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Isolation and amplification DNA of *Elaeis guineensis* from Bangun Bandar Plantation, North Sumatra

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Abstract. *Elaeis guineensis* is the primary commodity that supports the economy of Indonesian country. Lately, several reported diseases of oil palm caused by fungi, have declined in the quality of oil palm plantation also caused the death of several mature oil palm plants. The disease damages roots or tree trunk tissue is known as Basal Stem Rot (BSR). The present study aimed to report explains of the PCR and UV-tex method for detecting Ganoderma in mature palm plants. PCR can detect disease and primer EgMT used to produce various sizes of PCR products. Each sample has different brightness amplification depending on the reaction of the plant to the BSR fungus. The primer is expected to be suitable for detecting Ganoderma fungus in dura, pisifera and tenera oil palm mature plants. The length of the DNA fragment is visible with UV-1D after being irradiated with UV light. DNA amplification ranged from a value of 10,526-114,289 bp. The type of dura plant detected by amplified BSR disease was 34,211-114,289 bp. On the other hand, pisifera is only 21,053 bp and tenera 10,526-104,844 bp.

1. Introduction

Elaeis guineensis is the main commodity that supports the economy of Indonesian country, where these plants have three types of fruit, they were dura, pisifera, and tenera (a hybrid between dura and pisifera) [1]. The quality of palm oil is a determinant the success of the CPO industry. Physicochemical parameters have been studied recently, precisely the quality of palm oils of defining factor by color, carotene content, fatty acid, dirt, moisture, and deterioration of bleachability index (DOBI) [2]. On the other hand, the diversity of oil palm fruit has also been reported based on a molecular approach, where different types of the dura, tenera, and pisifera have different carbon chain-length of polyprenol and dolichols [3].

Lately, several reported diseases of oil palm caused by fungi, have declined in the quality of oil palm plantation also caused the death of several mature oil palm plants. The disease damages roots or tree trunk tissue is known as Basal Stem Rot (BSR) [4]. The development of the parental plant of oil palm plants is important for plantation breeding, methods to controlling caused Ganoderma pathogens have also been developed, such as inoculation techniques and to rubberwood block methods [5-6]. The



present study aimed to report explains of the PCR and UV-tex method for detecting *Ganoderma* in mature palm plants.

2. Materials and method

2.1. Material

The material of the genetic used is the root DNA of mature types of the dura, pisifera, and tenera from the experimental plantation of oil palm at PT. Socfin Indonesia Bangun Bandar, Sub. District Dolok Masihul, North Sumatra Regency. The specific primer used is EgMT (assumption to play a role in plant stress [7] including fungi).

2.2. Method

The method used for amplification DNA at the SSPL DNA Laboratory PT. Socfin Indonesia, Dolok Masihul North Sumatra. Furthermore, the data were analyzed by software UV-1D (UV-Tex Cambridge ver v16,09b).

2.2.1. DNA extraction

DNA isolation used the CTAB method based on our previous for research by a little modification [8]. Shortly, DNA isolation from the root sample used was taken from the field washed and dried with liquid nitrogen. ± 0.5 g of the root is ground by a mortar when adding PVPP of liquid nitrogen. The samples were put over a centrifuge filled with 1 ml with CTAB buffer extraction and ten μ l β -mercaptoethanol, next stirred using vortex and incubated during bathing water for 30 min at 65°C. Every 10 minutes the sampling returns by slowly. Incubated at room temperature for 4-5 minutes, added (24: 1) of chloroform: isoamyl alcohol.

Next step, the sample was centrifuged at 13,000 rpm at room temperature for 10 min. The supernatant result was transferred to another centrifuge tube, and vortex also centrifuged at 13,000 rpm at 4°C for 10 min. The supernatant has been homogenization by flipping the tube, stored in the refrigerator (4°C) for 30 min, centrifuged at 13,000 rpm at 4°C for 10 min. Supernatant had to get, is removed then the pellets and drying. Dry pellets are dissolved with TE 100 μ l buffer, added absolute cold ethanol, till flipped and homogeneous. Then incubated in the freezer (-20°C) for 30 min and centrifuged at 13,000 rpm at 4°C for 10 min. The supernatant was removed while the pellet was washed using 70% ethanol and dried with air. A dry DNA pellet was dissolved with 100 μ l buffer TE, and finally, DNA was stored in a freezer (-20 °C).

2.2.2. Polymerase Chain Reaction (PCR)

Three μ l DNA templates for each PCR single tube were mixed with a mixture of 7 μ l PCR (2.5 μ l Gotaq master 0.5 μ l EgMT F and 0.5 EgMT R, and 3.5 μ l ddH₂O). PCR amplification was in Eppendorf Mastercycler ep 384 (Eppendorf, Westbury, New York, USA). The amplification program consisted of the first denaturation cycle for 4 min at 94°C, cycle 35 denaturation at 94°C, for 30 sec, annealing 52°C for 1 min 15 sec, extension 72°C for 1 min 30 sec, and step extension at 72°C for 8 min.

2.2.3. Agarose gel electrophoresis

PCR products were analyzed by electrophoresis in a 1% agarose gel stained with a Red Gel and visualized by Ultraviolet translation (UVP).

2.2.4. Data analysis

Amplification of DNA readings was analyzed by software UV-1D (UV-Tex ver v16,09b).

3. Results and Discussions

Figure 1 shows amplification and each sample from *Elaeis guineensis*, only 1 sample is blurred (non-detection). Each sample has different brightness amplification depending on the reaction of the plant

to the BSR fungus. The EgMT primer is expected to be suitable for detecting *Ganoderma* fungus in dura, pisifera and tenera oil palm plants.

PCR can detect disease and primer used to produce various sizes of PCR products. The development of PCR-based tests has also been reported to identify several fungi from wood reported from hardwood tree species, such as *Armillaria spp.*, *Ganoderma spp.*, *Schizophyllum spp.*, *Stereum spp.* and *Trametes spp* [9].

M	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
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Figure 1. UV-1D doc from PCR mix with DNA of root mature of *E.guineensis* (M=Marka, Line 1-10= Sampling of PCR mix)

The length of the DNA fragment is visible with UV-1D after being irradiated with UV light. DNA amplification ranged from a value of 10,526-114,289 bp (Table 1). The type of dura detected by amplified BSR disease was 34,211-114,289 bp. On the other hand, pisifera is only 21,053 bp, and tenera is 10,526-104,844 bp.

Table 1. Amplifikasi DNA for *Elaeis guineensis*

Line	Material Genetic	Amplification DNA (Base pair)
1	Dura	nd
2	Dura	114,289
3	Dura	109,500
4	Tenera	104,844
5	Dura	95,047
6	Dura	87,427
7	Tenera	87,427
8	Tenera	76,976
9	Dura	66,283
10	Tenera	60,876
11	Tenera	44,737
12	Dura	44,737
13	Dura	34,211
14	Pisifera	21,053
15	Tenera	10,526
16	Tenera	10,526

The coding of the metallothionein gene is found to be decreased regulated in infected *Ganoderma boninense* at the root of oil palm [10]. EgMT included in *metallothionein-like protein* has also been reported to function as redox-homeostatic in root and leaves tissue for expression of resistance genes of fungus *Ganoderma sp* [11-12].

4. Conclusions

The UV-1D method can read the length of the PCR product of the DNA fragment. The EgMT primer used in PCR testing thus provides a diagnostic tool for positive *Elaeis guineensis* due to BSR disease.

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