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Candidate Genes Identification of Oil Palm (*Elaeis guineensis* Jacq.) Interest Characters using Published Database

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Abstract

Palm oil has supplied more than 30% of vegetable oil consumption worldwide. Rising demand has pushed oil palm plantations to increase the yield. It is well known that genetic has played a significant role in phenotypic performance. Moreover, in recent years, genomic data has emerged tremendously. Unfortunately on the gene related to oil palm yield. Therefore, a preliminary study to classify and select oil palm candidate genes associated with characteristic by scanning existing genes in oil palm or other in-silico species were conducted. Based on Blast2Go results, 22 genes related to oil biosynthesis, two specifically related to fruit number and fruit weight were analysed. Furthermore, 19 candidate genes were able to amplify.

Keyword : homology; in-silico; known genes; production traits; oil palm; validation

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1. Introduction

Oil palm (*Elaeis guineensis* Jacq.) is a perennial tropical oil tree that accumulates palm oil up to 90% (base on dry weight) in it is mesocarp [1][3]. In a small plantation, oil yields have been achieved 12 t ha−1 yr−1, while base on theoretical, the yields measured using simulation models are 18.5 tonnes of oil per hectare per year [4], [5]. To increase the oil palm yield close to theoretical estimation, it requires a technology such as genetic marker.

Genetic markers have been able to increased breeding efficiency approximately 2-fold in soybean (Glycine max) and sunflower (*Helianthus annuus*) populations [6], [7] Genetic marker can be generated through genomic data. In recent years, genomic data has been generated abundantly in several plants, and the most advance and publicly available is in Arabidopsis (a plant model). The whole-genome sequence of Arabidopsis was sequence by the Arabidopsis Genome Initiative (AGI) in 2000. It has identified five chromosomes of Arabidopsis thaliana with total length 135 Mb [8].

The E. oleifera has 37% of similar to *E. guineensis* in terms of guanine-cytosine. The genome data were released in 2013 with a size around 1.8 Gb [9]. Unfortunately the *E. guineensis* data has minimum information regarding the candidate gene.

Therefore, this study aims to identify and select oil palm candidate genes that influence some production traits by scanning via in-silico mining and confirming it through sequencing. In the study presented here, sample sequence was compared with the an-notated *E. guineensis* and Arabidopsis genome sequence to assess which kind of sequences show conservation between these closely related species.

2. Materials and Methods

2.1 Plant Material and DNA Extraction

In this study, a test of sample was used for validation candidate gene. Six samples of oil palm young leaves were used to collect the total DNA using the DNeasy Plant Mini Kit (Qiagen, Germany) following the manufacturer's protocol with some modifications [10]. The concentration of DNA was assessed with a Thermo Scientific nanodrop 2000 spectrophotometer. The DNA sample was diluted into 10µg/µl.

2.2 Searching potential candidate genes (CG)

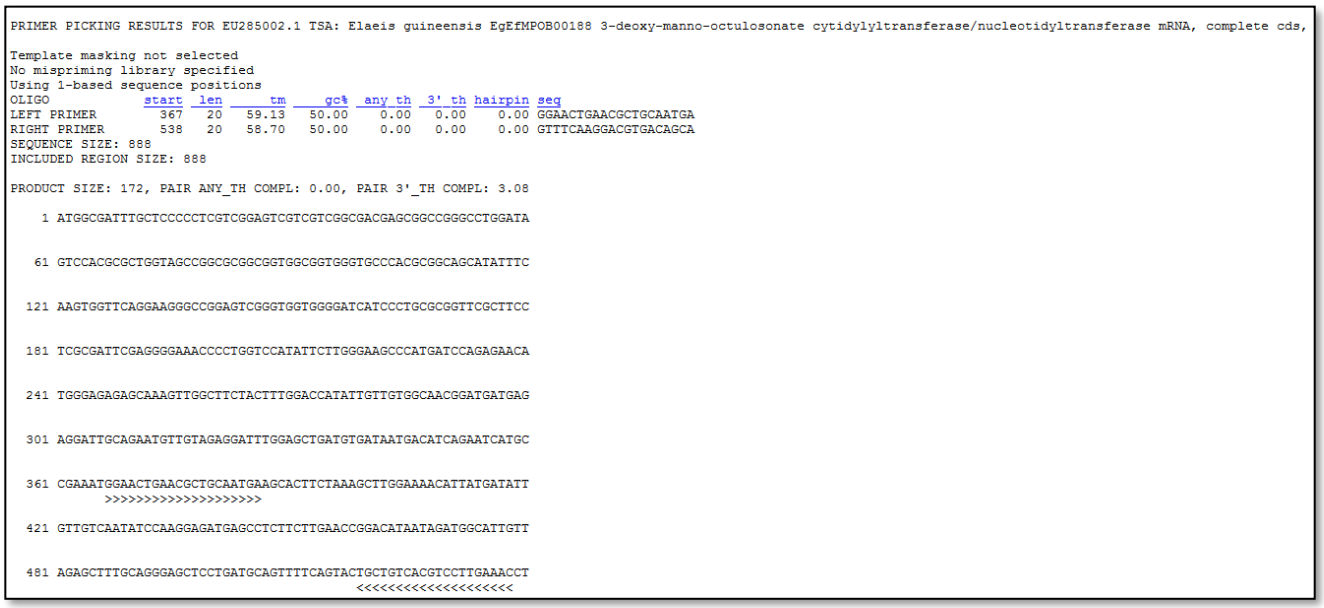
Candidate genes (CG) related to oil yield production were identified by in silico mining in the publicly available databases (Table 1.) for production characters. The potential CGs were subjected to homology sequence searching in *E. guineensis* and *E. oleifera* published sequences using Basic Local Alignment Search Tool (BLAST 2.0) [11]. The high homology was considered as potential candidate genes [12].

Table 1. Common website to search publish genome database

No	Organization name	Website
1	National Center of Biotechnology Information (NCBI)	http://www.ncbi.nlm.nih.gov/
2	Kyoto Encyclopaedia of Genes Genomes (KEGG)	http://www.genome.jp/kegg/

2.3 Primer design

The sequence of potential CG which was confirmed from previous step will be used as the template to design primer using Primer3 (http://bioinfo.ut.ee/primer3-0.4./). The primers for these CG were designed in exons (Figure 1). In total 35 pair of primer has been designed for 28 candidate genes (Table 2).



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PRIMER PICKING RESULTS FOR EU285002.1 TSA: Elaeis guineensis EgEfMPOB00188 3-deoxy-manno-octulosonate cytidylyltransferase/nucleotidyltransferase mRNA, complete cds,

Template masking not selected
No mispriming library specified
Using 1-based sequence positions
OLIGO            start  len      tm     gc%   any th  3' th hairpin seq
LEFT PRIMER        367   20   59.13   50.00     0.00   0.00    0.00 GGAACTGAACGCTGCAATGA
RIGHT PRIMER       538   20   58.70   50.00     0.00   0.00    0.00 GTTTCAAGGACGTGACAGCA
SEQUENCE SIZE: 888
INCLUDED REGION SIZE: 888

PRODUCT SIZE: 172, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 3.08

    1 ATGGCGATTTGCTCCCCCTCGTCGGAGTCGTCGTCGGCGACGAGCGGCCGGGCCTGGATA

   61 GTCCACGCGCTGGTAGCCGGCGCGGCGGTGGCGGTGGGTGCCCACGCGGCAGCATATTTC

  121 AAGTGGTTCAGGAAGGGCCGGAGTCGGGTGGTGGGGATCATCCCTGCGCGGTTCGCTTCC

  181 TCGCGATTCGAGGGGAAACCCCTGGTCCATATTCTTGGGAAGCCCATGATCCAGAGAACA

  241 TGGGAGAGAGCAAAGTTGGCTTCTACTTTGGACCATATTGTTGTGGCAACGGATGATGAG

  301 AGGATTGCAGAATGTTGTAGAGGATTTGGAGCTGATGTGATAATGACATCAGAATCATGC

  361 CGAAATGGAACTGAACGCTGCAATGAAGCACTTCTAAAGCTTGGAAAACATTATGATATT
          >>>>>>>>>>>>>>>>>>>

  421 GTTGTCAATATCCAAGGAGATGAGCCTCTTCTTGAACCGGACATAATAGATGGCATTGTT

  481 AGAGCTTTGCAGGGAGCTCCTGATGCAGTTTTCAGTACTGCTGTCACGTCCTTGAAACCT
                                        <<<<<<<<<<<<<<<<<<<
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Figure 1. An example of Primer3 result for picking primer for KG46, sequence gi|192913005|gb|EU285002.1|.

2.4 Primer Validation and Sequencing

The validation of the primer was done via PCR amplification protocol. The PCR reaction contained; extracted DNA from previous step as the template 2µl (10 ng/µl), 2 µl dNTPs (2,5mM), Taq DNA polymerase 0,1 µl (per reaction 5U/m) (DFS-Taq Polymerase, Bioron), Primer Forward 0,2 µl, Primer Reverse 0,2 µl, 2,5 µl PCR buffer 10X and ddH_2O of 18µl.

The amplification was done with pre-denaturation 94°C (5 minutes) and 30 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 45 seconds. The last extension 72°C for 10 min and 12°C forever. This PCRs were performed in a Thermal Cycler ABI 2720 (Applied Biosystems, Foster, USA).

The result must be run in agarose gel 1.5%, and 4 µL gel-red in 100 mL 1xTAE. Running in the agarose gel used 2 µL LADDER, 4 µL PCR product and 5 µL loading buffer. Amplified candidate gene were used for library preparation following [13]. This library was sent to third party and analyses using the Ion Torrent Personal Genome Machine (PGM) with the 318 Chip [14].

3. Results and Discussion

Commonly studies of candidate gene was provided information of analysis data and result [15][17]. Twenty-eight possible candidate genes were detected from *Elaeis* and *A. thaliana*. These results were obtained by conducting bioinformatics analysis from published database. The sequences of nucleotide were obtained from KEGG and Gene Bank databases. The candidate genes were found related to interest characters which were Palm Oil Yield (CPO), Average Fruit Number (FN) and Average Fruit Weight (FW).

Relevant primers of each candidate gene were developed for validation and used in amplification of multiple genotypes to con-firm the existence of candidate genes. The amplification results were visualized in Agarose gel 1% (Figure 2).

Table 2. List of candidate genes selected from different references searching from related literature based on characters of interest.

N°	Ref Gene	Origen	Traits	Accession/ Sequence	Blast2 Go Analysis			Amp Validation
					Seq. Name	Seq. Description	Mean Similarity	
1	KG24	*Elaeis* (Oil Palm Biosynthesis)	CPO	AF424808	4_gi|16224226|gb|AF424808.1|	acyl acyl-carrier-protein thioesterase type b	88.90%	+
2	KG25	*Elaeis* (Oil Palm Biosynthesis)	CPO	AF490767	5_1gi|20067069|gb|AF490767.1|	palmitoyl-acyl carrier protein thioesterase	91.10%	+
3				AF541880	5_2gi|23305882|gb|AF541880.1|	palmitoyl-acyl carrier protein thioesterase	93.05%	
4	KG26	*Elaeis* (Oil Palm Biosynthesis)	CPO	AF430248	6_1gi|18378374|gb|AF430248.1|	acyl-acp thioesterase	70.40%	+
5	KG27	*Elaeis* (Oil Palm Biosynthesis)	CPO	AY089977	7_1gi|20135601|gb|AY089977.1|	beta-ketoacyl-acp synthase ii	94.60%	+
6				FJ940767	7_2gi|228478493|gb|FJ940767.1|	beta-ketoacyl-acp synthase ii	83.05%	
7	KG29	*Elaeis* (Oil Palm Biosynthesis)	CPO	AF261691	9_gi|7739790|gb|AF261691.1|	glutelin	66.50%	+
8	KG31	*Elaeis* (Oil Palm Biosynthesis)	CPO	AY012452	11_gi|13236803|gb|AY012452.1|	atp synthase beta subunit	99.05%	+
9	KG32	*Elaeis* (Oil Palm Biosynthesis)	CPO	AY550180	12_gi|45272579|gb|AY550180.1|	diacylglycerol kinase 5	90.80%	-
10	KG33	*Elaeis* (Oil Palm Biosynthesis)	CPO	DQ004687	13_gi|68159363|gb|DQ004687.1|	acetyl- carboxylase beta subunit	93.05%	+
11	KG35	*Elaeis* (Oil Palm Biosynthesis)	CPO	EU057620	15_1gi|154354068|gb|EU057620.1|	chloroplast omega-3 fatty acid desaturase	82.25%	+
12				EU057619	15_2gi|154354066|gb|EU057619.1|	chloroplast omega-3 fatty acid desaturase	90.65%	
13	KG38	*Elaeis* (Oil Palm Biosynthesis)	CPO	EU285005	18_gi|192913011|gb|EU285005.1|	holocarboxylase synthetase	75.45%	+
14	KG37	*Elaeis* (Oil Palm Biosynthesis)	CPO	AF273023	17_1gi|8515911|gb|AF273023.1|	16 kda oleosin	83.35%	-
15				AF147758	17_2gi|5231120|gb|AF147758.1|	16 kda oleosin	88.45%	
16	KG39	*Elaeis* (Oil Palm Biosynthesis)	CPO	AF169015	19_1gi|5616521|gb|AF169015.1|	3-oxoacyl-synthase iii	88.45%	+
17				DQ459442	19_2gi|92111313|gb|DQ459442.1|	3-oxoacyl-synthase iii	81.65%	
18		*Elaeis* (Oil Palm Biosynthesis)	CPO	AF143502	20_1gi|4929209|gb|AF143502.1|	3-oxoacyl-synthase iii	89.85%	
19				DQ459441	20_2gi|92111311|gb|DQ459441.1|	3-oxoacyl-synthase iii	81.10%	
20	KG41	*Elaeis* (Oil Palm Biosynthesis)	CPO	AF143501	21_1gi|4929207|gb|AF143501.1|	stearoyl-acyl-carrier protein partial	97.20%	+
21				AF507965	21_2gi|21245147|gb|AF507965.2|	stearoyl-acyl-carrier protein desaturase	94.70%	
22	KG45	*Elaeis* (Oil Palm Biosynthesis)	CPO	FJ796065	25_gi|225904446|gb|FJ796065.1|	3-hydroxybutyryl-dehydratase	84.05%	+
23	KG46	*Elaeis* (Oil Palm Biosynthesis)	CPO	EU285002	26_gi|192913005|gb|EU285002.1|	3-deoxy-manno-octulosonate cytidylyltransferase	92.30%	-
24	KG47	*Elaeis* (Oil Palm Biosynthesis)	CPO	FJ796069	27_gi|225904437|gb|FJ796069.1|	peroxisomal acyl-coenzyme a oxidase 1-like	87.75%	+
25	KG48	*Elaeis* (Oil Palm Biosynthesis)	CPO	FJ796067	28_gi|225904450|gb|FJ796067.1|	diacylglycerol acyltransferase type 2	82.55%	-
26	KG50	*Arabidopsis thaliana*	CPO	FJ751636	30_gi|225728848|gb|FJ751636.1|	1-aminocyclopropane-1-carboxylate oxidase	82.80%	+
27	KG52	*Arabidopsis thaliana*	CPO	JN003473	32_gi|353441063|gb|JN003473.1|	phospholipid:diacylglycerol acyltransferase 1-like	94.10%	+
28	KG54	*Arabidopsis thaliana*	CPO	JN003482.1	34_gi|353441077|gb|JN003482.1|	enolase	97.60%	-
29	KG55	*Arabidopsis thaliana*	CPO	DQ531848	35_gi|209168852|gb|DQ531848.1|	biotin carboxylase precursor	89.50%	-
30	KG60	*Arabidopsis thaliana*	FN	NM_119406	40	flavine-containing monoxygenase	83.65%	-
31	KG62	*Arabidopsis thaliana*	FN	NM_121170	42	flavin-binding monooxygenase family protein	84.20%	-
32	KG65	*Arabidopsis thaliana*	FN, FW	NM_106232	45	receptor protein kinase clavata1	84.10%	-

33	**KG153**	*Arabidopsis thaliana*	**FW-FN**	NM_119611.4	7_gi|186516235|ref|NM_119611.4|	guanine nucleotide-binding protein subunit beta	94.20%	+
34	**KG154**	*Arabidopsis thaliana*	**FW-FN**	NM_119266.6	9_gi|240256108|ref|NM_119266.6|	ddb1- and cul4-associated factor homolog 1-like	76.70%	+
35	**KG155**	*Arabidopsis thaliana*	**FW-FN**	NM_121371.2	13_gi|30684532|ref|NM_121371.2|	iki3 family protein isoform 1	79.95%	+

Ref. code = reference code; Seq. Name = sequence name; Seq. Description = sequence description; Amp. Validation = amplification validation; + = amplified; - = not amplified.

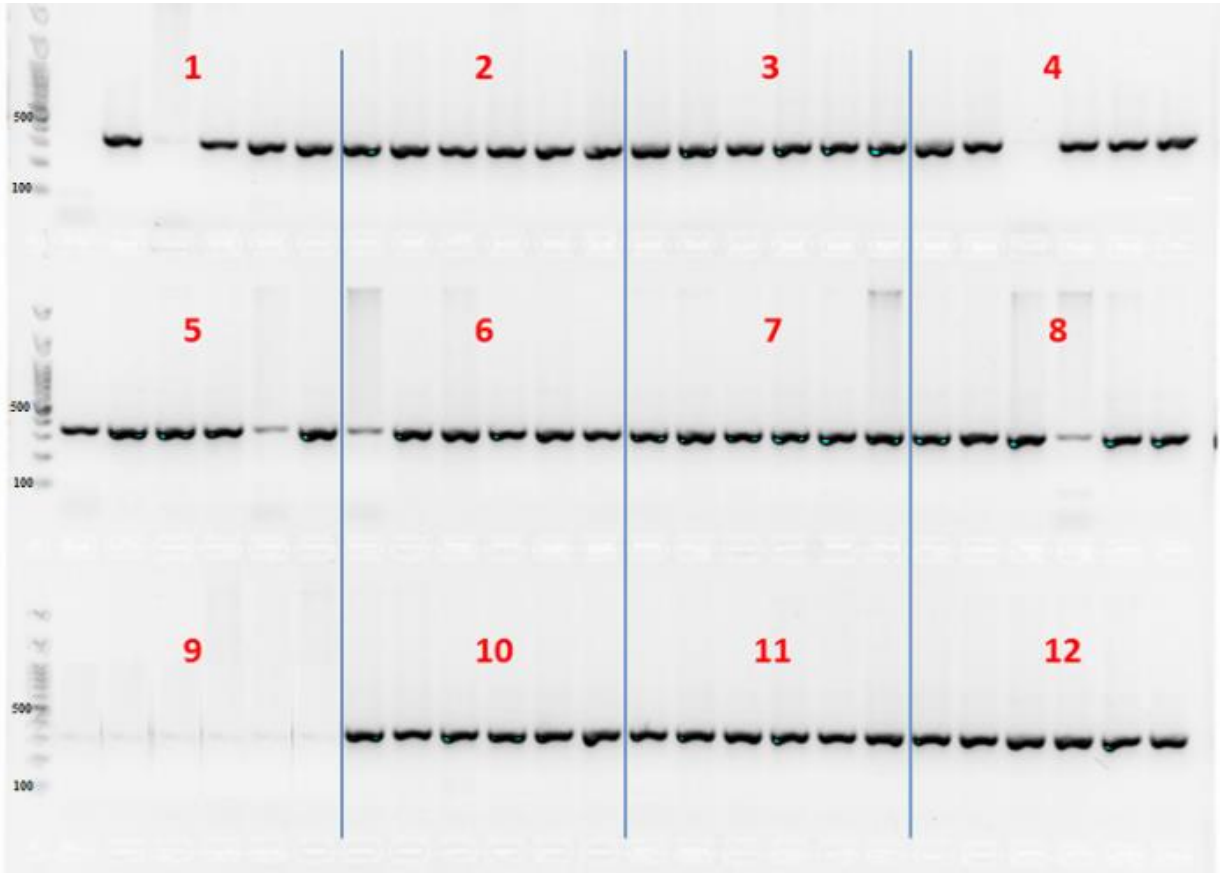

Figure 2. Marker validation that designed base on nucleotide sequence; 1)AF424808 (KG24), 2)AF490767 (KG25), 3)AF541880 (KG25), 4)AF430248 (KG26), 5)AY089977 (KG27), 6)FJ940767 (KG27), 7) AF261691 (KG29), 8)AY012452 (KG31), 9)AY550180 (KG32), 10)DQ004687 (KG33), 11) EU057620 (KG35), 12) EU057619 (KG35).

Variation is detected by comparing the samples tested and reference based on DNA sequence. Only nine candidate genes or 10 marker did not amplified in our genotypes. Several identified genes with large similarity to the *E. guineensis* database were not amplified in genotypes test. For example, KG54 which had 97.60% similarity analysis of Blast2Go but it did not amplify in the genotypes. Meanwhile, KG29 with only 66.50% similarity were amplified. This may occure due to the involvement of long intron [18] or some SNP in primer design process.The amplified candidate genes were used for sequencing. This sequences result have been blast to www.ncbi.nlm.nih. The example of number of variations in several KG result after aligment were explain in table 3. Those table showed that number of SNP and indel were varied.

Table 3. Number of variation result base on SNP and Indel.

Code	**Variation**	
	SNP	**Indel**
KG25	1 site	1 site
KG35	12 site	-

KG41	3 site	-
KG45	2 site	-
KG46	8 site	1 site
KG47	1 site	-

Based on the Blast2 Go analysis, the sequence description showed that 22 candidate genes were related to CPO and almost 82% of them were oil palm genes, the remaining 18.2% were known genes form *Arabidopsis thaliana*. We obtained six candidate genes for the other characters (FN and FW). The resemblance percentage based on Blast2Go findings vary from 65.50 to 99.05% (Table 2).

A broad range of heterogeneous biological data assemblies, including protein and genome sequence data and even expression profiles, were given in life science research. Instant detection of protein and gene identification is beneficial for expanding the annotation database coverage. The literature base is indeed useful in this analysis to recognize genetic variants of the traits associated with development of proteins References[19].

The traits of production primarily related to fruit development. The fruit formation and maturation are biologically complex and process specific in every plants. Interestingly, there was no detailed research into the molecular dimensions of palm oil processing and maturation [20]. Lately, knowledge of plant accumulation of triacyl-glycerol (TAG) was almost focused on studies of oil seeds that contain no more than 60 percent. Even so, genes discovered in the analysis on oil seeds were linked to oil biosynthesis.

The known genes originating from the *E. guineensis* genome database are mainly encoding enzymes or proteins that involve in palm oil biosynthesis directly or indirectly. This investigation has been conducted with genes related to fatty acid biosynthesis (FA) and derived of acyl lipids, specifically triacylglycerol (TAG). As acyl-CoA-esters in the endoplasmic reticulum, fatty acids synthesized by acetyl-CoA in plastid are transmitted through glycerol-lipid metabolisms [1]. The ACCase generated malonyl-COA is the essential carbon donor for fatty acid synthesis. The malonyl group is moved from COA to the protein cofactor acyl carrier protein (ACP) before joining the fatty acid synthesis pathway [21],[22]. Several enzymes connected to ACP and other related compounds participate in petroleum biosynthesis. On table 2, several ACP, acyl-CoA, acetyl-CoA and Ac-case associated genes have been identified. KG33, KG 39, KG41, KG45 and KG47 were related to this candidate genes. All of these 10 known genes have been amplified on DNA samples.

Diacylglycerol (DAG) in TAG is the last step of oil synthesis [2]. The catalytic activity is supposed to include diacyl-glycerol acyltransferase (DAT). In this observation found that the genes DGAT or DAG were either KG32 and KG48. Both genes reported not to be amplified in the DNA samples. These two genes were omitted, thus. Only thus which were amplified in DNA samples were selected as candidate genes.

In this work we used number of known genes from *Arabidopsis thaliana*, is a small flowering plant commonly used in plant biology as a model organism, which are also connected to oil biosynthesis as their genome is sequenced and annotated. Around 450 ontology were expressed in the oil palm monocarp from this plant metabolism acyl lipid genes during fruit ripening [2]. Ten known genes from this plant database were reported. Some genes, such as diacylglycerol transferase and ACCase have identical expression in the pathway of oil biosynthesis. These genes are identical to *E. guineensis* based on Blast2Go data (76–97%). Using the DNA samples, 50% of identified genes have been amplified.

4. Conclusion

The in silico approach was a good way to identified candidate genes. In this observation 67.86% of known genes were amplified in a small test genotypes using PCR technique. These potential candidate genes will be used in further studies. On the other hand, using this methodology could not find new gene, instead rediscover known gene from other species that might be expressed in the sample test.

5. Conflict of Interest

The authors declare that they have no competing interests.

6. Acknowledgement

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