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Molecular performances of oil palm (*Elaeis guineensis*) tolerance to *Ganoderma* sp.

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Abstract. Oil palm plantations in Asia, particularly in Indonesia and Malaysia face the threat of basal stem root diseases caused by *Ganoderma* sp. Various methods and approaches have been made to select the oil palm tolerant to *Ganoderma*, among others with genes selection. The purpose of this research is to analyze the molecular performance of some oil palm that tolerant to *Ganoderma* based specific SSR markers. The plant material used in this trial is two cross-series of genetic material belonging to PT Socfindo, which has been known the level of resistance to *Ganoderma*, first is cross-series of 15-year-old oil palm in the field, and secondly is a new cross-series for early detection in the nursery stage. The results showed all target genes are presented in all samples. There are differences in predicted gene expression between tolerant and susceptible palm in the healthy mature plant leave tissue. The five molecular markers (*EgMT*, *EgIFR*, *Rgen_Pto*, *Eg002*, and *Eg003*) in healthy palm leaf tissue can be considered as potential molecular markers for oil palm *Ganoderma* tolerant screening.

1. Introduction
Currently, oil palm is an important economic crop and has become one of the world's major vegetable oils as a potential source of biodiesel. Indonesia is presently the world's first palm oil producer with 36.5 million metric tons of palm oil production or nearly 70% of world palm oil production [1]. Unfortunately, oil palm plantations in Asia, particularly in Indonesia and Malaysia face the threat of basal stem root diseases caused by *Ganoderma* sp. pathogens [2].
The Ganoderma control that is widely studied and developed today is the use of tolerant plants. Various methods and approaches have been made to select oil palm tolerant to Ganoderma. That was observation oil palm infected in the field (commercial plantation, progeny trial, parental gardens, seed garden, and specific Ganoderma field trial), early screening test in the nursery stage, and selection at biochemical and molecular levels [3-5]. The study of genes related to Ganoderma resistance began to be done by several oil palm breeders in recent years. Several genes of resistance were identified, including EgPAL (phenylalanine ammonia lyase), EgC4H (cinnamic acid 4-hydroxylase), EgCHI (chalcone-flavone isomerase), EgIFR (isoflavone reductase). Furthermore EgEMLP1 (early methionine-labelled polypeptides), EgMT (metallothionein-like protein), EgVIR (Virescens protein) and EgSPI (Serine protease inhibitor) [4-8]. Several Resistance Genes Homologues (RGHs) also were identified in oil palm that is phenotypically resistant to Ganoderma [9]. These genes can be used as studies of molecular markers to select oil palm tolerant. In this study used a distinctive marker of several genes that have been known to be associated with tolerance to Ganoderma. Thus, this study aimed to analyze the molecular performance of some oil palm that tolerant to Ganoderma based specific SSR markers.

2. Materials and Method

2.1. Oil palm sample and DNA extraction

Oil palm planting material used in this trial is two cross-series of genetic material belonging to PT Socfindo, which has been known the status of tolerance to Ganoderma, first is cross-series of 15 years old oil palm in the field, and secondly is a new cross-series for early detection in the nursery stage. The mature oil palm was selected by infected and healthy palms, while in the nursery was chosen by inoculated and not inoculated seedling. Extraction and isolation of DNA samples on leaves and root tissue was performed using CTAB procedure [10] as developed in molecular laboratory of PT. Socfindo. The quality and quantity of the DNA was evaluated using 1% agarose gels and measured by UV-Spectrophotometer, respectively. All the samples of DNA extraction were stored at -20 °C before using for the next analysis.

2.2. Amplification of DNA with specific SSR primers

Seven specific primers were chosen and designed (EgPRP, EgMT, EgIFR, Rgen_Pto, Eg001, Eg002 and Eg003) from the selected oil palm resistance genes was used previously [4-5, 9]. The PCR product amplification banding profile was visualized using 2% agarose gels with a 1x TBE Buffer system at 70 V and electrophoresis for 1 hour. Gels were stained with GelRed (Biotium) and visualized by the Gel Doc UVITEC Cambridge (USA). The alleles each primer were identified based on base pair size.

2.3. Data analysis

The polymorphic DNA banding profile resulted by all different primers have measured the intensity of bands (as a predicted expression) using Gel Analyzer 2010 and scored according to above of average (1) and below average (0) to collect a binary matrix to put on cluster analysis.

3. Results and Discussion

Figure 1 show SSR banding profile using EgPRP primers (as representative) for tree level of resistance oil palm sample in the mature oil palm root tissue. The result showed identical banding profile each sample with each primer used. All the target genes are presented in almost all samples, indicating that the resistance gene identified in this study is present in the whole oil palm genomes. The level of resistance to Ganoderma is determined by gene expression [7] or has SNP (Single Nucleotide Polymorphism) in its DNA sequence.
Figure 1. DNA polymorphism of mature oil palm root tissue using SSR primer *EgPRP*.

M: marker, lane 1-23: individual samples from a different level of *Ganoderma* tolerance, 24: one of the tolerant parent.

The semi-quantitative approach was used to look at the difference of predicted gene expression from the presented band visualization by measuring the intensity of band using Gen Analyzer software (Figure 2). The result of the intensity measuring of the band shows the difference of intensity value of the sample. The intensity values of each sample of each primer are then scaled into high, medium and low intensity to illustrate the regulation of each gene in the observed tissue (Figure 3).
Figure 2. The intensity of band in EgPRP primer in mature oil palm root tissue (a) in the samples 1 (b), sample 2 (c) and sample 7 (d).

Figure 3. The predicted expression diagram of seven genes tested for resistance to Ganoderma tolerant (T) and susceptible (S) plants. The predicted abundance of transcripts was measured by the intensity of band visualized. The average value of the intensity counted from 3 replicates of each tissue. Values are represented in colours with low-intensity ranges (below average), intermediates (average range) and high intensity (above average).
From the predicted expression of the seven genes tested (Figure 3) can be grouped genes that have in regulating for resistance to *Ganoderma*. Genes that have the different appearance of the susceptible can be used as molecular markers for screening the oil palm that tolerant to *Ganoderma*. The genes that have the opposite expression with the susceptible are Eg001 in mature oil palm root tissue, EgMT, EgIFR, Rgen_Pto, Eg001, Eg002, and Eg003 in mature oil palm leaf tissue, EgMT and Eg002 in seedling root tissue. While in seedling, all genes have the same expression. This information can be used as a basis for screening of *Ganoderma* tolerance by using the molecular marker (MAS/Marker Assisted Selection). Many genes (polygenic) regulate resistance to *Ganoderma* in previous reports [7]. Therefore the using more of molecular markers will be more confident and precise results.

**Figure 4.** The dendrogram was depicting the genetic relationship of resistance to *Ganoderma* in healthy leaf tissue of mature oil palm using five selected SSR primers. With individual information; L: leaf tissue, M: Mature palm, T: tolerant, S: Susceptible, M: moderate, and h: healthy plant.

The results of cluster analysis based on predicted expression (the intensity of band) of SSR markers showed the grouping of plant resistance to *Ganoderma* occurred in healthy leaf tissue of mature palm using five selected SSR markers, i.e., EgMT, EgIFR, Rgen_Pto, Eg002, and Eg003 (Figure 4). The grouping of the population into two groups at the similarity coefficient is 0.60, i.e., I) the susceptible to moderate samples, and II) the moderate to tolerant samples. Tolerant palm samples within a group with tolerant parents (DD ITs_11) indicate that the resistance character is inherited.

4. **Conclusions**
All the target genes related to *Ganoderma* tolerance are present in all samples in this study. There are differences in predicted gene expression between tolerant and susceptible palm in the healthy mature plant leave tissue. The five molecular markers (EgMT, EgIFR, Rgen_Pto, Eg002, and Eg003) in healthy plants leaf tissue can be considered as potential molecular markers for oil palm *Ganoderma* tolerant screening.

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