

Identification of molecular performance from oil palm clones based on SSR markers

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Abstract. In Indonesia, the oil palms are an important economic crop, producing food and raw materials for the food, confectionary, cosmetics and oleo-chemical industrial demands of oil palm products. Clonal oil palm offers the potential for greater productivity because it is possible to establish uniform tree stands comprising identical copies (clones) of a limited number of highly productive oil palms. Unfortunately, tissue culture sometimes accentuates the expression of defects in oil palm, particularly when embryogenesis is induced in particular callus for prolonged periods. This research is conducted by taking individual tree sample of clone germplasm two years old. The purpose of this research is to molecular performance analysis of some oil palm clones based on SSR markers. A total of 30 trees oil palm clones were used for analysis. In this experiment, the DNA profile diversity was assessed using five loci of oil palm's specific SSR markers. The results of the experiment indicated out of 3 SSR markers (FR-0779, FR-3663 and FR-0782) showed monomorphic of PCR product and 2 SSR markers (FR-0783 and FR-3745) showed polymorphic of PCR product. There are 10 total number of PCR product. These preliminary results demonstrated SSR marker can be used to evaluate genetic relatedness among trees of oil palm clones.

1. Introduction

African oil palm (*Elaeis guineensis* Jacq.) has the highest productivity among cultivated oleaginous crops. Species can constitute a single crop capable to fulfill the growing demand for vegetable oils, which is estimated to reach 240 millions tons by 2050. Oil palm cultivation is one of the most profitable land uses in the humid tropics [1]. An important aspect to be considered when deriving perennial plants from micropropagation is the maintenance of genetic integrity with regard to the mother plant. In this regard, somaclonal variation has been reported at different levels (morphological, cytological, cytochemical, biochemical, and molecular) in micropropagated plants [2]. The economic consequence of somaclonal variation among regenerated plants is enormous in fruit crops and woody plants, because they have long life cycles. In consequence, the behaviour of micropropagated plants should be assessed after their long juvenile stage in field conditions. The occurrence of somaclonal



variation is a matter of great concern for any micropropagation system. In order to evaluate its presence several strategies were used to detect somaclonal variants, based on one or more determinants from among morphological traits, cytogenetic analysis (numerical and structural variation in the chromosomes), and molecular and biochemical markers [3]. Global methylation levels and methylation of specific sites are documented in several crops, e.g. oil palm [4], grapevine [5, 6] and apple [7]. In addition, epigenetic changes, such as DNA methylation and histone modifications, may be associated with the physiological responses of the plant cells to the conditions *in vitro* [8]. Studies on somaclonal variation are important for its control and possible suppression with the aim of producing genetically identical plants, and for its use as a tool to produce genetic variability, which will enable breeders the genetic improvement [1].

Microsatellite fingerprinting is based on high genetic variability and is one of the best molecular tools for elucidating the structure and genetic diversity of populations. 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) [9]. Their high information content, co-dominance, 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 [10]. The objective of the present investigation is to differentiate among 30 clones oil palm genotypes based on SSR markers. The description of molecular relationships was conformed using the constructing dendrogram.

2. Materials and methods

2.1. Plant material and DNA isolation

Fresh young leaves collected from 30 individual clones of 'BTC A - group' oil palm collections of PT. Socfindo. Total genomic DNA was isolated from the fresh leaf samples following modified method using CTAB by [11] with modification at *polyvinilpolypirrolidone* (PVPP) and *2-mercaptoethanol* concentration [12]. The quantity and quality of DNA were determined by spectrophotometer and electrophoresis on 1% agarose gel, respectively by *BioSpectrometer* (Eppendorf). The material genetics (genomic DNA) were stored at -20 °C.

2.2. *E. guineensis* microsatellite primers pairs and genotyping

Five independent microsatellite loci were chosen (FR-0779, FR-3663, FR-0782, FR-0783 and FR-3745) from the oil palm reference map published [13]. PCR products were run on 4% agarose gel with a 1x TBE Buffer system at 70 V and electrophoresed for 3-4 hours and stained with ethidium bromide. 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. Molecular sizes of amplified products were estimated using a 100bp DNA ladder marker (Promega).

3. Results and Discussions

The results will be discussed in two subsections, they are amplification of SSR marker and genetic-diversity structure of the clones *E. guineensis* type.

3.1. Amplification of SSR marker

Figure 1 shows the separation of the alleles of the microsatellite marker FR-3745 after electrophoresis at 60 V for about four hours. The banding pattern of FR-3745 differed by 53 bp. Longer electrophoresis period was required for better separation of the alleles with less difference. The reproducibility of the results was verified and the optimized conditions are described in material and methods. DNAs of the 30 oil palm clones genotypes were subject to PCR against SSR primers FR-3745 as described in Fig. (1) and illustrated in Tables (1). A total of 10 amplicons (amplified fragment) were generated by five SSR primers in which 3 of the primers were monomorphic, all as shown in Table 1. These preliminary results demonstrated SSR marker can be used to evaluate genetic relatedness among trees of oil palm clones genotype.

Table 1. Total number of DNA bands, number and size of polymorphic bands and their distribution among 30 oil palm clones genotypes

Primer	DNA bands		Size of polymorphic bands (bp)
	Total*	Polymorphic	
FR-0779	2	0	-
FR-3663	1	0	-
FR-0782	2	0	-
FR-0783	3	3	272, 300, 336
FR-3745	2	1	317

*total number of bands (polymorphic and non- polymorphic)

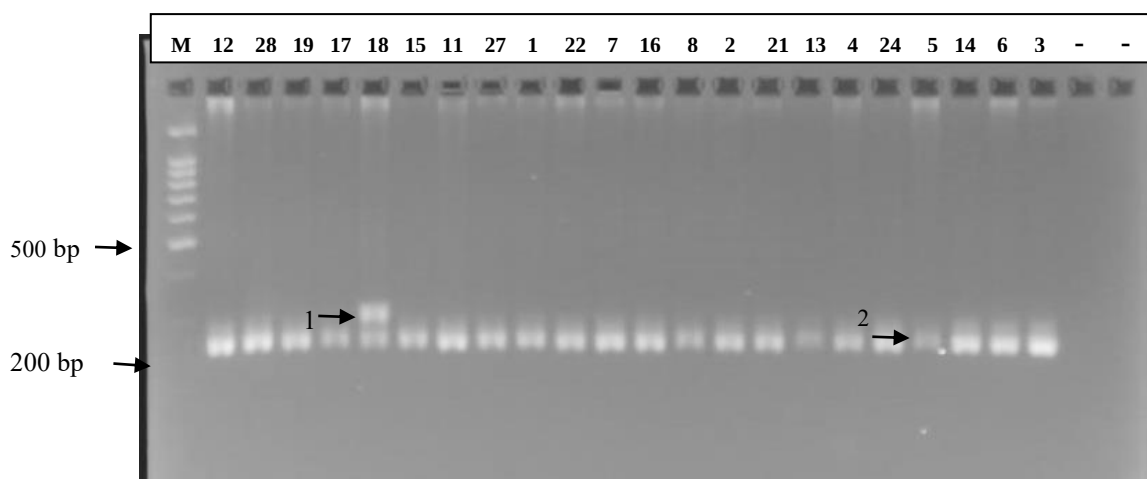


Figure 1. DNA polymorphism of 30 oil palm clone genotypes using SSR primer FR-3745
M: 100 bp DNA marker, 1-28: individu of oil palm clones
1=317 bp, 2=264 bp

3.2. Genetic diversity structure

The results of dendrogram by DARwin software indicated out of tree individuals have different profile from another (Figure 2). They are genotype 5, 18, and 29 of population. From that, we pre liminary indicated there are three of oil palm clones have somaclonal variation from different clones or

different to trait or mislabelled. From the research [4] there were variations in genomic DNA methylation during the longterm in vitro proliferation of oil palm embryogenic suspension cultures.



Figure 2. Representation of the unrooted *Neighbor-Joining* tree with five SSR genotypes using the simple matching distances under DARwin6

A comparison of the results obtained in this study (mean 2 alleles/ locus) was relatively lower than those earlier reported for MTG commercial oil palm variety (mean 3 alleles/locus) [14], relatively higher reported for two parents (LM2T and LM10T) of BRT10 first selection cycle oil palm population with an estimated average of 1.75 alleles/locus [15]. The number of alleles per locus is affected by the number of markers and sample size analyzed.

The five microsatellite loci of oil palm clone genotype were low polymorphic with 2 alleles per locus. At the population level, mean expected heterozygosity (H_E) 0.316. 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 expected heterozygosity ($H_e = 0.543$) was comparable to what was reported by [14] for MTG commercial oil palm variety from Indonesia (Socfindo).

When compared to the previous report of [16], higher values of expected heterozygosity ($H_e = 0.373$) for Deli NIFOR and ($H_e = 0.510$) for 6 Deli *dura* populations from different origins using 14 SSR markers. These figures are higher than the reported result in this study. This lower genetic diversity because of the oil palm clones population. The profile of microsatellite loci of oil palm clones genotype were in Table 2.

Table 2. Profile of microsatellite loci of oil palm clones genotype

Locus	N	Na	Ne	I	He
Pop-Level	1	2.000	1.627	0.460	0.316

Na = No. of Different Alleles Ne = No. of Effective Alleles, I = Shannon's Information Index, He = Expected Heterozygosity,

4. Conclusions

This preliminary conclusion is the SSR markers used in this study were able to classify the genetic background of clone type oil palm and represent an essential tool for genetic analysis of this main oil palm germplasm. Based on molecular results, some of 30 individual palms of clones oil palm type

have different DNA pattern from the others. The FR-0783 and FR-3745 marker can be used to evaluate genetic relatedness among trees of oil palm clones genotype in 'BTC A group'.

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