UNIVERSITI PUTRA MALAYSIA

TOWARDS THE DEVELOPMENT OF A PROTOCOL FOR CRYOPRESERVATION OF EMBRYOS OF A RECALCITRANT SEED (ARTOCARPUS HETEROPHYLLUS LAM.)

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By

BASKARAN KRISHNAPILLAY

Thesis Submitted in Partial Fulfilment of the
Requirements for the Degree of Doctor of Philosophy
in the Faculty of Agriculture
Universiti Pertanian Malaysia

July 1989

DEDICATED

to the memory of my late

Father

VARGHESE KRISHNAPILLAY

and

to my Mother whose patience and
understanding has been a
constant source of
inspiration for
me throughout
this study

ACKNOWLEDGEMENT

It pleases me to have this opportunity of expressing my sincere gratitude to Universiti Pertanian Malaysia and in particular the Faculty of Agriculture for enabling this study to be carried out. My sincere thanks are also due to the International Board for Plant Genetic Resources (IBPGR/FAO), Rome for financial support through their grant No. 85/114 (a) without which this study would not have been possible.

I am extremely grateful and greatly indebted to my supervisor Professor Chin Hoong Fong for his dedicated efforts, stimulating discussions, active participation and constant encouragement throughout the planning and execution of this research.

My sincere thanks also goes to my co-supervisor Dr Hor Yue Luan for his advice, constructive criticisms, constant encouragement and guidance throughout this study.

I take this opportunity also to thank Dr Zaliha Christine Alang of the Biotechnology Department for allowing me the use of the Plant Tissue Culture Laboratory facilities for carrying out a major portion of this research and also for her constant assistance provided throughout this study. Thanks are also due to Dr Gan Yik Yuan for her help and guidance during protein separation studies using electrophoresis.

My sincere appreciation is extended to Mr Ho Oi Kuan for his technical assistance in the preparation of the electron micrographs; Mr How Peng Guan for technical assistance in the preparation of the histological slides; Capt. Yap Yen Kiew for the construction of the starch-gel electrophoresis kit; Encik Abdul Ghani Hashim (Fakulti Sains dan Pengajian Alam Sekitar) for his professional photographic work; Encik Abdul Ghani Hashim (Unit Hydroponik) for the loan of his printer during the preparation of this manuscript; the Faculty of Forestry, U.P.M. for use of their misting and glasshouse facilities and Mr Ong Choon Hoe for his valuable friendship and assistance in the laboratory during the course of the study.

Thanks are also due to the Director, Dr M M Guha of Agricultural Research and Advisory Bureau for allowing the use of his office facilities during the entire course of this study.

Sincere thanks are due to Mr and Mrs Ramachandran for help provided for proof reading of the original drafts and in the preparation of the final manuscript.

Lastly but not the least, I wish to thank all my family members especially Rajan, Richard, Sara and George Krishnapillay for being very understanding and for their constant encouragement and support throughout the course of 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
TBA	Tertiary Butyl Alcohol
w/w	weight by weight
v/v	volume by volume
min	minutes
°C	Degree centigrade
mg	milligram
g	gram
l	litre
µl	microlitre
Kg/cm^2	Kilogram per square centimetre
µS	Microsemens
g/l	grams per litre
mg/l	miligram per litre
MS	Murashige and Skoog Medium formulation
%	percentage
DMSO	Dimethysulfoxide
Kg	Kilogram
rpm	revolution per minute
GA_3	Gibberellic acid

ABSTRACT

Abstract of the thesis submitted to the Senate of Universiti Pertanian Malaysia in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

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BASKARAN KRISHNAPILLAY

July, 1989

Supervisor	:	Professor Chin Hoong Fong
Co-Supervisor	:	Dr Hor Yue Luan
Faculty	:	Agriculture

Long term storage of recalcitrant seeds by conventional storage methods have by far been unsuccessful. In this study the excised embryos of seeds of jackfruit (Artocarpus heterophyllus). a recalcitrant species has been attempted at for long term preservation using cryogenic means.

Stage of fruit maturity was critical in the study where embryos excised from mature unripe fruits were found to be the most appropriate.

Selection of uniform sized embryos (4.0 - 5.0 mm in length) minimised variability in embryo fresh weight and moisture content to a considerable extent.

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

The safe critical moisture limit for excised jackfruit embryos was found to be around 14-15%. Storage of embryos dried to this moisture limit by conventional storage methods with a view to long term storage was unsuccessful. The maximum storage period achieved was only one month. Attempts at direct storage in liquid nitrogen after desiccation to near critical moisture limit was also found to be unsuccessful.

Embryos cryoprotected with 10% DMSO + 0.5% proline for 12 hours followed by a partial desiccation to moisture contents of 29-33% and slowly frozen to -40°C at approximately 1°C/min before plunging into liquid nitrogen survived cryopreservation and gave approximately 60% normal recovery. No significant decline in the viability percentage was observed in those embryos stored by this method for a period of one month.

For normal recovery growth of embryos after cryopreservation, addition of other growth promoting substances namely GA_3, glutamine, argenine, asparagine and adenine sulphate to the initially established medium was found to be beneficial.

Only 50% of the well developed plantlets transferred to polythene bags were able to survive the transplanting shock and resume normal growth in the glasshouse. The plantlets required a hardening period of five weeks in a high humidity chamber before they were able to withstand the ambient conditions in the glasshouse.

ABSTRAK

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia bagi memenuhi sebahagian daripada syarat-syarat untuk memperolehi Ijazah Doktor Falsafah.

TOWARDS THE DEVELOPMENT OF A PROTOCOL FOR CRYOPRESERVATION OF EMBRYOS OF A RECALCITRANT SEED (ARTOCARPUS HETEROPHYLLUS LAM.)

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BASKARAN KRISHNAPILLAY

July, 1989

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Fakulti	:	Pertanian

Penyimpanan biji benih tempoh panjang melalui kaedah penyimpanan biasa, belum berjaya setakat ini. Dalam kajian ini, embrio-embrio yang telah diasingkan dari biji benih nangka (Artocarpus heterophyllus), sejenis spesies rekalsitran, telah dikaji sebagai bahagian tumbuhan untuk penyimpanan tempoh panjang melalui kaedah kriogenik.

Peringkat kematangan buah didapati sangat penting. Embrio dari biji benih dalam buah yang matang tetapi belum masak, didapati yang sesuai sekali. Pemilihan embrio yang saiznya seragam (4.0 - 5.0 mm panjangnya) didapati mengurangkan variabiliti dalam berat segar dan kandungan kelembapan embrio.

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

Untuk embrio nangka, had kritikal kelembapan didapati lebih kurang 14-15%. Ujian untuk menyimpan embrio yang dikeringkan kepada kelembapan ini, melalui kaedah biasa tidak berjaya. Tempoh penyimpanan maksimum yang dicapai hanyalah selama sebulan sahaja. Percubaan untuk menyimpan embrio secara terus dalam nitrogen cecair selepas dikeringkan sehingga hampir had kritikal, juga didapati tidak sesuai.

Embrio yang dirawat dengan DMSO 10% + 0.5% prolin selama 12 jam diikuti oleh separuh kekeringan sehingga 29-33% kelembapan dan dibekukan dengan perlahan pada kadar 1°C/min sehingga -40°C sebelum diletakkan kedalam nitrogen cecair, didapati terus hidup dan menunjukkan kepulihan lebih kurang 60%. Tiada penurunan yang bererti dalam peratus viabiliti bagi embrio yang disimpan melalui kaedah ini selama sebulan.

Untuk memperolehi tumbesaran normal bagi embrio selepas krioawetan, penambahan bahan penggalakan tumbesaran (iaitu GA_3,