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Identication of Variance Moleculer Genotype Commercial Oil Palm (*Elaeis guineensis* Jacq.) based on Random Amplified Polymorpism DNA (RAPD) Markers

Arnen Pasaribu[1,*], Lollie Agustina P.Putri1, Dwi Suryanto[1]

Abstract —Commercial oil palm is one of plant genotype have a inbreeding so that it will be genetic depression. Genetic depression can cause difficulties to identification of genetic trait in palm oil, so that identification of moleculer variance is necessary to know the genetic potential of commercial palm oil to obtain initial information about genetic diversity. The objectives of this study was to find genetic diversity in commercial oil palm (*E. guineensis* **Jacq.***)* by using five RAPD primers (OPC-12, OPH-12, OPC-7, OPI-20 and OPD-16). The results of this study showed that Polymorpism Information Content (PIC) was 0.338. *PCoA analysis showed that the moleculer varian was 43,72%* and according to profil radial neighbour joining tree (NJtree) showed that three main groups.

Keywords—*Genetic Variation,Oil Palm , RAPD*

I. INTRODUCTION

Oil palm **(***E. guineensis* **)** is a species from family *Araceaceae*. It's a tropical crop which is mainly grown for the industrial production of vegetable oil and palm oil have gave economical value for Indonesia. It's strategic commodities. Oil palm produce Crude Palm Oil (CPO), CPO is very important for food (edible oil), industrial (oleochemical), and alternative energy sources-based biodiesel.

Commercial oil palm is a plant of breading program. The process of plant breeding can cause genetic depressionor loss genetic variability [1]. It was happened because selfing process belong to breeding program. The breeding process make genetic variation of plant will decrease. Genetic variance can be seen according to value of heterozygosity and value of homozygosity. If the value of heterozygosity is higher than the value of homozygosity, so that the genetic variance is high [2][3]. If genetic variance between wild population compare with commercial oil palm can be found there were genetic depression. Inbreeding depression on growth and yield of oil palm was happened on some character like yield of brunches, mean bunch weight and bunch number, leaf production leaf anf leaf area reatio [4].

Breeding programs have done to find best variety, high-yielding, good oil quality and tolerance to pests and diseases. The proccess of plant breeding can make inbreeding depression [5]. Inbreeding depression is caused by increased homozygosity of individuals. It's important to understand the genetic basic of these effects.

Observation to find the best genetic character is needed. There are two kinds how to find genetic characters they are morphological and moleculer identification but observation in the morphological level very difficult because environmental effect especially in low varieties. Identication of morphological characteristic at the plant which it have a low genetic variance is very difficult. So that, the identification of characteristic must be evaluated at molecular level. The identification at the molecular level is the activity to evaluate genetic diversity in DNA level at the genom of plant. The evaluate at DNA level will be give a good result because in DNA level is can not influence by environment factor. In other hand, evaluate of plant charachteristic use physiological and morphological need high cost of time and labor during measurenment [6].

The commercial palm oil have low variety so that observation to find difference of characters are very difficult. Genetic study in moleculer level can solve this problem. Moleculer marker can show diffrenciation between low varieties. The genetic moleculer markers is more accurate to study about genetic diversity than morphology characterization because they are independent of environmental effects [7] and allow cultivar identification in earlt stages of plant development [8].

Information of the genetic moleculer level is very important to study about identification of species, breeding programs, phylogenetic study, genetic variation and preservation of genetic diversity [9,10]. Diversity analyses require a large number of polymorphic markers to measure genetic relationships and genetic diversity in a reliable manner [11].

Many kinds of genetic markers have used to study of genetic divercity. They are random Amplified Polymorphism DNA (RAPD), Simple Sequences Repeats (SSR), Restriction Fragment Length Polymorpism (RFLP), Amplified Fragment Length Polymorpism (AFLP) and Single-Nucleotide Polymorpism (SNPs) [12].

The PCR amplification of genomic DNA using random primers detected DNA polymorphism and genetic relatedness between cultivars and varieties [13]. RAPD is very helpful in detecting genetic variation, evaluation of genetic diversity and we can indentifying germaplasm in a number of many species[14]. This technique relatively cheap, independent of environmental factors, effectively, and very easy to used [15].

RAPD is a more realible for genetic characterization of organism especially of plant [16]. The identification in molecular level need a sensitive primer to show the different between the object which evaluated. RAPD primer is the one of primer which can use to show genetic diversity. The advantage of this primer are polymorphism, spread all the locus, RAPD technique are quickly, easly generated by PCR and no prior sequence information and many study was use this primer for the last research. The objective of this study to find genetic diversity in commercial oil palm in polymorphic DNA level.

[1]Departement of Agrotechnology, University of North Sumatera, Medan, Indonesia *Correspondence to T. Author, email : pasaribu30@gmail.com, Tel.+62-853-62414111

II. Materials And Methods

A. Plant Materials and DNA extraction

Thirty individuals germ palm of commercial palm oil were used. Total genomic DNA was extracted from fresh leaf samples of each germ palm using a conventional CTAB method [17] with some modifications. Germ plant observed were 2-3 weeks old. The genomic DNA concentration was estimated with a nanophotometer (NanoPhotometer® P-Class-330 Implen GmbH-Germany) and the DNA quality was checked using gel agarose on 1.5 % agarose gel in 1X TAE (Tris-acid-EDTA) buffer at 75 V for 45 minutes.

B. RAPD Marker Analysis and PCR Program

There are five primers (Operon Tchnology, Almaeda, USA) (Table 1) were used for the amplification reactions. The PCR mixture (25µL) contained 2 µL DNA, 1 µL primer, 9.5 µL nucleus free water and 12.5 µL Go Taq ® Green Master Mix. PCR reaction was carried out in a DNA Thermal Cycler (The Veriti™ Thermal Cycler). The program of amplification reaction was used 45 cycles of 2 minutes at 94°C for 2 min, 94°C for 1 min, 36°C for 1 minutes, 72°C for 2 min and a final 10-min extension at 72°C. The RAPD products were electophorased on 2% agarose gel in 1X TAE buffer at 75 V for 1 h. The gel were stained in 0.5 ug ethidium bromide and photographed using gel documentation system (Gel Doc UVITEC Cambridge).

Table 1. RAPD primers used in study, the number and the percentage polymorphic fragments

Primer	Sequences (5' -3')
OPC-12	TGTCATCCCC
OPH-12	AAAGTGCGGG
OPC-7	GACGCCACAC
OPI-20	ACGCGCATGT
OPD-16	AGGGCGTAAG

III. Data Analysis

The analysis is based on the scoring results of the DNA bands in agarose gel. The bands were manually scored as present (1) or absence (0) bands. Polymorpism Information Content (PIC) value was calculated [18] with the formula $PIC_i = 2f_i\ (1\text{-}f_i)$, where PIC_i is the polymorphic informantion content of marker-i, f_i is the frequency of the marker band present and $(1\text{-}f_i)$ is the frequency of absent marker bands. Dominant marker as RAPD have a maximum of 0.5 [19]. Two types of descriptive analysis on genetic diversity were performed under DARwin 6.0.4 [20], such as: (i) a Principal Coordinates Analysis (PCoA), the factor analysis type to investigate the main groups and (ii) a Radial neighbour-Joining tree [21] to gain a clearer picture of relations among individuals.

IV. Results and discussion

Five primers were used in this study to identification of genetic variance of commercial oil palm. The analysis of PCoA is needed to measure of the capability of the marker to show the molecular variance. The analysis of PCoA was used to estimate of moleculer variatiaon [Figure 1].

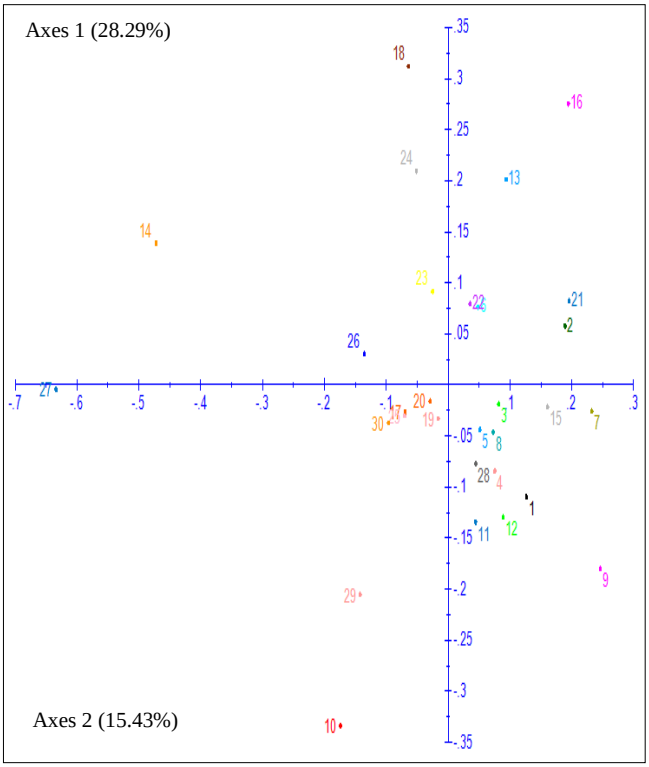

Fig. 1. Principal Coordinates Analysis (PCoA) using dissimiliarity simple matching

The analysis of PCoA showed that the distinct groups were discriminated, with axes 1 and 2 explaining 43.72% of the total molecular variance. It means the marker was used in this study can show the difference among commercial palm although the variety was used came from the same variety.

From the analysis using 5 markers on commercial oil palm, it was found that the percentage of polymorphism generated for all primers were 100% [Table 2]. Compare with the other study reported 100% polymorphism for primer OPA-02, OPA-19, and OPM-06 [22]. It means RAPD have suitable to identified genetic variance because DNA can found genetic band from the random genom. RAPD markers provide a reliable method for identifying individuals by analysis of DNA polymorphism [23].

The number of fragment explain genetic variability, the highest number of fragment showed in OPI-20 (5 fragments) and lowest number of fragment showed in OPC-12 (1 fragment). The size of bands generated by OPC-12, OPH-12, OPC-7, OPI-20 and OPD-16 ranged from 274 bp to 3053 bp. The value of PIC obtained were 0.064 to 0.491 with an average of 0.338 (Table 2). OPH-12 have highest PIC (0.491) then the other primers. PIC values also was used to find the informative primers to see diversity. The PIC of RAPD have a maximum value of 0.5 [2]. A good RAPD primer can show a high percentage of polymorphic and a high score of PIC [23]. In other that, the observation of primer is necessary to find a good primer because in some case primers can not show the genetic variation. Primer requires well to well and run to run consistency [24]. In this study all of the primers were used can show the genetic variance. The genetic variety in oil palm was identified. This information explain that the population in same variety was difference so that explorating to find another alles can be done for the next plant breeding program.

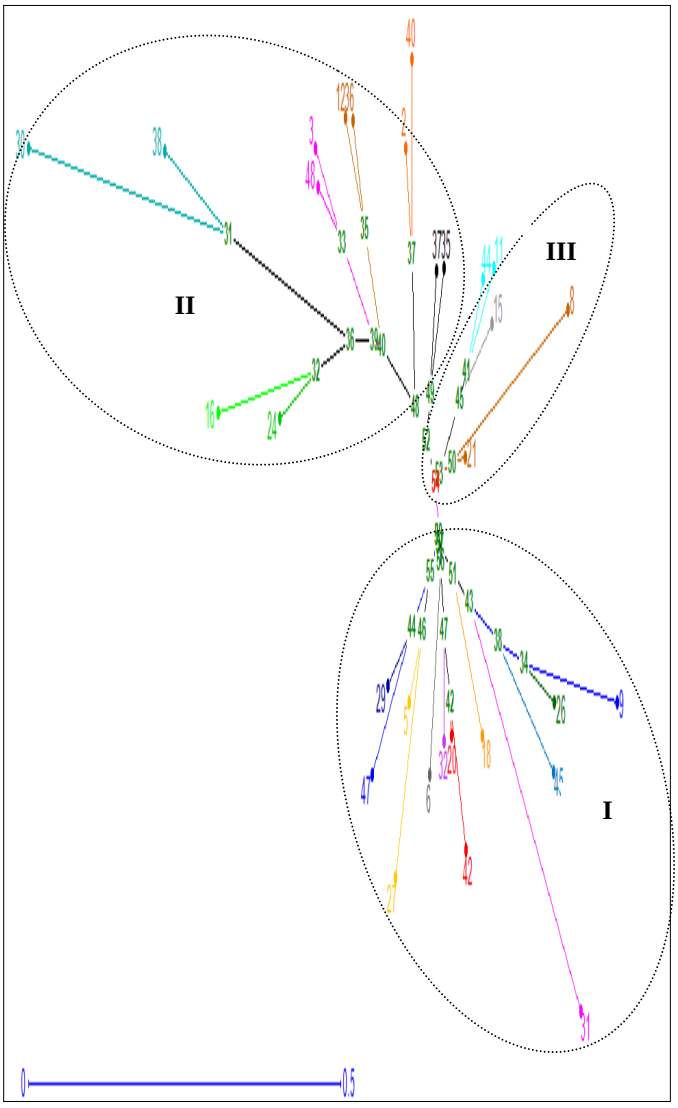

Fig. 2. Clustering of 30 individuals of commercials oil palm using radial neighbor-joining trees based simple matching dissimiliarity matrix analysis

Table 2. List of primers and their sequences used for RAPD analysis of commercial palm varieties

No	Primers	Size of fragnents (bp)	Number of Fragments	Number of Polymorphic fragments	Percentage of Polymorphic Fragments (%)	PIC
1	OPC -12	591	1	1	100	0.064
2	OPH-12	573-2153	4	4	100	0.491
3	OPC-7	455-807	3	3	100	0.215
4	OPI-20	439-3053	5	5	100	0.444
5	OPD-16	274-1810	4	4	100	0.480
	Total		18	18	-	
	Mean		3.6	3.6	100	0.338

This result showed that the primers were used in this study can show the genetic variation. In this study we can found the difference of individual samples. It means there were genetic variance in the same commercial palm oil according to primers were used. The phylogenetic analysis can show genetic distance of the species.

The radial neighbor-joining trees was used to found disting groups of individuals was used in this study [Figure 2]. Tree method was another approach for presenting diversity structure. According to this method there were 3 main groups. The first groups were #9, #26, #45, #31, #18, #42, #20, #32, #6, #27, #5, #47, and #29, the second groups were 24, #16, #38, #30, #48, #3, #12, #36, #2, 40#, #37 and #35 and the third groups were #44, #11, #15, #8 and #21. From this analysis, the next step for breeding program can choose the individual which come from the difference group, because the genetic distance for a difference group have a difference allel [25].

V. Conclusion

All these demonstrated that RAPD analysis were usefull for genetic diversity of commercial palm oil. The results of this study showed that Polymorpism Information Content (PIC) was 0.338. *PCoA analysis showed that the moleculer varian was 43,72%* and according to profil radial neighbour joining tree (NJtree) showed that three main grups.

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