

ESTABLISHMENT TISSUE CULTURE OF
NYFA FRUITIGANS

WONG PUI LIEN

FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA

2006

ESTABLISHMENT TISSUE CULTURE OF *NYPA FRUTICANS*

By

Wong Hui Lien

**Research Report submitted in partial fulfillment of
the requirements for the degree of
Bachelor of Science (Biological Sciences)**

**Department of Biological Sciences
Faculty of Science and Technology
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
2006**

This project should be cited as:

Wong, H. L. 2006. Establishment tissue culture of *Nypa fruticans*. Undergraduate thesis, Bachelor of Science in Biological Science, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia. Terengganu. 50p.

No part of this project report may be produced by any mechanical, photographic, or electronic process, or in form of phonographic recording, nor may be it be stored in a retrieval system, transmitted, or otherwise copied for public or private use, without written permission from the author and supervisor (s) of the project.

1100046069



**JABATAN SAINS BIOLOGI
FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA**

**PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II**

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: ESTABLISHMENT TISSUE CULTURE OF *Nypa fruticans* oleh WONG HUI LIEN no. matrik: ...UK7905...telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperoleh ijazah ...SARJANA MUDA SAINS (SAINS BIOLOGI)....., Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

Disahkan oleh:

Penyelia Utama

Nama:

Cop Rasmi:

DR AZIZ BIN AHMAD (Ph.D)
LECTURER
Dept of Biological Sciences
Faculty of Science and Technology
University College of Science
and Technology Malaysia
21030 Kuala Terengganu.

Tarikh: 27/4/2006

Penyelia Kedua (jika ada)

Nama:

Cop Rasmi

Kasawani Ibrahim
Pensyarah
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
21030 Kuala Terengganu.

Tarikh: 27/4/2006

Ketua Jabatan Sains Biologi

Nama:

Cop Rasmi:

PROF. MADYA DR. NAKISAH BT. MAT AMIN
Ketua
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
(KUSTEM)
21030 Kuala Terengganu.

Tarikh: 27/4/06

ACKNOWLEDGEMENTS

First of all, thanks God cause provide me with a lot of idea, to guide me with your bless until I got to complete my project with all of convenience. I hope this project on Establishment tissue culture of *Nypa fruticans* will be a pioneer to break the mystery that hiding behind this plant, will give a huge of benefit and a weath of profit to the other people.

I would like to take this golden opportunity to express my sincere gratitude and appreciations to my supervisor, Dr Aziz bin Ahmad for helping and supervising me along the way to complete my final project. His advices and his concern have given me a spectrum of light illuminates my direction to run my project accurately in order to achieve the objective given.

My appreciation also due to my co-supervisor En Kasawani, En Mazrul, and Cik Azlina for their dedication to help me and their contribution in completely my project hardly could not be denied.

Last but not least, last but the strength of my mind, the support of my soul, and the courage of my attempt, to my family, mum, father, without u the result will be nothing. Although it was hard to finish this project, but I have learnt and experienced many new things.

TABLE OF CONTENTS

	Page
ACKNOWLEDGMENT	ii
LIST OF TABLES	v
LIST OF FIGURES	vi
LIST OF SYMBOLS	vii
LIST OF APPENDICES	viii
ABSTRACT	ix
ABSTRAK	x
CHAPTER I INTRODUCTION	
1.1 <i>Nypa fruticans</i>	1
1.2 Problem statement of this study	1
1.3 Objective	3
CHAPTER II LITERATURE REVIEW	
2.1 A history of Nipa palm	4
2.2 Control of Nipa palm	6
2.3 Properties of Nipa palm	6
2.4 Palm Tissue Culture	7
2.4.1 Date Palm	8
2.4.2 Coconut Palm	14
2.4.3 Oil Palm	15

2.5 Used of Plant Growth Regulator in Tissue Culture	17
--	----

CHAPTER III METHODOLOGY

3.1 Source of Explant	20
3.2 Sterilization of Explant	20
3.3 Shoot Induction Media	23
3.4 Callus Induction Media	23
3.5 Establishment of Shoot Culture	24
3.6 Establishment of Callus Culture	24

CHAPTER IV RESULT

4.1 Establishment of Sterile Culture	25
4.2 Establishment of Shoot Cultures	27
4.3 Induction of Callus Cultures	29

CHAPTER V DISCUSSION

32

CHAPTER VII CONCLUSION

36

REFERENCES

37

APPENDIX

46

CURICULUM VITAE

50

LIST OF TABLES

Table		Page
1	The percentage of explants free of contamination after treatment with concentration of Clorox in different times of immersion.	25
2	Percentage of shoot was survived in different concentration of cytokinin	27
3	Percentage shoot was survived and dead shoot subculture with different concentration of Zeatin (PGR's).	29
4	Number of explant survive and dead with different concentration of 2,4-D (PGR's).	29

LIST OF FIGURES

Figure		Page
1	The picture of <i>Nypa fruticans</i> plant.	2
2	The sterilization procedure.	21
3	The dissection of Explant	22
4.1	The contaminated explant by fungus of bacteria and rarely damaged by tissue browning, healty shoot meristem and adventitious shoots.	26
4.2	The best media treatment for culture growth of <i>N. fruticans</i>	28
4.3	The 50% shoot were survived in the media MS contain 5mg/l Zeatin.	30
4.4	That low percentage of callus growth in Media MS contain 2,4-D	31

LIST OF SYMBOLS

cm	-	centimeter
Mm	-	millimeter
Mg	-	milligram
M	-	Meter
cm ³	-	centimeter square
%	-	percentage
°C	-	degree centigrade
v.v ⁻¹	-	volume pervolume
NaOH	-	sodium hydroxide
HCl	-	hydrochloride acid
PGR	-	plant growth regulator
BAP	-	benzylaminopurine
NAA	-	naphthalene acetic acid
IAA	-	indoleacetic acid
MS	-	media Murashige and Skoog
2-ip,	-	isopentenyl adenine
2,4-D	-	2,4-dichlorophenoxyacetic acid

LIST OF APPENDICES

Page

- | | | |
|----|---|----|
| 1. | The notes on solutions of B5 major salt and B5 minor salt. | 44 |
| 2. | The notes on solution of MS major salt and MS minor salt and vitamin. | 45 |
| 3. | The stock solutions of Growth Regulator. | 46 |
| 4. | The preparation of basic MS medium and B5 medium 1L. | 47 |

Wong. H. L. 2006. Establishment Tissue Culture of *Nypa fruticans*. Undergraduate thesis, Bachelor of Applied Science (Biology Science) Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Terengganu. 50p

ABSTRACT

A mangrove palm *Nypa fruticans* was successfully cultured *in-vitro*. Several sterilization treatments had been tested. The most suitable sterilization technique for the shoot tips where by using 100% Clorox (v/v) and immersed for 30 minutes. The explants were cultured in Murashige and Skoog (MS) medium containing either BAP, Kinetin, 2ip, Zeatin at various concentrations. The best medium for shoot tips cultures were on MS medium containing 5.0 mg/l Zeatin. The addition of 5ppt seawater was also enhance the growth of shoot and callus cultures about 50% of shoot were survived in the media MS containing 5.0 mg/l Zeatin, however, rarely damaged by tissue browning in subcultured. The best media for callus induction was MS added with 100mg/L of 2,4-D. Addition of sea water into the media did not significantly enhance the growth of callus.

ABSTRAK

Palma paya bakau *Nypa fruiticans* telah berjaya dikultur secara *in vitro*. Pelbagai rawatan pensterilan telah diujikan. Teknik pensterilan yang paling sesuai bagi tunas pucuk adalah menggunakan 100% klorox (v/v) dan direndamkan selama 30 minit. Eksplan telah dikultur atas media Murashige and Skoog (MS) yang mengandungi BAP, 2ip, Kinetin dan Zeatin dalam pelbagai kepekatan. Media lebih baik bagi kultur tunas pucuk adalah MS yang mengandungi 5.0 mg/L Zeatin. Pertambahan 5ppt air laut juga telah meningkatkan pertumbuhan kultur tisu pucuk dan kallus kira-kira 50% daripada pucuk eksplan telah hidup dalam MS media yang mengandungi 5.0 mg/L Zeatin, bagaimanapun terdapat sedikit tisu mengalami rosak akibat keperangan dalam subkultur. Media yang paling sesuai untuk pengurangan kallus adalah yang ditambah dengan 100mg/L 2,4-D. Penambahan air laut ke dalam media tidak membawa kesan yang ketara merangsang pertumbuhan callus.