



UNIVERSITI PUTRA MALAYSIA

**HYOSCYAMINE AND SCOPOLAMINE PRODUCTION IN
TRANSFORMED ROOT CULTURES OF
*DATURA METEL L***

AZIZ BIN AHMAD

FSAS 2000 40

**HYOSCYAMINE AND SCOPOLAMINE PRODUCTION IN
TRANSFORMED ROOT CULTURES OF
*DATURA METEL L.***

By

AZIZ BIN AHMAD

**Thesis Submitted in Fulfilment of the Requirement for the Degree of Doctor of
Philosophy in the Faculty of Science and Environmental Studies
Universiti Putra Malaysia**

December 2000



Dedicated to my Family.....



Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

HYOSCYAMINE AND SCOPOLAMINE PRODUCTION IN TRANSFORMED ROOT CULTURES OF *DATURA METEL* L.

By

AZIZ BIN AHMAD

December 2000

Chairperson: Prof. Dr. Marziah Mahmood

Faculty: Science and Environmental Studies

The transformed root cultures of *Datura metel* L (kecubung) was successfully established via *Agrobacterium rhizogenes*-mediated, which contained the pBI 121 plasmid harbouring the GUS and kanamycin coding genes. The transformation was biochemically confirmed with GUS assay indicated by the presence of blue spot on roots, Southern blotting and the resistance of transformed root to Kanamycin. Transformed roots were showed a typical character of transformed root, which is sensitive to exogenous IAA (auxin). Sustained root cultures appeared to produce hyoscyamine and scopolamine ten times higher than that produced in the intact plant.

The ability of the transformed roots to produce hyoscyamine and scopolamine in different types of basal media used was examined. It was observed that Gamborg's B5 basal medium was the best medium for root growth as well as the hyoscyamine and scopolamine production. Gamborg's B5 medium was used for subsequent studies. Among the carbon source tested, sucrose appeared to be the best carbon source for root growth. Consequently, the effect of Gamborg's B5 medium ionic strength and sucrose concentration was examined. Gamborg's B5 medium was



used in quarter, half and full strength, and supplemented with sucrose concentration in the range of 1 – to 8 % (w/v). Full strength Gamborg's B5 medium with 4 % sucrose was observed to enhance the root growth as well as hyoscyamine and scopolamine production. Studies were also carried out to examine the effect of various concentrations of macro and microelements on root growth, hyoscyamine and scopolamine production. The macro elements used were nitrogen (ammonium and nitrate balance), magnesium, calcium and phosphate. Amongst the macro element tested, nitrogen, which is in the form of nitrate and/or ammonium, was found to have a significant effect on the hyoscyamine and scopolamine production. Meanwhile, the microelements that have been studied were copper, ferric, manganese, zinc and boron. Ferric and copper appeared to have the greater effect on hyoscyamine and scopolamine production than other elements. Roots cultured in medium with lower concentration of microelement than that present in Gamborg's B5 medium was also observed to enhance the hyoscyamine and scopolamine content.

Feeding of each precursors i.e. putrescine, l-ornithine, arginine, l-phenylalanine, hyoscyamine and scopolamine at lower concentration (less than 0.2 mM) into treatment medium was observed to reduce the root growth as well as the hyoscyamine and scopolamine production. Combination of l-phenylalanine with putrescine appeared the best precursor for both hyoscyamine and scopolamine production.



Abstrak thesis dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGHASILAN HIOSIAMINA DAN SKOPOLAMINA DALAM KULTURA AKAR *DATURA METEL* L. YANG TELAH DITRANSFORMASI

Oleh

AZIZ BIN AHMAD

Disember 2000

Pengerusi: Prof. Dr. Marziah Mahmood

Fakulti: Sains dan Pengajian Alam Sekitar

Kultura akar rerambut kecubung (*Datura metel*) telah berjaya dihasilkan melalui perantaraan *Agrobacterium rhizogenes*, yang mengandungi plasmid pBI 121 yang membawa kod genetik bagi GUS dan Kanamycin. Transformasi telah disahkan dengan pembentukan warna biru pada akar oleh ujian GUS, Southern blot dan ketahanan akar ditransformasi pada Kanamycin. Akar ditransformasi juga menunjukkan ciri istimewa iaitu peka terhadap IAA luaran. Kultur akar yang dihasilkan juga menghasilkan hiosiamina dan skopolamina sepuluh kali lebih tinggi daripada yang dihasilkan oleh induknya.

Keupayaan akar untuk menghasilkan hiosiamina dan skopolamina dalam media asas yang berbeza telah diuji. Media Gamborg's B5 telah dikenalpasti sebagai media yang terbaik untuk pertumbuhan akar, begitu juga penghasilan hiosiamina dan skopolamina. Justeru itu, media Gamborg's B5 telah dipilih untuk kajian selanjutnya. Diantara gula yang diuji, sukrosa merupakan sumber karbon terbaik untuk pertumbuhan akar. Sebagai urutan, kesan kepekatan ionik media Gamborg's B5 dan sukrosa telah diuji. Media Gamborg's B5 telah digunakan pada kepekatan



suku, separuh dan penuh dan dibekalkan dengan sukrosa pada julat 1 - ke 8 % (b/i). Kepekatan penuh media Gamborg's B5 dengan 4 % sukrosa didapati telah menggalakkan pertumbuhan akar, begitu juga penghasilan hiosiamina dan skopolamina. Kajian juga telah dijalankan terhadap kesan pelbagai kepekatan unsur makro dan mikro terhadap pertumbuhan akar, penghasilan hiosiamina dan skopolamina. Unsur makro yang telah digunakan ialah nitrogen (ammonia dan nitrat), magnesium, kalsium dan fosfat. Di antara unsur makro yang telah diuji, nitrogen dalam bentuk nitrat dan/atau ammonia didapati boleh menunjukkan kesan yang signifikan terhadap penghasilan hiosiamina dan skopolamina. Sementara itu, unsur mikro yang telah diuji adalah kuprum, ferik, mangan, zink dan boron. Ferik dan kuprum menunjukkan kesan yang besar terhadap penghasilan hiosiamina dan skopolamina. Kultura akar dalam media dengan kepekatan unsur mikro yang lebih rendah daripada unsur di dalam medium Gamborg's B5 didapati merangsang penghasilan hiosiamina dan skopolamina.

Rawatan penambahan setiap prekursor berikut; putrescina, l-ornithin, arginina, l-fenilalanina, hiosiamina dan skopolamina ke dalam medium pada kepekatan yang lebih rendah (kurang daripada 0.2 mM) didapati memberi kesan terhadap pertumbuhan akar dan penghasilan hiosiamina serta skopolamina. Kombinasi l-fenilalanina dengan putrescine merupakan prekursor terbaik bagi penghasilan hiosiamina dan skopolamina.

ACKNOWLEDGEMENTS

All praise is to the Almighty **ALLAH**, the most Merciful and the Compassionate due to His willingness, that the completion of this study was made possible.

I would like to express my deep appreciation and gratitude to the Chairman of my Supervisor Committee, Professor Dr. Hj. Marziah Mahmood for her help, guidance and constant support in making the completion of this thesis a success. Thank also due to Associate Professor Dr. Radzali Muse and Dr. Halimi Mohd Saud my Supervisor Committee Members, for their guidance and help.

I'm gratefully to present and ex-members of the Plant Stress Laboratory at Department of Biochemistry and Microbiology, UPM; Dr. Rosmin and Dr. Kow (ex-members), Ibu Iteu, Azlan, Cik Radziah, Janna, Rammani, Rohaida and Suzita. My high regard also due to all my friends may I not mentioned in this scribe.

Last but not least, my heartiest appreciation to my parent, my brothers and sisters for giving the support and strength during my studies.

!! May ALLAH bless us always !!



I certify that an Examination Committee met on 20th December, 2000 to conduct the final examination of Aziz bin Ahmad on his Doctor of Philosophy thesis entitled “Hyoscyamine and Scopolamine Production in Transformed Root Cultures of *Datura metel* L.” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows.

Maheran binti Aziz, Ph.D.
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Marziah Mahmood, Ph.D.
Professor
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

Radzali bin Muse, Ph.D.
Associate Professor
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

Halimi Mohd Saud, Ph.D.
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Normah Mohd Noor, Ph.D.
Professor
School of BioScience and Biotechnology
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(Independent Examiner)



MOHD. GHAZALI MOHAYIDIN, Ph.D.
Professor/Deputy Dean of Graduate School,
Universiti Putra Malaysia

Date: 03 JAN 2001

The thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.

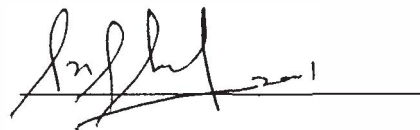


KAMIS AWANG, Ph.D.
Associate Professor,
Dean of Graduate School,
Universiti Putra Malaysia

Date: 11 JAN 2001

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 currently submitted for any other degree at UPM or other institutions.



(AZIZ BIN AHMAD)

Date: 3 | 1 | 2007 .

TABLE OF CONTENTS

	Page
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENTS	vii
APPROVAL SHEETS	viii
DECLARATION FORM	x
LIST OF TABLES	xv
LIST OF FIGURE	xvi
LIST OF PLATES	xix
LIST OF ABBREVIATIONS	xx
CHAPTER	
I	
INTRODUCTION	1
Use of Hyoscyamine and Scopolamine.....	1
Plant Tissue Culture	2
Objective	3
II	
LITERATURE REVIEW	4
Root Properties	4
Root Culture	4
Transformation Methods	5
<i>Agrobacterium rhizogenes</i> Transformation	6
Transformed Root Cultures	8
Advantages of Transformed Roots	11
Rapid Growth	12
Genetic and Biochemical Stability	12
Bioreactor Technology and Large Scale Production	13
Genetic Manipulation and Modification.....	14
Biotransformations.....	16
Limitation of Transformed root Cultures	16
Factors Influencing Growth of Transformed Root Cultures	17
Media and Ionic Strength	18
Effect of Carbon Sources	18
Ammonium and Nitrate Balance	19
Plant Growth Regulators (PGRs)	20
Initial pH of Media	22
Feeding of Precursor.....	22
Temperature	23
Light Exposures	23



Oxygen and Carbon dioxide	23
Magnetic Fields.....	25
Factors Influencing Secondary Metabolite Production	25
Effect of Carbon and Sugar Sources	25
Ammonium and Nitrate Balances.....	26
Plant Growth Regulators (PGRs)	27
Initial pH of Media	28
Feeding of Precursors	29
Temperature	30
Light Exposure	30
Oxygen and Carbon dioxide	31
Sources of Explant (Intact Plant)	32
Osmotic Stress	32
Elicitors	33
Biosynthesis of Tropane Alkaloids	34
Biosynthesis of Hyoscyamine	35
Biosynthesis of Scopolamine.....	35

III

ESTABLISHMENT AND CHARACTERIZATION OF *DATURA METEL* TRANSFORMED ROOT CULTURES 38

Introduction	38
Materials and Methods	39
Plant Sources	39
Bacterial Culture	40
Induction and Establishment of Root Culture	40
Confirmation of Transformation	41
β -Glucuronidase assay	41
Southern Blot Analysis	41
Effect of Kanamycin (Km)	42
Effect of Exogenous IAA on Growth of Root Cultures.....	42
Growth Pattern of Transformed Root Cultures	43
Distribution of Hyoscyamine and Scopolamine	43
Extraction of Hyoscyamine and Scopolamine	43
Analysis of Hyoscyamine and Scopolamine	44
Results and Discussion	44
Establishment of Transformed Roots	44
Confirmation of Transformation	45
β -Glucuronidase assay	45
Southern Blotting	49
Effect of Kanamycin (Km)	49
Effect of Exogenous IAA on Growth of <i>Datura metel</i> Root Cultures	49
Growth Pattern of Transformed Root Cultures	56
Distribution of Hyoscyamine and Scopolamine	57
Conclusions	61



IV	THE INFLUENCE OF MEDIA COMPOSITION AND CARBON SOURCES ON GROWTH, HYOSCYAMINE AND SCOPOLAMINE CONTENT OF <i>DATURA METEL</i> TRANSFORMED ROOT CULTURES	63
	Introduction	63
	Materials and Methods	64
	Transformed Root Cultures	64
	Effect of Basal Medium.	64
	Effect of Carbon Sources	64
	Alkaloid extraction and Analysed	65
	Results and Discussion	65
	Effect of Basal Medium.....	65
	Root Growth, Scopolamine and Hyoscyamine Production in the Culture	66
	Effect of Carbon Sources on Growth	68
	Effect of Gamborg's B5 Concentrations	72
	Conclusion	75
V	THE INFLUENCE OF MACRO AND MICRO ELEMENTS ON HYOSCYAMINE AND SCOPOLAMINE CONTENT IN <i>DATURA METEL</i> TRANSFORMED ROOT CULTURES	77
	Introduction	77
	Materials and Methods.....	78
	Media Preparations	78
	Influence of Macro and Micro Elements Deficiencies	78
	Effect of Different Concentrations of Macro element and Micro element on Root Growth	79
	Effect of Different Concentration of Macro Elements on Root Growth, Hyoscyamine and Scopolamine Production	79
	Nitrate (NO ₃ ⁻) and Ammonium (NH ₄ ⁺) Ratio.....	79
	Phosphate	79
	Calcium	79
	Magnesium	79
	Potassium	80
	Effect of Different Concentrations of Micro elements on Root Growth, Hyoscyamine and Scopolamine	80
	Copper	80
	Ferric	80
	Manganese	80
	Zinc	80
	Boron	81
	Statistical Analysis	81
	Results and Discussion	81
	Influence of Macro and Micro Element Deficiencies	81
	Effect Doubling Macro elements	87



Effect of Different Concentration of Macro elements	88
Nitrate (NO ₃ ⁻) and Ammonium Ratio (NH ₄ ⁺)	88
Effect of Phosphate (PO ₄)	93
Effect of Calcium (Ca ²⁺).....	95
Effect of Magnesium (Mg ²⁺)	98
Effect of Potassium (K ⁺).....	98
Effect of Different Concentration of Micro Elements	102
Copper (Cu ²⁺).....	102
Ferric (Fe ³⁺)	104
Manganese (Mn ²⁺).....	106
Zinc (Zn ²⁺).....	108
Boron (B).....	110
Effect of Quarter Concentrations of Macro and Micro Elements .	112
Conclusion	113

VI	THE INFLUENCE OF SELECTED AMINO ACIDS, PUTRESCINE, HYOSCYAMINE AND SCOPOLAMINE ON HYOSCYAMINE AND SCOPOLAMINE CONTENT IN <i>DATURA METEL</i> TRANSFORMED ROOT CULTURES.....	116
	Introduction	116
	Materials and Methods	117
	Root Cultures	117
	Media and Precursor Preparation	117
	Putrescine	117
	Ornithine	117
	Arginine	118
	L-Phenylalanine	118
	Hyoscyamine	118
	Scopolamine	118
	Growth, Hyoscyamine and Scopolamine Analyses	118
	Statistical Analysis	118
	Results and Discussion	119
	Effect of Putrescine	119
	Effect of <i>l</i> -Ornithine	122
	Effect of Arginine	123
	Effect of <i>l</i> -Phenylalanine	125
	Effect of Hyoscyamine	129
	Effect of Scopolamine	131
	Effect of Putrescine and Phenylalanine	133
	Conclusion	133
VII	SUMMARY, GENERAL DISCUSSION AND CONCLUSSION	135
	REFERENCES	138
	APPENDIX.....	162
	BIOGRAPHICAL SKETCH	167



LIST OF TABLES

Tables	Page
1. The effect of macro element deficiencies on growth of <i>D. metel</i> transformed root cultures	82
2. The effect of micro element deficiencies on growth of <i>D. metel</i> transformed root cultures.....	85
3. The effect of doubling the macro element concentration In B5 medium on growth of <i>D. metel</i> transformed root cultures	88
4. The effect of macro and micro element on fresh and dry weight of <i>D. metel</i> transformed root cultures.....	113
5. The dry weight, hyoscyamine and scopolamine content in Transformed roots cultured in treatment medium supplemented With putrescine and phenylalanine	133



LIST OF FIGURE

Figure	Page
1. The pathway of tropane alkaloids biosynthesis	37
2. Growth of putative transformed root of <i>D. metel</i> cultured in solid and liquid Gamborg's B5 medium	48
3. Growth of non-transformed roots and transformed roots of <i>D. metel</i> cultured in different concentration of Kanamycin	51
4. Growth of transformed (TR) and non-transformed (NTR) of <i>D. metel</i> cultured separately in free-phytohormone Gamborg's B5 medium and medium supplemented with 0.5 mg/L IAA, respectively.....	55
5. The elongation rate of initially inoculated roots and number of branching of <i>D. metel</i> L transformed root cultures at different days of culture	58
6. The hyoscyamine and scopolamine content in transformed roots, nontransformed root and leaves of intact of <i>D. metel</i> L.....	60
7. Effect of Gamborg's B5, MS and White's media on <i>D. metel</i> transformed root cultures	67
8. Root growth, hyoscyamine and scopolamine content of <i>D. metel</i> transformed root cultured in Gamborg's B5 medium at different days of culture	69
9. Growth of <i>D. metel</i> transformed root cultured in Gamborg's B5 medium supplied with different concentrations of carbon sources	71
10. The effect of Gamborg's B5 medium and sucrose concentration on root dry weight, hyoscyamine and scopolamine content of <i>D. metel</i> in transformed root cultures	74



11.	The effect of different concentrations of nitrate on growth, hyoscyamine and scopolamine production in transformed root cultures of <i>D. metel</i>	90
12.	The effect of different concentrations of ammonium on growth, hyoscyamine and scopolamine production in transformed root cultures of <i>D. metel</i>	92
13.	The effect of different concentrations of phosphate on growth, hyoscyamine and scopolamine production in transformed root cultures of <i>D. metel</i>	94
14.	The effect of different concentrations of calcium on growth, hyoscyamine and scopolamine production in transformed root cultures of <i>D. metel</i>	97
15.	The effect different concentrations of magnesium on growth, hyoscyamine and scopolamine production in transformed root cultures of <i>D. metel</i>	99
16.	The effect different concentrations of potassium on growth, hyoscyamine and scopolamine production in transformed root cultures of <i>D. metel</i>	101
17.	The effect different concentrations of copper on growth, hyoscyamine and scopolamine production in transformed root cultures of <i>D. metel</i>	103
18.	The effect different concentrations of ferric on growth, hyoscyamine and scopolamine production in transformed root cultures of <i>D. metel</i>	105
19.	The effect different concentrations of manganese on growth, hyoscyamine and scopolamine production in transformed root cultures of <i>D. metel</i>	107
20.	The effect different concentrations of zinc on growth, hyoscyamine and scopolamine production in transformed root cultures of <i>D. metel</i>	109
21.	The effect different concentrations of boron on growth, hyoscyamine and scopolamine production in transformed root cultures of <i>D. metel</i>	111
22.	The effect different concentrations of supplied putrescine on growth of <i>D. metel</i> transformed root cultures	120



23.	The effect different concentrations of supplied putrescine on growth, hyoscyamine and scopolamine production in <i>D. metel</i> transformed root cultures of	121
24.	The effect of different concentrations of supplied ornithine on growth, hyoscyamine and scopolamine produced by <i>D. metel</i> transformed root cultures	124
25.	The effect of different concentrations of supplied arginine on growth, hyoscyamine and scopolamine produced by <i>D. metel</i> transformed root cultures	126
26.	The effect OF different concentrations of supplied phenylalanine on growth, hyoscyamine and scopolamine produced by <i>D. metel</i> transformed root cultures	128
27.	The effect of different concentrations of exogenous hyoscyamine on growth, hyoscyamine and scopolamine produced by <i>D. metel</i> transformed root cultures	130
28.	The effect of different concentrations of exogenous scopolamine on growth, hyoscyamine and scopolamine produced by <i>D. metel</i> transformed root cultures	132



LIST OF PLATES

Plates	Page
1. The formation of hairy roots on the surface of an infected young leaf and no root was produced from an infected old leaf of <i>D. metel</i> with <i>Agrobacterium rhizogenes</i>	46
2. Growth of putative transformed root of <i>D. metel</i> cultured in solid and liquid Gamborg's B5 medium	47
3. GUS histochemical assay of the transformed roots of <i>D. metel</i> showing the blue stain and Southern blotting of genomic DNA which putative transformed root showing insertion of 3 kb of GUS gene	50
4. Nontransformed root cultures of <i>D. metel</i> cultured in MS medium containing 0.5 mg/L IAA supplemented with different concentrations of Kanamycin	52
5. The transformed roots <i>D. metel</i> cultured in free-phytohormone Gamborg's B5 medium and transformed roots cultured in Gamborg's B5 medium supplemented with 0.5 mg/L IAA, nontransformed roots cultured in free-phytohormone B5 medium and nontransformed roots cultured in Gamborg's B5 supplemented with 0.5 mg/L of IAA	54
6. Transformed root of <i>D. metel</i> cultured in Gamborg's B5 medium containing high concentration of sucrose	73
7. The effect of macro element deficiencies on transformed root cultures of <i>D. metel</i>	83
8. The effect of micro element deficiencies on transformed root cultures of <i>D. metel</i>	86



LIST OF ABBREVIATIONS

BAP	benzyl-amino purine
DNA	deoxyribonucleic acid
DMRT	Duncan's Multiple Ranges Test
g	grams
GA ₃	gibberellic acid
GUS	β-Glucuronidase
HPLC	High Performance Liquid Chromatography
IAA	indole-acetic acid
mg	milligrams
mg/g	milligrams per gram
mg/L	milligrams per litre
mL	millilitre
mM	millimolar
N	normal
μg	micrograms
μg/g	micrograms per gram
μM	micro molar
wt.	weight
w/v	weight per volume
v/v	volume per volume
°C	degree of centigrade
%	percentage



CHAPTER I

INTRODUCTION

Use of Hyoscyamine and Scopolamine

Tropane alkaloids contain more than 150 members including hyoscyamine and scopolamine (Yamada and Tabata, 1997). They were found in many genera of Solanaceae family, e.g. *Atropa*, *Brugmansia*, *Datura*, *Duboisia*, *Hyoscyamus*, *Mandragora*, and *Scopolia* (Yamada and Tabata, 1997). *Datura* species are plants that produce the largest amount of hyoscyamine and scopolamine (Jung and Tepfer, 1987). Atropine (racemate of *d* and *l*-hyoscyamine) and scopolamine are anticholinergic agent or as parasympatholytic agents (Jauhikainen et al., 1999; Pitta-Alvarez and Giulietti, 1995). *l*-hyoscyamine first stimulates, then depresses the central nerve system (CNS), whereas *l*-scopolamine depresses it, because of this property scopolamine is preferred in clinical application and the demand in the world-market is 10 times more than that of *l*-hyoscyamine (Yamada and Tabata, 1997). A combination of scopolamine and hyoscyamine was used with ergotamine for treating acute migraine (Lewis and Elvin-Lewis, 1977). Scopolamine itself was used for treating motion sickness, sleeping pill psychosis and as anti-inflammatory on eyes (Pitta-Alvarez and Giulietti, 1995). Meanwhile, hyoscyamine was used for treating asthma, ulcer, Parkinson's disease and also used as anti spasmotic and analgesic (Lewis and Elvin-Lewis, 1977). However, the production of these particular compounds was extracted directly from any part of the plant. This accounted with the yield and the productivity was not constant due to uncertainty of supply and instability of raw materials (Yeoman and Yeoman, 1996). Therefore, to overcome this problem artificial chemical synthesis was introduced.

However, the biochemicals have complex structures and can be chiral molecules, which are difficult and expensive or even impossible to synthesize chemically (Waterman, 1992; Leete 1990). Subsequently, according to Hibi et al., (1992) only *l*-isomer of these tropane alkaloids is pharmacologically active.

Plant Tissue Culture

Plant tissue culture offers an alternative approach for the production and manufacturing of natural and foreign plant secondary products. Plant cell culture, however, was reported oftenly fail to produce the spectrum of the useful compounds in the higher plants and the yield was low which became a major barrier for their production (Yeoman and Yeoman, 1996; Oksman-Caldentey et al. 1994; Constabel, 1990). Development of plant organ cultures such as roots, shoots and leaves was shown to enhance the production of secondary metabolites *in vitro* (Subroto et al., 1996a,b; Ehmke et al., 1995; Alvarez et al., 1994; Sharp and Doran, 1990). In recent years, there has been an increasing interest in application of genetically transformed organs such as transformed root cultures for plant secondary metabolite production.

Transformed roots could be obtained following the infection of explant with *Agrobacterium rhizogenes*. This was due to the transfer and cooperation of Ri T-DNA from the bacteria into genomes of the host plant cells (Chilton et al., 1992). This T-DNA was indicated to alter auxin metabolism in plant cells (Hamill, 1993) and resulted in a rapid growth of roots called 'hairy roots'. This kind of culture was reported to produce higher secondary metabolite than normal plant with a similar spectrum (Jung and Tepfer, 1987). The ability of transformed root cultures to produce tropane alkaloids has been demonstrated for several genera in Solanaceae.

These include *Datura*, *Atropa*, *Scopolia*, *Hyoscyamus*, *Brugmansia* and *Duboisia* (Maldonado-Mendoza et al., 1995; 1993; Robins et al., 1990; Rhodes et al., 1989; Jaziri et al., 1988). These alkaloids are synthesised in the roots then transported to and accumulated in the leaves (Mano et al., 1989; Endo and Yamada, 1985). Therefore, using the plant root cultures is the best system for the producing these particular alkaloids.

Objectives

1. To establish the transformed hairy root culture of *Datura metel*.
2. To determine the effect of plant growth regulators and media manipulation on root growth, scopolamine and hyoscyamine production.
3. To investigate the influence of different macro and microelements and precursors on root growth, scopolamine and hyoscyamine production in hairy root cultures.

CHAPTER II

LITERATURE REVIEW

Root Properties

The main function of plant organ such as roots is as water and nutrient absorber (Canny, 1998). However, roots have been demonstrated to play an important role for biosynthesis of plant secondary metabolites. Studies have shown that roots was the main organ used for biosynthesis of terpenoids, steroids, alkaloids and phenolics in the higher plants (Harbone and Khan, 1993; Parr, 1989; Endo and Yamada, 1985). Hashimoto et al., (1991), reported that root is the main site for biosynthesis of tropane alkaloids. These particular alkaloids were then naturally transported through a vascular system-xylem and phloem (Kitamura et al., 1993) and accumulated in the leaves or other parts of plant (Mano et al., 1989; Ghani, 1986; Endo et al., 1987). The production of hyoscyamine is fulfilled in the roots of the plant, while its transformation (epoxide) to scopolamine is accomplished either in pericycle in some plants (Kanagae et al., 1994) or in the aerial parts in others (Maldonado-Mendoza and Loyola-Vargas, 1995; Yun et al., 1992; Parr, 1989).

Root Culture

The basic techniques required in initiating and maintaining excised root cultures were established as early as the 1930s. Butcher and Street (1964) reviewed further modification and refinements in the methodology (Charlwood et al., 1990). Auxins at lower concentrations were normally used for initiating and growth of the roots