UNIVERSITI PUTRA MALAYSIA

MORPHO-HISTOLOGICAL, PHYSIOLOGICAL AND MOLECULAR ANALYSIS OF "TRUNCATED LEAF SYNDROME" SOMACLONE IN TENERA OIL PALM (*Elaeis guineensis* Jacq.)

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By

SHEIKH HASNA HABIB

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfillment of the Requirement for the Degree of Doctor of Philosophy

November 2011

In the Name of Allah, Most Gracious, Most Merciful

Dedication

This thesis is dedicated to:

My Father Sheikh Md. Habibur Rahman

My Mother Rawsan Ara Begum

My Husband Md. Kausar Hossain

My Son A.M. Farhan Ishrak

Their Sacrifice and Infinitive Love Led Me to Present this Achievement.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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SHEIKH HASNA HABIB

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Chairman: Parameswari A/P Namasivayam, PhD

Faculty: Biotechnology and Biomolecular Sciences

Oil palm tissue culture is important for rapid multiplication of uniform planting materials, a % of tissue culture derived oil palm seedlings lead to a somaclonal variant termed as truncated leaf syndrome (TLS). The causes of this somaclone are unknown as no extensive research has been carried out. In order to identify the causes of TLS occurrence, it is essential to know the details of the morpho-histology, nutritional status, hormonal level and the genes that are expressed or suppressed in TLS seedlings. Therefore, the objectives of this study were to investigate the morpho-histological, physiological and molecular differences in TLS seedlings in comparison to normal. The TLS and normal oil palm seedlings were obtained from FELDA Agricultural Services Sdn Bhd, Malaysia. On the basis of the percentage of TLS occurence, the nine clones were categorized into: severely effected (>70 %), moderate (40-69 %), and mild (<40 %). Stunted growth, lower vigor, curled truncated and less green leaf, fewer number of roots and smaller shoot apical meristem (SAM) were found in TLS seedlings. The severity of TLS increased as the size of the SAM decreased indicating a clear relationship between SAM size and TLS abnormality. Crinkled and undulated leaf surfaces, coalesced mesophyll cells, sunken, deformed and fewer numbers of stomata and a smaller root cap were found

in TLS leaf by scanning electron microscopy. Longer epidermal cells and a depressed epidermal layer, bigger sub-epidermal cells with different shapes, loosely arranged mesophyll cells, undifferentiated leaf vascular bundle, impaired root tips, and smaller and compact SAM with defective leaf primordial at the distal end were also observed in TLS seedlings by light microscopy. Physiological analysis revealed that the levels of Boron (5.8, 5.4 and 6.1 mg/kg in severe, moderate and mild TLS respectively), Zinc (60.1 and 54.1 mg/kg in severe and moderate TLS respectively) and chlorophyll (0.49, 0.54 and 0.53 mg/g total chl in severe, moderate and mild TLS respectively) were lower and the levels of cytokinin (tZ: 10.1, tZR: 16.9, tZOG: 34.0, tZR5'MP: 15.6 and cZR5'MP: 10.3 pmol/g in severe TLS) and brassinosteroid (brasinolide: 7.5 pmol/g in severe TLS) were higher in TLS seedlings than normal. Phenotypic and morpho-histological study revealed that this abnormality might be due to smaller SAM in severe TLS palms. Therefore, to isolate the differentially expressed genes in TLS seedlings, a forward and a reverse suppressive subtractive hybridization (SSH) library were constructed for severe TLS SAM. From the suppressive SSH library, 387 and 173 transcripts were found to be up-regulated and down-regulated in severe TLS SAM respectively. The annotated 33 % and 14 % of unique genes sequences from the forward and reverse libraries, respectively, which had significant matches were further classified based on their putative functions Three transcripts pescadillo N-terminus family protein (EgPES), TLD family protein (EgTLD) and putative finger transcription factor (EgPFTF) were selected from the forward subtracted library for full-length cDNA isolation and characterization by Real-Time RT-PCR. EgPES and EgTLD are developmental related proteins and EgPFTF is a transcriptional activator. EgTLD and EgPFTF were up-regulated in severe and moderate TLS SAM but down-regulated in mild TLS SAM. EgPES was

up-regulated in severe TLS SAM but down-regulated both in moderate and mild TLS SAM. All three transcripts were predominantly expressed in leaf and root tissues of severe TLS SAM. Functional analyses are required for better understanding of the molecular functions of these transcripts in TLS somaclone.

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

ANALISIS MORPHO-HISTOLOGI , FISIOLOGI DAN MOLEKUL "TRUNCATED LEAF SYNDROME" SOMAKLON KELAPA SAWIT TENERA (*Elaeis guineensis* Jacq.)

Oleh

SHEIKH HASNA HABIB

November 2011

Pengerusi : Parameswari Namasivayam, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Kultur tisu kelapa sawit adalah penting untuk memperbanyakkan bahan tanaman yang seragam. Namun, satu peratus anak benih pokok kelapa sawit yang dihasilkan daripada proses kultur tisu menghasilkan variasi somaklon yang dikenali sebagai "truncated leaf syndrome (TLS)". Penyebab terjadinya somaklon ini masih tidak diketahui kerana tidak ada kajian yang luas telah dilakukan. Untuk mengenalpasti penyebab berlakunya TLS, adalah penting untuk mengetahui butiran morfo-histologi, status nutrisi, paras hormon dan gen yang diekspres ataupun direncat dalam anak benih TLS. Sehubungan dengan itu, objektif kajian ini adalah untuk mengkaji morfo-histologi, fisiologi dan perbezaan molekul antara anak benih TLS dan pokok semulajadi. Kedua-dua jenis anak benih pokok sawit ini telah diperolehi dari FELDA Agricultural Services Sdn Bhd, Malaysia. Berdasarkan peratusan berlakunya TLS, sembilan klon telah dikategorikan kepada: teruk (>70 %), sederhana (40-69 %), dan ringan (<40 %). Pertumbuhan yang terbantut, kurang "vigourity" daun yang cacat, kerinting dan kurang hijau, jumlah akar yang kurang dan meristem apeks pucuk (SAM) yang lebih kecil telah didapati dalam anak benih TLS. Keterukan TLS yang meningkat dengan berkurangnya saiz SAM telah

menunjukkan hubungan yang jelas antara saiz SAM dan ketidaknormalan TLS. Di bawah imbasan mikroskop elektron, anak benih TLS menunjukkan daun yang kerinting dan berombak, sel mesofilnya berkumpul bersama, kelihatan tenggelam, berbentuk tidak normal, kurang jumlah stomata dan jidal akarnya adalah lebih kecil. Di bawah mikroskop cahaya pula, anak benih TLS mempunyai sel epidermis yang panjang, sub-sel-epidermis yang berlainan bentuk, berkas vaskular yang tidak berbeza, akar yang rosak, SAM yang kecil, padat dan terdapatnya kerosakan pada hujung primordia tumbuhan. Analisis fisiologi mendedahkan bahawa tahap Boron (5.8, 5.4 dan 6.1 mg / kg dalam TLS serius, sederhana dan ringan masing-masing), Zink (60.1 dan 54.1 mg / kg dalam TLS serius dan sederhana) dan klorofil (0.49, 0.54 dan 0.53mg / g jumlah klorofil dalam TLS serius, sederhana dan ringan masing-masing) adalah lebih rendah dan tahap cytokinin (TZ: 10.1, tZR: 16.9, tZOG: 34.0, tZR5'MP: 15.6 danc ZR5'MP: 10.3 pmol / g dalam TLS serius) dan brassinosteroid (brasinolide: 7.5 pmol /g dalam TLS serius) adalah lebih tinggi dalam anak pokok TLS daripada biasa. Kajian fenotipik dan morpho-histologi menunjukkan bahawa keadaan ketidaknormalan ini mungkin disebabkan oleh SAM yang lebih kecil dalam pokok TLS serius. Daripada perpustakaan SSH, 387 dan 173 transkrips telah diekspreskan dalam anak benih TLS yang parah. Sebanyak 33 % dan 14 % anotasi jujukan gen yang unik daripada perpustakaan kehadapan dan kebelakang yang mempunyai persamaan yang signifikan, telah diklasifikasikan dengan lebih lanjut berdasarkan fungsi putatif. Tiga transkrip “pescadillo N-terminus family protein (EgPES)”, “TLD family protein (EgTLD)” dan “putative finger transcription factor (EgPFTF)” yang dipilih daripada perpustakaan kehadapan untuk pemencilan cDNA lengkap dan pencirian dengan “real-time RT-PCR”. EgPES dan EgTLD ialah protin

yang berkaitan dengan perkembangan manakala EgPFTF merupakan pangaktif transkripsi. EgTLD dan EgPFTF didapati diekspreskan dengan banyak dalam TLS SAM yang teruk dan sederhana manakala sedikit diekspreskan dalam TLS SAM yang ringan. EgPES pula diekspreskan dengan banyak dalam TLS SAM yang teruk tetapi sedikit diekspreskan dalam kedua-dua TLS SAM yang sederhana dan ringan. Ketiga-tiga transkrip ini lebih banyak diekspreskan pada tisu daun dan akar anak benih TLS SAM yang teruk. Analisis fungsi adalah diperlukan untuk lebih memahami fungsi molekul ketiga-tiga transkrip ini dalam somaklon TLS.

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I certify that a Thesis Examination Committee has met on 29 November 2011 to conduct the final examination of Sheikh Hasna Habib on her doctor of philosophy thesis entitled "**Morpho-histological, physiological and molecular analysis of "truncated leaf syndrome" somaclone in tenera oil palm (*Elaeis guineensis* jacq.)**" in accordance with the Universities and University Colleges 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 Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

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Date:

DECLARATION

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

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SHEIKH HASNA HABIB

Date: 29 November 2011

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