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 Poospooragi, 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

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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 μM 6-benzylaminopurine (BAP), while nodal explant was chosen for *T. semidecandra* shoot initiation in full strength MS medium supplemented with 20 μM BAP.

Shoot multiplication and elongation was optimal in half strength MS medium supplemented with 6 μM BAP for *T. semidecandra*, 9 μM BAP for *M. malabathricum* and 12 μM BAP for *M. decemfidum* while *M. dodecandrum* required quarter strength MS medium supplemented with 3 μM 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 μM BAP, followed by petiole at 6 μM BAP, and internode at 9 μM 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 μM dicamba and 2.5 μM kinetin for leaf explant, 10.0 μM NAA and 2.5 μM BAP for petiole explant, and 10.0 μM NAA and 2.5 μM kinetin for internode explant. Full

strength MS medium supplemented with 5.0 to 7.5 μM BAP alone had induced multiple shoots from the leaf-derived callus compared to 2.5 to 5.0 μM BAP for petiole-derived callus. A combination of 0.5 μM NAA and 5.0 μM BAP, however, was found to enhance shoot formation from the petiole-derived callus compared to when 5.0 μM 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. semidecandera*. 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.

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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 μM 6-bensilaminopurina (BAP) manakala eksplan buku batang dipilih untuk penghasilan pucuk bagi *T. semidecandra* bila dikultur dalam medium MS penuh yang mengandungi 20 μM BAP.

Pembiakan dan pemanjangan pucuk didapati paling sesuai dalam medium setengah MS yang mengandungi 6 μM BAP bagi *T. semidecandra*, 9 μM BAP bagi *M. malabathricum* dan 12 μM BAP bagi *M. decemfidum* manakala medium

seperempat MS yang mengandungi 3 μM BAP didapati sesuai bagi *Melastoma dodecandrum*. Medium MS tanpa pengawalatur pertumbuhan telah meningkatkan pengeluaran akar secara *in vitro* bagi pucuk berbanding medium yang mengandungi asid nafthalena 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 μM BAP, diikuti dengan petiol pada 6 μM BAP dan ruas batang pada 9 μM 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 mengandungi 2.5 μM dicamba dan 2.5 μM

kinetin bagi eksplan daun, 10.0 μM NAA dan 2.5 μM BAP bagi eksplan petiol dan 10.0 μM NAA dan 2.5 μM kinetin bagi eksplan ruas batang. Medium MS penuh yang mengandungi 5.0 hingga 7.5 μM BAP telah menghasilkan pucuk daripada kalus daun berbanding dengan 2.5 hingga 5.0 μM BAP bagi kalus petiol. Kombinasi 0.5 μM NAA dan 5.0 μM BAP telah meningkatkan penghasilan pucuk daripada kalus petiol berbanding bila hanya 5.0 μM 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*.

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I certify that an Examination Committee met on 17 January 2005 to conduct the final examination of Ramani Poospooragi 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:

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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 :

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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.

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RAMANI POOSPOORAGI

Date :

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES