
**UNIVERSITI PUTRA MALAYSIA**

***IN VITRO PLANT REGENERATION AND RAPD ASSESSMENT OF SOMACLONAL VARIATIONS IN SAFED MUSLI (Chlorophytum borivilianum Santapau & R.R.Fernandes)***

**NURASHIKIN BINTI KEMAT**

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**NURASHIKIN BINTI KEMAT**

**MASTER OF SCIENCE**

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**This thesis is dedicated to:**

*My dear mother, Norainon binti Arshad and my dear father, Kemat bin Adnan for their encouragement throughout my study,*

*my beloved husband, Sharul Nizam bin Ahmad for his sacrifies, understanding and commitment throughout my study*

*and my lovely son, Raes Zafran bin Sahrul Nizam for making my life more meaningful*

*words cannot express alone my gratitude to people above for their endless and boundless love,*

*and most of all for their ever continuous do'a for my life.*

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

**_IN VITRO_ PLANT REGENERATION AND RAPD ASSESSMENT OF SOMACLONAL VARIATIONS IN SAFED MUSLI (_Chlorophytum borivilianum_ Santapau & R.R.Fernandes)**

By

**NURASHIKIN BINTI KEMAT**

**May 2012**

**Chairman : Associate Professor Mihdzar Abdul Kadir, PhD**

**Faculty : Agriculture**

Safed musli (*Chlorophytum borivilianum*), is a traditional medicinal plant which belongs to liliaceae family and has an increasing demand due to its wide range of uses. It has an aphrodisiac property and form an important ingredient of herbal tonics prescribed in the Ayurvedic systems of medicine. Due to its medicinal properties and high demand in herbal industry, Safed musli has been introduced in Malaysia by Felda in 2008 under Felda Herbal Corporation Sdn. Bhd. The major constraint in the cultivation of Safed musli is long tuber dormancy at about 6 -7 months, so it's only one crop per year can be grown. Besides, the other constraints include shortage of quality planting material, lower tuber multiplication rate and impossible to obtain true to type and disease free plants either from seed tuber or seed. A study was carried out using *in vitro* propagation and

molecular characterization of Safed musli. *In vitro* culture has shown the advantage over the conventional methods of vegetative production as it ensures rapid rate of multiplication and disease free abundant plantlets. Young shoot buds of Safed musli were collected and sterilized. 100% of clean culture and plant survived in sterilization treatment with 70% alcohol (6 minutes), 50% Sodium hypochlorite (20 minutes), 0.1% aqueous mercuric chloride (15 minutes). Mass production of shoots was achieved on MS medium supplemented with 3 mg/L BAP which produced the highest mean number of shoots (18.9) and shoot length (6.0) cm. MS medium supplemented with 1 mg/L IBA which produced the highest mean number of roots (52.4) and root length (6.6) cm. In acclimatization, plantlets on media containing vermiculite : organic matter (1:1) showed the highest percentage of survival plants (83%) and mean number of shoot length (18.69) cm. Callus of Safed musli were induced from young shoot bud on MS medium containing 5 mg/L 2,4-D with the highest percentage of callus formation (66.66%) and mean weight of callus (10.65) g. For shoot regeneration, the highest percentage (66.67%) and the highest mean number of shoots per vial (9.6) was obtained on MS medium with 0.5 mg/L BAP . The RAPD assessment of somaclonal variations among Safed musli plantlet was performed. Out of 90 bands, 55 were polymorphic bands and 35 were monomorphic bands ranging between 115.43bp – 3388.24bp. RAPD marker effectively recognized the genetic difference among the *in vitro* shoots and the mother plant. From this study, it can improve the production efficiency and cost effectiveness in Safed musli.

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

**REGENERASI TANAMAN *IN VITRO* DAN PENILAIAN RAPD UNTUK VARIASI SOMAKLONAL PADA SAFED MUSLI (*Chlorophytum borivilianum* Santapau & R.R.Fernandes)**

Oleh

**NURASHIKIN BINTI KEMAT**

**Mei 2012**

**Pengerusi : Profesor Madya Mihdzar Abdul Kadir, PhD**

**Faculti : Pertanian**

Safed musli (*Chlorophytum borivilianum*), merupakan tumbuhan ubatan tradisional yang tergolong dalam keluarga liliaceae dan kini mempunyai permintaan yang semakin tinggi disebabkan oleh pelbagai kegunaannya. Ia mempunyai ciri-ciri *aphrodisiac* dan merupakan ramuan tonik herba yang penting di dalam sistem perubatan Ayurveda. Oleh kerana ciri-ciri perubatan dan permintaan yang tinggi dalam industri herba, tanaman Safed musli telah diperkenalkan di Malaysia oleh Felda pada tahun 2008 di bawah Felda Herbal Corporation Sdn. Bhd. Masalah utama dalam penanaman Safed musli ialah tempoh dormansi pada umbisi yang panjang iaitu 6-7 bulan jadi hanya satu pusingan tanaman sahaja dalam tempoh setahun. Antara masalah yang lain ialah kekurangan bahan tanaman yang berkualiti, kadar penggandaan umbisi yang lebih rendah dan masalah untuk mendapatkan kesaaman genetik sama seperti tanaman

induk dan bebas dari penyakit adalah sukar. Kajian telah dijalankan menggunakan pembiakan secara *in vitro* dan pencirian molekul tanaman Safed musli. Kultur *in vitro* telah menunjukkan kelebihan dalam pertumbuhan anak pokok, dengan penggandaaan yang tinggi dan menghasilkan tanaman bebas penyakit berbanding dengan kaedah penanaman konvensional. Pucuk muda Safed musli telah diambil dan steril. 100% kultur bersih dan pucuk tumbuh dalam rawatan pensterilan dengan menggunakan rawatan alkohol 70% (6 minit), 50% Natrium hipoklorit (20 minit), 0.1% larutan merkuri klorida (15 minit). Pengeluaran pucuk secara besar-besaran diperolehi pada medium MS yang ditambah dengan 3 mg/L BAP yang menghasilkan purata bilangan pucuk (18.9) dan panjang pucuk tertinggi (6.0) cm. MS Medium yang ditambah dengan 1 mg/L IBA menghasilkan purata bilangan akar (52.4) dan panjang akar (6.6) cm. Penyesuaian, anak pokok pada media yang mengandungi *vermiculite*: bahan organik (1:1) menunjukkan peratusan (83%) dan purata bilangan pucuk tertinggi (18.69) cm. Kalus Safed musli terhasil pada medium Murashige dan Skoog (MS) yang ditambah dengan 5 mg/L 2,4-D dan menghasilkan peratusan pembentukan kalus (66.66%) dan purata berat kalus tertinggi (10.65) g. Regenerasi pucuk daripada kalus diperolehi pada medium MS yang ditambah dengan 0.5 mg/L dengan peratusan penghasilan pucuk (66.67%), purata bilangan pucuk tertinggi (9.6) per vial. Penilaian RAPD untuk variasi somaklonal di kalangan anak pokok Safed musli telah dilakukan. Daripada 90 jalur yang diperolehi, 55 adalah jalur polimorfik dan 35 jalur monomorfik di antara 115.43 bp – 3388.24 bp. RAPD mengesan perbezaan genetik di antara pucuk *in vitro* dan induk tanaman (*mother plant*). Daripada kajian yang dilakukan ini, kecekapan pengeluaran dan keberkesanan kos bagi tanaman Safed musli dapat ditingkatkan

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Finally, I pray that I shall be a good steward of this honor.

I certify that a Thesis Examination Committee has met on 21st May 2012 to conduct the final examination of Nurashikin Binti Kemat on her thesis entitled "***In vitro* Plant Regeneration and RAPD Assessment of Somaclonal Variations in Safed musli (*Chlorophytum borivilianum*)"** in accordance wih the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master Science. The members of the Supervisory Committee were as follows:

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## DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or any other institution.

**NURASHIKIN BINTI KEMAT**

Date: 21 May 2012

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