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 ticker 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 PENGKULTURAN AKAR RERAMBUT *DATURA METEL* DAN PENGHASILAN SKOPOLAMIN

Oleh

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April 2004

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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 memyebabkan 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 skopolomin dalam kultur akar rerambut telah meningkat kepada 1.6 mg/g berat sel kering pada kepekatan awalan fostat 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 dicederakan 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 hyoscyamin. 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 21st 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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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Master of Science. The members of the Supervisory 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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