UNIVERSITI PUTRA MALAYSIA

PCR DETECTION OF Ganoderma spp. WITH TRANSLATION ELONGATION FACTOR 1 - ALPHA (tef1-α) GENE

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PCR DETECTION OF *Ganoderma* spp. WITH TRANSLATION ELONGATION FACTOR 1 - ALPHA (*tef1-α)* GENE

BY

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A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science

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ENDORSEMENT

This project entitled "PCR Detection of *Ganoderma* spp. with Translation Elongation Factor 1-alpha (*tef1-α)* gene" is prepared by Nur Sofnis Erina Binti Mohd Suhaimi and submitted to Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science.

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LIST OF ABBREVIATIONS

BSR	Basal stem rot
BLAST	Basic Local Alignment Search Tools
PCR	Polymerase chain reaction
DNA	Deoxyribonucleic acid
tef1-α	Translation elongation factor 1-alpha
MEA	Malt extract agar
PDB	Potato dextrose broth
CTAB	Centrimethyl ammonium bromide
Tris-HCl	Tris-Hydrochloric acid
PVP	Polivinylpyrrolidone
NaCl	Sodium Chloride
TE	Tris-EDTA
RNAse A	Ribonuclease A
TAE	Tris-Acetate-EDTA
bp	Base pair length
ITS	Internal Transcribe Spacer
E	Expect value
ELISA	Enzyme-linked Immunosorbent Assay

ABSTRACT

The most serious disease of oil palm in Malaysia all the while is basal stem rot (BSR). The losses can be accounted to about 50-85% over the life cycle of oil palm planting. There are several species of *Ganoderma* that are associated with BSR or isolated from oil palm trees but the major pathogen of this disease is *Ganoderma boninense*. The identification of *Ganoderma* species is difficult using morphological method. This will lead to a problem in disease management. The objectives of this study were 1) to extract genomic DNA from four *Ganoderma* species and 2) to develop a polymerase chain reaction (PCR) protocol using translation elongation factor 1-alpha (*tef1-α*) gene. To achieve objective 1, centrimethyl ammonium bromide (CTAB) method will be used for DNA extraction. To achieve objective 2, universal primers of *tef1-α* will be used to amplify *Ganoderma* species, sequenced the PCR products and identify the DNA sequences using Basic Local Alignment Search Tool (BLAST). For objective 1, only one genomic DNA species was obtained successfully with 295.0 ng/µl (A260/280) and the purity were 1.8 (A260/280) and 1.9 (A260/230). For objective 2, PCR protocol successfully amplified *tef1-α* gene for one species of *Ganoderma*. Primer pair *tef1-α* and *ef1* could be used to amplify *tef1-α* gene from *Ganoderma* spp. even though it was successfully amplified only one species in this project which was *Ganoderma boninense*. PCR amplification will enable for better identification of *Ganoderma* species by improving DNA extraction and taking all the precautions.

ABSTRAK

Penyakit yang paling serius dalam industri kelapa sawit Malaysia adalah reput pangkal batang. Penyakit ini menyumbang kerugian di antara 50-85% sepanjang kitaran hidup pokok kelapa sawit. Sebenarnya, terdapat beberapa spesies *Ganoderma* yang menjadi punca penyakit reput pangkal batang ini tetapi patogen utamanya ialah *Ganoderma boninense*. Spesies-spesies *Ganoderma* sukar dikenalpasti dengan kaedah morfologi. Hal ini akan menjadi punca kepada masalah pengurusan penyakit. Jadi, tujuan kajian ini adalah untuk membangunkan satu prosedur untuk pengesanan dan pembezaan spesies *Ganoderma* yang tepat. Objektif kajian ini adalah 1) untuk mengekstrak genomik DNA dari empat spesies *Ganoderma* dan 2) untuk membangunkan sistem pengesahan PCR dengan menggunakan faktor gen terjemahan pemanjangan 1-alfa (*tef1-α*). Untuk mencapai objektif 1, kaedah centrimethyl ammonium bromide (CTAB) akan digunakan untuk pengekstrakan DNA. Untuk mencapai objektif 2, primer sejagat *tef1-α* akan digunakan untuk mengenalpasti spesies *Ganoderma*, disusun produk PCR dan mengenalpasti susunan DNA menggunakan Basic Local Alignment Search Tool (BLAST). Untuk objektif 1, hanya satu spesies DNA genomic telah diperolehi dengan 295.0 ng/µl (A260/280) dan kadar puriti adalah 1.8 (A260/280) dan 1.9 (A260/230). Untuk objektif 2, protocol PCR berjaya mengenalpasti gen *tef1-α* untuk satu species *Ganoderma*. Pasangan primer *tef1-α* dan *ef1* boleh digunakan untuk mengenalpasti gen *tef1-α* dari *Ganoderma* spp. walaupun hanya berjaya mengenalpasti satu spesies sahaja dalam projek ini iaitu *Ganoderma boninense*. Penguatan PCR akan membolehkan pengenalpastian spesies *Ganoderma* lebih baik dengan meningkatkan pengekstrakan DNA dan mengambil semua langkah berjaga-jaga.

Chapter 1

INTRODUCTION

Oil palm (*Elaeis guineensis Jacq.*) is originated from West Africa and become one of the most important agricultural crops in Malaysia. Malaysia is one of the countries that contribute in world palm oil production. Oil palm trees can produce two types of oil which are from the flesh of fruit and palm kernel oil from seed or kernel. There are about 4.49 million hectares of land cover with oil palm plantation which can generate almost 17.73 million tonnes of oil palm and 2.13 tonnes of palm oil kernel. It is a monocious crop which has both male and female flowers on the same tree. The oil palm trees start to produce fruit 30 months after planting and its productivity can last for 20 to 30 years (Source:http://www.mpoc.org.my/Palm_Oil).

Basal stem rot (BSR) disease has becomes the most serious disease in oil palm industry. BSR disease can reduce the production of oil palm. This disease is caused by fungus *Ganoderma boninense*. Isolation of *Ganoderma* from diseased oil palm surround Peninsular Malaysia is concluded as a same species of *Ganoderma boninense* (Ho and Nawawi, 1985). There are four species that have been recorded recently which are *G. boninense, G. zonatum, G. miniatocinctum and G. tornatum* (Idris, 1999).

Earlier stage of oil palms tend to easily can be infected, especially the young plantings of less than 5 years old. The earliest symptoms of this disease occur in the foliage of lower frond that become yellowing. A dry rotting of internal tissues at stem base or root bole is produces by the infection of *Ganoderma boninense*. The infection and distribution of *Ganoderma* spp. can be through root (Turner, 1981). In recent studies showed that BSR can also be spread through spores (Flood *et al*., 2003; Flood and Hassan, 2004).

The invention of polymerase chain reaction (PCR) is by Kary Mullis and his colleagues in 1980s. Monoclonal and polyclonal antibodies had been used to detect pathogenic *Ganoderma* species. However, the assay of polyclonal antibodies showed cross reaction with saprophytic fungi commonly found on diseased oil palm roots and trunk although its percentage were lower (Utomo and Niepold, 2000; Idris and Rafidah, 2008). There is no distinction was found between pathogenic *Ganoderma* spp. isolated from oil palms and other pathogenic *Ganoderma* spp. that signified Enzyme-linked Immunosorbent Assay (ELISA) test cannot be used to distinguish among different *Ganoderma* species.

These conventional methods were time-consuming and their accuracy is not very high. For that reason, the availability of rapid, inexpensive and accurate diagnostic technique that is specific and readily adapted can give benefit decision-making for appropriate control (Utomo and Niepold, 2000). The identification of *Ganoderma* spp. can be detected using polymerase chain reaction (PCR).

The objectives of this study were 1) to extract genomic DNA from four *Ganoderma* species and 2) to develop a polymerase chain reaction (PCR) protocol using translation elongation factor 1-alpha (*tef1-α*) gene

The DNA genomic for all of four species of *Ganoderma* is successfully obtained. Polymerase chain reaction (PCR) protocol is developed to detect each species using translation elongation factor 1-alpha (*tef1-α*) gene and specific enough

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