

**DEVELOPMENT OF MEDIUM AND BIOREACTOR SYSTEM  
FOR THE CULTIVATION OF HAIRY ROOTS OF *DATURA METEL* AND  
SCOPOLAMINE PRODUCTION**

by

**LAW SEN YU**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirement for the Degree of Master of Science**

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## DEDICATION

*This thesis is dedicated to a very special person in my life, my wife, Ng Phek Lan, who gave me support, strength and courage to make this thesis possible in every way. I would also like to dedicate this thesis to my beloved grandmother, parents and all family members for their support.*

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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**Chairman: Profesor Abdul Manaf bin Ali, Ph.D.**

**Faculty: Food Science and Biotechnology**

The effects of phytohormones (auxin and cytokinin), sucrose and the ratios of ammonium to nitrate upon root branching and elongation of *Datura metel* hairy roots were studied on modified B5O medium. Addition of phytohormones in the medium is fatal to *Datura metel* hairy root cultures. However, root branching and root elongation were enhanced with addition of nitrate at a concentration of 9mM-36mM without any ammonium in the medium.

The effects of ammonium, nitrate, phosphate and *l*-ascorbic acid on the growth of *Datura metel* hairy root cultures were investigated under three different light conditions. The combination effects of ammonium and media strength at half and full strength of B5O media on the growth of *D. metel* hairy root cultures have also been studied. The root growth and morphology were significantly ( $p=0.05$ )

influenced by the light conditions. Light-grown cultures have thicker roots and less branching, thus had slower growth than dark-grown cultures. Root cultures grown in half strength media had a lower growth rate as compared to root cultures grown in full strength media at all concentrations of ammonium. High concentration of ammonium had an inhibitory effect on the roots. However, root cultures grown in full strength media received less impact from ammonium toxicity suggested that the presence of certain chemical at higher initial concentration suppressed the toxicity of ammonium on the root tissue.

The effect of nitrate on the growth of root cultures was not significantly ( $p=0.05$ ) influenced by light conditions. On the contrary, the effect of phosphate was greatly influenced by light conditions. The root growth was enhanced remarkably, up to 12.4 g/L at 4.4 mM of initial phosphate concentration in the presence of light. The induction of lateral roots was also enhanced dramatically at the concentration of initial phosphate higher than 4.4 mM. This suggested that the branching capability of the hairy root cultures could be improved by supplying phosphate in media in abundant. Scopolamine level in hairy root cultures was increased dramatically to 1.6 mg/g cell dry weight at 4.4 mM of initial phosphate concentration in the dark. The increment of scopolamine level in the root cultures may relate to the root branching.

A 1 liter aerated tank without agitation comprised of a polycarbonate cylinder with dimensions of 10 cm in internal diameter and 16.5 cm in height, containing a stainless steel mesh with a mesh size of 2 mm was designed for the cultivation of hairy roots of *Datura metel*. The effect of hydrodynamic stress that was caused by air flow to the root culture was determined by conductometry. The result of this

study shows that root cultures were easily injured by the air flow rate higher than 1.7 vvm. After 16 days of incubation, the root culture filled up almost 95% of the tank space, which gave the final biomass of 175 g fresh weight per liter (8.9 g dry weight per liter), 1.75 mg/g cell dry weight of scopolamine and 0.80 mg/L cell dry weight of hyoscyamine. The productivity of scopolamine in *D. metel* root cultures grown in the bioreactor is comparable to those cultures in the shake flask.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PEMBANGUNAN MEDIA DAN SISTEM BIOREAKTOR  
UNTUK PENKULTURAN AKAR RERAMPUT *DATURA METEL*  
DAN PENGHASILAN SKOPOLAMIN**

Oleh

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Kaji selidik terhadap kesan fitohormon (auksin and sitokinin), sukrosa dan nisbah ammonium kepada nitrat ke atas percabangan akar dan pemanjangan akar rerambut *Datura metel* telah dilakukan dengan menggunakan media B50 yang diubahsuaikan. Penambahan fitohormon dalam media didapati menyebabkan kematian kultur akar rerambut *Datura metel*. Walau bagaimanapun, percabangan dan pemanjangan akar rerambut telah dipertingkatkan dengan menambahkan nitrat dalam lingkungan kepekatan dari 9mM hingga 36 mM dan tanpa kehadiran ammonium dalam media.

Kesan ammonium, nitrat, fosfat and asid askorbik-*l* terhadap pertumbuhan kultur akar rerambut *Datura metel* telah dikajiselidik di bawah tiga keadaan cahaya yang berlainan. Kaji selidik terhadap kesan gabungan ammonium dan kekuatan media

B50 (separa kekuatan dan sepenuh kekuatan) ke atas pertumbuhan kultur akar rerambut *D. metel* juga telah dijalankan. Pertumbuhan dan morfologi akar adalah ketara kelihatan dipengaruhi oleh keadaan cahaya. Kultur akar rerambut yang ditumbuhkan di bawah keadaan bercahaya memperlihatkan akar yang lebih tebal dan kurang bercabang. Jadi, ia menunjukkan pertumbuhan kultur akar yang lebih lambat jika dibandingkan dengan kultur yang ditumbuhkan dalam keadaan gelap. Kultur akar dalam media separa kekuatan menunjukkan kadar pertumbuhan yang lebih rendah berbanding dengan kultur akar rerambut yang ditumbuhkan dalam media sepenuh kekuatan, pada semua kepekatan ammonium. Kepekatan ammonium yang tinggi menyebabkan kerencatan pertumbuhan dan ketoksikan pada kultur akar. Namun, kultur akar yang ditumbuhkan dalam media sepenuh kekuatan menunjukkan kesan yang kurang daripada ketoksikan ammonium. Fenomena ini menunjukkan bahawa terdapat sesuatu bahan kimia jika dalam kepekatan awalan yang tinggi dapat menyekat ketoksikan ammonium pada tisu akar.

Kesan nitrat terhadap pertumbuhan kultur akar tidak dipengaruhi oleh keadaan cahaya. Sebaliknya, kesan fosfat terhadap pertumbuhan kultur akar adalah sangat dipengaruhi oleh keadaan cahaya. Pertumbuhan akar bertambah dengan berkesan, sehingga 12.4 g/L pada kepekatan awalan fosfat 4.4 mM dalam keadaan bercahaya. Aruhan akar sisian juga dipertingkatkan dengan ketara pada kepekatan awalan fosfat yang lebih tinggi daripada 4.4 mM. Keputusan ini menunjukkan bahawa keupayaan bercabang bagi kultur akar rerambut boleh diperbaiki dengan membekalkan kuantiti fosfat yang banyak dalam media. Kepekatan skopolamin dalam kultur akar rerambut telah meningkat kepada 1.6 mg/g berat sel kering pada kepekatan awalan fosfat 4.4mM dalam keadaan gelap. Peningkatan kepekatan skopolamin ini mungkin

berkaitan dengan dengan percabangan akar. Kepekatan skopolamin dalam kultur akar telah meningkat dalam media yang ditambahkan 1 mg/L asid askorbik, tetapi, peningkatan ini adalah lebih rendah berbanding dengan kesan penambahan fosfat dalam media terhadap kultur akar rerambut.

Pengkulturan akar rerambut *D. metel* dalam bioreaktor telah dijalankan dengan menumbuhkan kultur akar rerambut dalam sebuah tangki berudara tanpa pengacauan. Tangki berisipadu 1 liter yang terdiri daripada silinder polikarbonat ini berukuran diameter dalaman 10 cm dan ketinggian 16.5 cm, serta mengandungi sebuah jaringan keluli tahan karat dengan saiz lubang 2 mm. Kesan tekanan hidrodinamik yang disebabkan oleh aliran udara terhadap kultur akar telah ditentukan dengan kaedah konduktometri. Keputusan daripada kajian menunjukkan kultur akar mudah dcederakan pada kadar aliran udara yang lebih tinggi daripada 1.7 vvm.

Selepas 16 hari, kultur akar memenuhi sebanyak 95% daripada ruangan bioreaktor, yang memberi biojisim akhir sebanyak 175 g berat basah per liter (8.9 g berat kering per liter), 1.75 mg/g berat sel kering skopolamin dan 0.80 mg/L berat sel kering hyoseyamin. Produktiviti skopolamin dalam kultur akar rerambut *D. metel* yang ditumbuhkan dalam bioreaktor adalah setanding dengan kultur dalam kelalang goncangan.



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I certify that an Examination Committee met on 21<sup>st</sup> April 2004 to conduct the final examination of Law Sen Yu on his Master of Science thesis entitled "Development of Medium and Bioreactor System for the Cultivation of Hairy Roots of *Datura metel* and Scopolamine Production" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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## **DECLARATION**

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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## TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL	x
DECLARATION	xii
LIST OF TABLES	xvi
LIST OF FIGURES	xviii
LIST OF PLATES	xxi
LIST OF ABBREVIATIONS	xxiii
<b>CHAPTER</b>	
1. INTRODUCTION	
2. LITERATURE REVIEW	4
2.1. Hairy Root Cultures	4
2.1.1. Hairy Roots: Root Induced by <i>Agrobacterium rhizogenes</i>	4
2.1.2. Properties of Hairy Root Cultures	5
2.2. Tropane Alkaloids	10
2.2.1. Biosynthesis of scopolamine	12
2.2.2. Metabolism of Nitrogen	17
2.2.2.1. Nitrate Reductase	17
2.2.2.2. Nitrite Reductase	18
2.2.2.3. Reduced Nitrogen Containing Compounds	19
2.3. Pharmacology of Scopolamine	19
2.3.1. Antispasmodic Effect	20
2.3.2. Antisecretory Effect	20
2.3.3. Mydratic Effect	20
2.3.4. Use against Nausea, Drooling, Alzheimer and Parkinson Disease	21
2.4. <i>Datura metel</i> (Kecubung)	21
2.5. Optimization of the Medium	22
2.5.1. Effect of Medium Strength	23
2.5.2. Effect of Inorganic Nitrogen: Ammonium and Nitrate	23
2.5.3. Effect of phosphate	25
2.5.3.1. Response to Phosphate Limitation	26
2.6. Response to Ascorbic Acid (AA)	27
2.6.1. Ascorbic Acid as Reducing Agent	28
2.6.2. Ascorbic Acid in Biosynthesis of Tropane Alkaloids	29
2.7. Effect of Phytohormone on Growth and Scopolamine Content	30
2.8. Response of Light Irradiation	32
2.9. Importance of Branching in Hairy Root Cultures	35
2.10. Studies on Bioreactor System	37

2.10.1. Development of Bioreactor System for Hairy Root Cultures	37
2.10.2. Problems and Solutions in Bioreactor Design for Hairy Root Cultures	40
3. MATERIALS AND METHODS	45
3.1. Root Maintenance	45
3.2. Studies on Branching and Elongation Properties of Hairy Root Cultures	45
3.2.1. Effect of Phytohormones	46
3.2.2. Effects of Sucrose Levels	46
3.2.3. Effects of Varying Relative Amounts of nitrate (NO <sub>3</sub> <sup>-</sup> ) and Ammonium (NH <sub>4</sub> <sup>+</sup> )	46
3.3. Development of Growth Medium and Production Medium	47
3.3.1. Effects of Ammonium Levels in Half Strength and Full Strength B50 Medium	47
3.3.2. Effect of Initial Concentration of Nitrate (NO <sub>3</sub> <sup>-</sup> )	48
3.3.3. Effect of Initial Concentration of Phosphate (PO <sub>4</sub> <sup>3-</sup> )	48
3.3.4. Effect of Ascorbic Acid Level	49
3.4. Effect of Water Levels in the Flask upon the Root Growth	49
3.5. Development of Bioreactor System for Root Cultures	49
3.5.1. Bioreactor Configuration	49
3.5.2. Effect of Hydrodynamic Stress Expressed by Electric Conductivity	51
3.5.3. Cultivation of root cultures in Bioreactor	51
3.6. Measurement of Root Growth (Dry Weight)	52
3.7. Measurement of Scopolamine Content (mg/g dry weight)	53
3.7.1. Extraction and Purification of Alkaloids from the Root	53
3.7.2. Extraction and Purification of Alkaloids from the Medium	53
3.7.3. HPLC Analysis	53
3.7.3.1. Chromatographic System	54
3.7.3.2. HPLC Mobile Phase	54
3.8. Statistical Analysis and Presentation of the Results	54
4. RESULTS AND DISCUSSION	55
4.1. Studies on Root Branching and Elongation	55
4.1.1. Effect of Phytohormones, Sucrose Levels and the Ratio of Ammonium to Nitrate	55
4.2. Development of Growth Medium and Production Medium	59
4.2.1. Effect of Ammonium in Half Strength and Full Strength B50 Medium	59
4.2.2. Effect of Initial Nitrate Concentration	65
4.2.3. Effect of Initial <i>l</i> -Ascorbic Acid Concentration	69
4.2.4. Effect of Initial Phosphate Concentration	75
4.3. Extending Studies of Root Branching and Elongation	81
4.3.1. Effect of Initial Phosphate Concentration	81
4.4. Development of Bioreactor System for Root Cultures	84
4.4.1. Configuration of Bioreactor	84

4.4.2. Effect of Hydrodynamic Stress Expressed by Eelectric Conductivity	84
4.4.3. Cultivation of <i>Datura metel</i> Hairy Root Cultures	86
4.4.4. Potential Pitfalls	90
5. CONCLUSION	92
REFERENCES	94
APPENDICES	111
BIODATA OF THE AUTHOR	150