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PROTEOMIC PROFILES OF FLORAL AND LEAF TISSUES OF Michelia alba DC.

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By

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

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia
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PROTEOMIC PROFILES OF FLORAL AND LEAF TISSUES OF *Michelia alba* DC.

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October 2012

Chairman : Associate Professor Mohd Puad Abdullah, PhD

Faculty : Biotechnology and Biomolecular Sciences

Michelia alba DC is a well known fragrant plant that is rich in high quality essential oils of economic importance especially for the perfumery industry. At biochemical level, the exact metabolic pathway that is responsible for the generation of the essential oils in *M. alba* and the regulatory mechanism is poorly understood. Therefore, profiling of proteomes from the organs that are involved in the formation of the oils is an approach to obtain the potential candidate proteins related to the oils production. This study was carried out as an attempt to identify differentially expressed proteins during the development of fragrant-producing organs by optimizing the methods of protein extraction and profiling the proteomes at different developmental stages. The objectives of this study were i) to optimize the protein extraction protocols for profiling of *M. alba* proteomes by two-dimensional gel electrophoresis (2-DGE), ii) to optimize the reproducibility of two-dimensional polyacrylamide gel electrophoresis (2-DGE) in flower and leaf tissues of *M. alba*, iii) to profile the proteomes of the leaf and flower of *M. alba* using 2-DGE at different developmental stages of the organs, iv) to find trends and correlation of the

proteomic profile in term of regulation of unique proteins and v) to identify uniquely proteins expressed for fragrance production throughout developmental stages.

Two-dimensional gel electrophoresis (2-DGE) is currently one of the methods that can offer a relatively good separation power for proteomics analysis. In this study, traditional 2-DGE was used to profile the proteomes of the leaf and flower organs of *M. alba* at different developmental stages. A prerequisite for a reproducible, high resolution of proteomic protein separation on the 2-DGE, is the availability of protein extraction protocol that is optimized for the plant especially for *M. alba* as a woody plant. Three different protein extraction methods were evaluated for their abilities to extract high amounts of soluble protein, to produce high resolution of protein separation of the crude extract on 1D and 2D gels, and to eliminate maximum amounts of non-protein contaminants from the extract. The protocol based on the use of Bis-Tris/acetone was the best protein extraction protocol for *M. alba* based on the criteria above and was significantly highest in total protein content in both flower and leaf tissues ($p<0.05$).

Using this protein extraction protocol, the proteomes of different developmental stages of M. *alba* leaf and flower were successfully resolved on 2-DGE. The numbers of protein spots and their expression levels were monitored during the development of the organs. Generally the numbers of protein spots increased during the development of the organs. During the development of *M. alba* flower, the numbers of protein spots reached its peak when the flower was about to open, but then decreased once the flower bloomed. Profiling of the leaf and flower proteomes from different developmental stages generated 80 to 210 spots, of which some

showed differentially-expressed patterns. Five protein spots from the flower proteome and eight from the leaf were successfully identified based on peptide mass fingerprinting method. These proteins are categorized into three major classes according to their functions: primary metabolism, developmental and fragrance-related proteins. Five of these are fragrance-related proteins namely 1-aminocyclopropane-1-carboxylic acid synthase, S-adenosylmethionine decarboxylase, guaiadiene synthase, acyl carrier protein 3 and caffeate o-methyltransferase. The findings of the study provide some fundamental information for a more comprehensive approach to analyze and then explain the mechanism involved in fragrance biosynthesis and its regulation in this plant.

Abstrak thesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMPROFILAN PROTEOMIK PADA TISU BUNGA DAN DAUN *Michelia alba* DC.

Oleh

HASLIZA BINTI HASSAN

Oktober 2012

Pengerusi : Professor Madya Mohd Puad Abdullah, PhD

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Michelia alba DC merupakan tumbuhan wangian yang terkenal dan kaya dengan minyak perlu yang berkualiti tinggi dan berkepentingan dari segi ekonomi terutamanya kepada industri wangian. Salah satu daripada komponen utama minyak perlu tersebut ialah monoterpena. Dalam tumbuhan, terpena disintesis dalam tapak jalan metabolik yang kompleks yang melibatkan kedua-dua metabolisma utama dan sekunder. Pada peringkat biokimia, tapak jalan metabolik yang tepat yang bertanggungjawab dalam penghasilan minyak perlu dalam *M. alba* dan mekanisma pengawalaturannya masih lagi kurang difahami. Oleh itu, pemprofilan menyeluruh ke atas proteome dari organ-organ yang terlibat dalam pembentukan minyak perlu ini merupakan salah satu pendekatan untuk memperoleh protein-protein yang berkaitan dan berpotensi dalam penghasilan minyak perlu tersebut. Secara amnya, kajian ini dijalankan untuk mengenalpasti protein yang berbeza pengekspresan semasa peringkat perkembangan melalui pengenalpastian kaedah pengekstrakan protein yang optimum dan pemprofilan proteome pada peringkat perkembangan yang berbeza.

Objektif kajian ini adalah untuk i) mengoptimumkan kaedah pengekstrakan protein untuk pemprofilan proteome *M. alba* dengan menggunakan elektroforesis gel dua dimensi (2-DGE), ii) mengoptimumkan kebolehulangan 2-DGE dalam tisu bunga dan daun *M. alba*, iii) memprofil proteome bunga dan daun *M. alba* pada peringkat perkembangan organ yang berbeza dengan menggunakan 2-DGE, iv) mengenalpasti arah aliran dan hubungkait profil proteomik dari sudut pengawalaturan protein yang unik dan v) mengenalpasti protein yang diekspress secara unik untuk penghasilan wangian semasa peringkat perkembangan.

Elektroforesis gel dua dimensi (2-DGE) adalah salah satu kaedah yang dapat menghasilkan pemisahan yang baik untuk analisis pengekspresan protein. Dalam kajian ini, kaedah 2-DGE yang tradisional telah digunakan untuk memprofil protein pada organ daun dan bunga *M. alba* di peringkat perkembangan yang berbeza. Pra-syarat untuk mendapatkan kebolehulangan dan resolusi yang tinggi dalam kaedah proteomik pemisahan protein 2-DGE adalah pengekstrakan protein tumbuhan yang dioptimumkan terutamanya tumbuhan berkayu seperti *M. alba*. Tiga kaedah pengekstrakan protein yang berbeza telah dinilai keupayaannya iaitu dari segi pengekstrakan jumlah protein larut yang tinggi, penghasilan pemisahan protein resolusi tinggi bagi ekstrak mentah pada gel 1D dan 2D dan dapat menyingkirkan pencemar bukan protein daripada ekstrak protein tersebut dengan maksimum. Protokol yang melibatkan penggunaan Bis-Tris/aseton merupakan kaedah pengekstrakan protein yang terbaik bagi *M. alba* berdasarkan ciri-ciri tersebut dan secara signifikannya ($p<0.05$) mempunyai kandungan protein yang paling tinggi dalam tisu bunga dan daun.

Dengan menggunakan protokol pengekstrakan ini, proteome pada peringkat perkembangan yang berbeza dalam daun dan bunga *M. alba* telah berjaya diperoleh melalui kaedah 2-DGE. Bilangan bintik protein dan tahap pengekspresannya telah dipantau semasa peringkat perkembangan organ-organ tersebut. Secara umumnya, bilangan bintik protein bertambah secara progresif semasa peringkat perkembangan organ-organ tersebut. Semasa perkembangan bunga *M. alba*, bilangan bintik protein adalah paling tinggi semasa bunga mula berkembang namun berkurangan setelah bunga berkembang penuh. Pemprofilan proteome untuk daun dan bunga daripada peringkat perkembangan yang berbeza menghasilkan 80 hingga 210 bintik yang menunjukkan pola pengekspresan yang berbeza, yang mana sebahagiannya telah berjaya dijujuk. Lima bintik protein daripada proteome bunga dan lapan daripada daun telah berjaya dikenalpasti identitinya dengan menggunakan kaedah pengenalpastian cap jari jisim peptida (PMF). Protein-protein ini dapat dikategorikan kepada tiga kelas utama mengikut fungsi masing-masing: metabolisme utama, perkembangan dan protein yang berkaitan dengan wangian. Lima daripada protein yang berkaitan dengan wangian ialah 1-aminocyclopropane-1-karboksilik asid synthase, S-adenosylmethionine dekarboksilase, guaiadiene synthase, acyl carrier protein 3 dan caffeate o-metiltransferase. Penemuan daripada kajian ini menyediakan maklumat asas untuk pendekatan analisa yang lebih komprehensif dan dapat menerangkan mekanisme yang terlibat dalam biosintesis wangian dan pengawalaturannya dalam tumbuhan ini.

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I certify that an Examination Committee has met on **4 October 2012** to conduct the final examination of **Hasliza Binti Hassan** on her **Master of Science** thesis entitled "**Proteomic Profiles of The Floral and Leaf Tissues of *Michelia alba* DC.**" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the degree of Master of Science.

Members of the Examination Committee were as follows:

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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 of Science. The members of 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 concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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HASLIZA BINTI HASSAN

Date: 4 OCTOBER 2012

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