DNA Profiles of MTG (Moderat Tahan Gano) Oil Palm Variety Based on SSR Marker

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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. The objectives of this study were to know DNA profile of commercial MTG (Moderat Tahan Gano) oil palm variety collections. A total of 10 trees MTG oil palm variety were used for analysis. In this experiment, the DNA profile diversity was assessed using mEgCIR0174 and SSR-1 loci of oil palm’s specific SSR markers. The results of the experiment indicated out of 3 alleles of PCR product of mEgCIR0174 (198, 203 and 208 bp) and SSR-1 (201, 217 and 232 bp). These preliminary results demonstrated SSR marker can be used to evaluate genetic relatedness among trees of MTG (Moderat Tahan Gano) oil palm variety derived from different crossing or difference to disease resistance trait or misslabeled.

1. Introduction

Oil palm (Elaeis guineensis Jacq.) is an important perennial crop in humid tropic areas and currently the most important plantation crop especially in Indonesia. Unfortunately, it now faces the threat of a devastating disease. Many researchers have identified Ganoderma boninense as the major pathogen that affects the oil palm tree and eventually kills it. Availability of Ganoderma resistance germplasm and its genetic diversity informations is main base of creating superior plant cultivar. Beside in optimization of germplasm utilization, use of the technology is able to identify early important plant traits.

Information on the genetic diversity within and among closely related oil palm varieties is essential for a rational use of genetic resources. The analysis of genetic variation both within and among elite breeding materials is of fundamental interest to plant breeders. It contributes to monitoring germplasm and can also be used to predict potential genetic gains [1].

Molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and among species. Molecular markers like RFLP,
RAPD and AFLP have also been used in oil palm [2-6]. Molecular markers provide an important technology for evaluating levels and patterns of genetic diversity and have been utilised in a variety of plant species [7]. Among the various DNA marker methods currently available that can be used to examine genetic diversity at the molecular level, the most informative polymorphic marker system to date is microsatellites, or SSRs (simple sequence repeats) [7]). Their high information content, codominance, and PCR based detection mean that SSRs are an ideal tool for many genetic applications. The advantages of microsatellite over other types of genetic markers will become more important, and more obvious, when they are used to track desirable traits in large-scale breeding programs and as anchor points for map-based gene cloning strategies. They are also preferred for high throughput mapping, genetic analyses and marker assisted plant improvement programmes [8]. In this study, we have utilised primers to amplify polymorphic SSR [9] in commercial MTG (Moderat Tahan Gano) oil palm variety collections.

2. Materials and methods

2.1. Plant material and DNA isolation
A total of 10 individuals of oil palm leaves were collected from commercial MTG oil palm variety. DNA from the leaf was extracted and purified using the CTAB method by [10] with modification at polyvinilpolypirrolidone (PVPP) and 2-mercaptoethanol concentration [11]. The quality of the DNA was evaluated using 1% agarose gels and then quantified by BioSpectrometer (Eppendorf). The material genetics were stored at -20 ºC.

2.2. E. guineensis microsatellite primers pairs and genotyping
Two independent microsatellite loci were chosen (mEgCIR00174 and SSR-1) from the oil palm reference map publishe [9]. PCR amplification of genomic DNAs using the SSR primers were carried out as per [9] and separation of the amplification products by horizontal electrophoresis with 1x TBE buffer and eletrophoresed for 3-4 hours at 60V using. The amplified products were mixed with 4 μl of 6X loading dye and resolved in 4 % agarose gel containing ethidium bromide in a horizontal electrophoresis tank. The amplified SSR allelic patterns were analysed with the Gel Doc UVITEC Cambridge (USA) and alleles were identified according to their base pair size. GENEalex ver 6.502 and DARwin ver 6.0 software were used in this experiment.

3. Results and Discussions
The results will be discussed in two subsections, they are amplification of SSR marker and genetic-diversity structure of the commercial MTG E. guineensis variety.

3.1. Amplification of SSR marker
Figure 1 shows the separation of the alleles of the microsatellite marker mEgCIR0174 after electrophoresis at 60 V for about four hours. The banding pattern of mEgCIR0174 differed by 5 bp. Longer electrophoresis period was required for better separation of the alleles with less difference. The difference of one versus two and four hour electrophoresis at 60 V for the separation of 95 and 100 bp alleles of marker MGHES-06 [12]. One or two hour of electrophoresis did not clearly separate the alleles while four hours of electrophoresis clearly separated the alleles. To ensure precision and reproducibility of fragments, DNA samples were amplified and analyzed at least twice from each individual sample. Amplicon of SSR-1 produced 3 alleles (201, 217 and 232 bp) from ten samples. These preliminary results demonstrated SSR marker can be used to evaluate genetic relatedness among trees of MTG (Moderat Tahan Gano) oil palm variety.

In the present research work we have effectively employed agarose gel electrophoresis for genotyping with microsatellite markers in oil palm. Electrophoresis of SSRs on agarose gels appeared to be easier and economical.
3.2. Genetic diversity structure
The results of clustering by DARwin software indicated out of ten samples divided to three clustering (Figure 2). Cluster I consisted of two individu plams (N, S); cluster II consisted of four individu palms (M, L, T, R) and cluster III consisted of four individu palms (O, P, Q, U). From that, we preliminarily indicated there are three profiles of commercial MTG variety derived from different crossing or different to MTG resistance trait.

A comparison of the results (Table 1) obtained in this study (mean 3 alleles per locus) was relatively higher than those earlier reported for two parents (LM2T and LM10T) of BRT10 first selection cycle oil palmpopulation with an estimated average of 1.75 alleles/locus [13]. The number of alleles per locus is affected by the number of markers and sample size analyzed.

The two microsatellite loci of MTG variety were low polymorphic with 3 alleles per locus. The observed heterozygosity ($H_o$) for each locus ranged from 0.400 to 0.875 with an average 0.638 (Table 1). At the population level, mean expected heterozygosity ($H_e$) 0.543. Nonetheless, comparisons were not on the same basis as the origin and number of samples was different coupled with the number of SSRs assayed. Similarly, the observed heterozygosity ($H_o = 0.683$) was comparable to what was reported by [14] for oil palm accessions from Ahoada ($H_o = 0.685$) and a bit higher than in Ayangba
(Ho = 0.673), but much higher than the results (0.516 to 0.551) of [15] for the five natural oil palm populations from Nigeria.

When compared to the previous report of [14], lower values of expected heterozygosity (He = 0.373) for Deli NIFOR and (He = 0.510) for 6 Deli dura populations from different origins using 14 SSR markers. Also, [16] revealed even lower values (He = 0.340) for Deli dura via 15 EST-SSRs. Recently, [15] reported Ho values (0.310 and 0.211) and He values (0.549 and 0.559) for Deli dura MPOB (Malaysia) and Deli dura Dabou (Côte d’Ivoire) with 16 microsatellite markers. These figures are higher than the reported result in this study. This low genetic diversity of the Deli breeding population reinforces the very narrow genetic base of the Deli materials having been selected from four palms introduced in Bogor (Indonesia) in 1848.

**Table 1. Profile of microsatellite loci of MTG oil palm variety**

| Locus      | N | Na | Ne | I   | Ho   | He   | F    |
|------------|---|----|----|-----|------|------|------|
| 174        | 10| 3.000 | 2.062 | 0.886 | 0.400 | 0.515 | 0.223 |
| SSR-1      | 8 | 3.000 | 2.327 | 0.947 | 0.875 | 0.570 | -0.534 |
| Pop-Level  | 3.000 | 2.195 | 0.917 | 0.638 | 0.543 | -0.155 |

Na = No. of Different Alleles Ne = No. of Effective Alleles, I = Shannon’s Information Index, Ho = Observed Heterozygosity, He = Expected Heterozygosity, F = Fixation Index

4. Conclusions
This preliminary conclusion is the SSR markers used in this study 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 10 individual palms of commercial MTG (Moderat Tahan Gano) oil palm variety have different DNA pattern from the others. The FR SSR-1 and mEgCIR 0174 marker can be used to evaluate genetic relatedness among trees of MTG (Moderat Tahan Gano) oil palm variety derived from different crossing or derived from different parent (different MTG resistance trait) and detecting either genetic variants or mislabelled.

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