah matang tetapi belum masak, didapati sesuai sekali diguna dalam kajian ini.

Pengurangan variabiliti bagi berat basah dan kandungan kelembapan embrio didapati amat penting dalam kajian ini. Pemilihan embrio yang saiznya seragam, berukuran luas permukaannya 3.0^2-4.0^2mm tiap sebelah ketulan kiub tisu kotiledon yang mengandungi embrio adalah amat perlu demi memastikan berat basah dan kandungan kelembapan embrio yang seragam diperolehi.

Medium Murashige dan Skoog diubahsuai, yang mengandungi 1.0 mg/l NAA dan Kinetin masing-masing, 2 g/l arang diaktifkan dan 170 mg/l $NaH_2PO_4.H_2O$ didapati sesuai untuk perkembangan dan tumbesaran embrio rambutan.

Untuk embrio rambutan, had kritikal kelembapan didapati lebih kurang 10-15%. Percubaan untuk menyimpan embrio secara terus dalam nitrogen cecair selepas dikeringkan sehingga hampir had kritikal, didapati tidak berjaya.

Embrio yang dirawat dengan DMSO 10% + Glycerol 10% selama 6 jam diikuti oleh separuh kekeringan sehingga 29-33% kelembapan dan dibekukan dengan perlahan pada kadar $1^{O}C$/min sehingga $-40^{O}C$ sebelum diletakkaan kedalam nitrogen cecair, didapati terus hidup dan menunjukkan viabiliti lebih kurang 40%. Walaubagaimana pun, hanya pertumbuhan embrio yang abnormal dengan perkembangan bahagian akar atau pucuk sahaja didapati.

Untuk memperolehi tumbesaran normal bagi embrio selepas krioawetan, penambahan bahan penggalakan tumbesaran, iaitu GA_3, glutamine, arginine, asparagine dan adenine sulphate kepada medium kultur yang ditentukan dahulu, telah dicuba. Walaubagaimana pun, pertumbuhan embrio selepas krioawetan dalam media dengan tambahan bahan penggalakan ini tidak berbeza dari yang terdahulu di mana pertumbuhan plantlet abnormal masih diperhatikan.

CHAPTER 1

INTRODUCTION

Seeds is one of the most important inputs in crop production; its quality greatly influences subsequent performance and yield. Thus, the maintenance of seed viability in storage is of particular importance. Roberts (1973) introduced the terms `orthodox' and `recalcitrant' to describe the storage behaviour of seeds.

Orthodox seeds are those that can tolerate desiccation and freezing temperatures. They can be generally dried to moisture contents in the range of 1-5% without damage. In dehydrated state, orthodox seeds can be stored for long periods with no serious loss of viability. Recalcitrant seeds on the other hand, are those seeds that are normally shed in a moist condition and do not undergo drying during maturation. Attempts to dry these seeds below some relatively high critical value of moisture content (12-31%) result in desiccation injury and death. Futhermore, recalcitrant seeds are also sensitive to freezing injury at low temperatures.

Many of the major plantation crops, fruit trees and timber species produce recalcitrant seeds. Little attention has been given to the storage of the seeds of tropical tree fruit species (Hanson, 1984) and there are many storage problems yet to be solved. Before considering the various storage methods available, it is necessary to identify those seeds species to which recalcitrant behaviour has been attributed (Roberts and King, 1982).

A number of list of recalcitrant seed species have been produced (Chin, 1978; Chin and Roberts, 1980; Harrington, 1972; Roberts, 1975; Roberts and King, 1982). Unfortunately, comprehensive compilation is hampered by incomplete information for many species. Furthermore, in some cases seed may have been reported to have been killed by drying, and therefore might be classified as recalcitrant, when it is possible that the drying method was at fault and that the seed is really orthodox. Inevitably, any catalogue of recalcitrant seed species is likely to be subjected to considerable modification as more recalcitrant species are identified and certain species provisionally recorded as recalcitrant are classified as orthodox (King and Roberts, 1980). In realizing the importance of identification of recalcitrant seeds, Chin *et al*. (1984) proposed a method