UNIVERSITI PUTRA MALAYSIA

ISOLATION AND CHARACTERISATION OF TRANSCRIPTS FOR Leafy, Globosa AND Drooping Leaf TRANSCRIPTION FACTORS FROM NORMAL AND MANTLED OIL PALM INFLORESCENCE

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FBSB 2018 46

ISOLATION AND CHARACTERISATION OF TRANSCRIPTS FOR *Leafy*, *Globosa* AND *Drooping Leaf* TRANSCRIPTION FACTORS FROM NORMAL AND MANTLED OIL PALM INFLORESCENCE

By

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

March 2018

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

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Flowering is a crucial process in plants to ensure the continuity of species. It is a highly regulated process of which transcription factors play a major role from initiation of the reproductive development until production of fruits. In oil palm clonal planting materials, somaclonal variation in flower development was observed, notably mantling abnormality in which feminization of male flower structures occur. Consequently, production of fruit is affected and oil yield is jeopardized. The objective of this study was to investigate the possible function and also association of LEAFY(LFY), GLOBOSA (GLO) and DROOPING LEAF(DL) transcription factor genes with the mantling phenomena. Transcripts and transcript variants of these genes were isolated and their expression patterns were characterised in oil palm organs, developing female inflorescence, as well as normal and mantled inflorescence via quantitative PCR. Furthermore, selected transcripts were analysed with RNA in situ hybridisation. Two transcripts each of EgLFY, EgGLO and EgDL were isolated. Additionally, a splice variant was isolated for both EgLFY and EgGLO respectively. Both splice variants, EgLFY2v and EgGLO1v, have an intron and a premature stop codon. Tissue-specific expression analysis showed that EgLFY, EgGLO and EgDL were expressed in apical meristem tissue, whereas EgLFY and EgGLO are expressed in both male and female inflorescences at stage 2 of development. Further analysis on expression trend of transcripts at stage 1 to stage 4 of developing female inflorescence series revealed accumulation of EgLFY transcripts are at its highest at stage 1, whereas EgDL expression begins at stage 3 and increases steeply at stage 4. Meanwhile, the expression of EgGLO was constant throughout the developmental stages. Comparative expression analysis between normal and mantled inflorescence showed that EgLFY1 transcript abundance observed in mantled inflorescence was only 75% of that of in normal inflorescence at stage 1 of development whereas, the expression of EgDL1 and EgDL2 was slightly higher than 2-fold in mantled inflorescence at stage 4 of development. However only differential expression of EgLFY1 was statistically significant. Furthermore, RNA in situ hybridisation of EgDL1 revealed expression of the transcript in the ectopic supplementary carpels of mantled inflorescence. Duplication of genes might have arisen through segmental duplication which is widespread in oil palm species. Expression of EgLFY, EgGLO and EgDL transcripts in apical meristem indicates that

these transcripts may play an additional role in vegetative growth in oil palm. All three TFs also play crucial role during initiation and development of oil palm inflorescence. Based on the qPCR and RNA in situ hybridisation expression results, it was postulated that EgLFY1 and EgDL1 are associated to the mantling abnormality in clonal palms. The findings enabled the identification of transcripts which has the potential to be developed as markers for mantling floral abnormality.

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

ISOLASI DAN PENCIRIAN TRANSKRIP UNTUK FAKTOR TRANSKRIPSI *Leafy, Globosa* DAN *Drooping Leaf* DARIPADA POKOK KELAPA SAWIT NORMAL DAN *MANTLE*

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Pembungaan adalah proses penting dalam tumbuhan untuk memastikan kemandirian spesies. Ia adalah proses yang terkawal ketat di mana faktor transkripsi memainkan peranan utama daripada permulaan proses pembiakan sehingga penghasilan buah .Di antara bahan tanaman klon kelapa sawit, variasi somaklonal dalam perkembangan bunga telah diperhatikan, terutamanya abnormaliti *mantle* di mana feminisasi organ reproduktif jantan berlaku. Akibatnya, penghasilan buah dan hasil minyak terjejas.Objektif kajian ini adalah untuk menyiasat fungsi dan juga penglibatan gen faktor transkripsi *LEAFY (LFY), GLOBOSA (GLO,)* dan *DROOPING LEAF (DL)* dengan fenomena *mantle*. Transkrip daripada gen-gen ini telah dipencilkan dan corak ekspresinya telah dicirikan dalam infloresen normal dan *mantle* melalui PCR kuantitatif. Seterusnya, transkrip yang dipilih telah dianalisis dengan hibridisasi *in situ* RNA. Dua salinan transkip berbeza daripada *EgLFY, EgGLO* dan *EgDL* telah diasingkan dan satu transkrip varian sambatan telah diasingkan daripada *EgLFY* dan *EgGLO.* Kedua-dua transkrip varian sambatan, *EgLFY2v* dan *EgGLO1v,* mempunyai intron dan kodon penamat prematur. Analisis ekspresi pada tisu pokok kelapa sawit menunjukkan ekspresi *EgLFY, EgGLO* dan *EgDL* dalam tisu meristem apikal dan juga ekspresi *EgLFY* dan *EgGLO* dalam infloresen jantan dan betina pada tahap 2 perkembangan. Analisis selanjutnya ke atas corak ekspresi transkrip pada tahap 1 hingga tahap 4 dalam perkembangan siri infloresen betina menunjukkan bahawa ekspresi transkripsi *EgLFY* adalah tertinggi pada tahap 1, manakala ekspresi *EgDL* bermula pada tahap 3 dan meningkat pada tahap 4. Sementara itu, ekspresi transkrip *EgGLO* berada pada tahap yang lebih kurang sama sepanjang peringkat perkembangan.Perbandingan analisis ekspresi antara infloresen normal dan *mantle* menunjukkan bahawa kuantiti transkrip *EgLFY1* di dalam infloresen *mantle* adalah hanya 75% daripada kuantiti transkrip di dalam infloresen normal pada tahap 1 perkembangan, manakala ekspresi transkrip *EgDL1* dan *EgDL2* adalah lebih sedikit daripada 2 kali ganda di dalam infloresen *mantle* pada tahap 4 perkembangan. Walau bagaimanapun, hanya perbezaan ekspresi *EgLFY1* adalah signifikan secara statistik. Tambahan pula, melalui analisis hibridasi *in situ* RNA, ekspresi transkrip *EgDL1* diperhatikan dalam karpel ektopik tambahan pada infloresen *mantle*. Dua salinan gen daripada setiap faktor transkripsi mungkin terhasil melalui proses duplikasi segmen

yang berlaku secara meluas dalam spesies kelapa sawit. Ekspresi transkripsi *EgLFY, EgGLO* dan *EgDL* dalam meristem apikal menunjukkan bahawa transkrip-transkrip ini memainkan peranan tambahan dalam pertumbuhan vegetatif pokok kelapa sawit. Ketiga-tiga faktor transkripsi tersebut juga memainkan peranan penting semasa permulaan dan perkembangkan infloresen kelapa sawit. Berdasarkan hasil ekspresi qPCR dan *in situ* hibridisasi RNA, terdapat kemungkinan bahawa *EgLFY1* dan *EgDL1* terlibat dengan variasi *mantle* pada pokok kelapa sawit klon. Penemuan ini membolehkan pengenalpastian transkrip yang berpotensi untuk dijadikan sebagai penanda untuk variasi somaklonal *mantle*.

ACKNOWLEDGEMENTS

Firstly, I would like to thank my main supervisor, Dr. Parameswari Namasivayam for her encouragement, assistance and guidance for completion of my Masters work. I would like to also thank my co-supervisor, Dr. Ho Chai Ling, for providing helpful comments and constructive criticisms. A special thanks and sincere appreciation to my external supervisor and mentor, Dr. Kwan Yen Yen for the invaluable guidance, input and consistent motivation provided throughout the study.

My deepest appreciation to FELDA Agricultural Services Sendirian Berhad (FASSB) for funding my research project as well as for generously providing the oil palm samples for the completion of my study.

My sincere thanks to Dr. Ooi Siew Eng and Pn Rosna (MPOB) for sharing their knowledge and technical expertise on *in situ* hybridisation technique with me.

Special thanks to my close friend, Qistina, for always being there for me. Not forgetting my other friends, Suran, Puva, Reena, Bhanisha and Revathi for sharing their experiences and being supportive, thank you all.

Finally, I wish to thank my pillar of strength, my family. The words of support, patience and understanding of my wonderful parents and siblings have motivated me to complete this research. Last but not least, I wish to thank my beloved husband, Rueban for the kind understanding and encouragement throughout this journey.

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

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LIST OF ABBREVIATIONS

AP1	Apetala 1
AS	alternative splicing
ASF	accompanying staminate flower
BLAST	basic local alignment search tool
bp	base pair
cDNA	complementary DNA
CDS	coding sequences
Cq	quantification cycle
DBD	DNA-binding domain
DEPC	Diethyl pyrocarbonate
DNA	deoxyribonucleic acid
EAR	ethylene-responsive element binding factor-associated amphiphilic repression
GLO	GLOBOSA
GTFs	general transcription factors
LFY	LEAFY
LNA™	Locked Nucleic Acid
MADS	**M**CM1 **A**GAMOUS **D**EFICIENS **S**RF
ORF	open reading frame
QPCR	quantitative PCR
NCBI	National Centre for Biotechnology Information
NMD	non-sense mediated decay
RISH	RNA *in situ* hybridisation
RACE	rapid amplification of cDNA ends
siPEPs	small interfering peptides
TFs	site specific transcription factors

CHAPTER 1

INTRODUCTION

Elaeis guineensis, also known as the African oil palm, is the most efficient oil-bearing crop in the world. One hectare of oil palm yields about 3.7 tons/ha/year of oil and the same area will yield 0.7, 0.5 and 0.4 tons of oil if planted with rapeseed, sunflower and soy, respectively (Sumathi *et al*., 2008). Requirement for vegetable oil worldwide is predicted to be 240 Mt in the year 2050. Due to increased demand for edible oil and its potential to be used as raw material for biofuel production in future, and also limited availability of land for expansion of oil palm planting, it is necessary to increase the oil yield productivity (Corley, 2009). Hence, high yielding hybrid *tenera* oil palm planting materials are planted to maximize the productivity of each acreage. However, advanced commercial hybrids exhibited low heritability for oil yield (Corley & Tinker, 2003). To address the yield gap in field, one of the strategies is to plant clonal planting materials that have the potential to produce genetically uniform palms that could increase up to 30% yield compared to hybrids (Mutert & Fairhurst, 1999).

In vitro propagation is capable of multiplying a single elite palm into tens of thousands of ramets. However, incidence of mantled somaclonal variation remains the headwind for planters to embrace clonal planting materials commercially. Mantled somaclonal variation is characterized by the feminization of male counterparts in the flower, resulting in abnormal fruits containing supplementary carpel structures. Mantled phenotype is observed in 5% of the tissue-culture derived regenerants, affecting oil yields due to bunch failure (Jaligot *et al*., 2000). At the pre-nursery stage, an oil palm ramet is sold at approximately RM 22. Taking into consideration the price of ramet only, a 5% rate of mantling will result in RM 1.1 million loss in a company with 1 million production of ramet/year.

Recently, differences in the methylation status of *Karma* transposon located in the intron of *DEFICIENS* (*DEF*) gene have been identified as the culprit for mantled somaclonal variation. Loss of methylation of *Karma* region has introduced a new splicing site at *DEF* gene transcript that resulted in the production of a truncated transcript, *kDEF* (Ong-Abdullah *et al*., 2015). Expression of *kDEF* is restricted only on mantled inflorescence at stage 3 onwards, coinciding with initiation of the floral reproductive organs. However, not much information is available on what triggers the hypomethylation of *Karma* element which leads to this abnormality. Therefore, further investigations are necessary in order to identify other players involved in contributing to mantled condition to obtain a better understanding of the phenomena.

In line with the objective, three candidate genes which are involved in flower structure formation, *LEAFY (LFY)*, *GLOBOSA (GLO)* and *DROOPING LEAF (DL)*

were selected to be analysed in the present study. *LFY* gene functions both as a meristem identity gene and an upstream regulator of MADS-box genes (Liljegren *et al*., 1999). *GLO* is a member of B-class MADS-box gene, necessary for specification of reproductive organs, while *DL* is required for specification of carpel structure (Yamaguchi *et al*., 2004). Full-length transcripts and splice variants of these genes were isolated and the gene expression were characterized in oil palm. This provided an opportunity to both investigate the possible function of these genes in oil palm flower development as well as identify association of the genes in mantling abnormality. This fundamental study on the candidate genes comprises of the following objectives.

I. To isolate *EgLFY, EgGLO* and *EgDL* transcripts and associated splice variant transcripts;
II. To profile the isolated transcripts' expression level in different oil palm organs, inflorescence developmental stages and comparative expression level in both normal and mantled inflorescence;
III. To determine the localization of selected transcripts in normal and mantled inflorescence through *in situ* hybridization.

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