UNIVERSITI PUTRA MALAYSIA

ADVENTITIOUS ROOT FORMATION OF HARDWOOD AND SOFTWOOD STEM CUTTINGS OF *Pogostemon cablin* (NILAM) AS AFFECTED BY DIFFERENT INDOLE-3-BUTYRIC ACID (IBA) CONCENTRATIONS

NOR AINA AZRIN

FP 2015 82

ADVENTITIOUS ROOT FORMATION OF HARDWOOD AND SOFTWOOD STEM CUTTINGS OF *Pogostemon cablin* (NILAM) AS AFFECTED BY DIFFERENT INDOLE-3-BUTYRIC ACID (IBA) CONCENTRATIONS

NOR AINA BINTI AZRIN

FACULTY OF AGRICULTURE

UNIVERSITI PUTRA MALAYSIA

SERDANG, SELANGOR

2014/2015

ADVENTITIOUS ROOT FORMATION OF HARDWOOD AND SOFTWOOD STEM CUTTINGS OF *Pogostemon cablin* (NILAM) AS AFFECTED BY DIFFERENT INDOLE-3-BUTYRIC ACID (IBA) CONCENTRATIONS

by

NOR AINA BINTI AZRIN

A project submitted to the Faculty of Agriculture,
Universiti Putra Malaysia,
In fulfilment of the requirement of PRT 4999 (Project)
for the award of the degree of
Bachelor of Horticultural Science

FACULTY OF AGRICULTURE

UNIVERSITI PUTRA MALAYSIA

SERDANG, SELANGOR

2014/2015

CERTIFICATION

This project entitled '**ADVENTITIOUS ROOT FORMATION OF HARDWOOD AND SOFTWOOD STEM CUTTINGS OF Pogostemon cablin (NILAM) AS AFFECTED BY DIFFERENT INDOLE-3-BUTYRIC ACID (IBA) CONCENTRATIONS**' is prepared by Nor Aina binti Azrin and submitted to the Faculty of Agriculture in fulfilment of the requirement of the **PRT 4999 (Project)** for the award of the degree of **Bachelor of Horticultural Science**.

Student's name: Student's signature:

Nor Aina binti Azrin ----------

Certified by:

(**Assoc. Prof. Dr. Thohirah Lee Abdullah**)

Project Supervisor,

Department of Crop Science,

Universiti Putra Malaysia.

Date: ----------

ACKNOWLEDGEMENT

First of all, I would like to express my deepest gratitude to Allah S.B.T. for His grace that I managed to complete my final year project in these two semesters. Next, I would like to thank my supervisor, Assoc. Prof. Dr. Thohirah Lee Abdullah for her guidance, help, ideas and comments throughout the completion of this project. Thank you very much.

Also, my appreciations go to my lovely lecturers, Assoc. Prof. Dr. Saleh bin Kadzimin, Assoc. Prof. Dr. Adam Puteh, Prof Dr. Ghizan Saleh, Assoc. Prof. Dr. Che Fauziah Ishak, Assoc. Prof. Dr. Mohd Ridzwan A. Halim, Assoc. Prof. Dr. Siti Hajar Ahmad and Dr. Siti Zaharah Sakimin for their guidance. A lot of thanks to lab asistances, Mr. Mat Yusof Suki, Mr. Azahar Othman, Mr. Mazlan Bangi, Mr. Mohd Yusoff Mohd Yassin and Mr. Baharin Mohd Amin and also to the postgraduate students, Mr. Chen Xing Wei, Mr. Taweesak and Mr Kang Seong Hun for their kind helps.

Not forgetting, thank you to my friends who helped me a lot throughout this project. Thank you too to my beloved family who never fail to give me their supports and encouragements in order for me to complete this project.

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF ANOVA

ADVENTITIOUS ROOT FORMATION OF HARDWOOD AND SOFTWOOD STEM CUTTINGS OF *Pogostemon cablin* (NILAM) AS AFFECTED BY DIFFERENT INDOLE-3-BUTYRIC ACID (IBA) CONCENTRATIONS

ABSTRACT

Patchouli or its scientific name, *Pogostemon cablin* is an herbaceous perennial plant which is a native to India and Malaysia but nowadays, it is being cultivated in many tropical countries for the value of its essential oil which can be extracted to produce various profitable products. There are two methods in cultivating this plant which are by sexual or asexual propagation. Patchouli is a plant that rarely flowers and if it does, the flowering period is short and it is non-synchronized with other Patchouli plants. Because of this, propagation is done by asexual reproduction. Commonly, there are two methods of vegetative reproduction which are by stem cuttings and *in vitro* culture. Two studies were conducted in this project. Study one was done to observe the adventitious root formation of hardwood stem cuttings of *Pogostemon cablin* as affected by four different Indole-3-Butyric Acid (IBA) concentrations. These IBA concentrations were 0, 20, 40 and 60 mg/L. Study two was conducted to observe the adventitious root formation of softwood stem cuttings of *Pogostemon cablin* as affected by four different Indole-3-Butyric Acid (IBA) concentrations. These IBA concentrations were the same as the concentrations used in study one. The experimental design used in both studies was Randomized Complete Block Design (RCBD). Parameters used in both studies were the same which were days taken to root, root length, rooting percentage, root fresh weight and root dry weight. These experiments were done by treating a number of cuttings from study one and study two with these different IBA concentrations and

were left to produce their root in a favourable condition over a period of 6 weeks. All data were analysed by Analysis of Variance (ANOVA) using Statistical Analysis System (SAS). Means comparison were used to determine the best IBA concentration treatments for both studies by using Less Significant Difference (LSD). In study one, result showed that 20 mg/L IBA concentration is the best treatment for hardwood stem cuttings of *Pogostemon cablin*. Study two proved that 60 mg/L IBA concentration is the best treatment for softwood stem cuttings of *Pogostemon cablin*.

KESAN KEPEKATAN INDOLE-3-BUTYRIC ACID (IBA) TERHADAP PEMBENTUKAN AKAR OLEH TANAMAN *Pogostemon cablin* (NILAM) MELALUI KERATAN BATANG KAYU KERAS DAN KAYU LEMBUT

ABSTRAK

Nilam atau nama saintifiknya, *Pogostemon cablin* adalah tanaman jenis saka herba yang berasal dari India dan Malaysia tetapi pada hari ini, ia banyak ditanam di negara-negara beriklim tropika kerana nilai minyak patinya yang boleh diekstrak bagi menghasilkan pelbagai barangan dan mendatangkan keuntungan. Terdapat dua cara pembiakan tanaman ini iaitu melalui pembiakan seksual atau aseksual. Nilam merupakan tanaman yang susah untuk berbunga dan jika ia berbunga sekalipun, masa pembungaan adalah singkat dan tidak selaras dengan masa pembungaan pokok Nilam yang lain. Oleh kerana itu, pembiakan harus dilakukan melalui pembiakan aseksual. Pada kebiasaannya, dua cara pembiakan tampang dilakukan iaitu dengan menggunakan keratan batang dan kultur *in vitro*. Dua kajian telah dijalankan di dalam projek ini. Kajian pertama adalah mengenai pemerhatian terhadap pembentukan akar daripada keratan batang *Pogostemon cablin* berkayu keras setelah dipengaruhi empat konsentrasi Indole-3-Butyric Acid (IBA) yang berbeza yaitu 0, 20, 40 dan 60 mg/L. Kajian kedua adalah mengenai pemerhatian terhadap pembentukan akar daripada keratan batang *Pogostemon cablin* berkayu keras setelah dipengaruhi empat konsentrasi Indole-3-Butyric Acid (IBA) yang berbeza. Konsentrasi-konsentrasi IBA ini adalah sama seperti yang digunakan di dalam kajian satu. Reka bentuk eksperimen yang digunakan di dalam kedua-dua kajian ini adalah Rekabentuk Blok Rawak Lengkap (RCBD). Parameter-parameter yang digunakan di dalam kedua-dua kajian ini adalah sama iaitu bilangan hari bagi pembentukan akar,

panjang akar, peratus pengakaran, berat basah akar dan berat kering akar. Kajian-kajian ini dijalankan dengan merawat sejumlah keratan batang dari kajian pertama dan kajian kedua dengan konsentrasi IBA yang berbeza serta dibiarkan selama 6 minggu bagi proses pembentukan akar di dalam kondisi yang sesuai. Kesemua data dianalisis dengan Analysis of Variance (ANOVA) menggunakan Statistical Analysis System (SAS). Perbandingan purata digunakan bagi menentukan rawatan konsentrasi IBA terbaik untuk kedua-dua kajian dengan menggunakan Less Significant Difference (LSD). Kajian pertama menunjukkan konsentrasi IBA pada 20 mg/L adalah terbaik untuk keratan batang *Pogostemon cablin* berkayu keras. Kajian kedua membuktikan bahawa rawatan IBA pada konsentrasi 60 mg/L IBA adalah yang terbaik untuk keratan batang *Pogostemon cablin* berkayu lembut.

CHAPTER 1

1.0 INTRODUCTION

Pogostemon cablin is a species of herbaceous plant. Commonly, it is known as Patchouli or Nilam in Malaysia. This Malaysian and Indian originated plant comes from Lamiaceae family and from the genus of *Pogostemon* (Hu et al., 2005). Asian countries are known as 'the land of aromatic plants' as many aromatic plants grow in these countries because of the favourable conditions in tropical areas (Chomchalow, 2002). The environment has to be warm and moist. Nowadays, due to this plants' essential oil's value, it is being commercialized in other tropical countries with Indonesia as the top world producer (Miyazawa et al., 2000). It lives well especially on foothills of Himalayas (Maheswari et al., 1993). Even though it is originally came from Malaysia, due to pests and diseases outbreak, this species is no longer abundant in Malaysia (Meena, 1996) that sadly, some of the natives do not even know what Nilam Plant is.

Patchouli is an herbaceous plant that is categorized under bushy perennial plants with fragrant leaves (Thohirah, 2014). It has erect stems and can reach to a height of 1.09 m (Wu et al., 2011). It can live very healthy under areas with good Nitrogen (N) fertilizer application and good irrigation that it can revive quickly when water is supplied after dehydration due long exposure under sunlight. Even though it is suited to the tropical environment, it cannot be cultivated under direct sunlight (Singh et al., 2002). This plant is able to live well in areas with evenly distributed rainfall which is about 150-300 cm per year. It reaches its maturity after

6 months of cultivation. It is a fast growing plant that is able to generate one's economy.

Pogostemon cablin is being cultivated commercially as it contains essential oil that can be used in various ways. This essential oil is extracted from Patchouli's leaves by steam distillation method which requires the cell wall to be ruptured (Singh et al., 2002). One of the usages is to treat patients with various illnesses such as common cold, cerebral stroke, headache and other pains. Besides that, the oil is being used in perfumery industry as it has this scented smell to make soaps, cosmetics and oral hygiene products (Lu et al., 2009). Elderly people of Malaysia used the leaves as fragrances and place them in the Holy Quran (Thohirah, 2014). It also has therapeutic activities that can be used in treating people with nausea, diarrhoea and to stimulate one's appetite (Wan et al., 2009). Other than that, Patchouli's oil can be used to repel insects as it has the property of insecticides (Kim et al., 2008). It is also used in food and beverages as natural flavouring.

Methods in cultivating Patchouli are divided into two. They are sexual cultivation, which is by seeds and asexual cultivation, which is by using vegetative parts of Patchouli. However, records shown that there was not much sexual cultivation of this plant as it hardly flowers and if it does, it will be in a very short period and not synchronized with other Patchouli plant. That is why seed cultivation is rare. Normally, it is cultivated vegetatively by using stem cuttings or *in vitro* method (Hasanah dan Setiari, 2007). Either softwood or hardwood stem cuttings can be used for plant propagation. Any type of plant materials used has to

be maintained well in its favourable conditions in order to grow a well and healthy offspring of Patchouli.

In every plant, there are chemicals called plant hormones or plant growth regulators that regulate the plant's growths and developments. Basically, it consists of Auxin and Cytokinin where Auxin has the role of promoting roots while Cytokinin is used to promote shoots. There are endogenous Auxin such as Indole Acetic Acid (IAA) and exogenous Auxin such as Indole-3-Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA) which are produced by microorganisms. IBA can be applied to induce root initiation but the concentration has to be precise in order to produce optimum number of roots (Hasanah dan Setiari, 2007). In this study, experiments were conducted to find the best concentration of IBA to be applied on stem cuttings of *Pogostemon cablin*.

1.1 Research Objectives

1. To determine the optimum rate of Indole-3-Butyric Acid (IBA) affecting the hardwood stem cuttings.
2. To determine the optimum rate of Indole-3-Butyric Acid (IBA) affecting the softwood stem cuttings.

BIBLIOGRAPHY

Alvarez, R., Nissen, S.J. and Sutter, E.G. (1989). Relationship between Indole-3-Acetic Acid Levels in Apple (*Malus pumila* Mill) Rootstocks Cultured in Vitro and Adventitious Root Formation in the Presence of lndole-3-Butyric Acid. Plant Physiology. 89:439-443.

Chomchalow, N. (2002). Production of Aromatic Plants in Asia - An Overview. www.journal.au.edu/au_techno/2002/jul2002/article8.pdf. Retrieved 03 July 2012.

Davies, P.J. (1995). The plant hormones: Their nature, occurrence, and functions. In Plant Hormones and Their Roles in Plant Growth and Development, ed. P.J. Davies. pp.1-5. Ithaca, New York.

Djauhariya dan Rahardjo. (2004). Pengaruh Zat Pengatur Tumbuh terhadap Keberhasilan Perbanyakan Tanaman Mengkudu dengan Stek Batang. Prosiding Seminar Nasional XXV Tumbuhan Obat Indonesia. pp. 79-86.

Eipstein, E. and Lavee, S. (1984). Conversion of Indole-3-butyric Acid to Indole-3-acetic Acid by Cuttings of Grapevine *(Vitis vinifera)* and Olive *(Olea europea*). Plant & Cell Physiology. 25(5):697-703.

Epstein, E., Chen, K.H. and Cohen, J.D. (1989). Identification of indole-3-butyric acid as an endogenous constituent of maize kernels and leaves. Plant Growth Regulation. 8(3):215-223.

Federal Regulations Code. (2002). Food and Drugs Administration, from the U.S. Government Printing Office via GPO Access [CITE: 21CFR172.510], U.S.A., 3:49–52.

Hartmann, H.T., Kester, D.E., Davies, F.T. and Geneve, R. (1983). Plant Propagation: Principles and Practices. pp. 298-342. New Jersey: Prentice-Hall.

Hasanah, F.N. dan Setiari, N. (2007). Pembentukan Akar pada Stek Batang Nilam (*Pogostemon cablin* Benth.) setelah direndam Iba (Indol Butyric Acid) pada Konsentrasi Berbeda. Buletin Anatomi dan Fisiologi 15(2):1-6.

Hsu, H.C., Yang, W.C., Tsai, W.J., Chen, C.C., Huang, H.Y. and Tsai, Y.C., (2006). Alpha-bulnesene, a novel PAF receptor antagonist isolated from *Pogostemon cablin*. Biochemical Biophysical Research Communications. 345(3):1033-1038.

Hu, L.F., Li, S.P., Liu, J.J., Gao, J.L., Yang, F.Q. and Wang, Y.T. (2005). GC-MS fingerprint of *Pogostemn cablin* in China. Journal of Pharmaceutical and Biomedical Analysis. 42(2):200-206.

Irawati, H. (2005). Pertumbuhan Stek Batang Tanaman Daun Dewa (*Gynura pseudochina*) Setelah Direndam dengan IBA (*Indol Butyric Acid*). Universitas Diponegoro, Semarang.

Jones, L.H. and Krishnadethan, P.P.S. (1973). Factors influencing production of patchouli sesquiterpenes in cultured cells and regenerated plantlets. Phytochemistry. 12:1513-1514.

Kim, H.W., Cho, S.J., Kim, B.Y., Cho, S.I. and Kim, Y.K. (2008). *Pogostemon cablin* as ROS Scavenger in Oxidant-induced Cell Death of Human Neuroglioma Cells. Evidence-Based Complementary and Alternative Medicine (eCAM). 7(2):239-247.

Klerk, G.J.D., Krieken, W.V.D. and Jong, J.C.D. (1999). Review, The formation of adventitious roots: new concepts, new possibilities. In Vitro Cellular and Developmental Biology-Plant. 35:189–199.

Lestari, E.G. (2011). Peranan Zat Pengatur Tumbuh dalam Perbanyakan Tanaman melalui Kultur Jaringan. Jurnal Agro*Biogen* 7(1):63-68.

Lu, T.C., Liao, J.C., Huang, T.H., Lin, Y.C., Liu, C.Y., Chiu, Y.J. and Peng, W.H. (2009). Analgesic and Anti-Inflammatory Activities of the Methanol Extract from *Pogostemon cablin*. Evidence-Based Complementary and Alternative Medicine. 2011:Article ID 671741, 9 pages.

Maheswari, M.L., Kumar, V., Sharma, N. and Chandel, K.P.S. (1993). Patchouli-An Indian perspective. 37:9-11.

Meena, M. (1996). Regeneration of patchouli (*Pogostemn cablin* Benth.) plants from leaf and node callus, and evaluation after growth in the field. Plant Cell Reports. 15:991-994.

Miyazawa, M., Okuno, Y., Nakamura, S. and Kosaka, H. (2000). Antimutagenic activity of flavonoids from *Pogostemn cablin*. Journal of Agricultural and Food Chemistry. 48(3):642-647.

Nordström, A.C., Jacoks, F.A. and Eliasson, L. (1991). Effect of Exogenous Indole-3-Acetic Acid and Indole-3-Butyric Acid on Internal Levels of the Respective Auxins and Their Conjugation with Aspartic Acid during Adventitious Root Formation in Pea Cuttings. Plant Physiology. 96(3):856-861.

Prastowo, N.H., Roshetko, J.M., Maurung, G.E.S., Nugraha, E., Tukan, J.E. dan Harum, F. (2006). Tehnik Pembibitan dan Perbanyakan Vegetatif Tanaman Buah. Bogor: World Agroforestry Centre (ICRAF) & Winrock International.

Rahardja, P.C. dan Wiryanta, W. (2003). Aneka Memperbanyak Tanaman. Jakarta: Agromedia Pustaka.

Rismunandar. (1995). Budidaya Bunga Potong. Jakarta: Penebar Swadaya.

Rukmana, R. (2004). Nilam: Prospek Agribisnis dan Teknik Budi Daya. Yogyakarta: Penerbit Kanisius.

Santoso, H.B. (2000). Bertanam Nilam. Jakarta: Penerbit Kanisius.

Sharma, N., Chandel, K.P.S. and Maheshwari, M.L. (1992). Indian Perfumer. 36:70-74.

Sharma, R. (2003). Medicinal Plants of India- An encyclopedia. New Delhi: Daya Publishing House. pp. 197-198.

Singh, M., Sharma, S. and Ramesh, S. (2002). Herbage, oil yield and oil quality of patchouli [*Pogostemon cablin* (Blanco) Benth.] influenced by irrigation, organic mulch and nitrogen application in semi-arid tropical climate. Industrial Crops and Products. 16:101-107.

Srivastava, L.M. (2002). Plant Growth and Development. Hormones and the Environment. Oxford: Academic Press.

Sutter, E.G. and Cohen, J.D. (1992). Measurement of indolebutyric Acid in plant tissues by isotope dilution gas chromatography-mass spectrometry analysis. Plant Physiology. 99(4):1719–1722.

Suwandiyati, N.D. (2009). Pengaruh Asal Bahan Setek dan Dosis Pupuk Kandang Sapi terhadap Pertumbuhan Bibit Nilam (*Pogostemon Cablin* Benth). Universiti Sebelas Maret, Surakarta.

Swarup, R., Perry, P., Hagenbeek, D., Straeten, D.V.D., Beemster, G.T.S., Sandberg, G., Bhalerao, R., Ljung, K. and Bennett, M.J. (2007). Ethylene upregulates auxin biosynthesis in Arabidopsis seedlings to enhance inhibition of root cell elongation. The Plant Cell. 19:2186–2196.

Thohirah Lee Abdullah, Lecturer, Faculty of Agriculture, Universiti Putra Malaysia, personal communication. 1 September 2014.

Wan, J.B., Li, S.P., Wang, Y.T., Liu, J. and Kang, J.X. (2009). Quality Control of Chinese Meddicine Based on Technology Innovation. North American Journal of Medicine and Science. 2(4):152-155.

Wu, L.H., Wu, Y.G., Guo, Q.S., Li, S.P., Zhou, K.B. and Zhang, J.F. (2011). Comparison of genetic diversity in *Pogostemon cablin* from China revealed by RAPD, morphological and chemical analyses. Journal of Medicinal Plants Research. 5(18):4549-4559.

Zaim, M, Ali, A., Joseph, J. and Khan, F. (2013). Serological and Molecular Studies of a Novel Virus Isolate Causing Yellow Mosaic of Patchouli [*Pogostemon cablin* (Blanco) Benth]. Public Library of Science (PLoS ONE). 8(12):e83790.