Molecular performance of commercial MTG variety oil palm based on RAPD markers

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Abstract. The oil palm, an economically important tree in Indonesia, has been one of the world’s major sources of edible oil and a significant precursor of biodiesel fuel. This research is conducted by taking individual tree sample of commercial MTG variety germplasm oil palm one years old. The purpose of this research is to analyse molecular performance of some oil palm MTG variety based on RAPD markers. In this experiment, the DNA profile diversity was assessed using markers of oil palm’s random RAPD markers (OPD-20, SB-19, OPM-01 and OPO-11). A total of 15 trees commercial MTG oil palm variety were used for analysis. The results of the experiment indicated out of 4 RAPD markers (OPD-20, SB-19, OPM-01 and OPO-11) showed polymorphic of PCR product. These preliminary results demonstrated RAPD marker can be used to evaluate genetic relatedness among trees of commercial MTG variety oil palm and detecting either genetic variants or mislabelled.

1. Introduction

African oil palm is a prominent export commodity and contributes significantly to Indonesia's current economy and Indonesia is the world's largest producer and exporter of palm oil (Elaeis guineensis Jacq.). North Sumatra Province is one of the oil palm plantation centers in Indonesia, with CPO production in 2016 (crude palm oil) reaching 5,440,594 tons and is the second largest province after Riau for CPO production [1]. The high productivity is not only in the production parameters of FFB (Fresh Fruit Bunch), but also the high oil to extraction rate (OER), the quality and composition of the material contained in the oil, such as the content of unsaturated fatty acids and carotenoids.

Optimal yield production of oil palm in Malaysia and other countries of South East Asia are hampered by the presence of devastating Basal Stem Rot (BSR) disease caused by Ganoderma boninense. For more than 40 years, Ganoderma remained to be the most serious problem in many areas in Malaysia and Indonesia. But unfortunately, there is no single reliable application in suppressing or controlling either the disease severity or a promising resistant variety of oil palm to this
pathogen [2]. Many researchers claim that *Ganoderma* is an important disease in oil palm that causes significant losses in palm oil production. This disease is able to attack at various stages of oil palm development, ranging from germination stage to adult stadia. The symptoms are slow, but plants that have been infected will be death. The attack always starts from the root zone [3]. Genetic material (germplasm) is a key element in the use of molecular diagnostics to improve seed quality and productivity of oil palm. Currently, oil palm germplasm with resilience characteristic of *ganoderma* is already available from Indonesian producers of seedlings (eg, MTG varieties of Socfindo and PPKS) and can be commercially obtained, but this is of moderate resistance. Therefore, this applicative research is focused on the development and production of oil palm genotype with superior resistance to stem rot disease with molecular diagnostic approach.

The use of plant material resistant / resistant to diseases such as the use of plant material varieties MTG (Moderate Tahan Ganoderma) is one of the efforts to increase the productivity of oil palm plantations. For the long term, the use of resistant plant material is a great opportunity to control stem rot disease. Breeding for resistance is an obvious approach and a long-term solution for *Ganoderma* disease. It can also be used for controlling disease among oil palms by developing a variety of palm trees with high resistance to the disease. The most important factor in breeding for resistance is the source of resistance (the genes), which is available in the same gene pool that contains genes of all other inherited traits [4]. Breeding for *Ganoderma* disease tolerance is limited due to the lack of an effective screening method [5]. The use of DNA as a genetic material has been widely applied to know the diversity of a particular character in oil palm at the genomic level;[6-8]. Thus far, resistance to *Ganoderma* has not been clearly found yet in any oil palm germplasm. Furthermore, even when tolerance is present it may represent a relative level of tolerance, in the sense that the all palms get the disease, but the tolerant genotypes last longer.

2. Materials and methods

2.1. Plant material and DNA isolation

The fresh young leaves plant material used is palm oil from commercial MTG (Moderate Tahan *Ganoderma*) derived from PT. Socfindo. The extraction and isolation of genomic DNA was performed by genomic DNA isolation procedures adapted from the CTAB method by Orocho[9] with some modifications to the concentrations of polyvinilpolypirillidone (PVPP) and 2- mercaptoethanol [10-11]. The quantity of each isolated DNA was measured with a nanodrop spectrophotometer while its quality was electrophoresed on 0.8% electrophoresis gel. The material genetics (genomic DNA) were stored at -20 ⁰C.

2.2. DNA amplification

For DNA amplification, the method is based on [11]. Furthermore, PCR was performed on a total volume of 25 µl, 2 µl of DNA extract added to 12.5 µl reaction mix (Go Green Taq Promega), 9.5 µl nuclease free water and 1 µl random primer. Amplification using 6 primers ie OPD-20, SB-19, OPM-01, and OPO-11. PCR reaction using AB Biosystem thermocycler is programmed as follows: after 2 minutes of heating at 94 ⁰C, DNA amplification was performed at 45 cycles from 1 minute denaturation at 94 ⁰C, 1 minute at 36-37 ⁰C, and 2 minutes extension at 72 ⁰C. Forty-five cycles are terminated after 4 minutes of extension at 72 ⁰C and cooled to 4 ⁰C. The amplified products were mixed with 4 µl of 6X loading dye and resolved in 2 % agarose gel containing ethidium bromide in a 2 % agarose gel containing ethidium bromide in a horizontal electrophoresis tank. The amplified RAPD patterns were analysed with the Gel Doc UVITEC Cambridge (USA) and alleles were identified according to their base pair size. Molecular sizes of amplified products were estimated using a 1kb DNA ladder marker (Promega).

Each RAPD band is considered a single locus, only locus showing clearly defined used for scoring: there are (1) and empty (0). The distance matrix or genetic inequality for all combinations of individual pairs can be performed with two types of descriptive analysis of diversity: (1) Principal Coordinates Analysis (PCoA), a factorial analysis type in the inequality table for the main group origin.
and (ii) Neighbor-Joining Tree (NJtree) to obtain a picture of kinship among individuals. This calculation and descriptive analysis uses DARwin 6.05 software [12].

3. Results and Discussions

The use of molecular markers, revealing polymorphism at the DNA level, has been playing an increasing part in plant biotechnology and their genetic studies. In this work, the utility of RAPD markers for variation and identification of oil palm germplasm was studied.

3.1. Polymorphism detected using RAPD markers

Out of 4 primers tested, all primers were chosen for further studies based on clarity of the banding patterns (table 1). The number of bands generated among the 15 oil palm MTG accessions using these 4 primers was 20, which ranged from 2 (OPO-11) to 7 (SB-19), with an average of 5 bands/primer. Out of these fragments scored, 14 (70 %) were polymorphic. A total of 20 amplicons (amplified fragment) were generated by four RAPD primers in which all of the primers were polymorphic, all as shown in Table 1. These preliminary results demonstrated RAPD markers can be used to evaluate genetic relatedness among trees of MTG variety oil palm genotype.

The results of the present study, using 15 MTG oil palm accessions and 4 RAPD primers, indicated that 70 % of the scored fragments were polymorphic, which was relatively lower compared to earlier studies utilizing dominant marker systems like RAPD [13-15]. The number of amplicon per locus is affected by the number of markers and sample size analyzed.

Table 1. Sequence of RAPD primers, the number of scorable polymorphic bands and percentage of polymorphism of each primer

| Primer Name | Primer sequence | No. of amplified bands | No. of polymorphic bands | Polymorphism % |
|-------------|-----------------|------------------------|--------------------------|----------------|
| OPD-20      | ACCCGGTAC       | 5                      | 4                        | 80             |
| SB-19       | CAGCACCCAC      | 7                      | 4                        | 57.14          |
| OPM-01      | GTTGCTGGCT      | 6                      | 5                        | 83.33          |
| OPO-11      | GCAGGGAGGT      | 2                      | 1                        | 50             |
| Average     |                 | 5                      | 3.5                      | 70             |
| Total       |                 | 20                     | 14                       |                |

3.2. Genetic diversity analysis among the MTG oil palm accessions

The scored bands were used to calculate the genetic diversity among the 15 MTG oil palm accessions. The genetic similarity coefficient between the pair samples was evaluated by calculating the Jaccard’s dissimilarity coefficient based on the proportion of shared bands. The pairwise dissimilarity coefficient was lowest (0.00) between MTG-12 and MTG-11; MTG-10 and MTG-5; MTG-8 and MTG-17 (with yellow colour). The maximum genetic dissimilarity (1.00) was observed between MTG-5 and MTG-18; MTG-5 and MTG-6; MTG-6 and MTG-7; MTG-6 and MTG-10; MTG-10 and MTG-18 (with green colour) (table 2). Pair wise dissimilarity coefficient’s ranged from 0.01 to 1.00 indicating the capability of RAPD markers to detect high levels of genetic diversity among the MTG oil palm accessions analyzed.

A dendrogram constructed using corresponding genetic dissimilarity coefficients obtained from Neighbor Joining UPGMA analysis was used to determine the clustering pattern among the MTG oil palm accessions analysis (figure 1). Three main clusters (designated ‘A’ ‘B’ and ‘C’) were formed diverging at the dissimilarity coefficient of 0.28. The cluster (‘A’) was a large one comprising 9 accessions. Within this cluster (A), there were two sub-clusters (designated ‘a1’ and ‘a2’). Sub-cluster ‘a1’ had a single cluster—comprising one accessions. The second cluster within sub-cluster ‘a2’ had 8 accessions. The second main cluster (‘B’), had four MTG oil palm accessions. And the third main
cluster (‘C’) had two MTG oil palm accessions. The clades were supported by reliable bootstrap values.

**Table 2.** Dissimilarity matrix among 15 MTG oil palm accessions based on Jaccard’s coefficient using RAPD markers

|     | MTG4 | MTG5 | MTG6 | MTG7 | MTG8 | MTG9 | MTG10 | MTG11 | MTG12 | MTG13 | MTG14 | MTG15 | MTG16 | MTG17 | MTG18 | MTG19 |
|-----|------|------|------|------|------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| MTG4 | 0.63 | 1.00 | 0.61 | 0.63 | 0.35 | 0.45 | 0.60  | 0.20  | 0.20  | 0.45  | 0.55  | 0.28  | 0.35  | 0.42  | 0.65  | 0.42  |
| MTG5 |      | 0.63 | 0.67 | 0.35 | 0.65 | 0.15 | 0.70  | 0.55  | 0.55  | 0.70  | 0.70  | 0.60  | 0.70  | 0.75  | 0.65  | 0.75  |
| MTG6 |      |      | 0.61 | 0.35 | 0.47 | 0.65 |       |       |       |       |       |       |       |       |       |       |
| MTG7 |      |      |      | 0.63 | 0.00 | 1.00 | 0.67  | 0.85  | 0.65  | 0.15 | 0.54  | 0.71  | 0.81  | 0.50  | 0.54  | 0.35  |
| MTG8 |      |      |      |      | 0.63 | 0.00 | 1.00  | 0.67  | 0.85  |       |       |       |       |       |       |       |
| MTG9 |      |      |      |      |      |      |       |       |       |       |       |       |       |       |       |       |
| MTG10|      |      |      |      |      |      |       |       |       |       |       |       |       |       |       |       |
| MTG11|      |      |      |      |      |      |       |       |       |       |       |       |       |       |       |       |
| MTG12|      |      |      |      |      |      |       |       |       |       |       |       |       |       |       |       |
| MTG13|      |      |      |      |      |      |       |       |       |       |       |       |       |       |       |       |
| MTG14|      |      |      |      |      |      |       |       |       |       |       |       |       |       |       |       |
| MTG15|      |      |      |      |      |      |       |       |       |       |       |       |       |       |       |       |
| MTG16|      |      |      |      |      |      |       |       |       |       |       |       |       |       |       |       |
| MTG17|      |      |      |      |      |      |       |       |       |       |       |       |       |       |       |       |
| MTG18|      |      |      |      |      |      |       |       |       |       |       |       |       |       |       |       |
| MTG19|      |      |      |      |      |      |       |       |       |       |       |       |       |       |       |       |

**Figure 1.** Dendrogram generated from genetic dissimilarity, using Jaccard’s coefficient and UPGMA clustering method of 15 MTG oil palm accessions based on RAPD analysis

When compared to the previous report of Putri [16], we get the three main clustering of 10 MTG variety individual with 2 SSR markers. Ten samples in this report is the same as previous research [16]. From that, we preliminary indicated there are three profiles of this commercial MTG variety germplasm collection derived from different crossing or different to MTG resistance characters.

4. Conclusions

This preliminary study indicates that RAPD markers are informative, could be used to detect polymorphism among MTG commercial oil palm germplasm accessions and were able to classify the genetic background of MTG variety oil palm and represent an essential tool for genetic analysis of
this main oil palm germplasm. Based on molecular results, some of 15 individual MTG oil palm type have different crossing or derived from different parent (different MTG resistance trait) and detecting either genetic variants.

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