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MOLECULAR MARKER SCREENING OF OIL PALM TO IDENTIFY PALMS WITH LOW LIPASE ACTIVITY

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ABSTRACT

The mesocarp of oil palm fruit, which constitutes 45%-55% oil by weight, is the primary source of oil, and its quality directly impacts economic value. High lipase activity in the mesocarp raises free fatty acid (FFA) levels, which serve as critical indicators of oil quality. This study aimed to screen IOI oil palm materials with low acidity levels using three published SSR markers. Two markers, mEgCIR_LIP07 and DelEgCIR_C1E3, showed no polymorphism in four breeding crosses, while mEgCIR_LIP03 showed polymorphism only in the Ulu Remis Tenera (URT) cross. DNA sequencing of mEgCIR_LIP03 revealed two alleles: $(TG)_6$ $(TA)_7$ (281 bp) and $(TG)_0$ $(TA)_{29}$ (325 bp). The marker-trait correlation study showed a clear trend: The single marker allele (281 bp) was predominantly associated with lower FFA values, while the combined presence of two alleles (281 and 325 bp) or the single 325 bp allele was skewed towards higher FFA values. By using the mEgCIR_LIP03 marker, we can eliminate high lipase palms, allowing breeders to identify potential low lipase palms and develop an elite low lipase line of URT.

Keywords: *Elaeis guineensis*, free fatty acids, lipase, simple sequence repeats.

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INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is the most important crop in Malaysia. This perennial crop produces two types of oil: Palm oil or crude palm oil (CPO) and crude palm kernel oil (CPKO). The quality of CPO is extremely important in commerce. According to Local Malaysian Delivered CPO (PORAM/MPOA) standard, maximum quality requirement of CPO is free fatty acid (FFA) at 5%. Since FFA are less stable than neutral oil, they are more prone to oxidation and rancidity, which significantly limits the shelf life of the oil (Ngando-Ebongue *et al*., 2006). FFA content is therefore a key indicator of oil and fat quality and commercial value. Although FFA can be eliminated through energy-costly refining process, but diglycerides remain and have significant undesirable effects on crystallisation and fractionation (Corley & Tinker, 2016), resulting in significant economic losses (Gibon *et al*., 2007). The quality of palm oil can be affected by harvest and post-harvest activities, including storage time and processing delay time. Ideally, mature oil palm bunches should be harvested every 7-10 days and processed within 24 hr to stop the lipase enzyme from increasing FFA levels. However, smallholders often struggle with timely harvesting and extraction processes, especially those who lack access to nearby, efficient milling facilities. Additionally, the oil palm industry is grappling with a significant shortage of workers, exacerbating these challenges. This labour

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shortage means that harvesting and processing are often delayed, leading to overripe bunches may be rejected by mills (Morcillo *et al*., 2013). Harvesting too early results in oil loss, while delaying harvest leads to acidity and lower-quality oil. Addressing these challenges entails substantial additional costs for the oil palm industry. (Domonhédo *et al*., 2018).

Lipases in the mature palm mesocarp are enzymes that hydrolyse triglycerides into glycerol and fatty acids. Lipase plays an important role in lipid metabolism, regulating the synthesis and hydrolysis of lipids. They catalyse the hydrolysis of ester compounds, primarily neutral lipids such as triacylglycerols (TAG), at the oil-water interface, resulting in the release of long-chain aliphatic acids from glycerol (Brockman, 1984). Incomplete hydrolysis leads to the release of monoacylglycerols (MAG) and diacylglycerols (DAG) in addition to FFA. Researches have established a clear link between lipase activity and mesocarp oil acidity in oil palm fruit. Ngando-Ebongue *et al*. (2006) demonstrated that lipase activity was correlated with mesocarp oil acidity in palms whose fruit mesocarp contains either high or low lipase activity. A lipase gene expressed in the mesocarp, initially identified as *FLL1* (full-length lipase class 3) by Nurniwalis *et al*. (2007), was further studied by Tranbarger *et al*. (2011) and Morcillo *et al*. (2013). Subsequently, it was renamed as *EgLIP1* in oil palm. Morcillo *et al*. (2013) found that high gene expression was observed in ripening fruit with high acidity but absent in the genotype with low acidity. This observation aligns with findings from other studies that have reported segregation in lipase activity levels within various oil palm populations, with some individuals displaying high mesocarp lipase activity and others low activity (Angkat *et al*., 2021; Cadena *et al*., 2013; Likeng-Li-Ngue *et al*., 2016; Morcillo *et al*., 2013; Sambanthamurthi *et al*., 2000; Wong *et al*., 2015).

Morcillo *et al*. (2013) proposed a monogenic inheritance model for lipase activity in oil palm, identifying *EgLIP1* as the sole controlling gene. They developed the mEgCIR_LIP03 genetic marker through bulk segregant analysis (BSA), which co-segregated with the low/high lipase activity trait. However, this conclusion was challenged by Domonhédo *et al*. (2018), who suggested that *EgLIP1* may not be the only gene involved in regulating lipase activity. Their research identified three closely linked genes, including *EgLIP1*, that potentially contribute to oil hydrolysis, particularly in contexts where *EgLIP1* expression is absent. Consequently, they developed three SSR markers: mEgCIR_LIP03 (Morcillo *et al*., 2013), mEgCIR_LIP07 and DelEgCIR_C1E3 (Domonhédo *et al*., 2018). In the current study, these three markers were selected for analysis to investigate their amplification patterns and their association with variations in mesocarp FFA and lipase in oil palm fruit mesocarp.

The mEgCIR_LIP03 marker has been successfully employed in DNA intensity analysis to identify elite low-lipase oil palm lines (Angkat *et al*., 2021). These authors, along with Domonhédo *et al*. (2018) and Morcillo *et al*. (2013), highlight the importance of three validated SSR markers as critical tools for differentiating low and high lipase genotypes in oil palm breeding programs. This capability empowers breeders to make informed decisions in their breeding programs, aiming to develop oil palm varieties with optimal oil quality traits and low FFA. The objective of this study is to screen the IOI oil palm breeding materials characterised by low acidity levels, utilising the aforementioned three SSR markers. These SSR markers are closely linked to marker loci associated with lipase genes, offering valuable insights into the lipase activity of the examined oil palm materials.

MATERIALS AND METHODS

Plant Material

This study utilised oil palm samples from trial plots located at Regent Estate (Negeri Sembilan) and Pamol Timur Estate (Johor), Malaysia. A total of 353 palms were genotyped using three published SSR markers. The samples comprised: 310 palms from Trial O/10-135/R (Ulu Remis, UR origin) (169 *tenera*s, 63 *dura*s, 78 *pisifera*s), 14 palms of TSBT (Ulu Remis, UR origin), 16 palms of DxP control (Deli AVROS) and 13 palms of Trial DT (Ekona *Dura* material). The Trial O/10-135/R material (UR origin) represented the fourth generation, encompassing five families developed and selected through either full-sib mating or selfing. The TSBT material (UR origin) represented the third generation, with a single selected family. The Ekona *Dura* material consisted of four families. Notably, the Ekona *Dura* grandparental generation originated in Africa, while subsequent generations were introduced to Malaysia and planted at Pamol Timur Estate.

Assay for Lipase Activity Using the Cold-activated Titration Method

The Cold-activated Titration Method for lipase activity determination adopted throughout this article was a modification from Wong *et al.* (2015). A ripe bunch with 1-5 loose fruit (before harvest) was sampled from trial areas. The harvested bunch sample was kept inside a gunny sack and then tied and attached with a tag label with details such as trial code, palm number and number of loose

fruit. After chopping, only undamaged normal fruitlets (equal number of outer and inner) which showed no visual signs of fungal infection were detached from spikelets. The fruitlets from the same bunch were stored at 4°C for 21 hr to fully activate the lipase. Fruitlets were then pressure-cooked for 30 min at 10 psi to deactivate the lipase and soften the mesocarp. The water level for each pressured-cooker must be ensured to be the same. The oils were extracted by physical pressing. Oil samples were oven-dried for 1 hr at 105°C. After that the samples are left for cooling down for 30 min inside the desiccator. A volume of 50 mL of neutralised isopropanol was added to 1 g of oil. The resultant mixture was regulated to about 40°C to ensure that the oil was fully dissolved before titrated with 0.1 N sodium hydroxide (NaOH) (American Oil Chemists' Society [AOCS], 1998). The FFA content in the palm oil was expressed as a percentage based on palmitic acid. The % FFA (as palmitic acid) is equal to (25.6 x NNAOH x V)/m, with N is the normality of standardised 0.1 N NaOH; V is the volume of standard 0.1 N NaOH and m is the mass of the test portion (AOCS, 1998).

DNA Extraction and Quantification

Mature leaves were collected for DNA extraction. Total genomic DNA was extracted using the FavorPrep™ Plant Genomic DNA Extraction Mini Kit (Favorgen, Taiwan), following the manufacturer's protocol. DNA quantification and quality assessment were performed using a DeNovix DS-11 spectrophotometer (DeNovix Inc., Wilmington, Delaware, USA). Additionally, DNA quality was visually assessed via gel electrophoresis on a 1.0% agarose gel. Extracted genomic DNA samples were then stored at -20°C for short-term use and at -80°C for long-term storage.

PCR-based Marker Analysis

DNA targets associated with lipase activity in oil palm were detected using three previously published SSR markers (Domonhédo *et al*., 2018; Morcillo *et al*., 2013). Minor modifications including nucleotide correction, primer length extension or redesign of the primer priming site after aligned on the oil palm whole-genome sequence as published by Singh *et al*. (2013) to enhance the specificity of polymerase chain reaction (PCR) on IOI breeding materials. These markers, namely mEgCIR_LIP03, mEgCIR_LIP07 and DelEgCIR_C1E3, are listed in *Table 1*.

PCR reaction performed in 12 µL final volumes, with 30 ng of genomic DNA mixed with PCR mixture [1X Taq II buffer (1st Base, Singapore), 0.2 mM dNTP Mix, 1.5mM MgCl2, 0.5 µM of forward primer, 0.5 µM of reverse primer, 0.025U Taq II DNA polymerase and Nuclease-Free water]. The PCR was done on a Veriti™ 96-Well Thermal Cycler (Applied Biosystems™, USA). The PCR program for three markers was initial pre-denaturation stage at 94ºC for 5 mins followed by 35 cycles of denaturation stage at 94ºC for 45 s. The annealing stage was at 50ºC/56ºC for 60 s, the extension stage was at 72ºC for 60 s and the final extension stage was at 72ºC for 5 mins. PCR products were analysed using the DNA 500 kit on the Shimadzu microchip electrophoresis system MCE-202 MultiNA (Shimadzu, Japan), following the manufacturer's recommended protocol. Samples were run with the reagents from the DNA 500 kit: Separation buffer, DNA marker reagent and either a 100 bp DNA ladder or pUC19 DNA/MspI (HpaII) DNA ladder. Samples and reagents were loaded onto the MultiNA instrument, where on-chip mixing and electrophoresis were performed automatically. Data acquisition and analysis were conducted using the MultiNA Control and MultiNA Viewer software (Shimadzu, 2008). The PCR products were sent to Apical Scientific Sdn. Bhd., Malaysia, for sequencing.

RESULTS AND DISCUSSION

Primer Testing for Amplification and Optimisation

Four IOI oil palm breeding crosses - Ulu Remis *tenera* (URT), Deli *dura* x AVROS *pisifera* (DxP), Ekona *dura* and TSBT were evaluated with three SSR markers. Initial primer sequences and PCR conditions, following Domonhédo *et al*. (2018), resulted in non-specific amplification for two markers, mEgCIR_LIP03 and mEgCIR_LIP07.

TABLE 1. THREE MODIFIED SSR MARKERS SEQUENCE AND CONDITION FOR LIPASE ACTIVITY IN OIL PALM

Primer	Primer sequence (5′ – 3′)	Tm (ºC)	Ta (ºC)	Expected allele size
mEgCIR_LIP03	F: TCTGAAGGTGTGTATTGGATTATTG	53.0	56	281
	R:CCAGTAAGCTAACACACATATGTAGATAAA	54.8		
mEgCIR_LIP07	F:CAATCCCTCTCCCATTCTCAAGAG	56.8	50	206
	R:CAGTGGAGCCGCTAATCTCTATACC	58.3		
DelEgCIR_C1E3	F:GCCAGATCGATCAAGCAAAT	53.4	50	311
	R:CTTTGCCAAAAGAAATGCAA	50.2		

Note: Tm - melting temperature; Ta - annealing temperature.

To enhance specificity, these two pairs of markers were redesigned by increasing the primer length and melting temperature. Optimal PCR primer length typically ranges from 18-24 bases. Shorter primers are less specific during the annealing phase, resulting in more non-specific binding and amplification (Michael *et al.*, 2012). To determine the optimal annealing temperature for the PCR primers, gradient PCR was carried out. Conversely, increasing primer length and annealing temperature enhances the specificity of PCR and increases the yield of specific amplification products.

The three PCR-based markers - mEgCIR_LIP03 (Morcillo *et al*., 2013), mEgCIR_LIP07 and DelEgCIR_C1E3 (Domonhédo *et al*., 2018) target lipase genes: *EgLIP1*, *g0170* and *g0040*, respectively. Despite the high sequence identity among these genes, each marker effectively amplified its unique locus. The DelEgCIR_C1E3 marker identified an indel variant within exon three of *g0040*. The mEgCIR_LIP03 marker revealed a $(TG)_8(TA)_{16}$ microsatellite stretch located between 744 and 792 base pairs (bp) upstream of the ATG initiation codon of *g0050* (*EgLIP1*). The mEgCIR_LIP07 marker indicated a $(CT)_{10}$ microsatellite stretch within the 5′ untranslated region (UTR) of *g0170*. These loci, each exhibiting two alleles with minor size variations, were previously shown to be useful for tracking lipase gene allele co-segregation (Domonhédo *et al*., 2018). In this study, mEgCIR_LIP07 and DelEgCIR_C1E3 failed to show polymorphism across the four IOI breeding crosses (*Figure 1a, 1b*), showing consistent amplicon sizes of 302 and 220 bp (*Figure 2a-2b*), respectively. Consequently, these markers are not suitable for differentiating genotypes based on lipase activity. Furthermore, the mEgCIR_LIP03 primer pair reported by Morcillo *et al*. (2013) exhibited suboptimal PCR amplification. The mEgCIR_LIP03 forward primer was therefore redesigned to target a region 1,048 bp upstream of the *EgLIP1* ATG initiation codon, resulting in improved amplification. To establish the optimal PCR parameters for the modified mEgCIR_LIP03 marker, we performed gradient PCR to determine the ideal annealing temperature. In addition to primer redesign and gradient PCR, we explored various optimisation strategies, including adjustments to annealing time, magnesium chloride concentration, enzyme concentration, the implementation of touchdown PCR and the addition different concentration of dimethyl sulfoxide (DMSO), these efforts unfortunately did not enhance PCR amplification. The modified mEgCIR_LIP03 marker showed polymorphism only in the URT cross (*Figure 1d*), unlike the other three crosses only showed monomorphism (*Figure 1c*). Despite Angkat *et al*. (2021)'s successful use of mEgCIR_LIP03 and DNA intensity analysis to identify an elite low-lipase line, our application of this method did not reveal significant differences within these three IOI breeding crosses. Consequently, DNA intensity analysis appears unsuitable for identifying the low-lipase line in these particular crosses.

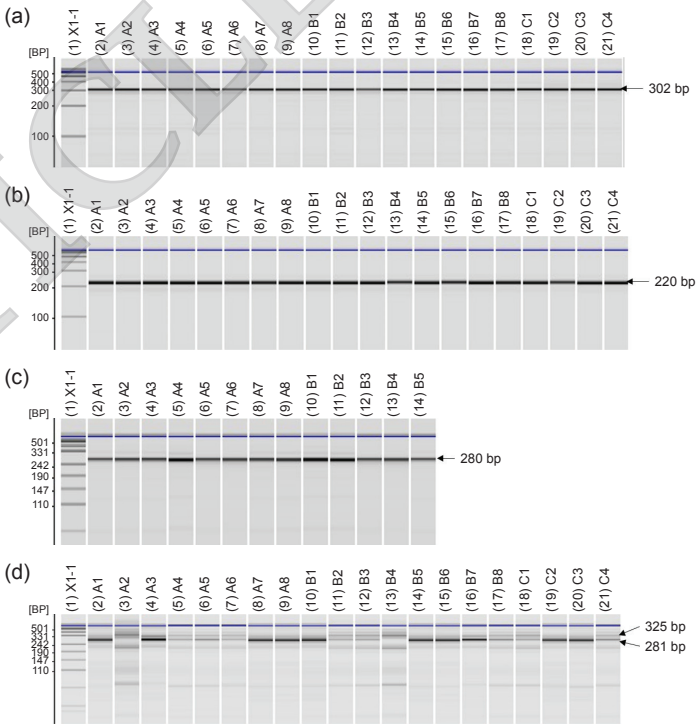

Figure 1. PCR amplification and visualisation of three markers loci in four IOI breeding crosses using Shimadzu MCE-202 MultiNA. (a) Amplification of DelEgCIR_C1E3 marker, (b) Amplification of mEgCIR_LIP07 marker, (c) Amplification of modified mEgCIR_LIP03 marker in all IOI breeding crosses except URT and (d) Amplification of modified mEgCIR_LIP03 marker in IOI URT cross. (1) X1-1: 100 bp DNA ladder or pUC19 DNA/MspI (HpaII); (2)-(21): number of samples.

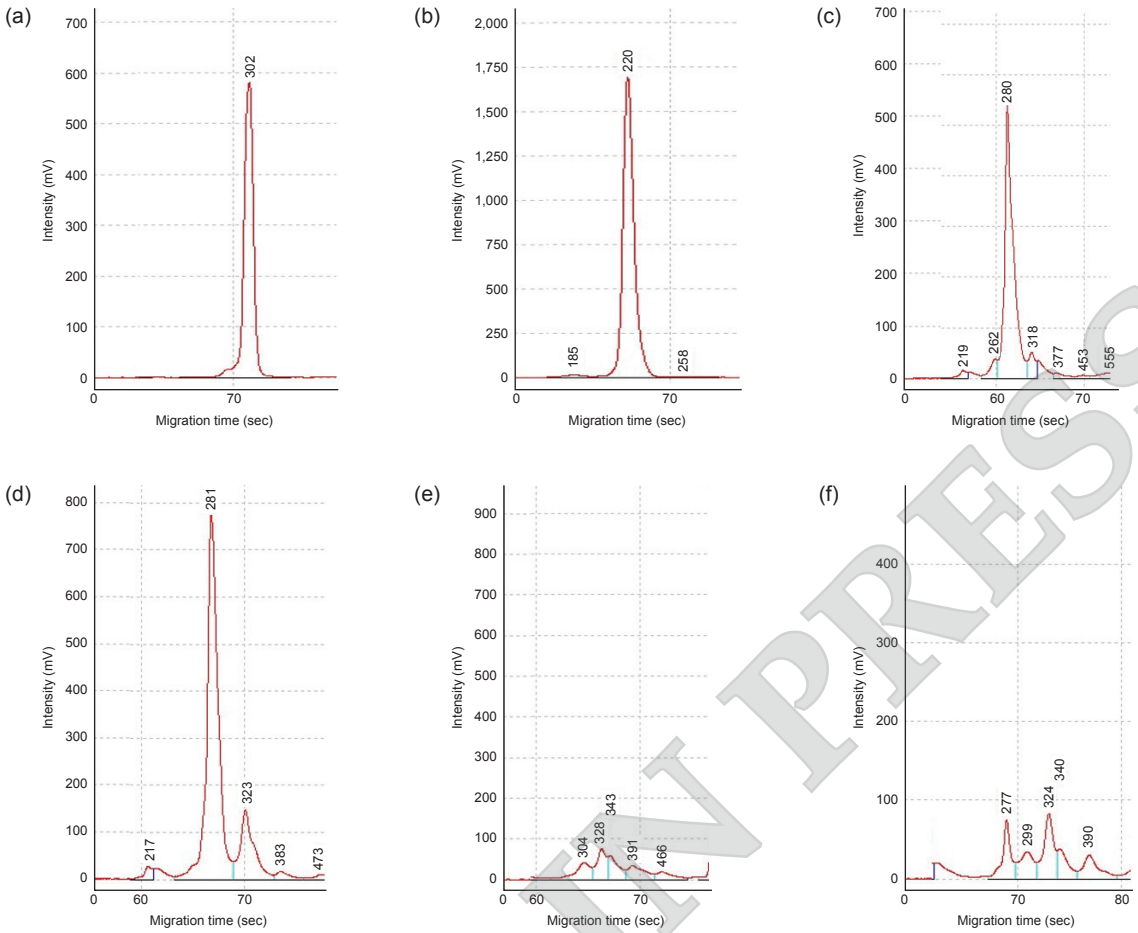

Figure 2. PCR amplicons size estimation of three markers loci in four IOI breeding crosses using Shimadzu MCE-202 MultiNA. (a) DelEgCIR_C1E3; (b) mEgCIR_LIP07; (c) Modified mEgCIR_LIP03 marker in all crosses except URT and (d) – (f) Modified mEgCIR_LIP03 in URT cross.

Polymorphism of mEgCIR_LIP03 Marker

In the study by Morcillo *et al*. (2013), analysis of genomic DNA revealed the existence of a $(TG)_8$ $(TA)_{16}$ microsatellite marker (mEgCIR_LIP03), with two alleles exhibiting minor base differences. Two marker alleles (267 and 269 bp) were present in the bulk for high lipase activity and one single marker allele (267 bp) in the bulk for low lipase activity. In this study, PCR amplicons were sent for DNA sequencing to examine the differences between the two amplicons. The allele size was determined by aligning the forward and reverse amplicon sequences to calculate the variations in size. In present study, modified mEgCIR_LIP03 marker also revealed two alleles (281 and 325 bp) (*Figure 2d-2f*). The DNA sequencing result with a single PCR amplicon band (281 bp) revealed a microsatellite repeat of $(TG)_6$ $(TA)_7$ (*Figure 3a*). In contrast, the single PCR amplicon band (325 bp) showed a $(TG)_0$ $(TA)_{29}$ microsatellite (*Figure 3b*). Using the modified mEgCIR_LIP03 marker PCR amplicons, sequence alignment of the 281 and 325 bp alleles revealed a difference of at least 44 base pairs between them. Given the significant base differences between the two alleles, genotyping results can be readily distinguished using electrophoresis.

While PCR amplification of SSRs typically yields polymorphic bands, replication slippage during *in vitro* amplification can generate stutter products of varying lengths from main product (Hosseinzadeh-Colagar *et al*., 2016). Factors such as amplification errors at dinucleotide repeats can lead to altered repeat lengths and incorrect PCR product sizes (Clarke *et al*., 2001). Hosseinzadeh-Colagar *et al*. (2016) observed that Taq DNA polymerase can slip during *in vitro* microsatellite amplification, causing insertions or deletions of repeat units in DNA strands. This leads to the generation of amplified fragments with different lengths, appearing as stutter bands in gel electrophoresis. Consequently, population studies employing SSR markers take into account the impact of replication slippage and the presence of stutter bands when interpreting their results.

The mEgCIR_LIP03 marker's $(TA)_{29}$ repeat, a long dinucleotide repeat that can reduce PCR amplification efficiency and contribute to allele

size discrepancies in the 325 bp allele. As a result, the homozygous 281 bp allele (*Figure 2d*) exhibits a higher amplicon intensity compared to the heterozygous (281 and 325 bp) and homozygous 325 bp alleles (*Figure 1d, 2e-2f*). To mitigate these inconsistencies, the MCE-202 MultiNa microchip electrophoresis system was used for PCR genotyping analysis. MultiNa provides high-sensitivity detection and quantification of DNA fragments, providing superior separation, sensitivity, reproducibility and quantitative accuracy compared to agarose gel electrophoresis. Notably, it enables the visualisation of low-intensity amplicons that are undetectable with traditional agarose gels. However, this system may overestimate amplicon sizes relative to the theoretical size (Burrell *et al*., 2011; Vega *et al*., 2014). Consequently, allele sizes may fluctuate by 1-5 bp between runs. Therefore, the amplicon sizes measured using the Shimadzu MCE-202 MultiNA may slightly differ from the actual sizes (*Figure 2d-2f*).

Further marker analysis, using the mEgCIR_LIP03 marker, was conducted on all 310 palms of IOI's URT materials. These URT materials comprise four crosses (two T-sibling: URTxT/3 and URTxT/9 and two T-self: URTxT/7 and URTxT/10) developed from the O/10-135/R trial, derived from two parental palms (Pkg1160/12/1 and Pkg1160/44/8) (*Table* 2). Regrettably, only the Pkg1160/12/1 parent remains in the field. The mEgCIR_LIP03 marker locus was successfully amplified in this parent. Pkg1160/12/1 exhibited both the 281 and 325 bp alleles (*Figure 4*), indicating heterozygosity. The genotyped URT crosses displayed segregating banding patterns consistent with parental genotypes. When both parents are heterozygous, their progeny have a 25% chance of inheriting the dominant allele (associated with high lipase activity), a 50% chance of inheriting both alleles (high and medium lipase activity) and a 25% chance of inheriting the recessive allele (low lipase activity). Based on the progeny genotyping results, it is highly probable that the now-absent parent, Pkg1160/44/8, also carried both the 281 and 325 bp alleles.

TABLE 2. THE O/10-135/R TRIAL CROSSES UTILISED IN ANALYSIS

Program code	Female parent	Male parent
URTxT/10	Pkg1160/12/1	Pkg1160/12/1
URTxT/3	Pkg1160/12/1	Pkg1160/44/8
URTxT/7	Pkg1160/44/8	Pkg1160/44/8
URTxT/9	Pkg1160/44/8	Pkg1160/12/1

Marker-trait Correlation Study of mEgCIR_LIP03 Marker

Of the 310 URT palms genotyped with the modified mEgCIR_LIP03 marker to assess lipase activity-related genetic variation, 59 *tenera* palms (each with at least three biological replicates of FFA data) were selected for a marker-trait correlation study focused on bunch analysis. The oil extraction ratio was 23.73 ± 2.11% in the low lipase line and 24.67 ± 2.38% in the high lipase line, showing no significant difference between the lines at $p<0.05$ (*Table 3*). This indicates that the low lipase trait does not impact oil yield. Similarly, no significant differences at $p<0.05$ were observed between the lines for fruit to bunch ratio, wet mesocarp to bunch ratio, or oil to dry mesocarp ratio (*Table 3*), consistent with previous findings by

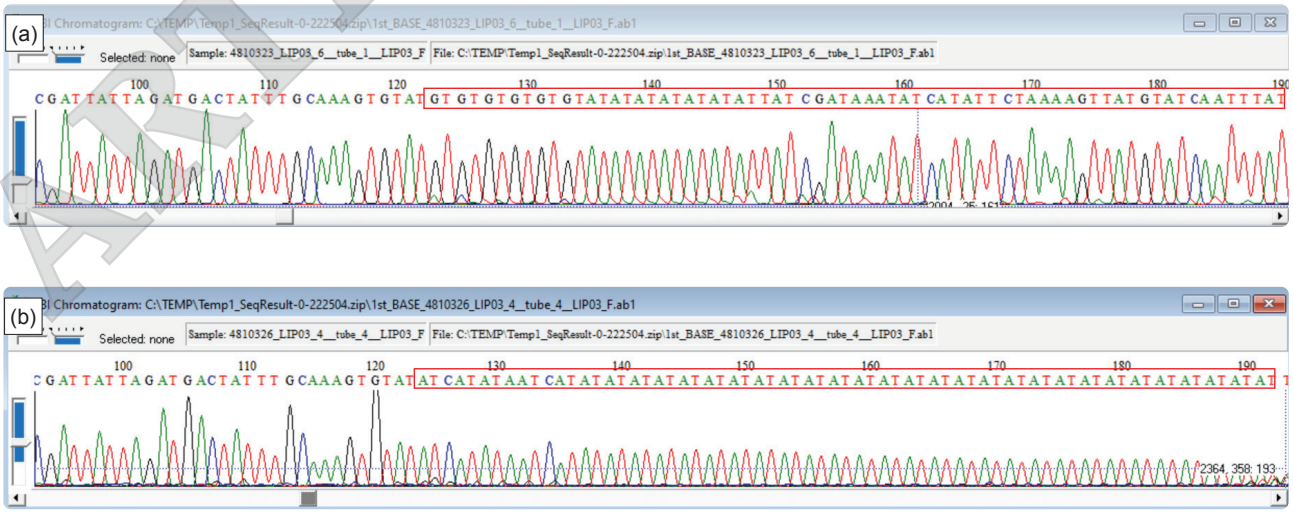

Figure 3. DNA sequencing chromatograms of two distinct PCR amplicons (281 and 325 bp) generated using the modified mEgCIR_LIP03 marker. Chromatogram of the (a) 281 bp allele and (b) 325 bp allele. Sequence differences between the two amplicons are highlighted in red boxes.

Morcillo *et al*. (2013). The URT oil palm population, comprising *tenera*, *pisifera* and *dura* fruit types, exhibited substantial variation in palm oil acidity levels. Analysis of 85 palms (60 *tenera*s, 17 fertile *pisifera*s, 8 *dura*s) with FFA data revealed a wide range of acidity, spanning from 9.78%-55.23%, with an average acidity of 36.78%. Statistical analysis using ANOVA demonstrated significant differences at $p<0.05$ in oil acidity within both the low and high lipase palm groups (*Table 4*). This finding highlights the presence of considerable variability in oil acidity even within groups categorised by lipase activity levels.

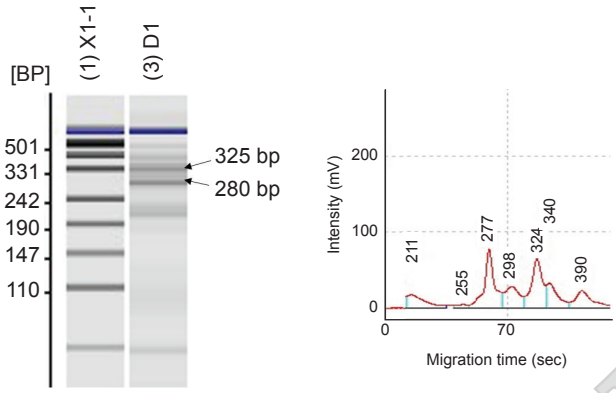

Figure 4. PCR amplification and visualisation of Pkg1160/12/1 parent by modified mEgCIR_LIP03 marker and their estimated amplicon size using Shimadzu MCE-202 MultiNA. (1) X1-1: pUC19 DNA/MspI (HpaII) DNA ladder; (3) D1: Pkg1160/12/1 parent.

Ratios of main components of yield are in ± se. Means are from 276 bunches from 35 *tenera* palms of single allele (281 bp) and 157 bunches from 24 *tenera* palms of two marker alleles (281 bp and 325 bp) and a single marker allele (325 bp). All the results are not significant at $p<0.05$.

Morcillo *et al*. (2013) reported a monogenic inheritance pattern for lipase activity (and consequently, oil acidity) in oil palm. Their findings indicated that two marker alleles (267 and 269 bp) were associated with high lipase activity (>40% FFA) in Deli and Deli x LaMe crosses, while a single allele (267 bp) was linked to low lipase activity (<20% FFA), suggesting low acidity as a recessive trait. In the current study, the URT *Dura* material did not display low lipase levels, and no clear allelic segregation was observed. Furthermore, the observed band sizes differed from those reported previously (Domonhédo *et al*., 2018; Morcillo *et al*., 2013). These discrepancies are likely attributable to the use of modified primers and the different genetic background of the oil palm materials examined in this study.

In this marker-trait correlation study, following the general principle outlined by Morcillo *et al*. (2013), palms carrying either two heterozygous alleles (281 and 325 bp) or a homozygous allele (325 bp) were classified as having high lipase activity. Conversely, palms with the homozygous allele (281 bp) were categorised as having low lipase activity. However, unlike Morcillo *et al*. (2013), bulk segregant analysis (BSA) was not performed.

TABLE 3. BUNCH ANALYSIS OF 59 URT PALMS

Item	Line			
	Low lipase	High lipase		
	Allele (281 bp)	Allele (325 bp or 281 & 325 bp)	*f*-ratio	*p*-value
Fruit to bunch	61.32 ± 3.76	62.07 ± 3.00	0.67	0.42
Wet mesocarp to bunch	82.33 ± 3.80	81.97 ± 3.54	0.14	0.71
Oil to dry mesocarp	78.66 ± 1.81	78.74 ± 1.67	0.03	0.87
Oil to bunch	23.73 ± 2.11	24.67 ± 2.38	2.52	0.12

TABLE 4. FREE FATTY ACID (FFA) ANALYSIS OF URT PALMS

Item	FFA (%)		
	Low lipase	High lipase	
	Allele (281 bp)	Allele (325 bp or 281 & 325 bp)	Overall
N	50	35	85
Mean	32.58	42.77	36.78
Std. Deviation	5.66	6.34	7.77
Minimum	9.78	28.76	9.78
Maximum	42.31	55.23	55.23
CV (%)	17.37	14.82	21.13

Note: N - number of palms; CV - coefficient of variation. The *f*-ratio value is 60.40. The *p*-value is <0.00001. The result is significant at $p<0.05$.

Instead, due to the absence of a defined genetic threshold for low lipase activity in oil palm, the URT crosses were divided into seven groups based on FFA percentages: Group 1 (<20%), Group 2 (20%-25%), Group 3 (25%-30%), Group 4 (30%-35%), Group 5 (35%-40%), Group 6 (40%-45%) and Group 7 (>45%) (*Table 5*). This grouping was implemented because there is no established genetic standard for identifying low lipase palms. In the oil palm industry, "low" FFA is typically defined as below 5%, a standard set by the Codex Alimentarius and the Palm Oil Refiners Association of Malaysia (PORAM) to maintain acceptable oil quality at the milling stage.

Of the 50 single marker alleles (281 bp) analysed, 48 (96.0%) were associated with FFA levels below 40.0%. Conversely, 26 out of 35 (74.3%) of the combined two marker alleles (281 and 325 bp) and single marker allele (325 bp) were associated with FFA levels above 40.0% (*Table 5*). The distribution of the single marker allele (281 bp) was skewed towards lower FFA values, while the distribution for the combined two marker alleles (281 and 325 bp) and single marker allele (325 bp) was skewed towards higher FFA values. While the 281 bp allele effectively identified palms with FFA levels below 25.0%, the presence of individuals in other FFA categories reduced overall accuracy. These results suggest that the marker, while useful, has limitations. They are particularly effective for excluding high lipase palms (FFA >40.0%), making them a valuable tool for breeders seeking to avoid high FFA levels in their selections. However, their ability to precisely identify low lipase palms for breeding purposes is less accurate due to overlap and noise from intermediate FFA categories. Despite this limitation, the markers can still be a helpful tool to eliminate high lipase palms, allowing breeders to focus their phenotyping efforts on the remaining palms to identify ideal candidates for subsequent breeding.

These findings are consistent with the current understanding of the genetic of lipase activity in oil palm. High lipase activity, and its associated high FFA levels, are more readily identifiable, allowing for effective selection against these undesirable traits. This selective breeding strategy contributes to maintaining acceptable oil quality, as high FFA content negatively impacts oil stability and shelf life. The results of this study offer a practical application of genetic markers in oil palm breeding programs, emphasising the improvement of oil quality by minimising high lipase activity.

Lipase Gene Effect and Molecular Marker for Breeding Application

The *EgLIP1* gene is primarily expressed in the mesocarp of oil palm fruit during ripening (Morcillo *et al*., 2013; Nurniwalis *et al*., 2007). The protein encoded by this gene has been isolated and found to exhibit high lipase activity in certain genotypes of oil palm with highly acidic oil, but it is absent in genotypes with low acidity (Morcillo *et al*., 2013). These studies also showed that oil acidity was correlated with the level of transcripts encoding the *EgLIP1* lipase. Additional screenings for lipase activity and acidity by various researchers revealed segregation among existing oil palm materials, with some palms displaying high lipase activity or high acidity in the mesocarp, while others showed low lipase activity or low acidity (Angkat *et al*., 2021; Cadena *et al*., 2013; Likeng-Li-Ngue *et al*., 2016; Morcillo *et al*., 2013; Sambanthamurthi *et al*., 2000; Wong *et al*., 2015). Although *EgLIP1* exhibits the highest expression levels in the fruit mesocarp, as shown by transcriptome analysis, the other two genes (*g0170* and *g0040*) could also contribute to oil acidity, particularly *g0170*, which was shown to be expressed in the study by Domonhédo *et al*. (2018). Therefore, despite the co-segregation of the low-acidity trait with *EgLIP1*, the trait's inheritance

TABLE 5. MARKER-TRAIT CORRELATION STUDY OF PCR GENOTYPING OF mEgCIR_LIP03 MARKER WITH URT FREE FATTY ACID (FFA) PERCENTAGE

FFA category	Total palms	PCR genotyping			
		Allele (281 bp)		Allele (325 bp or 281 & 325 bp)	
		No. of palm	%	No. of palm	%
Group 1 (<20%)	1	1	100.0	0	0.0
Group 2 (20%-25%)	1	1	100.0	0	0.0
Group 3 (25%-30%)	12	11	91.7	1	8.3
Group 4 (30%-35%)	22	19	86.4	3	13.6
Group 5 (35%-40%)	21	16	76.2	5	23.8
Group 6 (40%-45%)	18	2	11.1	16	88.9
Group 7 (>45%)	10	0	0.0	10	100.0
Total	**85**	**50**		**35**	

may not be strictly monogenic, but rather polygenic. Domonhédo *et al*. (2018) suggested that oil acidity variation is controlled by a genomic region containing multiple lipase genes, implying a cumulative effect of these genes on acidity levels. In the current study, the lack of polymorphism for the markers associated with the other two genes suggests that additional lipase genes, beyond those investigated, may also play a role in determining oil acidity.

CONCLUSION

Oil palm's perennial nature and long-life cycle necessitate considerable space and time for conventional breeding to select promising crosses, especially when expanding parental biodiversity (Cardona *et al*., 2018; Herrero *et al*., 2020). Therefore, a rapid and cost-effective screening method, such as using SSR markers as valuable tools for linking phenotypic traits, potentially including those related to lipase activity, with underlying genetic backgrounds, allowing for the identification of promising crosses based on marker associations. This study, consistent with Domonhédo *et al*. (2018), indicates that *EgLIP1* is primarily responsible for oil acidity in high lipase genotypes. The mEgCIR_LIP03 SSR marker, being located at the promoter region of *EgLIP1*, likely affects gene expression due to its variable length and sequence. Utilising the mEgCIR_LIP03 SSR marker enables the identification and elimination of high lipase palms, allowing breeders to focus on potential low lipase individuals and develop elite low lipase lines from URT crosses. This targeted selection leads to a skewed FFA distribution towards lower lipase activity, ultimately improving overall palm oil quality. Such breeding strategies offer significant potential for enhancing economic returns for both growers and mills.

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REFERENCES

American Oil Chemists' Society (AOCS). (1998). Method Ca 5a-40. In D. Firestone (Ed.), *Official methods and recommended practices of the AOCS* (4th–6th ed.). American Oil Chemists' Society.

Angkat, N. U., Siregar, L. A. M., 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 Journal of Biological Diversity, 22*(2), 900–905. https://doi.org/10.13057/biodiv/d220245

Brockman, H. L. (1984). General features of lipolysis: Reaction scheme, interfacial structure and experimental approaches. In B. Borgstrom & H. L. Brockman (Eds.), *Lipases* (pp. 3–46). Elsevier Science.

Burrell, A., Foy, C., & Burns, M. (2011). Applicability of three alternative instruments for food authenticity analysis: GMO identification. *Biotechnology Research International*, *2011*(1), 838232. https://doi.org/10.4061/2011/838232

Cadena, T., Prada, F., Perea, A., & Romero, H. M. (2013). Lipase activity, mesocarp oil content, and iodine value in oil palm fruits of *Elaeis guineensis*, *Elaeis oleifera*, and the interspecific hybrid O×G (*E. oleifera* × *E. guineensis*). *Journal of the Science of Food and Agriculture, 93*(3), 674–680. https://doi.org/10.1002/jsfa.5940

Cardona, C. C. C., Coronado, Y. M., Conronado, A. C. M., & Ochoa, I. (2018). Genetic diversity in oil palm (*Elaeis guineensis* Jacq.) using RAM (Random Amplified Microsatellites). *Bragantia*, *77*(4), 546–556. https://doi.org/10.1590/1678-4499.2017385

Clarke, L. A., Rebelo, C. S., Gonçalves, J., Boavida, M. G., & Jordan, P. (2001). PCR amplification introduces errors into mononucleotide and dinucleotide repeat sequences. *Molecular Pathology, 54*(5), 351–353. https://doi.org/10.1136/mp.54.5.351

Corley, R. H. V., & Tinker, P. B. (2016). *The oil palm* (5th ed.). Wiley.

Domonhédo, H., Cuéllar, T., Espeout, S., Droc, G., Summo, M., Rivallan, R., Cros, D., Nouy, B., Omore, A., Nodichao, L., Arondel, V., Ahanhanzo, C., & Bilotte, N. (2018). Genomic structure, QTL mapping, and molecular markers of lipase genes responsible for palm oil acidity in the oil palm (*Elaeis guineensis* Jacq.). *Tree Genetics & Genomes, 14*, 69. https://doi.org/10.1007/s11295-018-1284-7

Gibon, V., De Greyt, W., & Kellens, M. (2007). Palm oil refining. *European Journal of Lipid Science and Technology, 109*(4), 315–335. https://doi.org/10.1002/ejlt.200600307

Herrero, J., Santika, B., Herrán, A., Erika, P., Sarimana, U., Wendra, F., Sembiring, Z., Asmono, D., & Ritter, E. (2020). Construction of a high density linkage map in oil palm using SPET markers. *Scientific Reports*, *10*, 9998. https://doi.org/10.1038/s41598-020-67118-y

Hosseinzadeh-Colagar, A., Haghighatnia, M. J., Amiri, Z., Mohadjerani, M., & Tafrihi, M. (2016). Microsatellite (SSR) amplification by PCR usually led to polymorphic bands: Evidence which shows replication slippage occurs in extending or nascent DNA strands. *Molecular Biology Research Communications, 5*(3), 167–174.

Likeng-Li-Ngue, B. C., Bell, J. M., Ngando-Ebongue, G. F., Ntsemboh, G. N., & Ngalle, H. B. (2016). Genetic determinism of oil acidity among some DELI oil palm (*Elaeis guineensis* Jacq.) progenies. *African Journal of Biotechnology, 15*(36), 1841–1845. https://doi.org/10.5897/AJB2016.15461

Michael, A. I., David, H. G., John, J. S., & Thomas, J. W. (2012). *PCR protocols: A guide to methods and applications*. Academic Press.

Morcillo, F., Cros, D., Billotte, N., Ngando-Ebongue, G. F., Domonhédo, H., Pizot, M., Cuéllar, T., Espéout, S., Dhouib, R., Bourgis, F., Claverol, S., Tranbarger, T. J., Nouy, B., & Arondel, V. (2013). Improving palm oil quality through identification and mapping of the lipase gene causing oil deterioration. *Nature Communications, 4*, 2160. https://doi.org/10.1038/ncomms3160

Ngando-Ebongue, G. F., Dhouib, R., Carrière, F., Amvam Zollo, P. H., & Arondel, V. (2006). Assaying lipase activity from oil palm fruit (*Elaeis guineensis* Jacq.) mesocarp. *Plant Physiology and Biochemistry, 44*(10), 611–617. https://doi.org/10.1016/j.plaphy.2006.09.006

Nurniwalis, A. W., Siti Nor Akmar, A., Chan, P. L., & Manaf, M. A. (2007). Isolation of a cDNA encoding a lipase class 3 family protein from oil palm (*Elaeis guineensis* Jacq.). In *Proceedings of the PIPOC 2007 International Palm Oil Congress: Agriculture, Biotechnology & Sustainability* (pp. 1011–1020). Malaysian Palm Oil Board.

Okoye, M. N., Uguru, M. I., Bakoumé, C., Singh, R., & Okwuagwu, C. O. (2016). Assessment of genetic diversity of NIFOR oil palm main breeding parent genotypes using microsatellite markers. *American Journal of Plant Sciences, 7*(1), 218–237. https://doi.org/10.4236/ajps.2016.71022

Sambanthamurthi, R., Rajanaidu, N., & Parman, S. H. (2000). Screening for lipase activity in the oil palm. *Biochemical Society Transactions, 28*(6), 769–770. https://doi.org/10.1042/bst0280769

Shimadzu. (2008). *Microchip electrophoresis system for DNA/RNA, MCE-202 MultiNA instruction manual operating procedure* (No. 292-28464D).

Singh, R., Ong-Abdullah, M., Low, E. L., Manaf, M. A. A., Rosli, R., Nookiah, R., Ooi, L. C., Ooi, S., Chan, K., Halim, M. A., Azizi, N., Nagappan, J., Bacher, B., Lakey, N., Smith, S. W., He, D., Hogan, M., Budiman, M. A., Lee, E. K., … Sambanthamurthi, R. (2013). Oil palm genome sequence reveals divergence of interfertile species in old and new worlds. *Nature, 500*(7462), 335–339. https://doi.org/10.1038/nature12309

Tranbarger, T. J., Dussert, S., Joët, T., Argout, X., Summo, M., Champion, A., Cros, D., Omore, A., Nouy, B., & Morcillo, F. (2011). Regulatory mechanisms underlying oil palm fruit mesocarp maturation, ripening, and functional specialization in lipid and carotenoid metabolism. *Plant Physiology, 156*(2), 564–584. https://doi.org/10.1104/pp.111.175141

Vega, E. D., & Marina, M. L. (2014). Characterization and study of transgenic cultivars by capillary and microchip electrophoresis. *International Journal of Molecular Sciences, 15*(12), 23851–23877. https://doi.org/10.3390/ijms151223851

Wong, Y. T., Kushairi, A., Rajanaidu, N., Osman, M., Wickneswari, R., & Sambanthamurthi, R. (2015). Screening of wild oil palm (*Elaeis guineensis*) germplasm for lipase activity. *The Journal of Agricultural Science, 154*(7), 1241–1252. https://doi.org/10.1017/S0021859615001112