

**MICROPROPAGATION AND EFFECT OF GROWTH RETARDANTS ON  
SELECTED SPECIES OF MELASTOMATACEAE**

**By**

**RAMANI POOSPOORAGI**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in  
Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

**January 2005**

**Dedicated to :**

My beloved father Poosporagi, mother Muniammah

My dearest sister Thavamalar and brother Suntharam

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

# MICROPROPAGATION AND EFFECT OF GROWTH RETARDANTS ON SELECTED SPECIES OF MELASTOMATACEAE

By

RAMANI POOSPOORAGI

January 2005

**Chairman : Professor Marziah Mahmood, Ph.D.**

**Faculty : Biotechnology and Biomolecular Sciences**

This study consists of four parts. The first part was to develop an efficient *in vitro* micropropagation protocol for *Melastoma malabathricum*, *Melastoma decemfidum*, *Melastoma dodecandrum* and *Tibouchina semidecandra*. These plants are locally known as 'senduduk'. Nodal segment and shoot tip of each species were used as explants for shoot initiation. Shoot tip was a more suitable explant for *M. malabathricum*, *M. dodecandrum* and *M. decemfidum* shoot initiation performed in full strength Murashige and Skoog (MS) medium supplemented with 30  $\mu\text{M}$  6-benzylaminopurine (BAP), while nodal explant was chosen for *T. semidecandra* shoot initiation in full strength MS medium supplemented with 20  $\mu\text{M}$  BAP.

Shoot multiplication and elongation was optimal in half strength MS medium supplemented with 6  $\mu\text{M}$  BAP for *T. semidecandra*, 9  $\mu\text{M}$  BAP for *M. malabathricum* and 12  $\mu\text{M}$  BAP for *M. decemfidum* while *M. dodecandrum* required quarter strength MS medium supplemented with 3  $\mu\text{M}$  BAP. Shoots

cultured on MS medium without any growth regulators supplementation was found to have higher *in vitro* rooting compared to medium supplemented with naphthalene acetic acid (NAA), indole butyric acid (IBA) and indole acetic acid (IAA). Full strength MS medium was suitable for *in vitro* rooting of *T. semidecandra* and *M. decemfidum*, opposed to half strength MS medium for *M. malabathricum* and quarter strength MS medium for *M. dodecandrum*. Rooting in the solid medium was better than liquid medium. A higher percentage of plantlets survived when they were acclimatized for one week compared to plantlets that were directly transferred from tissue culture medium to the soil.

The second part of this study was to regenerate shoots directly from the leaf, petiole and internode explants of *M. malabathricum*. Explants obtained from the most apical part of the plant formed a higher number of shoots compared to those below the apical end. Quarter strength MS medium was the most suitable medium strength for shoot regeneration of all explants tested. The highest number of shoots was formed from the leaf explant at 9  $\mu\text{M}$  BAP, followed by petiole at 6  $\mu\text{M}$  BAP, and internode at 9  $\mu\text{M}$  BAP.

The third part of this study was to regenerate shoots from leaf-, petiole- and internode-derived calli of *M. malabathricum*. A suitable callus induction medium was found to be a full strength MS medium supplemented with 2.5  $\mu\text{M}$  dicamba and 2.5  $\mu\text{M}$  kinetin for leaf explant, 10.0  $\mu\text{M}$  NAA and 2.5  $\mu\text{M}$  BAP for petiole explant, and 10.0  $\mu\text{M}$  NAA and 2.5  $\mu\text{M}$  kinetin for internode explant. Full

strength MS medium supplemented with 5.0 to 7.5  $\mu\text{M}$  BAP alone had induced multiple shoots from the leaf-derived callus compared to 2.5 to 5.0  $\mu\text{M}$  BAP for petiole-derived callus. A combination of 0.5  $\mu\text{M}$  NAA and 5.0  $\mu\text{M}$  BAP, however, was found to enhance shoot formation from the petiole-derived callus compared to when 5.0  $\mu\text{M}$  BAP was used alone.

The final part of this study was to evaluate the effects of growth retardants on vegetative growth and the flowering of *M. malabathricum*, *M. decemfidum* and *T. semidecandra*. Growth retardants (paclobutrazol and flurprimidol) significantly reduced the plant size, induced early flowering and increased the number of flowers formed unlike the untreated plants. Paclobutrazol applied at 200 mg/L (w/v) was found to be suitable for *M. malabathricum* compared to 300 mg/L (w/v) for *M. decemfidum*. Flurprimidol applied at 50 mg/L (w/v) concentration was suitable for *T. semidecandra*.

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

**PEMBIAKAN DAN KESAN PERENCAT PERTUMBUHAN TERHADAP  
BEBERAPA SPESIS TERPILIH DARIPADA MELASTOMATACEAE**

**Oleh**

**RAMANI POOSPOORAGI**

**Januari 2005**

**Pengerusi : Profesor Marziah Mahmood, Ph.D.**

**Fakulti : Bioteknologi dan Sains Biomolekul**

Kajian ini merangkumi empat bahagian. Bahagian pertama bertujuan mendapatkan protokol yang sesuai untuk pembiakan *Melastoma malabathricum*, *Melastoma decemfidum*, *Melastoma dodecandrum* dan *Tibouchina semidecandra* secara *in vitro*. Tumbuhan ini lebih dikenali dengan nama tempatannya sebagai senduduk. Dalam kajian ini, bahagian hujung pucuk dan buku batang bagi setiap spesis digunakan sebagai eksplan untuk penghasilan pucuk. Hujung pucuk didapati lebih sesuai untuk penghasilan pucuk bagi *M. malabathricum*, *M. decemfidum*, *M. dodecandrum* bila dikultur dalam medium Murashige dan Skoog (MS) penuh yang mengandungi 30  $\mu\text{M}$  6-bensilaminopurina (BAP) manakala eksplan buku batang dipilih untuk penghasilan pucuk bagi *T. semidecandra* bila dikultur dalam medium MS penuh yang mengandungi 20  $\mu\text{M}$  BAP.

Pembiakan dan pemanjangan pucuk didapati paling sesuai dalam medium setengah MS yang mengandungi 6  $\mu\text{M}$  BAP bagi *T. semidecandra*, 9  $\mu\text{M}$  BAP bagi *M. malabathricum* dan 12  $\mu\text{M}$  BAP bagi *M. decemfidum* manakala medium

seperempat MS yang mengandung 3  $\mu\text{M}$  BAP didapati sesuai bagi *Melastoma dodecandrum*. Medium MS tanpa pengawalatur pertumbuhan telah meningkatkan pengeluaran akar secara *in vitro* bagi pucuk berbanding medium yang mengandung asid naphthalena asetik (NAA), asid indolabutirik (IBA) dan asid indolasetik (IAA). Medium MS penuh didapati paling sesuai untuk pengeluaran akar secara *in vitro* bagi *T. semidecandra* dan *M. decemfidum* berbanding dengan medium setengah MS bagi *M. malabathricum* dan medium seperempat MS bagi *M. dodecandrum*. Pengeluaran akar dalam medium pepejal didapati lebih sesuai berbanding medium cecair. Peratusan pokok yang hidup selepas seminggu dalam proses aklimasi didapati lebih tinggi berbanding dengan pokok yang dipindahkan secara terus dari medium kultur tisu ke tanah.

Dalam bahagian kedua, regenerasi pucuk secara langsung daripada eksplan daun, petiol and ruas batang *M. malabathricum* telah dikaji. Eksplan yang diambil daripada bahagian paling atas pokok telah menghasilkan bilangan pucuk yang lebih tinggi berbanding dengan eksplan daripada bahagian bawah. Medium seperempat MS didapati paling sesuai untuk regenerasi pucuk bagi semua eksplan yang dikaji. Bilangan pucuk tertinggi didapati bagi eksplan daun pada 9  $\mu\text{M}$  BAP, diikuti dengan petiol pada 6  $\mu\text{M}$  BAP dan ruas batang pada 9  $\mu\text{M}$  BAP.

Dalam bahagian ketiga, regenerasi pucuk daripada kalus daun, petiol dan ruas batang *M. malabathricum* telah dikaji. Medium yang paling sesuai untuk induksi kalus adalah medium MS penuh yang mengandung 2.5  $\mu\text{M}$  dicamba dan 2.5  $\mu\text{M}$

kinetin bagi eksplan daun, 10.0  $\mu\text{M}$  NAA dan 2.5  $\mu\text{M}$  BAP bagi eksplan petiol dan 10.0  $\mu\text{M}$  NAA dan 2.5  $\mu\text{M}$  kinetin bagi eksplan ruas batang. Medium MS penuh yang mengandungi 5.0 hingga 7.5  $\mu\text{M}$  BAP telah menghasilkan pucuk daripada kalus daun berbanding dengan 2.5 hingga 5.0  $\mu\text{M}$  BAP bagi kalus petiol. Kombinasi 0.5  $\mu\text{M}$  NAA dan 5.0  $\mu\text{M}$  BAP telah meningkatkan penghasilan pucuk daripada kalus petiol berbanding bila hanya 5.0  $\mu\text{M}$  BAP digunakan.

Dalam bahagian terakhir, kesan bahan perencat pertumbuhan terhadap pertumbuhan vegetatif dan pembungaan *M. malabathricum*, *M. decemfidum* dan *T. semidecandra* telah dikaji. Perencat pertumbuhan (paclobutrazol dan flurprimidol) telah berjaya mengurangkan saiz pokok, mempercepatkan pengeluaran bunga dan meningkatkan bilangan bunga secara ketara berbanding dengan pokok kawalan. Paclobutrazol yang digunakan pada kepekatan 200 mg/L (b/i) amat sesuai bagi *M. malabathricum* berbanding dengan 300 mg/L (b/i) bagi *M. decemfidum*. Rawatan dengan flurprimidol pada kepekatan 50 mg/L (b/i) didapati sesuai bagi *Tibouchina semidecandra*.



## **ACKNOWLEDGEMENTS**

I would like to express my gratitude to Prof. Dr. Maziah Mahmood, Dr. Janna Ong Abdullah and Dr. Mohd Puad Abdullah for their invaluable guidance and encouragement during the course of my study and the preparation of this thesis.

I would like to thank Universiti Putra Malaysia for financial support (PASCA) and my supervisor Dr. Maziah Mahmood which enable this study to be completed.

I would also like to express my deepest thanks to my father Poosporagi, my mother Muniammah, my sister Thavamalar, my brother Suntharam for their encouragement, patience and moral support during the period of studies. Finally, I would also like to thanks my friends Tee, Rosli, Sri, CY, Ida, Sobri, Anna, Janna, Dorene, Saras and Judy who always help, give advice and motivate during the studies.

I certify that an Examination Committee met on 17 January 2005 to conduct the final examination of Ramani Poosporagi on her Doctor of Philosophy thesis entitled 'Micropropagation and Effect of Growth Retardants on Vegetative Growth and Flowering of Selected Species of Melastomataceae Family' 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:

**Siti Khalijah Daud, Ph.D.**

Associate Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Chairman)

**Radzali Muse, Ph.D.**

Associate Professor  
Faculty of Biotechnology and Biomolecular Sciences,  
Universiti Putra Malaysia.  
(Member)

**Mohd. Arif Syed, Ph.D.**

Professor  
Faculty of Biotechnology and Biomolecular Sciences,  
Universiti Putra Malaysia.  
(Member)

**Chan Lai Keng, Ph.D**

Professor  
School of Biological Sciences,  
Universiti Sains Malaysia, Penang  
(Independent Examiner)

---

**GULAM RUSUL RAHMAT ALI, Ph.D.**

Professor/Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date :

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows :

**Marziah Mahmood, Ph.D.**

Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Chairman)

**Mohd. Puad Abdullah, Ph.D.**

Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Member)

**Janna Ong Abdullah, Ph.D.**

Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Member)

---

**AINI IDERIS, Ph.D.**

Professor/Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date :

## **DECLARATION**

I hereby declare that this 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.

RAMANI POOSPOORAGI

Date :

## TABLE OF CONTENTS

	<b>Page</b>
<b>DEDICATION</b>	ii
<b>ABSTRACT</b>	iii
<b>ABSTRAK</b>	vi
<b>ACKNOWLEDGEMENTS</b>	ix
<b>APPROVAL</b>	x
<b>DECLARATION</b>	xii
<b>LIST OF TABLES</b>	xvii
<b>LIST OF FIGURES</b>	xxi
<b>LIST OF ABBREVIATIONS/NOTATIONS</b>	xxx
<b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	<b>1</b>
1.1 Background	1
1.2 Objectives	11
<b>2 LITERATURE REVIEW</b>	<b>12</b>
2.1 Floriculture Industry in Malaysia	12
2.1.1 Production	12
2.1.2 Export Market	15
2.1.3 Issues and Problems in Floriculture Industry in Malaysia	15
2.2 Flowers of Malaysia	18
2.2.1 Malaysian ornamental plants	18
2.3 Melastomataceae family	20
2.3.1 Description and distribution	20
2.3.2 <i>Melastoma malabathricum</i>	21
2.3.3 <i>Melastoma decemfidum</i>	23
2.3.4 <i>Melastoma dodecandrum</i>	24
2.3.5 <i>Tibouchina semidecandra</i>	25
2.4 Use of tissue culture techniques for plant propagation	26
2.5 Methods of micropropagation	28
2.5.1 Axillary shoot proliferation	28
2.5.2 Adventitious shoot regeneration	29
2.5.2.1 Direct plant regeneration	31
2.5.2.2 Indirect regeneration from callus	32
2.5.3 Somatic embryogenesis	33
2.6 Factors influencing plant regeneration	35
2.6.1 Effect of explant	35
2.6.2 Effect of plant growth regulators	37
2.6.3 Effect of medium	40
2.6.4 Effect of carbon source	41

	2.6.5	Effects of environment	42
2.7		Rooting and Acclimatization	43
	2.7.1	Rooting	43
	2.7.2	Acclimatization of plantlets	46
2.8		Growth retardants	47
	2.8.1	Paclbutrazol	49
	2.8.2	Flurprimidol	50
<b>3</b>		<b>MICROPROPAGATION OF THE SELECTED PLANT SPECIES OF THE MELASTOMATACEAE FAMILY</b>	<b>52</b>
	3.1	Introduction	52
	3.2	Materials and Methods	55
	3.2.1	Establishment of <i>in vitro</i> plants of <i>Melastoma malabathricum</i> , <i>M. dodecandrum</i> , <i>M. decemfidum</i> and <i>Tibouchina semidecandra</i>	55
	3.2.1.1	Preparation of tissue culture media	55
	3.2.1.2	Culture conditions	56
	3.2.1.3	Surface sterilization test	56
	3.2.1.4	Shoot initiation	57
	3.2.1.5	Shoot multiplication and elongation	59
	3.2.1.5.1	Effect of medium strength and BAP concentration	59
	3.2.1.5.2	Effect of solid and liquid medium	60
	3.2.1.5.3	Effect of medium type on shoot proliferation of <i>M. dodecandrum</i>	61
	3.2.1.5.4	Effect of sucrose on shoot proliferation of <i>M. dodecandrum</i>	62
	3.2.1.5.5	Effect of casein hydrolysate on shoot proliferation of <i>M. dodecandrum</i>	63
	3.2.1.6	Rooting of <i>in vitro</i> plants	64
	3.2.1.6.1	Effect of auxins	64
	3.2.1.6.2	Effect of different medium strength	65
	3.2.1.6.3	Effect of solid and liquid medium	65
	3.2.1.7	Acclimatization of <i>in vitro</i> plants	66
	3.2.2	Direct shoot regeneration from leaf, petiole and internode explants of <i>Melastoma malabathricum</i>	68
	3.2.2.1	Plant material	68
	3.2.2.2	Culture medium for shoot induction	68
	3.2.3	Indirect shoot regeneration from leaf, petiole and Internode explants of <i>Melastoma malabathricum</i>	69
	3.2.3.1	Callus induction medium	70

	3.2.3.2 Callus maintenance medium	71
	3.2.3.2.1 Measurement of callus growth	72
	3.2.3.3 Regeneration from callus	73
3.3	Results and discussions	74
3.3.1	Establishment of <i>in vitro</i> plants of <i>Melastoma malabathricum</i> , <i>M. dodecandrum</i> , <i>M. decemfidum</i> and <i>Tibouchina semidecandra</i>	74
	3.3.1.1 Surface sterilization test	74
	3.3.1.2 Shoot initiation	79
	3.3.1.3 Shoot multiplication and elongation	90
	3.3.1.3.1 Effects of growth regulator and medium strength	90
	3.3.1.3.2 Effect of solid and liquid MS medium	107
	3.3.1.3.3 Effect of different types of media on shoot proliferation of <i>M. dodecandrum</i>	112
	3.3.1.3.4 Effect of sucrose on shoot proliferation of <i>M. dodecandrum</i>	117
	3.3.1.3.5 Effect of casein hydrolysate on shoot proliferation of <i>M. dodecandrum</i>	123
	3.3.1.4 Rooting of <i>in vitro</i> plant	128
	3.3.1.4.1 Effect of auxin	128
	3.3.1.4.2 Effect of different media strength	135
	3.3.1.4.3 Effect of solid and liquid medium	140
	3.3.1.5 Acclimatization of the <i>in vitro</i> plant	150
	3.3.1.6 Micropropagation protocol	153
3.3.2	Direct shoot regeneration from leaf, petiole and internode explants of <i>Melastoma malabathricum</i>	157
	3.3.2.1 Effect of media strength	157
	3.3.2.1.1 Leaf explants	157
	3.3.2.1.2 Petiole explants	166
	3.3.2.1.3 Internode explant	171
	3.3.2.2 Effect of growth regulators	175
	3.3.2.2.1 Leaf explants	175
	3.3.2.2.2 Petiole explants	177
	3.3.2.2.3 Internode explants	178
	3.3.2.3 Effect of explant position	179
	3.3.2.3.1 Leaf explant	179
	3.3.2.3.2 Petiole explant	180
	3.3.2.3.3 Internode explant	181
	3.3.2.4 Effect of explant type	181
	3.3.2.5 Direct shoot regeneration protocol	183

3.3.3	Indirect shoot regeneration from the leaf, petiole and internode explant of <i>Melastoma malabathricum</i>	183
3.3.3.1	Callus induction from different explants	183
3.3.3.1.1	Leaf explant	183
3.3.3.1.2	Internode explant	194
3.3.3.1.3	Petiole explant	207
3.3.3.2	Callus maintenance medium	220
3.3.3.2.1	Leaf derived callus	220
3.3.3.2.2	Petiole derived callus	223
3.3.3.2.3	Internode derived callus	226
3.3.3.3	Regeneration from callus	233
3.3.3.3.1	Effect of cytokinin alone	233
3.3.3.3.2	Effect of cytokinin and auxin	239
3.3.3.4	Indirect shoot regeneration protocol	242
<b>4</b>	<b>THE EFFECT OF GROWTH RETARDANTS ON THE ESTABLISHED <i>IN VITRO</i> PLANTLETS OF THE SELECTED SPECIES OF THE MELASTOMATACEAE FAMILY</b>	<b>244</b>
4.1	Introduction	244
4.2	Materials and Methods	245
4.2.1	Research location	245
4.2.2	Application of growth retardants	245
4.2.3	Data collection	246
4.2.4	Experimental design	249
4.3	Results	249
4.3.1	<i>Melastoma malabathricum</i>	249
4.3.2	<i>Melastoma decemfidum</i>	255
4.3.3	<i>Tibouchina semidecandra</i>	258
4.4	Discussions	263
4.4.1	Effect on the vegetative growth	263
4.4.2	Effect on the flowering	267
<b>5</b>	<b>CONCLUSION</b>	<b>269</b>
	<b>REFERENCES</b>	<b>276</b>
	<b>APPENDICES</b>	<b>301</b>
	<b>BIODATA OF THE AUTHOR</b>	<b>305</b>



## LIST OF TABLES

<b>Table</b>	<b>Page</b>
1.1 Gross Domestic Product (GDP) by industrial origin <sup>1</sup> in 1987 constant prices, Malaysia (RM million)	2
1.2 Distribution of orchid flower producers and production according to name of flowers, Malaysia 2000	4
1.3 Distribution of non-orchid flower producers and production According to name of flowers, Malaysia 2000	5
1.4 Distribution of ornamental plants producers and production according to name of ornamental plants, Malaysia 2000	6
1.5 Distribution of foliage producers and production according to name of foliage, Malaysia 2000	7
2.1 Production of flowers according to category of producers and states, 2000	13
2.2 Main growing areas of flowers according to districts and states, Malaysia 2000	14
2.3 Exports of floriculture product 1995 - 2001 (RM'000)	16
2.4 Export of floriculture commodity according to destination in 2001	17
3.1 Effect of different percentages of Clorox <sup>®</sup> (5.25% sodium hypochlorite) and exposure time on percentage of aseptic shoot tips and nodal segment of <i>Tibouchina semidecandra</i> , <i>Melastoma malabathricum</i> , <i>M. dodecandrum</i> and <i>M. decemfidum</i> after 4 weeks of culture	76
3.2 Effect of various concentrations of BAP and kinetin alone on shoot tips and nodal explant of <i>Tibouchina semidecandra</i> , <i>Melastoma malabathricum</i> , <i>Melastoma dodecandrum</i> and <i>Melastoma decemfidum</i>	80

3.3	Effect of different MS media strength and BAP supplied concentration on shoot multiplication and length for <i>Tibouchina semidecandra</i> , <i>Melastoma malabathricum</i> , <i>M. dodecandrum</i> and <i>M. decemfidum</i>	91
3.4	Effect of different types of media on shoot morphology of <i>Melastoma dodecandrum</i> . Data were recorded after 4 weeks of culture	115
3.5	Effect of different concentrations of sucrose on shoot morphology of <i>Melastoma dodecandrum</i> . Data were recorded after 4 weeks of culture	121
3.6	Effect of different concentrations of casein hydrolysate on shoot morphology of <i>Melastoma dodecandrum</i> . Data were recorded after 4 weeks of culture	125
3.7	Effect of different auxins (IBA, NAA and IAA) on <i>in vitro</i> rooting of <i>Tibouchina semidecandra</i>	129
3.8	Effect of different auxins (IBA, NAA and IAA) on <i>in vitro</i> rooting of <i>Melastoma malabathricum</i>	131
3.9	Effect of different auxins (IBA, NAA and IAA) on <i>in vitro</i> rooting of <i>Melastoma dodecandrum</i>	132
3.10	Effect of different auxins (IBA, NAA and IAA) on <i>in vitro</i> rooting of <i>Melastoma decemfidum</i>	133
3.11	Effect of different MS media strength on rooting of <i>in vitro</i> developed shoots of <i>Tibouchina semidecandra</i>	136
3.12	Effect of different MS media strength on rooting of <i>in vitro</i> developed shoots of <i>Melastoma malabathricum</i>	136
3.13	Effect of different MS media strength on rooting of <i>in vitro</i> developed shoots of <i>Melastoma dodecandrum</i>	138
3.14	Effect of different MS media strength on rooting of <i>in vitro</i> developed shoots of <i>Melastoma decemfidum</i>	138
3.15	Effect of different formulations of MS media on rooting of <i>in vitro</i> developed shoots of <i>Tibouchina semidecandra</i>	141
3.16	Effect of different formulations of MS media on rooting of <i>in vitro</i> developed shoots of <i>Melastoma malabathricum</i>	144

3.17	Effect of different formulations of MS media on rooting of <i>in vitro</i> developed shoots of <i>Melastoma decemfidum</i>	144
3.18	Effect of different formulations of MS media on rooting of <i>in vitro</i> developed shoots of <i>Melastoma dodecandrum</i>	144
3.19	The percentage of plantlet survival for four different species after 4 weeks transferred into soil	152
3.20	Effect of MS media strength, growth regulator (BAP and kinetin) and explant position on shoot formation from the leaf of <i>Melastoma malabathricum</i>	163
3.21	Effect of MS media strength, growth regulator (BAP and kinetin) and explant position on shoot formation from petiole of <i>Melastoma malabathricum</i>	168
3.22	Effect of MS media strength, growth regulator (BAP and kinetin) and explant position on shoot formation from internode of <i>Melastoma malabathricum</i>	172
3.23	Effect of different combinations of NAA and kinetin or BAP on callus induction from the leaf explants of <i>Melastoma malabathricum</i> after 4 weeks of culture	185
3.24	Effect of different combinations of dicamba and kinetin or BAP on callus induction from the leaf explants of <i>Melastoma malabathricum</i> after 4 weeks of culture	188
3.25	Effect of different combinations of picloram and kinetin or BAP on callus from the leaf explants of <i>Melastoma malabathricum</i> after 4 weeks of culture	190
3.26	Effect of different combinations of 2,4-D and kinetin or BAP on callus induction from the leaf explants of <i>Melastoma malabathricum</i> after 4 weeks of culture	193
3.27	Effect of different combinations of NAA and kinetin or BAP on callus induction from the internode explants of <i>Melastoma malabathricum</i> after 4 weeks of culture	196
3.28	Effect of different combinations of dicamba and kinetin or BAP on callus induction from the internode explants of <i>Melastoma malabathricum</i> after 4 weeks of culture	199

3.29	Effect of different combinations of picloram and kinetin or BAP on callus induction from the internode explants of <i>Melastoma malabathricum</i> after 4 weeks of culture	202
3.30	Effect of different combinations of 2,4-D and kinetin or BAP on callus induction from the internode explants of <i>Melastoma malabathricum</i> after 4 weeks of culture	205
3.31	Effect of different combinations of NAA and kinetin or BAP on callus induction from the petiole explants of <i>Melastoma malabathricum</i> after 4 weeks of culture	208
3.32	Effect of different combinations of dicamba and kinetin or BAP on callus induction from the petiole explants of <i>Melastoma malabathricum</i> after 4 weeks of culture	211
3.33	Effect of different combinations of picloram and kinetin or BAP on callus induction from the petiole explants of <i>Melastoma malabathricum</i> after 4 weeks of culture	214
3.34	Effect of different combinations of 2,4-D and kinetin or BAP on callus induction from the petiole explants of <i>Melastoma malabathricum</i> after 4 weeks of culture	217
3.35	Effect of cytokinin (BAP and kinetin) on shoot regeneration from the leaf derived callus of <i>Melastoma malabathricum</i>	234
3.36	Effect of cytokinin (BAP and kinetin) on shoot regeneration from petiole derived callus of <i>Melastoma malabathricum</i>	236
3.37	Effect of NAA and BAP on shoot regeneration from petiole derived callus of <i>Melastoma malabathricum</i>	240
4.1	The effect of paclobutrazol and flurprimidol on growth and flowering of <i>Melastoma malabathricum</i>	250
4.2	The effect of paclobutrazol and flurprimidol on growth and flowering of <i>Melastoma decemfidum</i>	256
4.3	The effect of paclobutrazol and flurprimidol on growth and flowering of <i>Tibouchina semidecandra</i>	261

## LIST OF FIGURES

<b>Figure</b>	<b>Page</b>
1.1 Flowering plant of Melastomataceae.	9
3.1 Shoot initiation from shoot tip and nodal segment of <i>Tibouchina semidecandra</i> after 4 weeks of culture.	81
3.2 Shoot initiation from shoot tip and nodal segment of <i>Melastoma malabathricum</i> after 4 weeks of culture	83
3.3 Shoot initiation from shoot tip and nodal segment of <i>Melastoma dodecandrum</i> after 4 weeks of culture	85
3.4 Shoot initiation from shoot tip and nodal segment of <i>Melastoma decemfidum</i> after weeks of culture	87
3.5 Shoot multiplication of <i>Tibouchina semidecandra</i> in full strength MS medium supplemented with (a) 0 (Bar = 0.3 cm), (b) 3 $\mu$ M BAP (Bar = 0.6 cm), (c) 6 $\mu$ M BAP (Bar = 0.3 cm), (d) 9 $\mu$ M BAP (Bar = 0.6 cm), (e) 12 $\mu$ M BAP (Bar = 0.3 cm), (f) 15 $\mu$ M BAP (Bar = 0.6 cm)	93
3.6 Shoot multiplication of <i>Tibouchina semidecandra</i> in half strength MS medium supplemented with (a) 0 (Bar = 1.0 cm), (b) 3 $\mu$ M BAP (Bar = 0.5 cm), (c) 6 $\mu$ M BAP (Bar = 0.5 cm), (d) 9 $\mu$ M BAP (Bar = 0.5 cm), (e) 12 $\mu$ M BAP (Bar = 0.6 cm), (f) 15 $\mu$ M BAP (Bar = 0.5 cm)	94
3.7 Shoot multiplication of <i>Tibouchina semidecandra</i> in quarter strength MS medium supplemented with (a) 0 (Bar = 0.4 cm), (b) 3 $\mu$ M BAP (Bar = 0.4 cm), (c) 6 $\mu$ M BAP (Bar = 0.5 cm), (d) 9 $\mu$ M BAP (Bar = 1.0 cm), (e) 12 $\mu$ M BAP (Bar = 0.5 cm), (f) 15 $\mu$ M BAP (Bar = 0.5 cm)	95
3.8 Shoot multiplication of <i>Melastoma malabathricum</i> in full strength MS medium supplemented with (a) 0 (Bar = 1.0 cm), (b) 3 $\mu$ M BAP (Bar = 0.8 cm), (c) 6 $\mu$ M BAP (Bar = 0.5 cm), (d) 9 $\mu$ M BAP (Bar = 0.5 cm), (e) 12 $\mu$ M BAP (Bar = 0.5 cm), (f) 15 $\mu$ M BAP (Bar = 0.5 cm)	96

- 3.9 Shoot multiplication of *Melastoma malabathricum* in half strength MS medium supplemented with (a) 0 (Bar = 0.6 cm), (b) 3  $\mu$ M BAP (Bar = 0.6 cm), (c) 6  $\mu$ M BAP (Bar = 0.6 cm), (d) 9  $\mu$ M BAP (Bar = 0.5 cm), (e) 12  $\mu$ M BAP (Bar = 0.6 cm), (f) 15  $\mu$ M BAP (Bar = 0.3 cm) 97
- 3.10 Shoot multiplication of *Melastoma malabathricum* in quarter strength MS medium supplemented with (a) 0 (Bar = 1.0 cm), (b) 3  $\mu$ M BAP (Bar = 0.6 cm), (c) 6  $\mu$ M BAP (Bar = 0.4 cm), (d) 9  $\mu$ M BAP (Bar = 0.4 cm), (e) 12  $\mu$ M BAP (Bar = 0.4 cm), (f) 15  $\mu$ M BAP (Bar = 0.4 cm) 98
- 3.11 Shoot multiplication of *Melastoma dodecandrum* in full strength MS medium supplemented with (a) 0 (Bar = 0.5 cm), (b) 3  $\mu$ M BAP (Bar = 0.3 cm), (c) 6  $\mu$ M BAP (Bar = 0.3 cm), (d) 9  $\mu$ M BAP (Bar = 0.3 cm), (e) 12  $\mu$ M BAP (Bar = 0.3 cm), (f) 15  $\mu$ M BAP (Bar = 0.3 cm) 100
- 3.12 Shoot multiplication of *Melastoma dodecandrum* in half strength MS medium supplemented with (a) 0 (Bar = 0.5 cm), (b) 3  $\mu$ M BAP (Bar = 0.5 cm), (c) 6  $\mu$ M BAP (Bar = 0.5 cm), (d) 9  $\mu$ M BAP (Bar = 0.5 cm), (e) 12  $\mu$ M BAP (Bar = 0.5 cm), (f) 15  $\mu$ M BAP (Bar = 0.5 cm) 101
- 3.13 Shoot multiplication of *Melastoma dodecandrum* in quarter strength MS medium supplemented with (a) 0 (Bar = 1.2 cm), (b) 3  $\mu$ M BAP (Bar = 0.6 cm), (c) 6  $\mu$ M BAP (Bar = 0.5 cm), (d) 9  $\mu$ M BAP (Bar = 0.5 cm), (e) 12  $\mu$ M BAP (Bar = 0.5 cm), (f) 15  $\mu$ M BAP (Bar = 0.5 cm) 102
- 3.14 Shoot multiplication of *Melastoma decemfidum* in full strength MS medium supplemented with (a) 0 (Bar = 1.0 cm), (b) 3  $\mu$ M BAP (Bar = 0.6 cm), (c) 6  $\mu$ M BAP (Bar = 0.6 cm), (d) 9  $\mu$ M BAP (Bar = 0.7 cm), (e) 12  $\mu$ M BAP (Bar = 0.6 cm), (f) 15  $\mu$ M BAP (Bar = 0.6 cm) 103
- 3.15 Shoot multiplication of *Melastoma decemfidum* in half strength MS medium supplemented with (a) 0 (Bar = 0.7 cm), (b) 3  $\mu$ M BAP (Bar = 0.6 cm), (c) 6  $\mu$ M BAP (Bar = 0.6 cm), (d) 9  $\mu$ M BAP (Bar = 0.5 cm), (e) 12  $\mu$ M BAP (Bar = 0.5 cm), (f) 15  $\mu$ M BAP (Bar = 0.5 cm) 104

3.16	Shoot multiplication of <i>Melastoma decemfidum</i> in quarter strength MS medium supplemented with (a) 0 (Bar = 1.0 cm), (b) 3 $\mu$ M BAP (Bar = 1.0 cm), (c) 6 $\mu$ M BAP (Bar = 1.0 cm), (d) 9 $\mu$ M BAP (Bar = 1.0 cm), (e) 12 $\mu$ M BAP (Bar = 0.3 cm), (f) 15 $\mu$ M BAP (Bar = 1.0 cm)	105
3.17	Effect of solid and liquid MS medium on the shoot number and length after 4 weeks of culture for (A) <i>Tibouchina semidecandra</i> , (B) <i>Melastoma malabathricum</i> , (C) <i>Melastoma dodecandrum</i> and (D) <i>Melastoma decemfidum</i> at different BAP concentration	109
3.18	Shoot multiplication in liquid medium.	110
3.19	Effect of different media on shoot number and length for <i>Melastoma dodecandrum</i> .	114
3.20	Shoot multiplication of <i>Melastoma dodecandrum</i> at different types of media	116
3.21	Effect of different concentration of sucrose concentration (%) on shoot number and length for <i>Melastoma dodecandrum</i> .	119
3.22	Shoot multiplication of <i>Melastoma dodecandrum</i> at different concentrations of sucrose in MS medium	120
3.23	Effect of different concentrations of BAP ( $\mu$ M) and casein hydrolysate (g/L) on shoot number and length for <i>Melastoma dodecandrum</i>	124
3.24	Shoot multiplication of <i>Melastoma dodecandrum</i> at different concentrations of casein hydrolysate	126
3.25	Rooting of <i>in vitro</i> plant of <i>Tibouchina semidecandra</i> in (A) solid (Bar = 1 cm) and (B) liquid (Bar = 0.8 cm) full strength MS medium with no growth regulator	143
3.26	Rooting of <i>in vitro</i> plant of <i>Melastoma malabathricum</i> in (A) solid (Bar = 0.8 cm) and (B) liquid (Bar = 0.8 cm) half strength MS medium with no growth regulator	145
3.27	Rooting of <i>in vitro</i> plant of <i>Melastoma decemfidum</i> in (A) solid (Bar = 0.8 cm) and (B) liquid (Bar = 0.8 cm) full strength MS medium with no growth regulator	147

3.28	Rooting of <i>in vitro</i> plant of <i>Melastoma dodecandrum</i> in (A) solid (Bar = 0.8 cm) and (B) liquid (Bar = 0.8 cm) quarter strength MS medium with no growth regulator	148
3.29	Rooting of <i>in vitro</i> plant of (A) <i>Tibouchina semidecandra</i> (Bar = 1.8 cm), (B) <i>Melastoma malabathricum</i> (Bar = 1.8 cm), (C) <i>Melastoma dodecandrum</i> (Bar = 1.6 cm) and (D) <i>Melastoma decemfidum</i> (Bar = 1.5 cm) in solid MS medium after 4 weeks of culture	149
3.30	Plantlets of (A) <i>Tibouchina semidecandra</i> (Bar = 2.0 cm), (B) <i>Melastoma malabathricum</i> (Bar = 1.6 cm), (C) <i>Melastoma dodecandrum</i> (Bar = 1.6 cm) and (D) <i>Melastoma decemfidum</i> (Bar = 1.6 cm) after 6 weeks of culture in rooting MS medium which are ready to be transferred to the soil	151
3.31	Successfully acclimatized plantlet growing in soil after one month of transfer to soil (A) <i>Tibouchina semidecandra</i> (Bar = 1.4 cm), (B) <i>Melastoma malabathricum</i> (Bar = 1.4 cm), (C) <i>Melastoma dodecandrum</i> (Bar = 1.8 cm) and (D) <i>Melastoma decemfidum</i> (Bar = 1.4 cm)	154
3.32	A two-month-old plantlets of (A) <i>Tibouchina semidecandra</i> (Bar = 1.3 cm), (B) <i>Melastoma malabathricum</i> (Bar = 1.6 cm), (C) <i>Melastoma dodecandrum</i> (Bar = 2.0 cm) and (D) <i>Melastoma decemfidum</i> (Bar = 1.5 cm) growing in the soil	155
3.33	Flowering of (A) <i>Tibouchina semidecandra</i> (Bar = 8.0 cm), (B) <i>Melastoma decemfidum</i> (Bar = 10.0 cm), (C) <i>Melastoma malabathricum</i> (Bar = 10.0 cm) and (D) <i>Melastoma dodecandrum</i> (Bar = 8.0 cm)	156
3.34	Protocol for rapid micropropagation of <i>Tibouchina semidecandra</i>	158
3.35	Protocol for rapid micropropagation of <i>Melastoma malabathricum</i>	159
3.36	Protocol for rapid micropropagation of <i>Melastoma dodecandrum</i>	160
3.37	Protocol for rapid micropropagation of <i>Melastoma decemfidum</i>	161