UNIVERSITI PUTRA MALAYSIA

MICROPROPAGATION OF *MICHELIA CHAMPACA* L.

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MICROPROPAGATION OF *MICHELIA CHAMPACA* L.

By

ARMIYANTI

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

October 2009

Specially Dedicated

To

My Late Father and Late Mother:
Ansari Itam and Arifah

…….. who inspired, supported and gave me tremendous courage to be a good person

All my relatives and my beloved husband

…… who inspired, supported, patience and understanding during the period of my study

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

MICROPROPAGATION OF *MICHELIA CHAMPACA* L.

By

ARMIYANTI

October 2009

Chairman : Mihdzar Abdul Kadir, PhD

Faculty : Agriculture

The expansion in Champaca industry has led to an increasing demand for planting materials. A study was conducted with the objective of developing plant regeneration system for *Michelia champaca* L. through organogenesis and somatic embryogenesis. The study was conducted in consideration of the potential economic importance of the species. It consisted of development of sterilization procedure for field grown *M. champaca* shoot tip and nodal segment explants and plant regeneration through organogenesis and somatic embryogenesis of *M. champaca* either through solid culture medium and cell suspension culture technique. This was the first report on the establishment of *M. champaca* cell suspension culture.

All experiments in this study were conducted in a Completely Randomized Design (CRD). In the development of sterilization procedure for field grown shoot tips and nodal segments of *M. champaca* sterilization with 20% of clorox (20 minutes) + 70% alcohol (2 minutes) + 10% clorox (5 minutes) and 0.1% $HgCl_2$ (5 minutes)

successfully produced non-contaminated explants up to 80% for field-grown shoot tip and 50% for nodal explants.

In the experiment of determination of *M. champaca* seed viability, different tetrazolium concentrations (0.1, 0.5, and 1 % (w/v)) in combination with different immersion times (1, 2, 4, 6 and 8 hours) were used as treatments. The highest percentage of seed viability (100%) was obtained from all tetrazolium concentrations tested in combination with eight hours of immersion time.

Results on plant regeneration via organogenesis showed that MS (Murashige and Skoog, 1962) medium supplemented with 0.2 gL^{-1} of charcoal, 30 gL^{-1} (w/v) of sucrose and solidified with 3.9 gL^{-1} gelrite agar containing BAP at 6 mgL^{-1} in combination with 0.5 mgL^{-1}NAA was the most suitable for shoot induction from shoot tip derived from seedling explants with the highest mean number of shoots produced per explant (1.8) and mean shoot height of 4.53 cm. Meanwhile treatment containing 0.5 mgL^{-1} NAA was the most suitable for rooting with a percentage of shoot producing root at 70%, with mean number of roots formed per shoot at 2.5 and mean root length was 1.07 cm.

For plant regeneration through somatic embryogenesis using immature seed, the highest percentage of explants that responded to form somatic embryos (30%) and mean number of somatic embryos produced per explant (87.3) were obtained on treatment containing 2 mgL^{-1} NAA. However, higher frequency of somatic embryo

formation was achieved through cell suspension culture system. Liquid full strength MS medium supplemented with 2 mgL^{-1} (w/v) NAA produced a mean number 47.67±4.53 per ml cotyledonary somatic embryos.

For germination of somatic embryos produced from solid medium and through cell suspension culture, hormone free MS medium gave the highest percentage of somatic embryo germination at 56% and 34%, and normal plantlet production at 45% and 29% respectively.

Plantlet regeneration protocols through organogenesis and somatic embryogenesis of *M. champaca* which were successfully established in this study could be used for mass production of planting materials of this crop.

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

PEMBIAKAN MIKRO *MICHELIA CHAMPACA* L.

Oleh

ARMIYANTI

Oktober 2009

Pengerusi : Mihdzar Abdul Kadir, PhD

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Perluasan industri Champaca menyebabkan peningkatan permintaan kepada bahan tanaman. Suatu kajian telah dijalankan dengan matlamat untuk mengembangkan kaedah regenerasi tumbuhan keatas *Michelia champaca* L. melalui organogenesis dan embriogenesis soma. Kajian ini dijalankan berdasarkan potensi kepentingan ekonomi spesies ini. Kajian ini terdiri daripada pembinaan kaedah pensterilan keatas eksplan mercu pucuk dan keratan ruas *M. champaca* yang ditanam di lapangan dan regenerasi tumbuhan melalui organogenesis dan embriogenesis soma samada melalui media kultur pepejal dan teknik kultur ampaian sel. Kajian ini adalah laporan pertama seumpama terhadap penentuan kultur ampaian sel daripada *M. champaca*.

Kesemua kajian ini disusun dalam reka bentuk berawak penuh (CRD). Dalam pembinaan kaedah pensterilan keatas eksplan mercu pucuk dan keratan ruas *M. champaca* yang ditanam di lapangan, kaedah pensterilan dengan clorox 20% (20 minit) + alkohol 70% (2 minit) + clorox 10% (5 minit) dan $HgCl_2$ 0.1% (5 minit)

telah berjaya menghasilkan eksplan mercu pucuk yang ditanam di lapangan terbebas daripada kontaminasi sebanyak 80% dan 50% keatas eksplan buku ruas.

Pada kajian penentuan viabiliti biji *M. champaca*, kepekatan tetrazolium yang berbeza (0.1, 0.5, dan 1 % (b/v)) yang dikombinasikan dengan masa perendaman yang berbeza (1, 2, 4, 6 dan 8 jam) telah digunakan sebagai rawatan. Peratusan viabiliti biji tertinggi (100%) diperolehi dari keseluruhan kepekatan tetrazolium yang dicuba dengan kombinasi lapan jam perendaman.

Hasil keatas regenerasi tumbuhan melalui organogenesis telah menunjukkan bahawa media MS (Murashige and Skoog, 1962) yang ditambahkan 0.2 gL^{-1} arang, 30 gL^{-1} (b/v) sukrosa dan dipejalkan dengan 3.9 gL^{-1} agar gelrite dan mengandungi BAP 6 mgL^{-1} dikombinasikan dengan 0.5 mgL^{-1}NAA adalah yang paling sesuai untuk induksi tunas daripada eksplan mercu pucuk yang diperolehi daripada anak benih dengan purata bilangan tunas terhasil yang paling tinggi per eksplan (1.8) dan purata tinggi tunas sebesar 4.53 cm. Manakala, untuk pengakaran, rawatan yang mengandungi 0.5 mgL^{-1} NAA adalah paling sesuai untuk pengakaran dengan peratusan tunas yang menghasilkan akar sebanyak 70% dengan purata bilangan akar terbentuk setiap tunas adalah 2.5 dan purata panjang akar adalah 1.07 cm.

Untuk regenerasi tumbuhan melalui embriogenesis soma dengan menggunakan biji muda, peratusan paling tinggi daripada eksplan membentuk embrio soma (30%) dan purata bilangan embrio soma yang terhasil per eksplan (87.3) telah diperoleh

daripada rawatan yang mengandungi 2 mgL^{-1} NAA. Walau bagaimanapun, frekuensi pembentukan embrio soma yang lebih tinggi telah dicapai melalui kaedah kultur ampaian sel. Medium penuh cecair MS yang mengandungi 2 mgL^{-1} (b/v) NAA telah menghasilkan kotiledon embrio soma sebanyak 47.67±4.53 per ml.

Untuk percambahan embrio soma yang dihasilkan daripada kaedah media pejal dan kaedah kultur ampaian sel, medium MS yang tidak mengandungi hormon telah memberikan peratusan embrio soma bercambah paling tinggi masing-masing pada 56% dan 34% dan penghasilan anak pokok normal masing-masing pada 45% dan 29%.

Kaedah regenerasi anak pokok melalui organogenesis dan embriogenesis soma daripada *M. champaca* yang telah berjaya dilaksanakan dalam kajian ini dapat diguna pakai untuk pengeluaran besar-besaran bahan tanaman daripada tumbuhan ini.

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I certify that a Thesis Examination Committee has met on 9th October 2009 to conduct the final examination of Armiyanti on her thesis entitled "Micropropagation of *Michelia champaca* L." in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Pertanian Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

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Date : 24 December 2009

The thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science. Members of the Supervisory Committee were as follows:

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Date : 14 January 2010

DECLARATION

I hereby declare that this thesis is based on my original work except 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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ARMIYANTI

Date : 9th October 2009

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