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DNA intensity and genetic diversity of oil palm (*Elaeis guineensis*) to determine an elite low lipase line

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Abstract. *Angkat NU, Siregar LAM, Basyuni M, Afandi D, Syahputra I. 2021. DNA intensity and genetic diversity of oil palm (Elaeis guineensis) to determine an elite low lipase line. Biodiversitas 22: 900-905.* The acidification of palm oil due to lipase activity in the mesocarp is assessed under genetic control. Three molecular markers have been established to gauge the lipase gene in oil palm. Lower lipase activity is desired for good quality edible oil. This study aims to identify the genetic diversity by screening groups/families to determine an elite low lipase genotype of oil palm. Genetic diversity and population structure of 15 groups of oil palm were investigated by using three specific markers with GenAlex 6.502 software. Results show that the Polymorphic Informative Content (PIC) value of markers was around 0,985-0,993, which indicates that these markers are effective in determining the diversity of lipase activity in oil palm. Analysis of molecular variance (AMOVA) revealed that genetic diversity varies within individuals (54%), among individuals (31%), and among population (15%). The value of number of alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), and number of allele migration (Nm) indicate that the genetic diversity in this population is relatively low. Phylogenetic analysis identified two main groups as high lipase and low lipase activity groups based on DNA intensity.

Keywords: AMOVA, lipase, microsatellite, oil palm

INTRODUCTION

Oil palm is the most important edible oil globally (Singh et al. 2013). According to the United States Department of Agriculture (2019), Indonesia is one of the largest producers and exporter of palm oil globally, producing a total of 43 million tons in 2019. Globally, the total vegetable oil production was 207.06 million MT, of which palm oil is the highest (75.69 million MT or 36.6%), followed by soybean oil (56.73 Million MT) and rapeseed oil (27.04 Million MT). The quality of crude palm is extremely important in commerce. According to Indonesia National Standard SNI (2006), the maximum quality requirements of CPO are dirt count, free fatty acid, moisture at 0.5%, and Iodine number at 55 grams Iodine per 100 grams.

The quality of palm oil can be influenced by harvest and postharvest activities, including storage time and processing delay time. The nature of raw materials and palm oil is closely related to quality components such as free fatty acid (FFA) content (Sharif et al. 2017; Oettli et al. 2018). Nonetheless, studies have confirmed that FFA formation is strongly affected by endogenous lipase under genetic control (Sambhantamurthi et al. 2000; Morcillo et al. 2013; Wong et al. 2015; Domonhedo et al. 2018). Previous studies demonstrated that a FLL1 gene in oil palm has been established as a mesocarp lipase regulating gene (Nurniwalis et al. 2017). Genes encoded lipase activity were mostly observed in ripening fruit with high acidity and lacked genotype with low acidity (Morcillo et al. 2013). Lipase plays an important role in catalyzing biochemical reactions such as esterification, interesterification, and transesterification in nonaqueous media. Incomplete hydrolysis of triglycerides results in monoacylglycerol release into diacylglycerols, increasing free fatty acid content (Singh and Sanjay 2013).

The efforts have been studied as alternative ways to control acidity. The utilization of bleaching earth adsorbent, synthetic magnesium silicate, and magnesium oxide (MgO) has not shown significant reduction of diacylglycerol and free fatty acid levels in CPO within or without vacuum (Bariyah et al. 2017). The cost-refining process can help remove free fatty acid, but diglycerol still remains, and fatty acid becomes more susceptible to peroxidation that is a trigger to produce aldehydes and ketone responsible for rancidity (Corley and Tinker 2016; Likeng et al. 2017).

In the perennial characteristic and long life cycle of oil palm cultivation, conventional breeding might need more space and time (Cardona et al. 2018) to select promising crosses especially when increasing parental biodiversity (Herrero et al. 2020). Therefore, an effective way to screen

oil palm lines is to determine if enzyme lipase is present in its DNA via microsatellite marker. Microsatellites or simple sequence repeats (SSRs) are widely used in genetic map construction, genetic diversity assessment, quantitative trait loci (QTL) mapping, and genetic structure determination (Ge et al. 2019; Yoichi et al. 2016). It has been used to identify plant genotype in the last 20 years due to its very informative and reproducible multi-allelic genetic marker (Mason 2015), and to evaluate oil palm genetic diversity and population structures of breeding materials and parental lines (Thongthawee et al. 2010; Okoye et al. 2016). The three validated SSR markers detect the presence of lipase activity (Domonhedo et al. 2018) and allow the breeders to screen the low and high lipase genotype of oil palm. The present study aims to identify the genetic diversity of oil palm group and examine the oil palm genotypes as a desirable elite of oil palm with a microsatellite marker synchronized to oil palm estimated characterization by CIRAD.

MATERIALS AND METHODS

Study area

This study was conducted from August 2019 to March 2020 with a sample of oil palm leaf collected from progeny trial field of *E. guineensis* which is grown in Aek Loba, Asahan District, North Sumatra Province, Indonesia. There are 45 genotypes from 15 different oil palm groups/families as derived from a cross between low lipase line x high lipase line by CIRAD. CIRAD has distinguished oil palm groups into three characters ― low, moderate and high lipase ― by their origin (Table 1). Leaflet samples were used for DNA isolation for molecular analysis at DNA Molecular Laboratory of Socfin Indonesia, Dolok Masihul, Indonesia.

The DNA extraction

Total DNA samples from fresh leaflets were isolated using CTAB (Cetyl Trimethyl Ammonium Bromide) method Orozco-Castillo (1994) with PVPP (Polivynilpolypirrolidone) modification method. The quality and quantity of isolated DNA were evaluated using 2% agarose gels on UV-illuminator documented using Gel Doc and by spectrophotometer method using wavelengths (λ) 260/280 nm. Product PCR molecule fragment size was analyzed by UVITEC Cambridge FineReader with 50 bp DNA ladder standard.

PCR amplification and visualization

Detection of DNA target to lipase activity in oil palm was expressed by 3 validated markers from a previous study (Domonhedo et al. 2018). They were mEgCIR_LIP03, mEgCIR_LIP07, and DelEgCIR_C1E3 shown in Table 2. Every PCR reaction consists of 3 µl DNA template with Mix PCR (1µl buffer PCR 10x; 0,3 µl $MgCl_2$; 0,5 µl primer forward; 0,5 µl primer reverse; 3,7 µl ddH_2O; 0,2 µl U Taq DNA polymerase dan 0,8 dNTPS). The PCR program for both mEgCIRLIP03 and mEgCIRLIP07 was initial pre-denaturation stage at 95ºC for 10 mins followed by denaturation stage at 94ºC for 30s. The annealing stage was at 55ºC for 1 min 15s, the extension stage was at 72ºC for 1 min 30s, and the final extension stage was at 72ºC for 30 mins. The process was run with 35 cycles. Primer DelEgCIR_C1E3 was by pre-denaturation stage at 94ºC for 4 mins. 10 "touchdown" cycles of denaturation stage were at 94ºC for 45s. The annealing stage was at 55ºC (-0,5 ºC per cycle) for 1 min while the extension stage was at 72ºC for 1 min. This was followed by 25 cycles of denaturation at 94ºC for 45s, extension stage at 72ºC for 60s, and final extension at 72ºC for 30 mins (Domonhedo et al. 2018).

PCR products were expressed by 2% gel electrophoresis in 375 mL TAE buffer, stained with $GelRed_{TM}$ for visualization. Every 3µL DNA was mixed with 2µl loading dye. Electrophoresis was carried out at 70 Volts for 70 mins and 50 bp DNA ladder was used as the fragment size standard. The DNA was documented with UV-transilluminator (UV-Doc) and Gel-Doc (U Doc).

Table 1. Estimated character of oil palm based on the origin by CIRAD

Group/family	Character estimated
PLL010	High lipase
PLL045	High lipase
PLL796	High lipase
PLL210	High lipase
PLL444	High lipase
PLL682	High lipase
PLL993	Moderate
PLL737	Moderate
PLL133	Moderate
PLL507	Moderate
PLL157	High lipase
PLL181	Low lipase
PLL966	Low lipase
PLL260	Low lipase
PLL233	Low lipase

Source: CIRAD (unpublished data)

Table 2. Characteristic of molecular markers for lipase activity in oil palm

Primer	Primer sequence	Reference allele size	Source
mEgCIRLIP03	F:TCAAAGAAACTTGTAGCATATATCAAA R:CATCCAGTAAGCTAACACACAAATG	272	Domonhedo et al. (2018)
mEgCIRLIP07	F:CAATCCCTCTCCCATTCTCA R:CAGTGGAGCCGCTAATCTCT	206	
DelEgCIRC1E3	F:GCCAGATCGATCAAGCAAAT R:CTTTGCCAAAAGAAATGCAA	311	

Molecular data analysis

Genetic diversity parameters were estimated using GenAlEx 6.5 such as effective number of alleles (Ne), number of different allele frequencies (Na), non-randomating coefficient (Fis), inbreeding coefficient (Fit), coefficient of differentiation (Fst), and total migrants (Nm). The polymorphism for each population and locus was assessed by calculating the observed average heterozygosity (Ho) and expected heterozygosity (He) (Nei 1978). Analysis of molecular variance (AMOVA) was calculated using GenAlEx version 6.3 (Peakall and Smouse 2012).

DNA intensity analysis

The DNA band profile resulted by all different markers have measured the intensity of bands (as a predicted expression) using Gel Analyzer 2010 and scored according to above of average as (1) and below-average as (0) to collect a binary matrix to put on cluster analysis (Afandi et al. 2018).

Dendrogram

Genetic distance was constructed using (Unweighted Pair Group Method using Arithmetic Average) UPGMA implemented in Multivariate Statistical Package (MVSP) ver 3.2 software to identify the phylogenetic relationship (Basyuni et al. 2018). Two dendrograms were constructed. The first one was to identify the phylogenetic of 45 total palms, and the other one was to identify phylogenetic of 15 groups.

RESULTS AND DISCUSSION

DNA expression

The expression of the DNA band profile in gel agarose was shown in all samples and has been categorized as low, moderate, and high lipase (Figure 1). Our study shows that samples 33-36 and 43-45 were found to be high lipase, 40-42 were low lipase, and 37-39 were moderate lipase. It was observed that the lipase gene was exclusively expressed in all mature oil palm.

To obtain more evidence of lipase genes in oil palm, a semi-quantitative approach was taken to significantly determine an elite low lipase genotype among the population. We measured DNA intensity using Gen Analyzer software based on gene expression from the presented band visualization (Figure 2).

DNA intensity measurement by Gel Analyzer was to show the difference between genotypes. Intensity value of DNA varies based on their characterization. The intensity of DNA in mEgCIRLIP03 shows that low lipase genotype was dominated with high value of DNA intensity followed by moderate lipase genotype while high lipase genotype has lower DNA intensity.

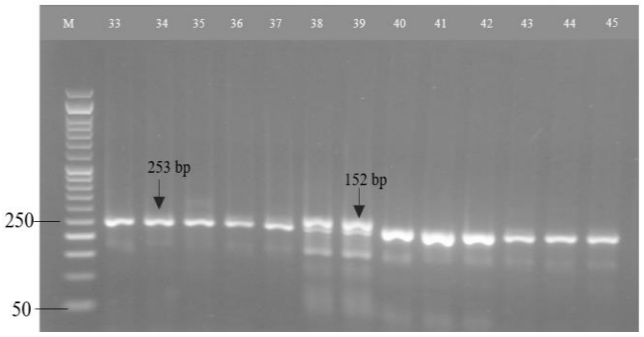

Figure 1. DNA visualization using marker mEgCIRLIP03 on agarose gel. M: DNA Ladder; 33-45: number of samples

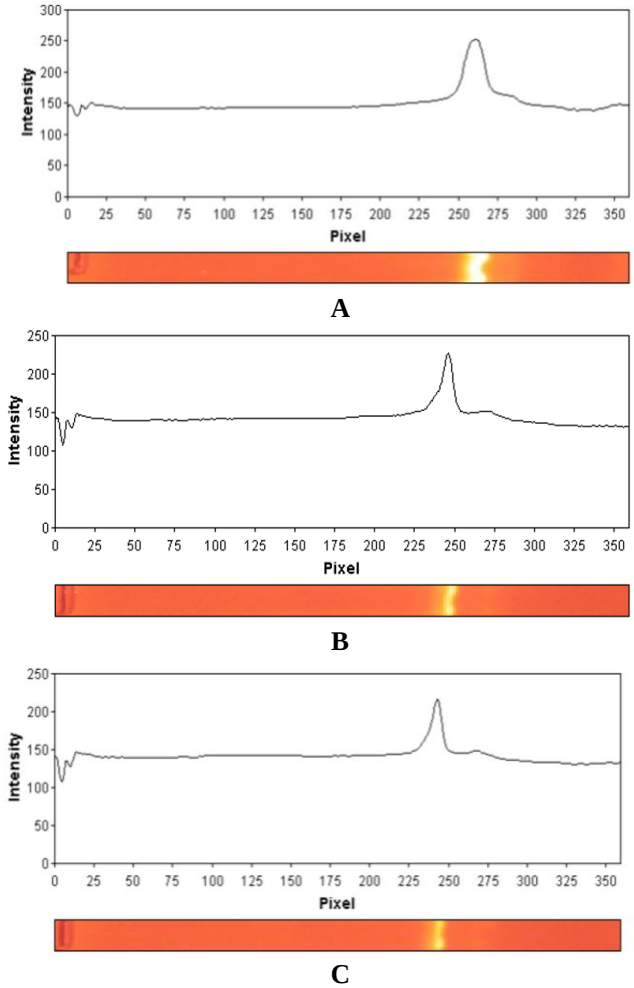

Figure 2. The intensity of DNA in mEgCIRLIP03 marker in sample 41 (A) samples 37 (B), sample 44 (C)

Polymorphism markers of oil palm

All the 45 genotypes were evaluated with 3 SSR markers loci resulting in various alleles about 22-36 and the average was 30.77 allele per locus (Table 3.). Total of 45 genotypes from 15 groups of oil palm was identified in three specific loci (mEgCIRLIP_03, mEgCIR_LIP_07, DeLEgCIR_C1E3). The PIC value (polymorphic

information content) of each markers respectively are mEgCIRLIP07 (0.985), DelEgCIRC1E3 (0.990), mEgCIRLIP03 (0.993). The total number of alleles determined the PIC value of each marker and the frequency of distribution within a population, wherein high PIC was > 0.5, moderate 0.5 > PIC > 0.25, and low PIC was < 0.25 (Bhattacharya et al. 2010).

Furthermore, these markers could be useful for genetic mapping according to Roubos et al. (2010) that PIC > 0.7. The probability (PI) in this study is lower than prevoius study (Val et al. 2012) indicates the lower of PI value, the more efficient the markers (Val et al. 2012). Genetic diversity also assessed by Fis, Fit, and Fst value. According to Soltis and Soltis (1989) the frequency of population genotype deviation could be demonstrated by Fis ranging from -1 (indicate the high heterozygosity) to 1 (indicate the low heterozygosity). Our study demonstrated the higher heterozygosity by value of Fis (-0.028 - 1.00) compared with the study of Bakoumé et al. (2014) the Fis value ranging from 0.007-1.00 for all loci, expressing a deficiency of heterozygosity, indicates deviation from the Hardy-Weinberg equilibrium (HWE), mostly due to inbreeding. Meantime average Fst value in this study (0.383) was higher than Diaz et al. (2014) reported that the Fst value of 311 oil palm tested using 10 microsatellite markers from the Republic of Cameroon was 0.03. The authors also mentioned that Fst value could be associated with the high number of migrants between population. Therefore, 15 groups of oil palm evaluated quietly similarly yet there is a variation among individuals. This variation allows for screening the low lipase activity trait in oil palm, in line with Astari (2016) the higher variation among individuals is potentially for soybean salinity tolerant screening. Average Fit value in this study (0.049) was higher than Arias et al. (2012) the Fit value of populations from the Republic of Cameroon evaluated using 31 microsatellites molecular marker was 0.015.

Genetic diversity

The genetic parameters of 15 groups of evaluated oil palm are shown in Tables 3 and 4. A greater value of Ho (observed heterozygosity) indicates that the loci in the population have a high level of heterozygosity while a greater value of He (expected heterozygosity) indicates a low level of heterozygosity (Govindaraj et al. 2015). In this study, the average observed heterozygosity (Ho) 0.526 was lower than the expected heterozygosity (He) 0.585, indicating that the genetic diversity was relatively low. Bakoumé et al. (2014) evaluated 49 populations of oil palm using 16 microsatellite markers which resulted in Ho and He means values being 0.46 and 0.64 respectively. Number of allele migration of 15 oil palm groups in this study was 1.429. The number of migration estimates gene flow as many individual genes migrate from one population to the other and per generation (Nm). Its influence is significant in the distribution of genetic materials. Nm values in this study were 1.43 higher than Budiman et al. (2019), who reported the Nm value of oil palm from six populations in Cameroon was low (0.41).

Overall, this study identified that the low level of genetic diversity in genotype of oil palm may be due to the breeding program, which agrees with Putri (2010) selfing and selection programs were able to decrease the gene diversity of plants while it is tested on the same markers.

Genetic structure of oil palm

Genetic structure of this present study was carried out by hierarchical analysis of molecular variance (AMOVA) using the infinite alleles models (F-Statistic) (Purba et al. 2020). The AMOVA of the distance matrix for 15 groups of oil palm leads the overall variation into three levels. The diversity of this population is highly influenced by variation within individuals (54%), variation among individuals (31%), and the lowest variation being among population (15%) (Table 5). This low value of variation among populations indicates the quite high similarity among 15 groups, supported by Hou and Lou (2011). The higher genetic variation within populations than among populations may partly explain why the northern populations were always clustered together.

Table 3. F-Statistik, total migrant and polymorphic information of population

Loci	Fis	Fit	Fst	Nm	PI	PIC
mEgCIRLIP_03	-0.356	0.067	0.312	0.552	0.993	0.082
mEgCIR_LIP_07	1	1	0.538	0.214	0.985	0.119
DeLEgCIR_C1E3	-0.028	0.279	0.88	0.588	0.990	0.097
Mean	0.205	0,449	0.383	0.452	-	-
SE	0.408	0.282	0.078	0.119	-	-

Note: Allele frequency correlation between indviduals in subpopulation (Fis), allele frequency correlation between population (Fst) , allele frequency in the population caused by Fis and Fst (Fit), total migrants (Nm), Probability Identity (PI), Polymorphic Information Content.

Table 4. Profile of microsatellite loci for 15 groups of oil palm

Population		Na	Ne	Ho	He	Nm
15 groups	Mean	3.178	2.970	0.526	0.585	1.429
	SE	0.197	0.185	0.065	0.033	-

Note Na: number of allele per locus, Ne: number of effective allele; Ho: observed heterozygosity; He: estimated heterozygosity; Nm: number of allele migration.

Table 5. Summary analysis of molecular variance (AMOVA) of 15 groups of oil palm population

Source	df	SS	MS	Est. Var	% Var
Among population	14	42.32	3.023	0.219	15
Among individuals	30	51.2	1.711	0.461	31
Within individuals	45	35.5	0.789	0.789	54
Total	89	129.16		1.469	100

Note: df: degree of freedom; SS: Sumsquare; MS: MeanSquare; Est.Var: estimation variation; Var: variant; Fis: inbreeding coefficient; Fit: non-randomating coefficient, Fst: different coefficient

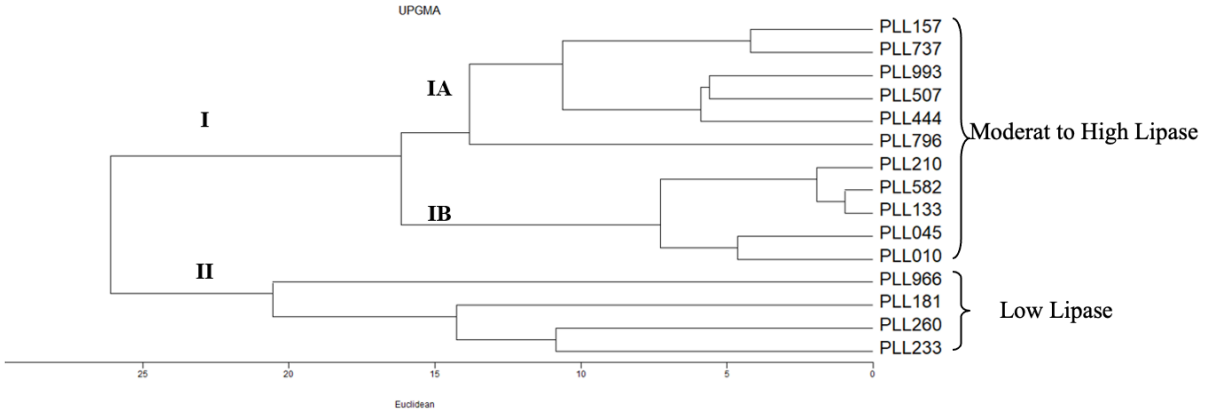

Figure 3. Cluster analysis of 15 groups oil palm based on DNA intensity

Phylogenetic of oil palm groups

Cluster analysis depicts that there are two main groups in population based on the DNA intensity measurement (Figure 3). The first group consists of 11 groups: PLL157, PLL737, PLL993, PLL507, PLL444, PLL796, PLL210, PLL582, PLL133, PLL045, and PLL010. The second group consists of PLL966, PLL181, PLL260, PLL233, which have lower FFA content. The first group is divided into two sub-clusters. The first cluster IA consists of PLL157, PL737, PLL933, PL507, PL744, and PL796 while the second sub-cluster IB consists of PLL210, PLL582, and PLL133, PLL045, PLL010.

Molecular analysis with 3 validated markers shows compatibility between our study and CIRAD estimate characterization (Table1.) This study analyzes the DNA intensity among 45 genotypes of 15 groups and the resulting four groups in the same clustering were PLL266, PLL230, PLL266, and PLL181, which are estimated as low lipase group by CIRAD. CIRAD estimating of oil palm characterization is based on the crosses of the origin (unpublished data). Val et al. (2012) mention that the membership of olive oil germplasms strongly suggests the existence of common ancestors in the genetic background. Our four selected groups have the same crosses of origin, where DA115D were female parents. Ebongue et al. (2008) evaluated the acidity of oil palm from different origins and it resulted in the progeny from the crosses between LM2T as female parents x DA115D as male parents having higher value of acidity (24%-30%). In line with our study, Domonhedo et al. (2018) mentions that all offspring of DA115D, from which numerous elite palms used were female parents for seed production originate, were found to be low lipase progenies.

Our study also detected the progenies as high lipase according to CIRAD characterization but only three groups from seven groups they had estimated, such as PLL157, PLL444, and PLL6796. The phylogenetic based on DNA intensity by three markers used show that three groups were in the same cluster. Generally, in our study, the difference between moderate and high lipase groups was not significant. The avarage value of DNA intensity of DelEgCIR_C1E3 shows that only two characteristics were detected: groups with DNA intensity value higher than average value 247,89 as a high lipase and groups with DNA intensity value lower than average values assumed as low lipase genotype.

However, four groups of oil palm were indicated as an elite low lipase line based on DNA intensity from molecular amplified band. PLL966, PLL181, PLL260, and PLL233 only groups were evident as high lipase according to CIRAD characterization. It was deduced that the lipase activity had low genetic diversity at population level. The most informative markers mEgCIRLIP03 and DelEgCIRC1E3 were relevant to determine two different lipase genotypes for developing oil quality in mesocarp.

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