Candidate genes linked to QTL regions associated with fatty acid composition in oil palm

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Abstract
The present study searched for candidate genes in five linkage groups (LGs) - T2, T3, OT4, OT6 and T9 hosting the QTLs associated with iodine value (IV) and fatty acid composition (FAC) in an oil palm interspecific hybrid population. Each of the five LGs was successfully anchored to its corresponding chromosomal segment where, a wider repertoire of candidate genes was identified. This study further revealed a total of 19 candidate genes and four transcription factors involved in biosynthesis of fatty acids, lipids (including triacylglycerol) and acetyl-CoA, glycosylation and degradation of fatty acids. Their possible involvement in regulating the levels of saturation are discussed. In addition, 22 candidate genes located outside the QTL intervals were also identified across the interspecific hybrid genome. A total of 92 SSR markers were developed to tag the presence of these candidate genes and 50 were successfully mapped onto their respective positions on the genome. The data obtained here complements the previous studies, and collectively, these QTL-linked candidate gene markers could help breeders in more precisely selecting palms with the desired FAC.

Keywords Elaeis guineensis · Interspecific hybrid · Fatty acid biosynthesis · Triacylglycerol biosynthesis · Transcription factors · Genetic linkage map

Abbreviations

| Abbreviation | Definition                      |
|--------------|---------------------------------|
| 4CLL1        | 4-coumarate–CoA ligase 1        |
| AACT         | acetoacetyl-CoA thiolase         |
| acbd4        | acyl-CoA-binding domain-containing protein 4 |
| ACX4         | acyl-CoA oxidase 4               |
| C14:0        | myristic acid                   |
| C16:0        | palmitic acid                   |
| C16:1        | palmitoleic acid                |
| C18:0        | stearic acid                    |

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Introduction

The oil palm industry is a major contributor to the global vegetable oils and fats market. The production of palm and palm kernel oil is at about 75.2 million tonnes accounting for almost one-third of the world’s vegetable oil production (Kushairi et al. 2018). Interestingly, over 84.0% of production is in South-east Asia (USDA, Oil crops yearbook, world vegetable oils supply and distribution 2012). The high demand for palm oil reflects its position as the most consumed vegetable oil, with India, China and the European Union among the main importing countries (Index Mundi 2016; Kushairi et al. 2018). Despite concerns about sustainable practices, especially in the European Union which the industry is addressing aggressively, the supply of palm oil needs to rise in order to meet increasing demand from the growing population worldwide.

Palm oil is produced and accumulates in the fruit mesocarp tissue and is referred to as crude palm oil (CPO). The physical and chemical characteristics (e.g. melting, crystallization and morphology) of CPO are mainly attributed to its fatty acid composition (FAC). In the commercial *Elaeis guineensis* CPO, FAC comprises a balanced combination of saturated and unsaturated fatty acids (FAs). The saturated FAs consist primarily of palmitic (C16:0; ~ 44.0%) and stearic (C18:0; ~ 4.5%) acids whereas the unsaturated FAs consist mainly of oleic (C18:1; ~ 39.0%) and linoleic (C18:2; ~ 10.0%) acids. The iodine value (IV) which measures the total level of unsaturation is on average about 53.0 in commercial materials. In comparison, CPO from *E. oleifera* has much higher levels of unsaturated FAs (IV: 70.0 – 93.0) due to high levels of C18:1 (ranges from 47.0 – 69.0%) and C18:2 (ranges from 2.0 – 22.0%). In contrast, saturated FAs in the *E. oleifera* CPO only range from 15.0 – 30.0% for C16:0 and 0.2 – 2.0% for C18:0 (Mohd Din et al. 2000; Montoya et al. 2014; Corley and Tinker 2016). Increasing the unsaturated FA levels in commercial *E. guineensis* CPO has advantages, especially for marketing palm oil in temperate countries. As such, conventional breeding programmes have been directed at selecting high IV oil palm. A sure way to achieve this is via interspecific hybrid breeding, where there is a desire for the unsaturated characteristic from *E. oleifera* to be introgressed into the commercial planting materials. Interspecific breeding crosses have been created using selected high IV *E. oleifera* palms (> 70.0) and Nigerian *E. guineensis* palms (~ 64.0) which appears close to the upper limit that can be achieved in pure *E. guineensis* materials. Early results showed an additive effect in the interspecific hybrids, which possess an
intermediate level of unsaturated FAs compared to both the *E. oleifera* and *E. guineensis* parental palms (Rajanaidu et al. 1989; Rajanaidu et al. 2000; Corley and Tinker 2016).

The application of marker-assisted selection (MAS), especially in interspecific hybrid breeding programmes, can expedite the introgression of unsaturated FAs into elite *E. guineensis* line. In this respect, a number of quantitative trait loci (QTLs) affecting FAC have been previously identified by Singh et al. (2009), Montoya et al. (2013, 2014) and Ting et al. (2016). Markers associated with these QTLs can help breeders in selecting palms with desired FAC, at least in specific genetic backgrounds. In addition to the identification of QTL-linked markers, efforts are also focussed on identifying the genes responsible for the variation in FAC as well as other important economic traits (e.g. yield) in oil palm (Kalyana Babu et al. 2020; Xia et al. 2019; Ting et al. 2018; 2016). The availability of the oil palm genome sequence (Singh et al. 2013) can facilitate the identification of the genes.

In the present study, the *E. guineensis* genome build 5 (EG5) successfully revealed a number of important genes and transcription factors (TFs) involved in biosynthesis of FAs and triacylglycerols (TAGs) within the QTL intervals, associated with FAC in an *E. oleifera* × *E. guineensis* (OxG) interspecific hybrid population (Ting et al. 2016). The authors had identified a total of 12 QTLs distributed across six linkage groups (LGs) - OT1, T2, T3, OT4, OT6 and T9 that were linked to IV, myristic acid (C14:0), C16:0, palmitoleic acid (C16:1), C18:0, C18:1 and C18:2. However, previous search for candidate genes was restricted to the QTL intervals in LGOT1. The method was efficient at revealing potential regulatory genes and as such, a similar approach was extended in the present study to mine for candidate genes from QTL intervals on the five other identified LGs. Interestingly, a number of the genes identified in the QTL intervals were similar to those described in other independent studies as regulating the synthesis of FAs and TAGs in the oil palm mesocarp (Sambanthamurthi et al. 1999; Tranbarger et al. 2011; Bourgis et al. 2011; Dussert et al. 2013; Guerin et al. 2016).

### Materials and methods

#### The OxG mapping population

The OxG mapping population is an oil palm interspecific hybrid cross between the maternal Colombian *E. oleifera* (coded as UP1026) and a paternal Nigerian *E. guineensis tenera* (coded as T128). The OxG cross consists of 108 F2 hybrid progenies. F2 mapping populations have been routinely utilized in genetic linkage and QTL analysis of important economic traits in oil palm as reported by Ong et al. (2019), Bai et al. (2017, 2018), Seng et al. (2016), Lee et al. (2015) and Jeennor and Volkaert (2014).

The spear leaves were sampled and stored at −80 °C. The frozen leaves were ground into powder in liquid nitrogen and DNA extraction was carried out using the modified cetyltrimonium bromide (CTAB) method (Doyle and Doyle 1990). DNA purity was assessed using a NanoDrop spectrophotometer (NanoDrop Technologies Inc. DE) and an A260/A280 OD ratio of at least 1.8 was considered acceptable. Quality of the extracted DNA was further verified by comparing DNA digested with *EcoRI* and HaeIII with undigested DNA on a 0.9% agarose gel in 1X TPE buffer (90 mM tris-phosphate buffer and 2 mM EDTA pH 8.0) after electrophoresed at 80 – 100 V for 3 h. The DNA was diluted to 50 ng/μL for genotyping with simple sequence repeats (SSR) markers.

#### Mining candidate genes and development of candidate markers

Sequence information of the single nucleotide polymorphism (SNP) markers linked to QTLs for IV and FAC (reported by Ting et al. 2016) was downloaded from the publicly accessible Genomsawit database at [http://genomsawit.mpob.gov.my](http://genomsawit.mpob.gov.my). The QTL linked SNP markers were then mapped to the published oil palm reference genome (EG5) (Singh et al. 2013) using BLASTN (Altschul et al. 1997) based on sequence similarity <1e-5. Markers linked to QTLs and candidate genes reported by Bourgis et al. (2011), Montoya et al. (2013) and Jeennor and Volkaert (2014) were also mapped to EG5. Subsequently, the genomic sequences of the entire chromosomal fragment corresponding to each QTL interval were extracted from EG5 and searched for significant homology (BLASTN and BLASTX) to known genes of interest in the National Center for Biotechnology Information (NCBI) databases ([https://blast.ncbi.nlm.nih.gov/Blast.cgi](https://blast.ncbi.nlm.nih.gov/Blast.cgi)). Relevant information associated with the biological functions of the genes and TFs was obtained from published literature and the Universal Protein Resource (Uniprot) database ([http://www.uniprot.org/uniprot/](http://www.uniprot.org/uniprot/)). Genes and TFs involved in regulation of biosynthesis of FAs and TAGs, glycolysis and other possible influential factors were shortlisted as candidates.

The identified candidate genes and TFs were mined for SSRs of various repeats (e.g. mono- di-, tri-, tetra-, penta- and hexa-nucleotides) using default parameters in the MicroSatellite identification tool (Thiel et al. 2003). One to five SSRs were selected for each candidate gene and forward (Fwd) and reverse (Rvs) primers (18–24 nucleotides) were designed using the default parameters in PRIMER 3 ([http://www-genome.wi.mit.edu/genome_software/other/primer3.html](http://www-genome.wi.mit.edu/genome_software/other/primer3.html)). A common M13-tail (5′-CACGACGTTGTAAAACGACGGCTC-3′) was attached to the forward primer (Fwd 5′-M13) whereas, another M13 primer was attached to each of the fluorescent dyes (FAM-, NED-, PET- and VIC-M13). Nomenclatures sPSc and _oSSR were used for these candidate SSR markers.
**Candidate SSR marker analysis**

The genotyping of the SSR markers on the 108 F1 hybrid progenies and two parental palms was carried out as described previously (Ting et al. 2013, 2014; Ting et al. 2016). PCR amplification of each SSR marker was carried out in a 10.0 μL mixture containing genomic DNA (50 ng/μL), Fwd 5'-M13 primer (2.5 μM), Rvs primer (2.5 μM), one fluorescent dye-M13 (2.5 μM), 1X PCR buffer (NEB, USA), 2 mM of each dNTP (NEB, USA) and 0.5 U Taq DNA polymerase (NEB, USA). The PCR conditions were as follows: 95 °C for denaturation (3 min); 35 cycles consisting of 95 °C (30 s), 52–56 °C (depending on primers, 30 s) and 72 °C (30 s) and a final extension at 72 °C (5 min). Prior to fragment analysis, multiplexing of four to eight PCR products was carried out, depending on the sizes of the expected amplicons. PCR fragments were analysed using capillary electrophoresis and subsequently detected using an ABI3730XL DNA analyser (Applied Biosystems, USA). Sizing and scoring of the SSR alleles were executed using the GeneMapper® 4.1 software (Applied Biosystems, USA).

The genotype profile of the markers was determined as originally described by Billotte et al. (2005). The four segregation profiles that were observed in the OxG mapping population previously (Ting et al. 2016) are illustrated in Online Resource 1: Fig. 1. For profile 1, a polymorphic locus is inherited from one of the parental palms and was scored as ab and aa for the heterozygous and homozygous genotypes, respectively, with an expected ratio of 1:1. For profile 2, polymorphism involved two common segregating alleles (observed as ab in both parents) and was scored as aa, ab and bb in the progenies with the expected ratio of 1:2:1. For polymorphisms that involved three segregating alleles (profile 3), the parental genotypes were scored as ab and ac whereas, the progenies were scored as aa, ab, ac and bc, which are expected to fit a 1:1:1:1 ratio. Finally, for profile 4, the four segregating alleles were scored as ab and cd in the two parents and were expected to segregate as ac, bc, ad and bd in the progenies, also in a 1:1:1:1 ratio.

**Mapping candidate SSR markers to the OxG linkage map and QTL analysis**

The F1 interspecific hybrid population was analyzed essentially as a pseudo-testcross (Grattapaglia and Sederoff 1994). The SSR loci coded as “cross pollinator” (CP) were incorporated into the existing data set (Ting et al. 2016) and linkage phases between the SSR alleles were determined using JoinMap® 4.1 (van Ooijen 2006). Segregation of the SSR marker alleles according to expected Mendelian ratios was evaluated using a built-in chi-square analysis and severely distorted markers ($p < 0.0001$) were excluded from linkage analysis. The existing OxG linkage map was used as the backbone in the analysis. These markers were mapped using MapQTL 6. Only QTLs that were consistently observed in all three methods were considered significant in this study.

**Results**

**Candidate genes underlying QTL intervals**

Eleven QTLs on LGs T2, T3, OT4, OT6 and T9 were previously associated with C14:0 ($\geq$ LOD3.7, GW), C16:1 ($\geq$ LOD4.3, GW), C18:0 ($\geq$ LOD3.8, GW) and C18:2 ($\geq$ LOD1.7 on LGT3 and LOD2.7 on LGOT6, CW) in the OxG mapping population (Ting et al. 2016). Each of the LGs was successfully anchored to the oil palm EG5 genome build on its corresponding pseudo-chromosome (CHR) – 8, 14, 2, 7 and 13, respectively. Markers associated with QTLs, including a number of candidate genes reported previously (Bourgis et al. 2011; Montoya et al. 2013; Jeennor and Volkaert 2014) were also aligned to CHR 2, 5, 6, 7, 8, 10, 13, 15 and 16. In total 45 candidate genes including four TFs, were identified, most of which are directly involved in biosynthesis of FAs and TAGs (Fig. 1, Table 1 and Online Resource 2: Table 1).

**Integration of candidate gene-linked SSR markers into existing genetic map**

A total of 45 FA and TAG related genes were utilized for development of SSR markers. One to five SSR markers were selected for each candidate gene, resulting in 92 SSR primer-pairs being designed (Table 1). Genotyping of these SSR markers in the OxG mapping population resulted in 50 polymorphic SSR markers, of which 47 were scored according to profile 1 (45 inherited from T128 and two inherited from UP1026) and three were scored as having profile 4. The allelic
segregation ratios for the 50 SSR markers met the expected Mendelian ratios at $p \geq 0.0005$. These 50 markers were then included into the existing marker data set for constructing the genetic map and all were successfully mapped into the existing OxG genetic map (Fig. 2 and Online Resource 3: Fig. 2).

Mapping of candidate gene markers to the respective QTLs

The candidate gene markers identified in this study were successfully mapped back to the respective QTL intervals in LGs T2, OT3, OT4, OT6 and T9. In LGT2, the 1.0 cM interval (61.2–62.1 cM) related to QTL for C14:0 was mapped to CHR08, but clear candidate genes related to biosynthesis of FAs or TAGs were not detected in the QTL interval. Therefore, the search was extended towards the left and right of the QTL interval and an oleoyl-ACP thioesterase (OTE/FATA), stearoyl-ACP desaturase (SAD) and hydroxyacyl glutathione hydrolase 2 (HAGH) gene were detected flanking both sides of the interval (Fig. 2). A similar chromosomal region corresponding to the QTL for C14:0 was also reported in an interspecific BC$_1$ mapping population (Montoya et al. 2014). This was revealed by two common SSR markers, namely megCIR3592 and megCIR3787 that were located within/near the similar QTLs reported by Montoya et al. (2014) (Fig. 2). Taking the regions containing all three QTLs, an interval ranging from 2.9–32.6 cM was examined, which identified four potential genes and three TFs. The four candidate genes were beta-ketoacyl-ACP synthases II and III (KASII, KASIII), malate dehydrogenase (MDH) and acetoacetyl-CoA thiolase (AACT) whereas, the TFs were myb family PHL8 (MYB), TCP15 (TCP15) and homeobox-leucine zipper protein ATHB-13 (HD-Zip) (Table 1).

In LGT9, the QTLs for C14:0, C16:1 and C18:0 were located at regions spanning 17.2–32.6, 2.9–32.6 and 13.4–24.8 cM, respectively (Ting et al. 2016). The QTLs for C14:0 and C18:0 were also found to be located very close to that reported previously in a tenera x dura mapping population (Montoya et al. 2014). This was revealed by two common SSR markers, namely sPSc00554, sPSc00571 and sPSc00574, associated with the candidate genes AACT, TCP15 and HD-Zip respectively, were successfully mapped within the QTL interval. The LOD5 linked sPSc00571 was mapped closest to the QTL peak (LOD5.2–12.7) and explained 24.5–49.8% the variation for C14:0, C16:1 and C18:0. The LOD score and phenotypic variation explained (PVE) after mapping of the candidate gene markers were higher than that observed before fine-mapping (LOD4.5–10.7 and 21.9–44.2% PVE) with the candidate markers (Ting et al. 2016). Unfortunately, the SSR markers developed...
| Linkage group (LG) | EG5 chromosome (CHR) | QTL# | Candidate SSR marker | Gene/TF | Source |
|-------------------|----------------------|------|----------------------|---------|--------|
| T2                | 8                    |      | sPSc00417; sPSc00418 | Oleoyl-ACP thioesterase (OTE/FATA) | Bourgis et al. (2011); Montoya et al. (2013); Jeennor and Volkaert (2014); NCBI |
|                   |                      |      | sPSc00647*; sPSc00648 | Stearoyl-ACP desaturase (SAD) | Bourgis et al. (2011); Montoya et al. (2013) |
|                   |                      |      | sPSc00607            | Enol-ACP reductase 1 (ENR1/EAR1) | Jeennor and Volkaert (2014); NCBI |
|                   |                      |      | sPSc00631            | Hydroxacyl glutathione hydratase 2 (HAGH) | Singh et al. (2013); NCBI |
| OT3               | 14                   | C182 | sPSc00654*; sPSc00655 | CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase 2 (pgsA) | Singh et al. (2013); NCBI |
|                   |                      | C182 | sPSc00664; sPSc00665; sPSc00666 | Omega-3 fatty acid desaturase (FAD3/7/8) | Singh et al. (2013); NCBI |
|                   |                      | C182 | sPSc00667; sPSc00668*; sPSc00669B | Acyl-coenzyme A oxidase 4 (ACX4) | Singh et al. (2013); NCBI |
|                   |                      | C182 | sPSc00679*          | Zinc finger protein 588 (ZNF588) | Singh et al. (2013); NCBI |
|                   |                      | C182 | sPSc00681*; sPSc00682* | Glycerol-3-phosphate acyltransferase 3 (GPAT3) | Singh et al. (2013); NCBI |
|                   |                      | C182 | sPSc00685*; sPSc00686* | Acyl-CoA binding domain-containing protein 4 (aclh4) | Singh et al. (2013); NCBI |
|                   |                      | C182 | sPSc00683*          | ATP--citrate synthase beta chain protein 1 (ACLB-1) | Singh et al. (2013); NCBI |
|                   |                      | C182 | sPSc00694            | lysophosphatic acid acyltransferase 1 (LPAAT1) | Singh et al. (2013); NCBI |
|                   |                      | C182 | sPSc00695            | Acetate/butyrate--CoA ligase (AE7) | Singh et al. (2013); NCBI |
| OT4               | 2                    | C180 | sPSc00583A          | Outer envelope protein 16–3 (OEPI63) | Singh et al. (2013); NCBI |
|                   |                      | C180 | sPSc00584A; sPSc00585* | beta-ketoacyl-ACP reductase (KAR) | Singh et al. (2013); NCBI |
|                   |                      | C180 | sPSc00587; sPSc00588 | 4-cozyme-CoA ligase 1 (4CLL1) | Singh et al. (2013); NCBI |
|                   |                      | C180 | sPSc00591*          | Fructose--1,6-bisphosphate aldolase (FBA) | Singh et al. (2013); NCBI |
|                   |                      | C180 | sPSc00593; sPSc00594* | UDP--glucose--transferase (UGT) | Singh et al. (2013); NCBI |
|                   |                      |      | sPSc00422; sPSc00423* | Stearoyl-ACP desaturase (SAD) | Bourgis et al. (2011); Montoya et al. (2013) |
|                   |                      |      | sPSc00430; sPSc00431* | Indole-3-glycerol phosphate synthase (IGPS) | Jeennor and Volkaert (2014); NCBI |
|                   |                      |      | sPSc00450A; sPSc00450B | Triacylglycerol lipase 2 (LIPC2) | Jeennor and Volkaert (2014); NCBI |
| T5                | 16                   |      | sPSc00432; sPSc00433 | Acyl-cozyme A oxidase 1 (ACX1) | Jeennor and Volkaert (2014); NCBI |
| OT6               | 7                    | C182 | sPSc00709            | Phospholipase Al-Igammal (Al-Igammal) | Singh et al. (2013); NCBI |
|                   |                      | C182 | sPSc00696*; sPSc00697*; sPSc00698* | Palmitoyl-acyl carrier protein thioesterase (PATE/FATB) | Singh et al. (2013); NCBI |

* Candidate SSR markers with an asterisk (*) indicate additional markers not listed in the main table.
| Linkage group (LG) | EG5 chromosome (CHR) | QTL# | Candidate SSR marker | Gene/TF | Source |
|--------------------|---------------------|------|----------------------|---------|--------|
| T9                 | 13                  |      | sPSc00554            | Acetoacetyl-CoA thiolase (AACT) | Bourgis et al. (2011); Montoya et al. (2013) |
|                    |                     |      | sPSc00556*; sPSc00557*; sPSc00558* | Beta-ketoacyl-ACP synthase II (KASI) | Singh et al. (2013); NCBI |
|                    |                     |      | sPSc00564*; sPSc00565* | TF myb family PH18 (MYB) | Jeennor and Volktaer (2014) |
|                    |                     |      | sPSc00566*; sPSc00567* | Malate dehydrogenase (MDH) | Singh et al. (2013); NCBI |
|                    |                     |      | sPSc00568*; sPSc00569* | Beta-ketoacyl-ACP synthase III (KASIII) | Singh et al. (2013); NCBI |
|                    |                     |      | sPSc00570*; sPSc00571 | TF TCP15 (TCP15) | Singh et al. (2013); NCBI |
| OT10               | 6                   |      | sPSc00439; sPSc00440* | Lysophosphatidase 2 (LYPLA2) | Jeennor and Volktaer (2014) |
|                    |                     |      | sPSc00436; sPSc00437*; sPSc00438 | Phosphoenolpyruvate/phosphate translocator 1 (PPT) | Jeennor and Volktaer (2014); NCBI |
| OT12               | 5                   |      | sPSc00399*; sPSc00400*; sPSc00401B | Beta-ketoacyl-ACP synthase II (KASI) | Bourgis et al. (2011); NCBI |
|                    |                     |      | sPSc00441*; sPSc00442 | Homeobox protein GLABRA (GLABRA) | Jeennor and Volktaer (2014); NCBI |
|                    |                     |      | sPSc00443; sPSc00444* | Glycerol-3-phosphate dehydrogenase (GPDH) | Jeennor and Volktaer (2014); NCBI |
|                    |                     |      | sPSc00445; sPSc00446 | Plastid-linoleate desaturase (FAD7) | Jeennor and Volktaer (2014); NCBI |
|                    |                     |      | sPSc00447; sPSc00448 | Malonyl-CoA ACP transacylase (MACP/MAT) | Jeennor and Volktaer (2014); NCBI |
| T14                | 11                  |      | sPSc00449 | Triacylglycerol lipase 2 (LIPT2) | Jeennor and Volktaer (2014) |
|                    |                     |      | SA1; oSSR | Stearoyl-ACP desaturase (SAD) | Singh et al. (2013); NCBI |
| OT15               | 10                  |      | sPSc00390*; sPSc00391; sPSc00392*; sPSc00393* | Beta-ketoacyl-ACP synthase II (KASI) | Bourgis et al. (2011); Montoya et al. (2013); NCBI |
|                    |                     |      | sPSc00394; sPSc00395; sPSc00396* | Beta-ketoacyl-ACP synthase I (KASI) | Bourgis et al. (2011); Montoya et al. (2013); NCBI |
| T16                | 15                  |      | sPSc00402*; sPSc00403*; sPSc00404*; sPSc00405 | Beta-ketoacyl-ACP synthase II (KASI) | Bourgis et al. (2011); Montoya et al. (2013); NCBI |

*Non-polymorphic markers; *QTLs reported by Ting et al. (2016)
for KASII, KASIII, MDH and MYB, were not polymorphic (*), and thus could not be mapped onto LGT9. They were placed on the LG based on their relative order compared to other markers (and genes) in CHR08, but their exact map positions could not be determined (Fig. 2).

On LG04 (CHR02), UDP-glycosyltransferase (UGT) was found located underlying the QTL peak for C18:0, defined by SNPM00121 (LOD5.0). UGTs are not involved in FA or TAG biosynthesis. They however, catalyse the covalent addition of sugars to a wide range of lipophilic molecules by transferring the glycosyl group from nucleoside diphosphate-activated sugars (e.g. UDP-sugars), and control the levels of many signalling molecules. The molecules include a broad array of hormones (including phytohormones), secondary metabolites and xenobiotics for maintaining good growth and development in plants (Ross et al. 2001; Barvkar et al. 2012; Ostrowski and Jakubowska 2014). Other genes from the QTL interval were fructose-bisphosphate aldolase (FBA), outer envelope pore protein 16–3 (OEP163), 4-coumarate–CoA ligase 1 (4CLL1) and beta-ketoacyl-ACP reductase (KAR). Among these, KAR is involved in the de novo FA chain elongation cycle and the SSR marker associated with this gene, sPSc00584A (LOD4.5) mapped closest to UGT. The PVE explained by sPSc00584A at 22.5% was similar to that observed for SNPM00121 (23.8%). In fact, another important FA gene namely stearoyl-ACP desaturase (SAD) which

![Fig. 2](image-url)
converts C18:0 to C18:1-ACP was identified on LGOT4 but, at a distance of about 36.0 cM from the QTL interval (Fig. 2).

Two putative QTLs for C18:2 were reported on LGs OT3 and OT6, at intervals 46.9–65.2 and 38.9–54.5 cM, respectively. Three candidate markers - sPSc00664, sPSc00665 and sPSc00666 associated with *omega-3 fatty acid desaturase* (*FAD3*/7/8), were developed within the QTL interval at LGOT3 (corresponded to CHR14). All three SSR markers were successfully mapped back to the QTL peak. The second gene within close proximity was *acyl-CoA oxidase 4* (*ACX4*) which is involved in the peroxisomal degradation of short-chain FAs (C4:0–8:0) during beta-oxidation. This process also recycles acetyl-CoA as a carbon and energy source for FA synthesis and plant growth (Poirier et al. 2006; Geopfert and Poirier 2007). Other genes involved in FA and TAG synthesis activities were also detected at the QTL interval in LGOT3. These include *lysophosphatidic acid acyltransferase 1* (*LPAAT1*), *acyl-CoA-binding domain-containing protein 4* (*acbd4*) and *glycerol-3-phosphate acyltransferase 3* (*GPAT3*). A candidate SSR marker, sPSc00694 was developed and mapped close to *LPAAT1* whereas, SSR markers for *acbd4* and *GPAT3* were not polymorphic (*) and could not be mapped (Table 1 and Fig. 2). For QTL-C18:2 on LGOT6 which corresponded to CHR07, the QTL interval hosted a *palmitoyl-ACP thioesterase* (*PATE/FATB*) gene. None of the markers developed from the gene were polymorphic (*). However, two important FA genes – *SAD* and *OTE/FATA* were identified at a distance of 4.4 cM from the QTL interval.

In addition to mining candidate genes from the QTLs identified by Ting et al. (2016), a number of candidate SSR markers were also developed for other published QTLs/genes for FAC and oil yield in oil palm. Other than the five LGs mentioned above, these markers were also mapped onto LGs T5, OT10, OT12, T14, OT15 and T16 (Table 1, Fig. 2 and Online Resource 3: Fig. 2). As an example, the *homeobox protein GLABRA* gene (*GLABRA*), *glycerol-3-phosphate dehydrogenase* (*GPDH*), *plastid-linoleate desaturase* (*FAD7*), *malonyl-CoA:ACP transacylase* (*MACP/MAT*) and *triacylglycerol lipase 2* (*LIPT2*) genes associated with QTLs for oil-to-fruit and oil-to-bunch traits (Jeennor and Volkaert 2014) were successfully mapped to the present LGOT12. The candidate SSR markers developed from these genes mapped close to each of the respective genes as follows: sPSc00442-GLABRA, sPSc00443-GPDH, sPSc00445-FAD7-sPSc00446, sPSc00447-MACP-sPSc00448 and sPSc00449-LIPT2.

**Discussion**

The present study builds on previous efforts in searching for candidate genes in the QTL intervals on LGs T2, T9, OT3, OT4 and OT6 (Ting et al. 2016). The use of SSR markers common to those utilized in other studies revealed that several of these QTLs were located near or within the genomic regions linked to FAC in previous studies using a BC1 and a *tenera x dura* mapping populations (Montoya et al. 2013, 2014). This provided confidence to search for candidate genes within the designated QTL intervals. In this study, there were 92 SSR markers developed from FA and TAG related genes, of which 50 (53.0%) were informative. The 50 SSR candidate markers followed two of the four segregation profiles observed in the OXG mapping population previously. All 50 were successfully mapped to the expected LG, corresponding to the genomic region from which they were designed, confirming the appropriateness of the mapping methodology applied in this study.

Each of the QTL intervals of interest in this study was successfully anchored to the corresponding pseudo-chromosomes and revealed a number of FA and lipid related genes. From the QTL regions associated with C14:0, C16:1 and C18:0 in LGT9 (CHR13), *MDH* and *KASIII* were identified. The *MDH* encoding enzyme can be found in a range of subcellular locations (e.g. cytosol and mitochondria) and it catalyses the interconversion of malate to oxaloacetate which subsequently can be converted to form phosphoenopyruvate or can be oxidized to form pyruvate (Wedding 1989; Minárík et al. 2002). This provides the pyruvate source to initiate the synthesis of FAs. In vitro experiments in castor bean demonstrated high FA synthesis rate when malate was provided as a precursor (Smith et al. 1992). The enzyme KASIII forms the acetoacetyl-ACP complex from acetyl-CoA and malonyl-ACP in preparation for FA-chain elongation. Both the MDH and KASIII-catalysed reactions take place at a very early stage even before the FA-chain elongation process starts. This suggests that MDH and KASIII activities are important prior to formation of various FAs and could explain the co-localization of the two genes within the same QTL interval associated with C14:0, C16:1 and C18:0 in LGT9.

Another gene, *KASII*, that plays a critical step in elongating C16:0-ACP to form C18:0-ACP was also detected in LGT9. This is one of the most important enzymatic activities for generating and supplying C18:0 for subsequent desaturation into unsaturated FAs by SAD and FADs. In oil palm, KASII activity was found to be positively correlated with unsaturated FA content. The observed relationship was particularly strong with C18:1 and C18:2, suggesting that increased levels of C18:0-ACP are efficiently converted to C18:1-ACP which subsequently is hydrolysed (Sambanthamurthi et al. 1999). The C18:1 released is activated to C18:1-CoA and channelled to endoplasmic reticulum (ER) for TAG assembly or further desaturated to C18:2 prior to TAG assembly. In contrast, KASIII activity was found to be negatively correlated with the saturated FAs (Sambanthamurthi et al. 1999). This was supported by the recent transcriptomic co-expression analysis in oil palm, where lower levels of C16:0 were the result of
increased KasII expression (Guerin et al. 2016). It has been suggested that lower rates of KasII activity increase accumulation of shorter FA chains such as C14:0- and C16:0-ACPs. The increased accumulation of C16:0-ACP allows C16:0-ACP to be desaturated to form C16:1-ACP. Increased accumulation of C16:0-ACP also results in increased hydrolysis by PATE/FATB, activation into C16:0-CoA and assembly of higher levels of C16:0 into TAG in the ER. In A. thaliana and cotton seed, silencing or down-regulating the KasII gene has led to two- to six-fold increase in C16:0 (Pidkowich et al. 2007; Liu et al. 2017). In Camelina, suppression of the KasII gene also led to higher accumulation of palmitate and further reduction of unsaturated FAs (Hu et al. 2017). This provides support for the involvement of KasII in the QTL interval linked to C14:0, C16:1 (produced from C16:0) and C18:0. However, the SSR markers designed to the KasII gene did not segregate in the mapping family. It will be interesting to extend the analysis in future to search for polymorphic SNPs, within or flanking the KasII gene. The identification of candidate genes that are required for the initiation of FA synthesis (MDH and KasIII) and in the accumulation of unsaturated FAs (KasIII) within the QTL interval in LGT9, suggests that it is an important genomic region influencing FAC in interspecific hybrids.

A number of genes encoding enzymes that show substrate specificity have also been identified in the confidence intervals of QTLs, in accordance with their respective FA preferences. These include FAD3/7/8, acbd4 and LpaAt1 that were associated with QTL for C18:2 in LGOT3 (CHR14). FAD3/7/8 encodes desaturase activity to convert C18:2 into C18:3 either in the plastids (by FAD7/8) or in the ER (by FAD3) (Song et al. 2004; Yurchenko et al. 2014). The acbd4 binds oleoyl (C18:1)-CoAs with high affinity and transports them from cytosol to ER for further modification of FAs or synthesis of TAGs (Leung et al. 2004; Xiao et al. 2008). Located next to acbd4 is Gpat which encodes the first step of enzymatic acylation to form TAGs in ER. Generally, Gpat is known to have preference for saturated FAs, especially towards C16:0-CoA (Griffiths et al. 1988; Griffiths and Harwood 1991; Xu et al. 2009). However, Sambanthamurthi et al. (2000), Manaf and Harwood (2000) and Dussert et al. (2013) suggested that oil palm Gpat can use both saturated and unsaturated acyl-CoAs (including C18:1-CoA) as substrates. Interestingly in Brassica napus, Gpat has a wider range of specificity, allowing addition of variety of fatty acyl-CoAs to the stereospecific number 1 (sn-1) position of glycerol-3-phosphate (Gly3P) (Larson et al. 2002). The subsequent acylation is catalysed by lysophosphatidic acid acyltransferase, an enzyme encoded by the LpaAt gene. In the current QTL interval, LpaAt1 was identified and interestingly it has been reported to show high specificity towards unsaturated fatty acyl-CoAs such as C18:1-CoA in humans and most plants (Shindou et al. 2009). In oil palm, LpaAt has also been reported to accept C16:0-CoA as the alternative substrate at the sn-2 position for producing phosphatidate (Sambanthamurthi et al. 2000). The QTL intervals essentially contain genes that regulate both FA synthesis in the plastid and TAG assembly in the ER. As such, the candidate genes and the SSR markers linked to these genes are ideal candidates to further investigate both FA and lipid biosynthesis in independent oil palm populations.

In this study, there were interesting candidate genes identified outside the QTL confidence intervals such as those for C18:0 in LGOT4 (CHR02) and C18:2 in LGOT6 (CHR07). Sad was located at a distance of about 36.0 cM from the QTL for C18:0 whereas on LGOT6, Sad and Ote/Fata were located at a position about 4.4 cM from the QTL for C18:2. Sad and Ote/Fata encode stearoyl-ACP desaturase and oleoyl-ACP thioestersases A, respectively and these two enzymes have high specificity towards C18 FA-ACPs. In the oil palm mesocarp, Sad modifies C18:0-ACP to C18:1-ACP while, Ote/Fata hydrolyses and releases C18:1 from C18:1-ACP. Detection of candidate genes outside the confidence interval has also been reported for QTLs associated with C18:1 and C18:2 in watermelon seeds (Meru and McGregor 2014). A point to consider is the observation by Raghavan and Collard (2012) that for a small mapping population (< 194 samples), there is a possibility that the QTL detected may actually be several cM away from its actual position. As such, even though the candidate genes were located outside the QTL confidence interval, they remain as good candidates for further evaluation.

**Conclusion**

The increasing availability of information on gene function and genome sequence data of plant species (including oil palm) that are accessible in public databases facilitated the present study to uncover potential candidate genes associated with fatty acid composition. This further facilitated development of markers closely linked to these candidate genes within the QTL confidence intervals. In this study, the candidate gene approach once again proved very efficient and was successfully applied to identify candidate genes and transcription factors from the QTL intervals. More importantly, biological functions of these candidate genes provided potential explanations for their possible involvement in the fatty acid and Kennedy pathways for lipid assembly. Both pathways play an important role in determining the levels of saturation and unsaturation in palm oil. The levels of saturation and unsaturation could possibly be regulated by the expression of these genes. More in-depth evaluation e.g. expression and functional studies will be required to confirm the regulatory effects of these candidate genes. This paper presents an atlas of candidate genes which may be involved in the oil saturation
differences between the high IV *E. oleifera* and lower IV *E. guineensis*. Introggression of the high IV character into the African oil palm could lead to new markets and applications for palm oil. The current work represents an important step towards realising these objectives.

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**Availability of data** The sequence information for the candidate SSR paper.

**Authors’ contributions** All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Ting Nguot-Chin. Bioinformatics analysis was supported by Chan Kuang-Lim and the plant materials used in this study was provided by Kandha Sridharan. The first draft of the manuscript was written by Ting Nguot-Chin and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**References**

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25:3389–3402. https://doi.org/10.1093/nar/25.17.3389

Bai B, Wang L, Lee M, Zhang YJ, Rahmadasyah R, Alfiko Y, Ye BQ, Wan ZY, Lim CH, Suwanto A, Chua N-H, Yue GH (2017) Genome-wide identification of markers for selecting higher oil content in oil palm. BMC Plant Biol 17:93. https://doi.org/10.1186/s12870-017-1045-z

Bai B, Wang L, Zhang YJ, Lee M, Rahmadasyah R, Alfiko Y, Ye BQ, Purwanto S, Suwanto A, Chua N-H, Yue GH (2018) Developing genome-wide SNPs and constructing an ultrahigh-density linkage map in oil palm. Sci Rep 8:691. https://doi.org/10.1038/s41598-018-1613-2

Barvkar VT, Pardeshi VC, Kale SM, Kadoo NY, Gupta VS (2012) Phylogenomic analysis of UDP glycosyltransferase 1 multigene family in *Limum usitatissimum* identified genes with varied expression patterns. BMC Genomics 13:175. https://doi.org/10.1186/1471-2164-13-175

Billotte N, Mansell C, Risterucci AM, Adon B, Broteire P, Baurens FC, Singh R, Herran A, Asmady H, Billot C, Ambiard P, Durrand-Gasselin T, Courtois B, Asmono D, Cheah SC, Rohde W, Charrier A (2005) Microsatellite-based high density linkage map in oil palm (*Elaeis guineensis* Jacq.). Theoretical and Applied Genetics 110:754–765. https://doi.org/10.1007/s00122-004-1901-8

Bourgis F, Kilaru A, Cao X, Ngando-Ehongue GF, Dirra N, Ohlrogge JB, Arondel V (2011) Comparative transcriptome and metabolite analysis of oil palm and date palm mesocarp that differ dramatically in carbon partitioning. Proc Natl Acad Sci U S A 108:12527–12532. https://doi.org/10.1073/pnas.1106502108

Corley RHV, Tinker PB (2016) The oil palm, 5th edn. Wiley, New Jersey

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. FOCUS 12:13–15

Dussert S, Guerin C, Andersson M, Joël T, Tranburger TJ, Pizot M, Sarah G, Omore A, Durand-Gasselin T, Morcillo F (2013) Comparative transcriptome analysis of three oil palm fruit and seed tissues that differ in oil content and fatty acid composition. Plant Physiol 162:1337–1358. https://doi.org/10.1104/pp.113.220525

El-Khouen K, Blangy S, Ortiz E, Gardies AM, Ferté N, Arondel V (2005) Identification and characterization of a triacylglycerol lipase in Arabidopsis homologous to mammalian acid lipases. FEBS Lett 579:6067–6073. https://doi.org/10.1016/j.febslet.2005.09.072

Goepfert S, Poirier Y (2007) Beta-oxidation in fatty acid degradation and beyond. Curr Opin Plant Biol 10:245–251. https://doi.org/10.1016/j.pbi.2007.04.007

Grattapaglia D, Sederoff R (1994) Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. Genetics 137:1121–1137

Griffiths G, Harwood JL (1991) The regulation of triacylglycerol biosynthesis in cocoa (*Theobroma cacao*) L. Planta 184:279–284. https://doi.org/10.1007/BF00197958

Griffiths G, Stymne S, Stobart AK (1988) The utilisation of fatty-acid substrates in triacylglycerol biosynthesis by tissue-slices of developing safflower (*Carthamus tactorius L.*) and sunflower (*Helianthus annuus L.*) cotyledons. Planta 173:309–316. https://doi.org/10.1007/BF00401017

Guerin C, Joël T, Serret J, Lashermes P, Vaissayre V, Aghessi MD, Beulé T, Severac D, Ambiard P, Tregear J, Durand-Gasselin T, Morcillo F, Dussert S (2016) Gene coexpression network analysis of oil biosynthesis in an interspecific backcross of oil palm. Plant J 87:423–441. https://doi.org/10.1111/tpj.13208

Hong SK, Kim KH, Park JK, Jeong KW, Kim Y, Kim EE (2010) New design platform for malonyl-CoA-acyl carrier protein transacylase. FEBS Lett 584:1240–1244. https://doi.org/10.1016/j.febslet.2010.02.038

Hu Z, Wu Q, Dalal J, Vasani N, Lopez HO, Sederoff HW, Qu R (2017) Accumulation of medium-chain, saturated fatty acyl moieties in seed oils of transgenic *Camelina sativa*. PLoS One 12:e0172296. https://doi.org/10.1371/journal.pone.0172296

Index Mundi (2016). Palm oil imports by country in 1000 mt. https://www.indexmundi.com/agriculture/?commodity=palm

Jeenor S, Volkaert H (2014) Mapping of quantitative trait loci (QTLs) for oil yield using SSRs and gene-based markers in African oil palm (*Elaeis guineensis* Jacq.). Tree Genet Genomes 10:1–14. https://doi.org/10.1007/s11295-013-0655-3

Kalyana Babu B, Mathur RK, Ravichandran G, Anita P, Venu MVP (2020) Genome wide association study (GWAS) and identification of candidate genes for yield and oil yield related traits in oil palm (*Elaeis guineensis*) using SNPs by genotyping-based sequencing. Genomics 112:1011–1020. https://doi.org/10.1016/j.ygeno.2019.06.018

Kushairi A, Loh SK, Azman I, Elinia H, Ong-Abullah M, Zanai Bidin MNI, Razmiah G, Shamsa S, Parveez GKa (2018) Oil palm economic performance in Malaysia and R&D progress in 2017. Journal of Oil Palm Research 30:163

Lee M, Xia JH, Zou Z, Ye J, Rahmadasyah AY, Jia J, Lianong J, Purnamasari MI, Lim CH, Suwanto A, Wong L, Chua NH, Yue GH (2015) A consensus linkage map of oil palm and a major QTL for stem height. Sci Rep 5:8232. https://doi.org/10.1038/srep08232
Leung K-C, Li H-Y, Mishra G, Chye M-L (2004) ACPBP4 and ACPBP5, novel Arabidopsis acyl-CoA-binding proteins with kelch motifs that bind oleoyl-CoA. Plant Mol Biol 55:297–309. https://doi.org/10.1007/s11103-004-0642-z

Liu Q, Wu M, Zhang B, Shrestha P, Petrie J, Green AG, Singh SP (2017) Genetic enhancement of palmitic acid accumulation in cotton seed oil through RNAi down-regulation of gkhKAS2 encoding β-ketoacyl-ACP synthase II (KASII). Plant Biotechnol J 15:132–143. https://doi.org/10.1111/pbi.12598

Manaf AM, Harwood JL (2000) Purification and characterisation of acyl-CoA, glycerol 3-phosphate acyltransferase from oil palm (Elaeis guineensis) tissues. Planta 210:318–328. https://doi.org/10.1007/pl00008140

Meru G, McGregor C (2014) Quantitative trait loci and candidate genes associated with fatty acid content of watermelon seed. J Am Soc Hortic Sci 139:433–441. https://doi.org/10.21273/jashs.139.4.433

Minárik P, Tómasskoviňová N, Kollárová M, Antalík M (2002) Malate dehydrogenases—structure and function. Gen Physiol Phys 21:257–265

Mohd Din A, Rajanaidu N, Jalani BS (2000) Performance of Elaeis oleifera from Panama, Costa Rica, Colombia and Honduras in Malaysia. Journal of Oil Palm Research 12:71–80

Montoya C, Lopes R, Flori A, Cros D, Cuellar T, Summo M, Espeout S, Rivallan R, Bittencourt D, Zambrano JR, Alarcón S, Tomásková N, Kollárová M, Antalík M (2002) Malate dehydrogenases—structure and function. Gen Physiol Phys 21:257–265

Montoya C, Lopes R, Flori A, Cros D, Cuellar T, Summo M, Espeout S, Rivallan R, Bittencourt D, Zambrano JR, Alarcón S, Tomásková N, Kollárová M, Antalík M (2002) Malate dehydrogenases—structure and function. Gen Physiol Phys 21:257–265

Ong A-L, The C-K, Kwong Q-B, Tangaya P, Appleton DR, Massawe F, Sing R, Ooi L-C-L, Ooi S-E, Chan K-L, Halim MA, Aziz N, Nagappan J, Bacher B, Lakey N, Smith SW, De H, Hogan M, Budiman MA, Lee EK, Desalle R, Kudrna D, Goicoechea JL, Wing RA, Wilson RK, Fulton RS, Ordway JM, Martienssen RA, Sambanthamurthi R (2013) Oil palm genome sequence reveals divergence of interfertile species in old and new worlds. Nature 500:335–339. https://doi.org/10.1038/nature12309

Smith RG, Gauthier DA, Dennis DT, Turpin DH (1992) Malate- and pyruvate-dependent fatty acid synthesis in leucoplasts from developing castor endosperm. Plant Physiol 98:1233–1238. https://doi.org/10.1104/pp.98.4.1233

Song SJ, Lee DE, Jung S, Kim H, Han O, Cho BH, Lee JH, Back K (2004) Characterization of transgenic rice plants expressing an Arabidopsis FAD7. Biol Plant 48:361–366. https://doi.org/10.1007/BF03354025

Thiel T, Michalek V, Warshney RK, Graner A (2003) Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (Hordeum vulgare L.). Theor Appl Genet 106:411–422. https://doi.org/10.1007/s00122-003-1031-0

Ting N-C, Jansen J, Nagappan J, Ishak Z, Chin CW, Tan S-G, Cheah S-C, Singh R (2013) Identification of QTLs associated with callogenesis and embryogenesis in oil palm using genetic linkage maps improved with SSR markers. PLoS One 8:e53076. https://doi.org/10.1371/journal.pone.0053076

Ting N-C, Jansen J, Mayes S, Massawe F, Sambanthamurthi R, Ooi L-C-L, Chin CW, Arulando X, Seng T-Y, Syed Alwee SSR, Ithnin M, Singh R (2014) High density SNP and SSR-based genetic maps of two independent oil palm hybrids. BMC Genomics 15:309. https://doi.org/10.1186/1471-2164-15-309

Ting N-C, Yaakub Z, Kumaruddin K, Mayes S, Massawe F, Sambanthamurthi R, Jansen J, Low ET, Ithnin M, Kushairi A, Arulando X, Rosli R, Chan K-L, Amiruddin M, Lim CC, Nookiah R, Mohd Din A, Singh R (2016) Fine-mapping and cross-validation of QTLs linked to fatty acid composition in multiple independent interspecific crosses of oil palm. BMC Genomics 17:289. https://doi.org/10.1186/s12864-016-2607-4

Ting NC, Mayes S, Massawe F, Sambanthamurthi R, Jansen J, Syed Alwee SSR, Seng TY, Ithnin M, Singh R (2018) Putative regulatory candidate genes for QTL linked to fruit traits in oil palm (Elaeis guineensis Jacq.). Euphytica 214:214. https://doi.org/10.1007/s10681-018-2296-y

Tranbarger TJ, Dussert S, Joët T, Argout X, Summo M, Champion A, Cros D, Omore A, Noub N, Morello F (2011) Regulatory mechanisms underlying oil palm fruit mesocarp maturation, ripening and functional specialization in lipid and carotenoid metabolism. Plant Physiol 156:564–584. https://doi.org/10.1104/pp.111.175141
USDA, Oil crops yearbook, world vegetable oils supply and distribution, (2012)/13–2016/17. https://www.ers.usda.gov/data-products/oil-crops-yearbook/oil-crops-yearbook/#Vegetable oils and animal fats.

van Ooijen JW (2006). JoinMap® 4.1, software for calculation of genetic linkage maps in experimental populations. Kyazma B.V., Wageningen, the Netherlands.

van Ooijen JW (2009). MapQTL® 6, software for the mapping of quantitative trait loci in experimental populations of diploid species. Kyazma B.V., Wageningen, the Netherlands.

Wedding RT (1989) Malic enzymes of higher plants, characteristics, regulation, and physiological function. Plant Physiol 90:367–371. https://doi.org/10.1104/pp.90.2.367

Xia W, Luo T, Dou Y, Zhang W, Mason AS, Huang D, Huang X, Tang W, Wang J, Zhang C, Xiao Y (2019) Identification and validation of candidate genes involved in fatty acid content in oil palm by genome-wide association analysis. Front Plant Sci 10:1263. https://doi.org/10.3389/fpls.2019.01263

Xiao S, Li HY, Zhang JP, Chan SW, Chye ML (2008) Arabidopsis acyl-CoA-binding proteins ACBP4 and ACBP5 are subcellularly localized to the cytosol and ACBP4 depletion affects membrane lipid composition. Plant Mol Biol 68:571–583. https://doi.org/10.1007/s11103-008-9392-7

Xu J, Zheng Z, Zou J (2009) A membrane-bound glycerol-3-phosphate acyltransferase from Thalassiosira pseudonana regulates acyl composition of glycerolipids. Botany 87:544–551. https://doi.org/10.1139/B08-145

Yurchenko OP, Park S, Ilut DC, Inmon JJ, Millhollon JC, Liechty Z, Page JT, Jenkins MA, Chapman KD, Udall JA, Gore MA, Dyer JM (2014) Genome-wide analysis of the omega-3 fatty acid desaturase gene family in Gossypium. BMC Plant Biol 14:312. https://doi.org/10.1186/s12870-014-0312-5

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