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DETECTION OF TLS PLANTLETS OF OIL PALM USING SPECIFIC GRDA MARKERS VIA PCR APPROACH

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By

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Thesis Submitted to the Department of Cell and Molecular Biology,

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Abstract of the thesis presented to the Department of Cell and Molecular Biology in fulfilment of the requirement for the degree of Bachelor of Science (Hons.) Cell and Molecular Biology

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Chair: Assoc. Prof. Dr Parameswari Namasivayam, PhD

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Truncated Leaf Syndrome (TLS) abnormality is one of the obstacles in the oil palm tissue culture as it is only observable after transferring to nursery which gives a drawback as it consumes a lot of time, expenses and labour. Therefore, early detection of the TLS plantlets while it is still in culture can save money, time and labour. This study aimed to investigate whether a previously isolated genomic RDA DNA marker can be used to distinguish TLS plantlets from normal plantlets. DNA extraction was carried out on leaves of five unknown clones and two known clones of normal and TLS plantlets using the modified method of Dellaporta. Smeared bands observed in the five unknown samples indicated degradation of DNA or low DNA quality which resulted from the poor sample quality, whereas samples from clones FC6432 and FC6516 showed a better DNA quality. The expected PCR product of 500 base pairs was successfully amplified using forward and reverse 1181BgI primers in all the tested samples. The amplified PCR product will have to be verified by Southern analysis or

sequencing. Time constraints limited the continuation of the southern analysis. Based on PCR results, this marker was not able to differentiate the four known plantlets since the expected bands were present both in the TLS and normal plantlets of clones FC6432 and FC6516. Hence, the 1181BgI primers are not suitable to be used as the DNA marker for detecting TLS plantlets.

Keywords: TLS, genomic RDA, abnormality, marker, tissue culture

Abstrak tesis yang dikemukakan kepada Jabatan Biologi Sel dan Molekul sebagai memenuhi keperluan untuk Ijazah Sarjana Muda Sains (Kepujian) Biologi Sel dan Molekul

PENGENALPASTIAN *TLS* DALAM ANAK POKOK KELAPA SAWIT MENGGUNAKAN SPESIFIK PENANDA *GRDA* MELALUI PENDEKATAN *PCR*

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Keadaan abnormal yang muncul pada sindrom pemendekkan daun (*TLS*) merupakan salah satu halangan dalam kultur tisu kelapa sawit kerana ia hanya dapat dikesan selepas dipindahkan ke tapak semaian. Ini menyebabkan pembaziran masa, wang dan tenaga buruh. Lantarannya, pengenalpastian awal anak pokok *TLS* ketika masih dalam kultur tisu boleh menjimatkan wang, masa dan tenaga buruh. Kajian ini bertujuan untuk mengenalpasti sama ada penanda genomik *DNA RDA* yang diasingkan sebelum ini boleh digunakan untuk membezakan anak pokok *TLS* daripada anak pokok biasa. Pengekstrakan *DNA* telah dijalankan ke atas lima helai daun klon yang tidak diketahui dan dua klon yang diketahui menggunakan kaedah yang diubahsuai daripada Dellaporta. Jalur yang tidak utuh telah diperhatikan dalam lima sampel yang tidak diketahui menunjukkan degradasi *DNA* atau kualiti *DNA* yang rendah yang berpunca daripada kualiti sampel yang kurang baik, sedangkan sampel daripada klon FC6432

dan FC6516 menunjukkan kualiti *DNA* yang lebih baik. Produk *PCR* yang bersaiz 500 pasangan bes telah berjaya diperbanyakkan dalam semua sampel dengan menggunakan pencetus depan dan belakang bernama 1181BgI. Produk *PCR* perlu disahkan melalui analisis *Southern* atau penjujukan. Kekangan masa menghadkan penerusan analisis *Southern*. Penanda ini tidak dapat membezakan empat anak pokok yang tidak diketahui tersebut kerana jalur yang dijangka diperolehi dalam kedua-dua anak pokok *TLS* dan biasa daripada klon FC6432 dan FC6516. Oleh itu, pencetus 1181BgI tidak sesuai untuk digunakan sebagai pencetus *DNA* untuk membezakan anak pokok *TLS*.

Kata kunci: *TLS*, genomik *RDA*, situasi abnormal, pencetus, kultur tisu

APPROVAL

This thesis was submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences and has been accepted as fulfilment of the requirement for the degree of Bachelor of Science (Hons.) Cell and Molecular Biology. The member of the Supervisory Committee was as follows:

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DECLARATION

Declaration by undergraduate student

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LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
bp	Base pairs
cDNA	Complementary DNA
DNA	Deoxyribonucleic acid
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
dGTP	Deoxyguanosine triphosphate
dNTPs	Deoxynucleotide triphosphates
dTTP	Deoxythymidine triphosphate
dUTP	Deoxyuridine triphosphate
EDTA	Ethylenediaminetetraacetic acid
gRDA	Genomic representational difference analysis
λ	Lambda
$MgCl_2$	Magnesium chloride
MAS	Marker-assisted selection
µl	Microliter
µM	Micromolar
mM	Millimolar
ng	Nanogram

PCR	Polymerase chain reaction
RAPD	Random amplified polymorphic DNA
RDA	Representational difference analysis
RFLP	Restriction fragment length polymorphism
SAM	Shoot apical meristem
SNP	Single nucleotide polymorphism
SSR	Single sequence repeat
NaCl	Sodium chloride
SDS	Sodium dodecyl sulfate
TLS	Truncated leaf syndrome
U	Unit
V	Volt

CHAPTER 1

INTRODUCTION

Oil palm, *Elaeis guineensis* Jacq., originated from West Africa (Ngando-Ebongue *et al*., 2012), is the most efficient oil-bearing crop providing the largest oil supply, and thus becoming one of the major agricultural crops in the world. The global demand of oil palm is highly increasing in the world such that palm oil consumption has reached to 52.1 million tonnes in 2012, due to its wide applications such as vegetable oil, food additive, cosmetics ingredient, pharmaceuticals, industrial lubricants, and biofuel (Oil World, 2013).

Oil palm should be largely produced to meet the high global demand. The development of genetic studies allows the introduction of tenera palm which is found to have an increase of 30% in its oil production due to its thicker mesocarp. Tenera palm is a better quality of oil palm results from the crosses of dura palm and pisifera palm. Since conventional breeding is extremely slow and costly because of its long breeding cycle (Low *et al*., 2008), therefore it is replaced by more reliable method which is tissue culture. Kushairi *et al*. (2010) reported that clonal palms were found to be highly uniform and yielded at least 25% higher than standard tenera plantlets, which has potential in improving productivity per unit area. Nevertheless, tissue culture encounters some major bottlenecks including somaclonal variation (Morcillo *et al*., 2006).

Somaclonal variation can either be genetic or epigenetic in origin (Larkin and Scowcroft, 1981). Genetic variation involves the changes in chromosome numbers (Karp *et al.*, 1989), chromosome structure (Philips *et al.*, 1994; Tremblay *et al.*, 1999) and DNA sequence (Brettell *et al.*, 1986); epigenetic variation refers to heritable

changes which are caused by DNA methylation (Kaeppler *et al*., 2000). Approximately 5% of somatic embryo-derived palms displayed mantled abnormality, an example of epigenetic (Corley *et al*., 1986) resulted in partial or complete flower sterility, and therefore, low oil production (Morcillo *et al.* 2006). TLS abnormality is another type of somaclonal variations which was first reported in the tissue culture-derived oil palm plantlets in 1989. This syndrome is observable in some plantlets within the first few weeks after transferring the plantlets to nursery. The symptoms include leaves showing mild necrosis across the lamina that will eventually lead to the complete breakage of the leaves. According to Habib *et al.* (2012), TLS plantlets displayed depressed and wavy leaf surface, sunken and distorted stomata, coalesced epidermal cells that could affect gaseous exchange and photosynthesis. They reported that the occurrence of truncated leaf syndrome probably due to the reduced shoot apical meristem (SAM) size.

Genetic marker is necessary to help in detection of TLS plantlets at early stage during tissue culture. Work dealing with molecular marker in oil palm started from the late 1980s, particularly in the studies of economically important traits like shell-thickness (Mayes *et al*., 1997), virescens and crown disease to improve the yield and quality of oil (Seng and Zaman, 2006). A more recent study reported on the development of biomarkers, $EgNB_3$ for screening the embryogenic potential of oil palm leaf explants (Ho *et al*., 2013). Chin *et al*. (2010) reported biomarkers are efficient screening tools in oil palm tissue culture, offering more advantages to oil palm industry, such as cost saving in terms of labor and time. In a previous work, Ms Kanagamalar (from Cell and Molecular Biology Lab) had used Representational Difference Analysis (RDA) method to detect genomic differences between Truncated Leaf Syndrome (TLS) and normal plantlets. From that study, previously isolated two

potential genomic RDA (gRDA) markers, 1181BgI-F and 1181BgI-R were identified. In this study, the previously isolated gRDA markers are tested if they can be used as markers to detect TLS plantlets.

Objective

1. To determine whether the gRDA DNA markers can be used to distinguish TLS plantlets from normal plantlets

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