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Sequence approach of *Elaeis guineensis* for early detection of *Ganoderma boninense* resistance

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Abstract. This study purposed to describe the product of Polymerase Chain Reaction (PCR) and sequencing of DNA from *Elaeis guineensis* root for *Ganoderma boninense* resistance in PT Socfin Indonesia. Based on PCR amplification a total of 18 sampling were detected, the percentage of positive results for *G. boninense* resistance was only 20%. The degree of sequence identity between DNA sequence known to be verified by the Blastx probability identity, *E-value* and total score. Only 11A genes that are available in GenBank and 15A and 16A gene found no similarity in the database of NCBI, therefore considered as new genes for probably *G. boninense* tolerance or resistance. The 11A gene showed the highest identity (98%) to predicted *metallothionein like-protein* with *E-value* 3e-19 and total scored 104. *Metallothionein* protein actives role in oil palm plants for resistance to *G. boninense*.

1. Introduction

Oil palm (*Elaeis guineensis*) is a significant economic crop and has become one of the world's major vegetable oils [1]. *Ganoderma* disease which is often known as stem rot is still a trending topic in the development of oil palm plants from Sumatra to Papua [2]. The pathogen will cause the plant to become chlorosis, necrosis and eventually the plant will be death [3]. Ganoderma fungus is hemibiotrophic, has intracellular hyphae which will give widespread necrotrophic and enzymatic attacks from infection of the cell wall layer in palm root plants [4].

Accurate identification and early detection of plant pathogens is an essential step in disease management programs. Selection with conventional methods will take a long time because it depends on physiological symptoms and requires extensive taxonomic expertise, where the most commonly used characteristics are morphological and physiological properties. The phenotype of a character is not only influenced by genetic factors, but also environmental factors [5]. Molecular laboratory studies with the discovery of DNA markers as a selection of tools based on genetic characteristics of plants supported by the *polymerase chain reaction* (PCR) methods currently being developed. This method is considered accurate for detecting pathogens of some plants because they will not be affected by the environment [6].

Plant breeding techniques can now be done using molecular approaches based on genetic information. Revolution in DNA and RNA sequencing has great promising in expanding genetic in

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achievement in both animals and plants [7]. Nucleotide base sequence or *Single Nucleotide Polymorphisms* (SNPs) obtained can help breeders find the target gene information with a specific purpose such as disease resistance, increasing production, etc. The present study aimed to analyses the DNA sequence from *E. guineensis* root resistance to *Ganoderma boninense.*

2. Materials and Methods

2.1. Materials

The genetic material used is DNA of the root *Elaesis guineensis* from the experimental farm in PT. Socfin Indonesia Bangun Bandar, Kec. Dolok Masihul Kab. Sumatera Utara. The total sampling used was 18 from adult root types of susceptible, moderate and tolerant Ganoderma. The specific primer used *EgMT* (that are genes thought roles in the resistance fungi of *Ganoderma* sp to *Elaeis guineensis*) [8].

2.2. Method

The methods used amplification in Laboratory DNA SSPL PT. Socfin Indonesia, Dolok Masihul Sumatera Utara. Furthermore, the PCR mix was sent for sequencing.

2.2.1. DNA extraction

DNA isolation used CTAB methods based on our previous study with minor modification [9]. Briefly, isolation of DNA from the root sampling used taken from the field was washed and dried with liquid nitrogen. ±0.5 gr of the root is ground with mortar while adding liquid nitrogen PVPP. The sample is inserted into a filled centrifuge 1 ml with buffer extraction CTAB and β-mercaptoethanol 10 µl, then stirred using vortex and incubated during water bath for 30 minutes at temperature 65ºC.

Every 10 min the sampling turned back slowly. Furthermore, the sample was incubated at room temperatures during 4-5 min, added 1 ml chloroform: isoamyl alcohol (24:1). Then, the sample was centrifuged at 13,000 rpm at room temperature for 10 min. The supernatant obtained is transferred to another centrifuge tube, vortex and centrifugeed at 13,000 rpm at a temperature 4ºC for 10 min. The supernatant was homageniced by flipping through the tube, stored in the refrigerator (ºC) for 30 min, centrifugeed at 13,000 rpm at 4ºC for 10 min. The supernatant obtained are discarded then the pellets are air-dried. Dry pellets were dissolved with a buffer TE 100 µl, added with absolute cold ethanol, then turned back and forth until homogenized. Then incubated in the freezer (20ºC) for 30 min and then centrifuged at 13,000 rpm at 4ºC for 10 min. The supernatant was removed while the pellets were washed using ethanol 70% and air-dried. The dried one DNA pellets are dissolved with 100 µl buffer TE, and finally, DNA stored in the freezer (-20ºC).

2.2.2. Polymerase Chain Reaction (PCR)

For each PCR tube, three µl of DNA templates are mixed with PCR mix 7 µl (2.5 µl Gotaq master 0.5 µl EgMT F and 0.5 EgMT R, and 3.5 µl ddH_2O). PCR amplification was on *Eppendorf Master Cycler* up 384 (Eppendorf, Westbury, New York, USA). The amplification program consists of a first denaturation cycle for 4 min at temperature 94ºC, 35 denaturetion cycle at 94ºC, during 30 sec, annealing 52ºC during 1 min 15 sec, extension 72ºC during 1 min 30 sec, and an elongation step at 72ºC during 8 min.

2.2.3. Electrophoresis of agars gel

The PCR product was analysed by electrophoresis in 2% agarose gel stained with Gel Red and visualised with Ultraviolet translumination as depicted in Figure 1.

2.2.4. DNA Sequencing and Analysis

PCR mix 30 all used specific primer *EgMT* sent to PT. Genetika Science and will be purified and then sequencing with ABI *Prism Genetic Analyzer*. DNA Electrophoresis will check the quality of the

sample before processing *cycle sequencing*. References sequence result is *single nucleotide polymorphisms* ((SNPs). As a result, the sequence (the new functional allele) will be matched with the nucleotide sequence based on Database at *National Center for Biotechnology Information* (NCBI) with BLAST (*Basic Local Alignment Search Tool*) program [10].

3. Results and Discussions

Biomarker used specific primer is EgMT with Sequence 5'-3' CTGTGATTCCTGCGGCTGTT-CTTGGATGCTTGGAAGGAGACC available in GenBank Accession No. XM_010922290.2 is a gene related to the resistance of Ganoderma pathogens that have been previously reported [8].

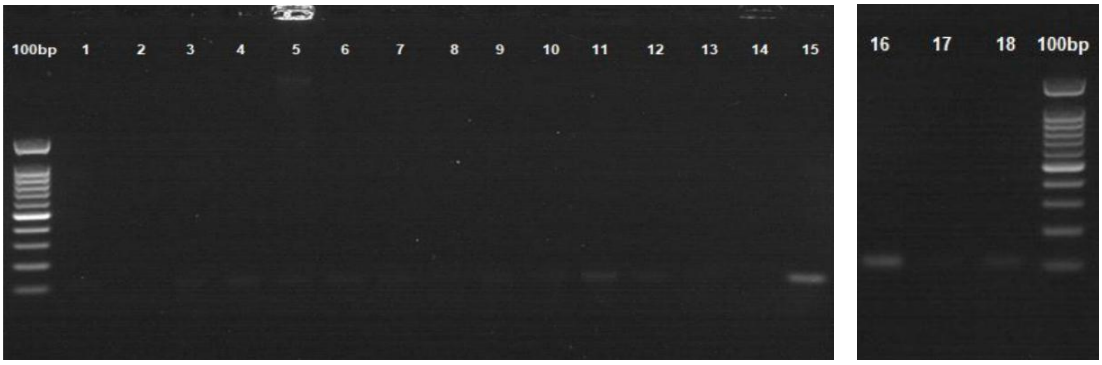


Figure 1. 1% Agarose gel shows PCR products from the roots of *Elaeis guineensis*

Based on PCR detection of a total of 18 sampling, the percentage of positive detection results was 20%. There is *a band* that appears on DNA amplification (Figure 1) using a specific primer of EgMT, and it is assumed that in some samples there are genes that are resistant to Ganoderma infection, otherwise *band* that does not appear or a suspected *smear* is positive for attacks on Ganoderma. The amplified *band* length almost looks the same. The results of the identification of 3 DNA sampling, line 11, 15 and 16 were amplified according to the specific primer EgMT gene target 100 bp and deserved to be sequencing.

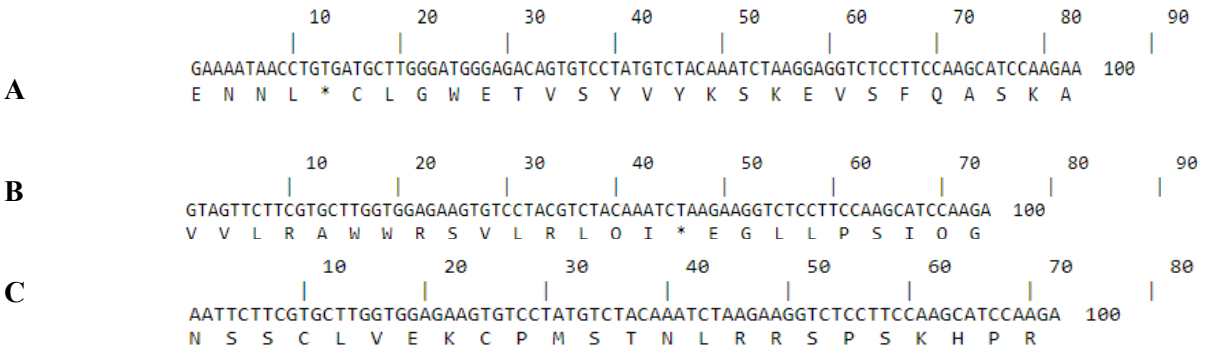


Figure 2. cDNA sequence of the roots *E. guineensis* A (11A) B (15A) and C (16A) and their translations to amino acid sequence.

Replication DNA fragments by PCR and sequencing informs the existence of nucleotide base sequences markers of Ganoderma resistance. Early detection based on PCR is an accurate method. cDNA sequence of *E. guineensis* 11A, 15A and 16A and its translation to amino acid sequence

(Figurea 2). This action is taken to save the trees, which can be able to spread to other trees through roots [11]. Sequencing analysis line 11 with the length of band 83 bp is the roots of a moderate healthy type of oil palm, line 15 with a range of 74 bp is susceptible attack and line 16 with a length of 72 bp is susceptible healthy type.

Sequence result of 3 sampling is matched with GenBank that is available at NCBI (Table 1). Only the 11A gene, which is *metallothionein-like protein*, and available in the database, while 15A and 16A gene found no similarity in the database of NCBI, therefore considered as new genes for probably *G. boninense* tolerance or resistance. *Metallothione*in is a common stress response protein with many significant roles in plants, such as regulation of normal growing and adaptation to changes in environmental conditions. *Metallothione*in promoter also active roles in *Elaeis guineensis* conform to abiotic stress such as disease resistance [12], likely even in resistance to Ganoderma.

Table 1. Sequencing result cDNA of the root *E. guineensis* in database NCBI.

Accession	Description	Identify	Total Score	*E*-Value
XM_010922290.2	Elaeis guineensis -like protein type 2 (LOC105044410), mRNA.	98%	104	3e-19

Genetic information was identified as a marker of oil palm genome sequence data. Genes 11A, 15A and 16A related to the defence which regulates pathogenic interactions of oil palm plants have been identified and have the potential to be developed as a biomarker for early detection of Ganoderma pathogen infections.

4. Conclusions

The total 18 roots of DNA samples from *E. guineensis* there were only three sampling (20%) that were successfully sequencing. The current study shows that the results of the 11A gene sequence are available in the NCBI database, while genes 15A and 16A are updates that are not yet available in the NCBI database. The genes succeeded in encoding *metallothionein-like protein* predictions from Elaeis guineensis.

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