

UNIVERSITI TEKNOLOGI MARA

***IN VITRO* PROPAGATION AND
COMPARATIVE STUDY ON
PHYTOCHEMICAL PROFILES OF *Pogostemon
cablin***

WAN NURUL HIDAYAH WAN ANUAR

Thesis submitted in fulfillment
of the requirements for the degree of
Master of Science

Faculty of Applied Sciences

April 2014

AUTHOR'S DECLARATION

I declare that the work in this thesis/dissertation was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

Name of student . Wan Nurul Hidayah Binti Wan Anuar

Student I.D. No. : 2009123981

Programme : Master of Science (AS780)

Faculty : Applied Sciences

Title : *In Vitro* Propagation and Comparative Study on
Phytochemical Profiles of *Pogostemon cablin*

Signature of Student :

Date : April 2014

ABSTRACT

Pogostemon cablin is an important aromatic plant producing patchouli oil from its leaves' extraction. The main objective of this study was to develop an effective method for *in vitro* propagation of Malaysian cultivated *P. cablin*. The effect of varying the strength of MS medium was investigated using node explants. Different concentrations of BAP and NAA were added to both full and half strength MS (Murashige and Skoog) medium for regeneration of this plant. The MS media supplemented with 0.25 mg/l BAP gave the highest number of shoots and length followed by the combinations of hormones 1.0 mg/l BAP and 0.25 mg/l NAA. The *in vitro* plantlets were then acclimatized in two different substrates that were soil and the soil mixture with vermiculite which was found with 100% survival rate obtained by *in vitro* grown *P. cablin* plants acclimatized in soil. The leaves part were harvested from both sources of *in vitro* and grown plants and *ex vitro* mother plant of *P. cablin* for further extraction of its essential oil via hydrodistillation and then were analyzed using GC-MS. The gas chromatogram of the extracted oils from both samples showed similar essential oil profiles with relative amount of patchouli alcohol were higher in the *in vitro* grown plants than that in *ex vitro* mother plant. The monomorphic banding pattern analyzed using two universal primers, cytochrome c oxidase (cox1) and maturase K (matK) indicated that the true to type plants of *P. cablin* were established from *in vitro* propagation through direct propagation from node explants. Rapid and high multiplication frequency as well as the essential oil content ensures the efficacy of the protocol developed for the production of this industrially important aromatic plant.

TABLE OF CONTENTS

| | Page |
|---|-------------|
| AUTHOR'S DECLARATION | ii |
| ABSTRACT | iii |
| ACKNOWLEDGEMENTS | iv |
| TABLE OF CONTENTS | v |
| LIST OF TABLES | ix |
| LIST OF FIGURES | x |
| LIST OF ABBREVIATIONS | xii |
| | |
| CHAPTER ONE: INTRODUCTION | |
| 1.1 Background and Problem Statement | 1 |
| 1.2 Significant of Study | 4 |
| 1.3 Objectives of Study | 5 |
| 1.4 Scope and Limitation | 5 |
| | |
| CHAPTER TWO: LITERATURE REVIEW | |
| 2.1 Medicinal and Aromatic Plant | 6 |
| 2.2 <i>Pogostemon cablin</i> | 7 |
| 2.2.1 Description of <i>P. cablin</i> | 7 |
| 2.2.2 Usages of Patchouli | 8 |
| 2.2.3 Commercial Importance of <i>P. cablin</i> | 10 |
| 2.3 Establishment of <i>In Vitro</i> Culture Technology | 11 |
| 2.3.1 <i>In Vitro</i> Technology | 11 |
| 2.3.1.1 Basal Media Strength | 12 |
| 2.3.1.2 Genotype | 13 |
| 2.3.1.3 Shoot and Root Induction | 13 |
| 2.3.1.4 Subculture | 15 |
| 2.4 Plant Growth Regulators | 15 |
| 2.4.1 Auxins | 16 |
| 2.4.2 Cytokinin | 17 |
| 2.5 Acclimatization | 17 |

| | | |
|---------|--|----|
| 2.6 | Plant Essential Oil | 18 |
| 2.7 | Phytochemical Extraction Process | 20 |
| 2.8 | Phytochemical Analysis Using Gas Chromatography- Mass Spectrometry (GC-MS) | 20 |
| 2.9 | Genetic Stability Of Tissue Culture Grown Plants | 22 |
| 2.9.1 | High Molecular Weight (HMW) DNA Isolation | 22 |
| 2.9.1.1 | Extraction of Genomic DNA | 23 |
| 2.9.2 | PCR Amplification of Plant Genes | 24 |
| 2.9.2.1 | Cytochrome c Oxidase (<i>cox1</i>) Primer | 24 |
| 2.9.3.2 | Maturase K (<i>matK</i>) Primer | 25 |

CHAPTER THREE: *IN VITRO* PROPAGATION OF *P. cablin*

| | | |
|-------|--|----|
| 3.1 | Introduction | 26 |
| 3.2 | Material and Method | 27 |
| 3.2.1 | Plant Material | 27 |
| 3.2.2 | Node Explants Sterilization | 27 |
| 3.2.3 | Media Preparation | 30 |
| 3.2.4 | Induction of Shoots and Roots | 30 |
| 3.2.5 | Subculture | 31 |
| 3.2.6 | Statistical Analysis | 31 |
| 3.3 | Result and Discussion | 31 |
| 3.3.1 | Effects on Sterilization Techniques in <i>P. cablin</i> Explants | 31 |
| 3.3.2 | Effects of Different Strength of MS Media on Propagation of <i>P. cablin</i> | 37 |
| 3.3.3 | Effects of BAP and NAA on Shoot and Root Development of <i>P. cablin</i> | 40 |
| 3.4 | Conclusion | 46 |

CHAPTER FOUR: ACCLIMATIZATION OF *P. cablin* PLANTLETS

| | | |
|---------|----------------------|----|
| 4.1 | Introduction | 48 |
| 4.2 | Materials and Method | 49 |
| 4.2.1 | Acclimatization | 49 |
| 4.2.1.1 | Plant Material | 50 |