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1 **Characterization and heterologous expression of three DGATs from oil palm (*Elaeis***
2 ***guineensis*) mesocarp in *Saccharomyces cerevisiae***

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14 2, Deceased June 23, 2018. This paper is dedicated to the memory of our talented
15 colleague.

16

17

18 **ABSTRACT**

19 Oil palm (*Elaeis guineensis*) can accumulate up to 88 % oil in fruit mesocarp. A previous
20 transcriptome study of oil palm fruits indicated that genes coding for three diacylglycerol
21 acyltransferases (DGATs), designated as EgDGAT1_3, EgDGAT2_2 and
22 EgWS/DGAT_1 (according to Rosli et al., 2018) were highly expressed in mesocarp
23 during oil accumulation. In the present study, the corresponding open reading frames
24 were isolated, and characterized by heterologous expression in the mutant yeast H1246,
25 which is devoid of neutral lipid synthesis. Expression of *EgDGAT1_3* or *EgDGAT2_2*
26 could restore TAG synthesis, confirming that both proteins are true DGAT. In contrast,
27 expression of *EgWS/DGAT_1* resulted in the synthesis of fatty acid isoamyl esters (FAIEs)

28 with saturated long-chain and very-long-chain fatty acids. In the presence of exogenously
29 supplied fatty alcohols, EgWS/DGAT_1 was able to produce wax esters, indicating that
30 *EgWS/DGAT_1* codes for an acyltransferase with wax ester synthase but no DGAT
31 activity. Finally, the complete wax ester biosynthetic pathway was reconstituted in yeast
32 by coexpressing *EgWS/DGAT_1* with a fatty acyl reductase from *Tetrahymena*
33 *thermophila*. Altogether, our results characterized two novel DGATs from oil palm as
34 well as a putative wax ester synthase that preferentially using medium chain fatty
35 alcohols and saturated very-long chain fatty acids as substrates.

36

37 Keywords:

38 *Elaeis guineensis*

39 DGAT

40 wax ester synthase

41 *Saccharomyces cerevisiae*

42

43 **1. Introduction**

44 Oil in the form of triacylglycerols (TAGs), which are composed of three fatty acids
45 esterified to glycerol, is a very common energy-dense storage strategy found in all
46 eukaryotes, and accumulated in many plant seeds and fruits [1,2]. The mesocarp of oil
47 palm (*Elaeis guineensis*) fruits can accumulate up to 88% (dry weight) oil [3], with TAGs
48 primarily made of 16:0 and 18:1 fatty acids. In addition, the kernel of palm fruits
49 produces about 50% (dry weight) oil, with TAGs mostly composed of saturated medium
50 chain fatty acid (MCFAs), especially lauric acid (12:0) [4]. Oil palm therefore represents
51 one of the most productive oil crops in the plant kingdom. The percentage of TAG in oil
52 palm is over 95%, the rest being composed of glycerolipids and phospholipids as well as
53 sterols, triterpene alcohols, tocopherol, carotenoids and chlorophylls [5].

54 All modern oilseed crops, like rapeseed (*Brassica napus*) or sunflower (*Helianthus*
55 *annuus*), accumulate storage lipids in the form of TAG in their seeds. Oilseed TAGs are
56 hydrolyzed upon germination to produce the energy necessary to fuel post-germinative
57 seedling growth before photosynthesis becomes active [1,6]. In fruits, TAGs are believed
58 to primarily serve as an effective strategy for attracting animal feeders in order to
59 increase seed dispersal and plant habitats. Wax esters (WEs, esters of primary fatty
60 alcohols and fatty acids) represent another kind of storage lipids synthesized in a wide
61 range of microorganisms [7], such as the bacteria *Acinetobacter calcoaceticus* ADP1 [8]
62 or the unicellular phytoflagellate protist *Euglena gracilis* [9,10]. Jojoba (*Simmondsia*
63 *chinensis*), a desert shrub from the south-western areas of North America, represents the

64 only plant known to accumulate WEs rather than TAGs as seed storage lipids [11,12]. In
65 contrast, the presence of WEs in the cuticular layer covering the aerial parts of land plants
66 has been reported in many species [13]. The cuticle plays a critical role in protecting
67 plants from desiccation, radiation, pathogens and insects herbivores [14].

68 The biosynthesis of TAGs has been widely studied in oilseeds, especially in
69 *Arabidopsis thaliana*, and was shown to rely on different and overlapping pathways. The
70 well-known and simplest way to produce TAGs is known as the Kennedy pathway,
71 where three fatty acids esterified to Coenzyme A are sequentially esterified to each
72 position of the glycerol backbone. First, the *sn*-1 and *sn*-2 positions of
73 glycerol-3-phosphate are respectively acylated by the acyl-CoA:glycerol-3-phosphate
74 acyltransferase (GPAT) and the acyl-CoA:lyso-phosphatidic acid acyltransferase (LPAT),
75 producing lyso-phosphatidic acid and subsequently phosphatidic acid (PA). The
76 phosphate of PA is then hydrolyzed by a PA phosphatase (PAP), yielding diacylglycerol
77 (DAG), which is further acylated by the acyl-CoA:diacylglycerol acyltransferase
78 (DGAT), leading to TAG. As GPAT, LPAT and PAP are also involved in phospholipid
79 biosynthesis, DGAT is the unique enzyme of the Kennedy pathway that is specific for
80 TAG biosynthesis. Similarly, PA and DAG are important intermediates for the
81 production of both TAGs and phospholipids. Three different types of DGAT enzymes
82 have been described in plants, and even though they similarly acylate DAG, they are
83 structurally unrelated [15,16]. DGAT1 and DGAT2 are membrane-associated enzymes
84 usually found in the endoplasmic reticulum but with different topologies. According to

85 hydrophathy analyses, DGAT1 enzymes are highly hydrophobic with 6 to 9
86 transmembrane domains spread over the entire protein while DGAT2 polypeptides
87 usually only contain at their N-terminus a single hydrophobic domain that could serve as
88 membrane anchor. The more recently discovered DGAT3 are in contrast soluble enzymes
89 involved in a cytosolic pathway for TAG biosynthesis that may be important to regulate
90 fluxes between the cytosolic acyl-CoA pool and TAGs at specific developmental stages
91 [16,17].

92 The WE biosynthetic pathway has first been elucidated in jojoba [11,18]. It relies on
93 two enzymatic activities catalyzed by a fatty acyl-CoA reductase (FAR) and an
94 acyl-CoA:fatty alcohol acyltransferase or WE synthase (WS). FAR is responsible for the
95 reduction of acyl-CoAs to fatty alcohols [18], while WS transfers the acyl group of
96 another acyl-CoA onto the former fatty alcohol, yielding WEs [11]. Following this
97 pioneering work in jojoba, the WE biosynthetic pathway has been characterized in many
98 other species like *Arabidopsis* or *Euglena* [9,10,19,20]. In *Arabidopsis*, both FAR and
99 WS enzymes are associated with membranes, strongly suggesting that WEs are
100 synthesized within the endoplasmic reticulum (ER) [21,22]. Interestingly, another type of
101 WS, which exhibits both WS and DGAT activity, has been identified in the bacteria
102 *Acinetobacter calcoaceticus* ADP1 [8]. This WS/DGAT bifunctional enzyme is unrelated
103 to the other known WS or DGAT proteins, and is essential for the production of both
104 TAGs and WEs in *A. calcoaceticus* [8]. The genome of *Arabidopsis* contains 11
105 sequences that are homologous to the *A. Calcoaceticus* WS/DGAT, and only one of them,

106 *AtWSD1*, has so far been characterized. *AtWSD1* was shown to play a key role in WE
107 synthesis, using LC and very-long-chain (VLC) primary alcohols together with
108 palmitoyl-CoA to produce the WEs found in *Arabidopsis* stem waxes [23].

109 A recent *in silico* survey of *Elaeis guineensis* DGAT-related genes identified 3
110 DGAT1s, 2 DGAT2s, 2 DGAT3s and 2 bifunctional WS/DGATs in the oil palm genome
111 [16]. Since some of these genes were annotated differently in several previous studies
112 [3,24–26], the most recent and complete nomenclature of Rosli et al. [16] will be
113 consistently used for clarity in the present report (see Table 1 for correspondences).
114 Transcriptomic analyses indicated that the expression of *EgDGAT1_1*, *EgDGAT3_1* and
115 *EgWS/DGAT_1* was highly up regulated in the mesocarp when oil accumulated, while
116 *EgDGAT1_2* was mostly expressed in the endosperm of the kernel. Up to now, only two
117 of these 9 putative DGAT-related genes have been studied and the encoded activity
118 characterized. By complementing a *Yarrowia lipolytica* mutant strain devoid of neutral
119 lipids, Aymé and coworkers [25] characterized *EgDGAT1_2* (annotated *EgDGAT1-1* in
120 their study) as an endoplasmic reticulum DGAT, and showed that it preferentially used
121 medium-chain fatty acid as substrates, in agreement with its high expression in palm
122 kernel. More recently, the characterization of *EgDGAT2_1* (annotated *EgDGAT2* in their
123 study) was achieved by Jin and coworkers [26] through the complementation of the
124 *Saccharomyces cerevisiae* TAG mutant H1246. Interestingly, seed-specific
125 overexpression of *DGAT2_1* in transgenic *Arabidopsis* did not increase the seed TAG
126 content but enhanced the levels of polyunsaturated fatty acids [26].

127 In a previous transcriptomic study [3], we had found that 3 putative DGAT genes
128 (*EgDGAT1_3*, *EgDGA2-2* and *EgWS/DGAT_1*) were highly expressed during fruit
129 maturation in oil palm (*Elaeis guineensis*) mesocarp but not in date palm (*Phoenix*
130 *dactylifera*), a closely related species that accumulates almost exclusive sugars. In the
131 present paper, we isolated the full-length cDNA of each gene and characterized the
132 function of the encoded proteins by heterologous expression in yeast. Our data confirmed
133 that *EgDGAT1_3* and *EgDGAT2_2* are active DGAT, and suggested that
134 *EgWS/DGAT_1* only displayed WS activity, with a preference towards saturated LC and
135 VLC acyl-CoAs and medium chain fatty alcohols (14OH and 16OH).

136

137 **2. Materials and methods**

138 *2.1. Materials*

139 Oil palm fruits were collected at CEREPAH La Dibamba (Cameroon) and immediately
140 frozen in liquid nitrogen upon harvest. Mesocarp was quickly fully dissected from
141 individual frozen fruit, ground in liquid nitrogen to a fine powder, and stored at -80°C
142 until used. All chemical reagents were from Sigma-Aldrich unless indicated otherwise.

143 *2.2. Cloning of oil palm DGATs*

144 Total RNA from oil palm mesocarp (22 and 23 weeks after pollination) was extracted
145 using the method described by Chirgwin and colleagues [27]. First-strain cDNA synthesis
146 was carried out using SuperScript™ II reverse transcriptase (Invitrogen) according to
147 manufacturer's instruction. The three oil palm *EgDGAT* open reading frames (ORF) were

148 then amplified using the specific primers listed in Table S1 and the Q5 high-fidelity DNA
149 polymerase (New England Biolabs) following manufacturer's protocol. PCR products
150 were gel-purified, digested with the corresponding restriction enzymes (see Table S1),
151 and cloned into the multiple cloning site 2 of pESC-URA by ligation. For co-expression
152 experiments, *TiFAR* from *Tetrahymena thermophila* [28] was cloned using the same
153 strategy (primers listed in Table S1) in the multiple cloning site 1 of pESC-URA and
154 *EgWS/DGAT_1*-pESC-URA. All generated constructs were verified by sequencing
155 (Beckman Coulter Genomics, GENEWIZ). Amino acid sequences alignments were
156 obtained using the CLUSTAL X program (gap opening 10, gap extension 0.05) and the
157 unrooted phylogenetic relationship constructed by the neighbor-joining method using Tree
158 View.

159 2.3. Yeast transformation and growth conditions

160 The yeast (*Saccharomyces cerevisiae*) strain H1246 (*MAT α ADE2-1 can1-100 ura3-1*
161 *are1- Δ ::HIS3 are2- Δ ::LEU2, dgal- Δ ::KanMX4 lro1- Δ ::TRP1*) [29], which is devoid of
162 neutral lipid synthesis, was used in this study. Three constructs *EgDGAT1_3*-pESC-URA,
163 *EgDGAT2_2*-pESC-URA and *EgWS/DGAT_1*-pESC-URA were separately transformed
164 into the yeast strain H1246 using the polyethylene glycol/lithium acetate method. The
165 empty vector pESC-URA was transformed into the H1246 and wild-type SCY62 [29]
166 yeast strains for comparison while the published *EgDGAT2_1* [26] was transformed into
167 the yeast strain H1246 as positive control. In coexpression studies using
168 *TiFAR-EgWS/DGAT_1*-pESC-URA in the yeast strain H1246, *TiFAR*-pESC-URA was

169 used as negative control. All transformants were selected on synthetic complete medium
170 lacking uracil (SC-U) supplemented with 2 % (w/v) glucose.

171 Three independent positive clones of each transformation were used for expression
172 studies. Pre-cultures were made in 2 ml SC-U medium containing 2 % (w/v) raffinose
173 and were grown overnight at 30 °C. For inducing the expression of the transgene(s),
174 cultures were collected, washed once with sterilized water and inoculated into 20 ml
175 SC-U induction medium with 2% galactose in 250mL flasks with a starting optical
176 density (OD) of 0.4. Flasks were incubated for 24 hours at 30 °C under continuous
177 agitation (250 rpm) and the yeast culture OD was measured before harvest. Usually, two
178 ml of culture were directly pelleted for total fatty acid quantification while the rest of the
179 culture (usually about 200 OD units) was collected by centrifugation in 8 mL glass tubes
180 with Teflon caps. Pellets were washed once with distilled water and immediately used for
181 lipid extraction, or frozen at -20 °C until used. When fatty alcohols were exogenously
182 supplied, 500 µM of fatty alcohol was added to the growing cells 24 hours after induction
183 and yeast cells were harvested 48 hours later.

184 *2.4. Lipid extraction and separation*

185 For total fatty acyl-chain profiling, 40 µg of heptadecanoic acid (C17:0) was added to
186 the 2 ml yeast pellets, and direct transmethylation with 2 ml of methanol containing 2.7%
187 (v/v) H₂SO₄ was performed for 2 hours at 80°C. After cooling down, 3 ml of hexane and
188 2 ml of NaCl (2.5%, w/v) were added for extracting fatty acid methyl esters (FAMEs) and
189 eventually fatty alcohols. Following phase separation by centrifugation, the hexane phase

190 was transferred in to a fresh tube, evaporated under nitrogen and the sample was
191 resuspended in 200 μ l of hexane for GC-MS analysis. For yeast cultures containing fatty
192 alcohols, the hexane extract was first washed with 2 ml of NaCl (0.09%, w/v) in 100 mM
193 Tris (pH 8.0). Following phase separation by centrifugation, the hexane phase was
194 collected in fresh tubes and evaporated under a gentle stream of nitrogen. The residue
195 was then dissolved in 150 μ l of *N,O*-bis(trimethylsilyl) trifluoroacetamide with
196 trimethylchlorosilane (BSTFA), and free hydroxyl groups were derivatized at 110°C for
197 20 min. The surplus BSTFA was evaporated (under nitrogen) and the sample was
198 dissolved in 200 μ l of hexane for GC-MS analysis.

199 For preparing the lipids extracts, 2 ml of chloroform/methanol (1:1; v/v) and glass
200 beads (180 μ m; about 3 ml, up to meniscus) were added to the yeast pellets (about 300 μ l)
201 and tubes were vigorously shaken 4 times for 30s. Following centrifugation, the organic
202 phase was collected, and beads were rinsed thoroughly once with 2ml of
203 chloroform/methanol (2:1; v/v) and once with 2 ml of chloroform. These two washes
204 were combined with the initial extract, and 2 ml of NaCl 2.5 % (w/v) was then added to
205 allow phase separation. Following centrifugation, the organic phase was collected while
206 the aqueous phase was re-extracted with 2 ml chloroform. This organic phase was
207 combined with the previous one and dried down. Finally, lipid extracts were dissolved in
208 250 μ l chloroform. For WE quantification, 8 μ g heptadecanyl heptadecanoate (WE
209 17OH-17:0; NU-CHEK-PREP) was added as internal standard before starting the lipid
210 extraction. Neutral lipids were isolated using solid phase extraction as previously

211 described [28], dried under nitrogen and resuspended in 200 μ l chloroform for GC-MS
212 analysis.

213 *2.5. TLC and GC-MS analyses*

214 For thin layer chromatography (TLC) analysis, total lipids equivalent to 5 or 10 OD
215 units and lipid standards were loaded on HPTLC Silica Gel 60 plates (Merck) pre-washed
216 with chloroform/methanol (1:1; v/v). PC, DAG, and TAG were separated by thin-layer
217 chromatography (TLC) and further quantified by GC-MS. Appropriate amounts of
218 internal standards PC 19:0 (2 μ g), DAG 15:0 (0.5 μ g), TAG 17:0 (5 μ g) were loaded
219 together with lipids samples (lipid extract from 10 OD units per lane for DAG and TAG,
220 4 OD units for PC) on TLC silica Gel 60 plates (Merck). The solvent system methyl
221 acetate/propanol/chloroform/methanol/KCl 0.25% (25:25:25:10:9, v/v/v/v/v) was used
222 for separating polar lipids, and hexane/diethyl ether/acetic acid (80:20:1, v/v/v) for
223 neutral lipids. Hexane/diethyl ether/acetic acid (90:7.5:1, v/v/v) was used as solvent
224 system for isolating isoamyl esters. Isoamyl esters were extracted from silica using
225 hexane and diethyl ether (1:1; v/v). Oleic acid, monoolein, diolein, triolein and WE
226 purchased from NU-CHEK-PREP were used as lipid standards.

227 GC-MS analyses were performed using an Agilent 6850 gas chromatograph equipped
228 with an HP-5MS column (30 m x 0.25 mm x 0.25 mm) and an Agilent 5975 mass
229 spectrometric detector (70 eV, mass-to-charge ratio 50-750), with helium (1.5 ml.min⁻¹)
230 as carrier gas. For the resolution of FAMES, the initial temperature of 50°C was held for
231 1 min, increased at 25°C min⁻¹ to 150°C, held for 2 min at 150°C, then increased at 10°C

232 min⁻¹ to 320°C, and held for 6 min at 320°C. Separation of the WEs was achieved with
233 the same program except that the final temperature (320°C) was held for 21 minutes.

234

235 **3. Results**

236 *3.1. Three putative DGATs from oil palm are steadily expressed in mesocarp during fruit* 237 *ripening*

238 Using the transcriptomic data from developing oil palm mesocarp [3], we found that
239 the expression of *EgDGAT1_3*, *EgDGA2_2* and *EgWS/DGAT_1* was correlated with oil
240 accumulation, which occurs from week 15 to week 23 after pollination. For each gene,
241 the expression level at any fruit development stage was at least similar, but often higher
242 than in leaf or kernel (Fig. 1). Globally, the expression levels in fruit mesocarp were
243 already high 15 weeks after pollination (WAP), remained nearly as high or steadily
244 increased during the next 4 weeks and became highest during the fruit ripening phase (21
245 to 23 WAP). The expression level of *EgDGAT2_2* was the lowest among the three genes.
246 It gradually increased during the first six weeks of oil accumulation (15 to 21 WAP),
247 before a sharp decrease at maturity (23 WAP). The expression of *EgDGAT1_3* was as
248 high in the first stages of mesocarp development (15 to 19 WAP) as in kernel but nearly
249 doubled during fruit ripening (21 to 23 WAP). Similarly, the expression of
250 *EgWS/DGAT_1* remained high between 15 to 21 WAP, before it more than doubled 23
251 WAP. However, in contrast to *EgDGAT1_3*, transcripts of *EgWS/DGAT_1* were barely
252 detected in the kernel (Fig. 1).

253 Using total RNA from the mesocarp of ripening fruits (22 and 23WAP) and
254 gene-specific primers (Table S1), the ORFs of *EgDGAT1_3*, *EgDGA2_2* and
255 *EgWS/DGAT_1* were isolated. *EgGATI_3* ORF (1710 bp) encoded for a protein of 569
256 amino acids which was 100 % identical to the Rosli et al. [16] sequence, but slightly
257 longer as it contained 29 amino acids more at the N-terminus, as in the GenBank
258 XP_010925472.1 sequence (Fig. S1A). *EgDGAT2_2* ORF was 978 bp long and coded for
259 a protein of 325 amino acids, which was 95 % identical to the GenBank sequence
260 XP_010919928.2 but only 70% identical to the Rosli et al. sequence [16]. As shown in
261 Fig. S1B, this *EgDGAT2_2* isoform did not contain the first 51 amino acids of the Rosli
262 sequence, and also differed at its C-terminal extremity, which was instead highly related
263 to the GenBank sequence XP_010919928. Finally, *EgWS/DGAT_1* ORF (1548 bp)
264 encoded for a protein of 515 amino acids which was 98 % identical to the Rosli et al. [16]
265 and GenBank XP_010924517.1 sequences (Fig. S1C). This *EgWS/DGAT_1* isoform
266 displayed three amino acid mismatches as well as a 4-amino acid shorter glutamic acid
267 repeat at its N-terminal extremity.

268 As shown in Fig. S1A, *EgDGAT1_3* contains the seven motifs corresponding to the
269 conserved regions of DGAT1 previously described by Cao [30] as well as a N-terminal
270 arginine motif (RXR) which confers ER localization according to Boulaflous et al., [31].
271 Similarly, both *EgDGAT2.2* (Fig. S1B) and *EgDGAT2_1* (Fig. S1D) displayed all six
272 motifs corresponding to the conserved regions of DGAT2 previously described by Cao
273 [30]. In addition, all three oil palm DGAT harbored the C-terminal pentapeptide

274 ER-retrieval motif described by Shockey et al. [32], strongly supporting their localization
275 in endomembranes. Finally, EgWS/DGAT_1 contained the wax ester synthase-like /
276 acyl-CoA acyltransferase domain (pfam03007) as well as is underlined as well as the
277 essential motif for acyl-transferase catalytic activity (HHXXXDG; Fig. S1C).

278 A phylogenetic analysis of the 9 oil palm DGATs with various DGATs and WS from
279 Arabidopsis and other species confirm their annotation and showed that if the 3 types of
280 DGAT form well-defined clades, WS are not grouped altogether but found in several
281 subgroups (Fig. 2). For example, EgDGAT2_2 and type-2 DGATs are more related to
282 mammalian wax synthases (MmWS) [33] than to type-1 or -3 DGATs. EgWS/DGAT_1
283 clusters with a WS from petunia (*Petunia hybrida*) [34] involved in the production of low
284 molecular wax-esters in petals as well as with *Arabidopsis* AtWSD1 (Fig. 2) [23]. It is
285 also closely related to the bifunctional enzyme AcWS/DGAT from the bacterium
286 *Acinetobacter calcoaceticus* ADP1, which is responsible for the production of both TAGs
287 and WEs under so-called storage conditions [8,35]. It is in contrast not related to the WS
288 from jojoba (*Simmondsia chinensis*) [11] and *Euglena* (*Euglena gracilis*) [9] which group
289 with the membrane bound O-acyl transferase from *Arabidopsis thaliana* (AtMBOAT) [36]
290 rather than with *Arabidopsis* AtWSD1.

291 3.2. Heterologous expression of EgDGAT1_3 and EgDGAT2-2 in the mutant yeast

292 H1246 restores TAG biosynthesis

293 In order to figure out whether EgDGAT1_3, EgDGA2_2 and EgWS/DGAT_1 act as
294 DGAT, their heterologous expression in the quadruple mutant yeast H1246, which is

295 devoid of neutral lipids [29], was first performed. Yeast transformed with an empty
296 vector (pESC-URA) or with a construct containing *EgDGAT2_1* [26] were respectively
297 used as negative and positive controls. After 2 days of expression, lipids were extracted
298 and neutral lipids were separated by TLC using hexane/diethyl ether/acetic acid (80:20:2;
299 v/v/v) as mobile phase. As shown in Fig. 3, the wild-type yeast strain SCY62 produced
300 both TAG and sterol esters (SE), while the quadruple mutant yeast H1246 transformed
301 with the empty vector (pESC-URA) produced no storage lipids (the slight band migrating
302 at the top corresponding to squalene). Similar to the positive control *EgDGAT2_1*,
303 expression of either *EgDGAT1_3* or *EgDGAT2_2* restored TAG, but not SE synthesis,
304 confirming that both proteins display DGAT activity. In contrast, expressing
305 *EgWS/DGAT_1* in the mutant yeast strain H1246 produced neither TAGs nor SE (Fig. 3).

306 The lipid content and composition of the H1246 complemented lines (*i.e.* those
307 expressing *EgDGAT2_1*, *EgDGAT1_3* and *EgDGA2_2*) and controls were then analyzed
308 in more details (Figures 4A and S2). The quadruple mutant yeast H1246, which is devoid
309 of TAG, contained 8 % less total fatty acids, but nearly 50 % more PC than the wild type
310 strain SCY62 (Fig. 4A). At the total fatty acid level, H1246 contained nearly 50 % less
311 16:0 and 25% more 18:1) than SCY62 (Fig. S2A). Upon expression of *EgDGA2_2*,
312 *EgDGAT2_1* and *EgDGAT1_3*, the total fatty acid content of H1246 was respectively
313 increased by 1.1, 1.25 and 1.4 folds (Fig. 4A). Whatever the DGAT expressed, the TAG
314 content reached levels close to those of the wild-type strain SCY62, while the PC loads
315 remained close to that of the H1246 control strain. Similarly, the lower 16:0 level

316 observed in H1246 was increased upon expression of DGAT1_3 and 2_2 but remained
317 lower than in SCY62 (Fig. S2A). Noteworthy, among the three DGATs, the expression of
318 *EgDGAT1_3* resulted in the highest increase in TAG loads (Fig. 4A). Analysis of the
319 TAG fatty acid composition (Fig. 4B) indicated that the expression of *EgDGAT1_3*
320 yielded a composition that was very close to that of the wild type strain SCY62. In
321 contrast, the expression of *EgDGAT2_1* and *EgDGAT2_2* resulted in higher levels of
322 monounsaturated fatty acids, especially 16:1 in the case of that of *EgDGA2_1*. In contrast,
323 whatever the DGAT expressed, the 16:1 levels were not significantly affected in either
324 PC or DAG (Fig. S2B and C).

325 *3.3. Heterologous expression of EgWS/DGAT_1 in the yeast mutant H1246 yields fatty* 326 *acid isoamyl esters with saturated LC and VLC fatty acids*

327 As shown by the back arrow in Fig. 3, the expression of *EgWS/DGAT_1* resulted in the
328 appearance of a new compound migrating just below squalene in neutral lipid separation.
329 Using a more polar solvent mixture (hexane/diethyl ether/acetic acid 90:7.5:0.1; v/v/v),
330 this compound was separated from squalene, and shown to migrate between SE and ethyl
331 esters (EE; Fig. 5A). Using a larger volume of culture and the same TLC separation
332 system, this new compound was purified, extracted from silica using hexane and diethyl
333 ether (1:1), and finally analyzed by GC-MS. MS fragmentation indicated that this new
334 compound corresponded to fatty acid isoamyl esters (FAIEs) with saturated fatty acids
335 ranging from 18:0 to 26:0 (Fig. 5; Fig. S3). Among the different molecular species, 20:0-,

336 22:0- and 26:0-IE were the most abundant, representing respectively 24.0, 30.8, and 26.7 %
337 of the total (Fig. 5B).

338 *3.4. Expression of EgWS/DGAT_1 in the presence of exogenously supplied fatty alcohols*
339 *results in WE synthesis*

340 As *Saccharomyces cerevisiae* has no capacity to produce fatty alcohols, an equimolar
341 mixture of fatty alcohols (14OH, 16OH and 18OH) was supplied to transgenic yeast
342 expressing *EgWS/DGAT_1* in order to determine if *EgWS/DGAT_1* displayed WS
343 activity. For these feeding experiments, a culture of the H1246 yeast strain transformed
344 with the empty vector was systematically grown in parallel and used for comparison
345 (thereafter referred to as control). Yeast expressing *EgWS/DGAT_1* showed a WE
346 productivity that was nearly 10 times that of the control (Fig. 6A). Looking at the
347 molecular species, WEs with 30 to 32 carbon atoms were most abundant, representing 70
348 to 80 % of the total in both control and *EgWS/DGAT_1* expressing strains (Fig. 6B). In
349 addition, for each WE chain length (composed of the same fatty acid and fatty alcohol, *i.e.*
350 16:1/16:0-14OH, 18:1/18:0-14OH, 16:1/16:0-16OH), there was about three times more
351 WEs with the unsaturated fatty acid than with the corresponding saturated one (compare
352 16:1-14OH, 18:1-14OH, 16:1-16OH with 16:0-14OH, 18:0-14OH and 16:0-16OH,
353 respectively). Interestingly, WEs containing VLCFAs (*i.e.* 20:0-14OH, 22:0-14OH and
354 22:0-14OH) were overrepresented upon *EgWS/DGAT_1* expression, suggesting that
355 *EgWS/DGAT_1* might prefer these fatty acids. Concerning the fatty alcohol part, 14OH
356 and 16OH were found in about 60 and 30 % of the total WE, respectively, whereas WE

357 containing 18OH represented less than 10 % of the total, indicating the preference of
358 *EgWS/DGAT_1* for medium chain fatty alcohols (Fig. 6B Insert).

359 3.5. Coexpression of *EgWS/DGAT_1* with *TtFAR* in the mutant yeast strain H1246

360 Finally, the complete WE biosynthetic pathway was reconstituted in the mutant yeast
361 H1246 by coexpressing *EgWS/DGAT_1* with the fatty acyl reductase *TtFAR* from
362 *Tetrahymena thermophila* which was shown to produce 16OH and 18OH when expressed
363 in yeast [28]. TLC analysis of lipids indicated that the heterologous expression of
364 *EgWS/DGAT_1* together with *TtFAR* in H1246 produced much more WEs than H1246
365 transformed with *TtFAR* alone (Fig. 7A). Using 17:0-17OH WE as internal standard, the
366 amount of WEs produced by coexpression in H1246 was about 7 times higher than that
367 obtained upon *TtFAR* expression alone (Fig. 7B). Concerning the WE composition, the
368 proportion of WEs containing saturated fatty acids (14:0, 16:0 and 18:0) were
369 systematically higher in yeast coexpressing *EgWS/DGAT_1* with *TtFAR* than in culture
370 expressing *TtFAR* alone (Fig. 7C). In addition, WEs containing VLCFAs (20:0 to 26:0)
371 were almost exclusively detected in the yeast coexpression *EgWS/DGAT_1* together with
372 *TtFAR* (Fig. 7C insert).

373 In order to get more insights on the substrate preference of *EgWS/DGAT_1*, we finally
374 compared the fatty acid and fatty alcohol profiles of the WEs produced by coexpressing
375 *TtFAR* and *EgWS/DGAT_1* yeast to those of the corresponding total lipid extracts (Fig. 8).
376 These later were obtained by directly transmethylating and silylating the lipid extracts as
377 indicated in the Material and Method section. Using yeast expressing *TtFAR* alone or in

378 combination with *EgWS/DGAT_1*, very similar total fatty acid and fatty alcohol profiles
379 were obtained (data not shown). As shown in Fig. 8A, the proportions of most of the
380 saturated fatty acids (14:0, 16:0, 18:0, 20:0 and 22:0) were higher in WEs than in total
381 lipids. In contrast, the level of 18:1 was 2.3 times higher in the total lipids than in WEs,
382 suggesting that 18:1 was severely discriminated by *EgWS/DGAT_1* (Fig. 8A).
383 Calculation of 16:1 to 16:0 and 18:1 to 18:0 ratios clearly illustrated that saturated fatty
384 acids are preferentially incorporated by *EgWS/DGAT_1* into WEs (Fig. 8B). Concerning
385 VLCFAs, 20:0 appeared as the favorite substrate for *EgWS/DGAT_1* (Fig. 8A insert). In
386 agreement with the feeding experiments previously described, analysis of the fatty
387 alcohol profiles indicated that *EgWS/DGAT_1* preferred 16OH over 18OH (Fig. 8C).

388

389

390 **4. Discussion**

391 In the present study, we characterized 3 putative DGATs from oil palm by
392 heterologous expression in the yeast mutant H1246. Whereas expressing either
393 *EgDGAT1_3* or *EgDGAT2_2* restored TAG biosynthesis to wild-type levels, the
394 expression of *EgWS/DGAT_1* failed to produce any TAG but resulted in the appearance
395 of FAIEs. Further experiments in the present of exogenously supplied or endogenously
396 produced fatty alcohols indicated that *EgWS/DGAT_1* can produce WE with preference
397 for 14OH and 16OH fatty alcohols, and saturated LC and VLC fatty acids.

398 The expression of *EgDGAT1_3*, *EgDGAT2_2* and *EgWS/DGAT_1* in fruit mesocarp
399 was shown to be correlated with oil accumulation and especially high during fruit
400 ripening (21 to 23 WAP; Fig. 1). A similar expression profile has been reported for
401 *EgDGAT2_1*, another DGAT gene from oil palm mesocarp recently characterized by Jin
402 et al. [26]. Nevertheless, these increases in expression levels were very moderate in
403 comparison to those reported for genes involved in fatty acid biosynthesis [3]. Similarly,
404 Rosli et al. [16] reported rather moderate changes in expression levels in fruit mesocarp
405 for all nine DGATs, with only *EgDGAT1_1* and *EgWS/DGAT_1* being slightly
406 up-regulated at the later stages of fruit development. In contrast, the same authors
407 reported significant changes in the levels of expression in the endosperm tissue (*i.e.* the
408 kernel), especially for *EgDGAT1_1*, *EgDGAT3_1* and *EgWS/DGAT_1* which expression
409 was sharply increased 12WAP. In agreement with our data, *EgDGAT1_3* and
410 *EgDGAT2_2* were reported to be highly and not very expressed in both tissues,
411 respectively. Interestingly, the expression of *EgDGAT1_2*, which is supposed to be
412 responsible for lauric acid accumulation in oil palm kernel [25], was high at all
413 endosperm developmental stages, but not increased during fruit maturation.

414 In the present study, we used the well-established complementation assay in the yeast
415 mutant H1246 [29] to confirm heterogously the DGAT activity of the enzymes encoded
416 by our candidate genes. The expression of *EgDGAT1_3* and *EgDGAT2_2* in the neutral
417 lipid mutant strain allowed for the production of TAG, but not SE (Fig. 3) indicating that
418 both enzymes display DGAT activity. While the expression of *EgDGAT2_2* restored the

419 total fatty acid and TAG levels to those of the wild type strain SCY62, expressing
420 *EgDGAT1_3* increased both above wild type levels (Fig. 4). Since the total fatty acid and
421 TAG compositions were very similar in both cases, *EgDGAT1_3* and *EgDGAT2_2* did
422 not display any substrate preference, accepting similarly saturated and monounsaturated
423 C16 and C18 fatty acids, at least in our assay conditions. Further studies in the presence
424 of exogenously supplied fatty acids or *in vitro* assays should be conducted to characterize
425 in more details the substrate specificity of these enzymes.

426 Unlike *EgDGAT1_3* and *EgDGAT2_2*, the expression of *EgWS/DGAT_1* failed to
427 produce any TAG (Fig. 3), indicating that *EgWS/DGAT_1* do not display DGAT activity,
428 at least in the conditions we used. According to our phylogenetic analysis (Fig. 2),
429 *EgWS/DGAT_1* from oil palm mesocarp is predicted to be related to the bifunctional
430 WS/DGAT from the bacterium *Acinetobacter calcoaceticus* ADP1 [7,8]. This protein
431 showed both DGAT and WS activities *in vivo*, producing TAGs and WEs in bacteria and
432 *S. cerevisiae* [8,36]. Arabidopsis contains 11 genes annotated as bifunctional WS/DGAT,
433 including *AtWSD1*. Interestingly, *in vitro* assays showed that the WS activity of
434 *AtWSD1* was approximately 10-fold higher than its DGAT activity, whereas its
435 expression in the yeast mutant strain H1246 produced WEs, but no TAGs [23]. In
436 agreement with *AtWSD1* primarily functioning as a WS *in planta*, Arabidopsis *wsd1*
437 mutants displayed reduced WE levels in the stem waxes [20]. The lack of *in vivo* DGAT
438 activity for WS/DGAT-like proteins expressed in yeast has already been reported in the
439 case of PhWS from *Petunia hybrida* [34], *EgWS2* and 5 from *Euglena* [10] as well as for

440 TrWSD4 and 5 from *Thraustochytrium* [37]. This might suggest that in contrast to
441 bacterial enzymes, bifunctional WS/DGAT proteins from plant and algae may not display
442 any DGAT activity when expressed in *S. cerevisiae*. If no TAG was produced, the
443 expression of *EgWS/DGAT_1* in the mutant yeast H1246 resulted in the production of
444 FAIEs with saturated LC and VLC fatty acids (Fig. 5). A similar result has been obtained
445 with WSs from the WS/DGAT family, including *AcWS/DGAT* from *A. calcoaceticus* and
446 *PhWS* from *Petunia hybrida* [34,38]. Interestingly, both *EgWS/DGAT_1* and *PhWS*
447 produced FAIEs with preferentially saturated LC and VLC acyl-groups when expressed
448 in the mutant yeast H1246, which is consistent with the results we obtained with
449 *EgWS/DGAT_1* (Fig. 5).

450 When *EgWS/DGAT_1* was expressed in yeast in the presence of exogenously supplied
451 fatty alcohols, WEs were systematically detected, indicating that *EgWS/DGAT_1*
452 displayed WS activity. In these conditions, *EgWS/DGAT_1* showed a slight preference
453 for medium chain fatty alcohols (14:0 and 16:0), unsaturated fatty acid (16:1 and 18:1) as
454 well as VLC fatty acids (Fig. 6). Nevertheless, these *in vivo* experiments showed that
455 *EgWS/DGAT_1* used as substrates a wide range of saturated and unsaturated acyl-CoAs
456 from C14 to C26, like the jojoba WS [11]. In similar assays, *AtWSD1* used palmitic acid
457 and 18:0, 24:0, or 28:0 fatty alcohols to produce WEs with 34 to 44 carbon atoms, in line
458 with the VLC-WEs found in *Arabidopsis* stem waxes [23]. When the WE biosynthetic
459 pathway was reconstituted in yeast using coexpression of *TtFAR* together with
460 *EgWS/DGAT_1*, the most abundant WE produced was 18:0-16OH. In addition, WEs

461 containing saturated VLC fatty acids were only detected in the presence of
462 EgWS/DGAT_1, indicating that the endogenous yeast activity generating WEs in these
463 conditions did not accept VLC fatty acids as substrates (Fig. 7). Conversely, these results
464 confirmed the preference of EgWS/DGAT_1 for saturated VLC acyl-CoAs (Fig. 7C).
465 Furthermore, when comparing the fatty acyl compositions of the WEs and total lipids
466 (Fig. 8), EgWS/DGAT_1 appeared to preferentially use saturated fatty acids, especially
467 16:0, 18:0 and 20:0 (Fig. 8A and B), and 16OH rather than 18OH (Fig. 8C).

468 Oil palm (*Elaeis guineensis*) is considered as the most productive plant oil crop with
469 over 50% and 85 % oil per dry weight in the endosperm and mesocarp, respectively [24].
470 The percentage of TAG in oil palm mesocarp is over 95% suggesting that the last and
471 specific step in TAG biosynthesis, *i.e.* the acylation of the third position of DAG, must be
472 highly efficient in this species. Unexpectedly, a comparative transcriptomic analysis of
473 mesocarp tissues from oil palm and date palm (*Phoenix dactylifera*), which does not store
474 oil, reported that if the plastidial pyruvate supply and fatty acids biosynthesis were
475 strongly up-regulated in oil palm, most enzymes involved in triacylglycerol assembly
476 were expressed at similar levels in both species [3]. Recently, an *in silico* analysis of
477 diacylglycerol acyltransferase genes (DGAT1-3 and WS/DGAT) using available
478 databases identified 7 DGATs and 2 WS/DGATs in oil palm [16], suggesting that the
479 presence of numerous isoforms and paralog genes, rather than a strong up-regulation,
480 could be responsible for the very high TAG levels present in oil palm. Up to now, only
481 the catalytic activity of EgDGAT1_2 and EgDGAT2_1 had been confirmed.

482 EgDGAT1_2 was characterized as a medium-chain fatty acid DGAT1 involved in lauric
483 acid accumulation in oil palm kernel [25] while EgDGAT2_1, which is highly expressed
484 in the mesocarp during oil accumulation, was shown to complement the yeast mutant
485 H1246 [26]. The present study functionally characterized two novel DGATs from oil
486 palm, EgDGAT1_3 and EgDGAT2_2. Transcriptomic data suggest that EgDGAT1_3
487 could be involved in both palm oil and palm kernel oil synthesis, while EgDGAT2_2 is
488 more mesocarp-specific. The exact function of EgWS/DGAT_1 remains more elusive.
489 Traces of 20:0, 22:0 and 24:0, representing about 0.28, 0.03 and 0.02 % of the total fatty
490 acids, could be detected when analyzing in detail the fatty acyl composition of oil palm
491 mesocarp during fruit ripening. These data suggest that the preference of EgWS/DGAT_1
492 for VLCFAs we observed in yeast could play a role in oil palm mesocarp. Unfortunately,
493 we could neither detect any WE in oil palm mesocarp, nor confirm with certainty the
494 presence of fatty alcohols, because traces of such compounds are often present in
495 commercially available solvents. Nevertheless, the presence of aliphatic alcohols in palm
496 oil has already been reported.[5] . In addition, high expression of EgWS/DGAT_1 was
497 found in pollen as well as in roots [16], suggesting that this protein may also play
498 important roles in these tissues.

499 In conclusion, this study presented the functional characterization of three new
500 enzymes from oil palm mesocarp potentially involved in neutral lipid biosynthesis. The
501 fact that the expression of *EgDGAT1_3* or *EgDGAT2_2* complemented the yeast neutral
502 lipid mutant H1246 strongly supports a role for the encoded proteins in storage lipid

503 accumulation in oil palm fruits. EgWS/DGAT_1, which belongs to the bifunctional
504 WS/DGAT family, lacked DGAT activity when expressed in yeast, but could produce
505 FAIEs and was able to use fatty alcohols for synthesizing WEs, suggesting that it rather
506 functions as wax synthase. Further studies are needed to unravel the exact role of these
507 different DGATs in oil palm lipid metabolism.

508

509 **Contributors**

510 V.A. and F.D. conceived and designed all experiments, and directed the project. Y.Y.
511 carried out most of the experiments with some contributions of V.A. and F.D. All authors
512 analyzed the data, interpreted the results and actively participated in the writing of the
513 manuscript. Y.Y. and F.D. generated the final versions of the figures and manuscript.

514

515 **Conflict of interest**

516 The authors declare no conflicts of interest.

517

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- 669

670 **Figure legends**

671

672 **Fig. 1. Temporal expression patterns of three DGATs in oil palm leaf, kernel and**
673 **mesocarp at five development stages.** Expression levels were obtained using the
674 transcriptome data from Bourgis et al. [3]. WAP, weeks after pollination. RKPM: reads
675 per kilobase per million reads.

676

677 **Fig. 2. Phylogenetic analysis of EgDGATs with WS and DGAT from other species.**

678 Unrooted phylogenetic tree was constructed using Tree View and an amino acid sequences
679 alignment generated with CLUSTAL X. Oil palm (*Elaeis guineensis*) DGAT sequences
680 were from Rosli et al., (2018) except for EgDGAT1_3, EgDGAT2_2 and EgWS/DGAT_1
681 that correspond to the sequences characterized in the present study (Supplemental Figure
682 1). The GenBank accession numbers of the sequences from other species are as follows:
683 *Simmondsia chinensis* ScWS (AAD38041.1), *Petunia hybrida* PhWS (AAZ08051.1),
684 *Mus musculus* MmWS (NP_808414.2), *Acinetobacter calcoaceticus* ADP1
685 AcWS/DGAT (AAO17391.1), *Euglena gracilis* EgWS (ADI60058.1), *Arabidopsis*
686 *thaliana* AtMBOAT (NP_174708), *Arabidopsis thaliana* AtDGAT1 (CAB45373.1),
687 AtDGAT2 (Q9ASU1.1), AtDGAT3 (Q9C5W0.2), AtWSD1 (NP_568547.1) and
688 AtGPAT9 (NP_568925.1).

689

690 **Fig. 3. Expression of EgDGAT1_3 and EgDGAT2_2 in the mutant yeast H1246**
691 **results in the production of TAG.** Neutral lipid content was analysed by TLC using
692 hexane/diethyl ether/acetic acid (80:20:2) for development. STD: 2.5 µg each (Oleic acid,
693 monoolein, diolein, triolein). Lipid from 10 OD units of each yeast culture was loaded on
694 TLC plate. MAG: monoacylglycerol; DAG: diacylglycerol; TAG: triacylglycerol; FFA:
695 free fatty acid; SE: sterol ester.

696

697 **Fig. 4. Total fatty acids, TAG, DAG and PC quantification in H1246 complemented**
698 **lines.** (A) Amounts of different lipids in yeast mutant strain complemented with oil palm
699 DGATs. TFA: total fatty acids determined by direct transmethylation from yeast pellets.
700 TAG, DAG and PC were isolated by TLC and their content and composition determined
701 by gas chromatography. Three replicates were made. (B) Fatty acid composition of
702 triacylglycerol from different yeast strains. Neutral lipids were separated by TLC and the
703 TAG content and composition determined by gas chromatography.
704 Results are expressed as the mean \pm standard deviation with each value corresponding to
705 three to five biological replicates (*i.e.* yeast transformants). Statistical significance of
706 differences was determined by Student's t-test (* P < 0.1, ** P < 0.05, *** P < 0.01)
707 using pESC-URA H1246 and pESC-URA SCY62 as control in 4A and 4B, respectively.

708

709

710 **Fig. 5. Expression of EgWS/DGAT_1 in the mutant yeast H1246 results in the**
711 **production of fatty acid isoamyl esters.** (A) TLC analysis of neutral lipids synthesized
712 by H1246 expressing *EgWS/DGAT_1* using hexane/diethyl ether/acetic acid (90:7.5:1;
713 v/v/v) as solvent mixture. Lipids corresponding to an equivalent of 10 OD units of yeast
714 culture cells were used. (B) The new compound indicated by an arrow in (A) was
715 identified by GC-MS as a mixture of Fatty Acid Isoamyl Esters (FAIEs), which
716 composition is showed. The vertical axis shows the relative percentage content of each
717 FAIE. pESC-URA: yeast strain H1246 transformed with the empty vector ESC-URA;
718 EgWDS1-like: yeast strain H1246 expressing EgWDS1-like; EgDGAT1_3: yeast strain
719 H1246 expressing EgDGAT1_3. Standards: STD: standard mixture containing 2.5 μ g of
720 each oleic acid (FFA), monoolein (MAG), diolein (DAG), triolein (TAG); TAG: triolein
721 (5 μ g); SE: cholestery oleate (10 μ g); EE: palmitoyl ethyl ester (10 μ g). This experiment
722 was repeated three times with similar results.

723

724 **Fig. 6. Wax esters produced by *EgWS/DGAT_1* expression in the mutant yeast**
725 **H1246 in the presence of an equimolar mixture of 14OH, 16OH and 18OH fatty**
726 **alcohols.** Yeast transformed with the empty vector pESC-URA (Control) or with
727 pESC-URA vector containing *EgWDS1-like* (*EgWDS1-like*) were grown for 48h in the
728 presence of an equimolar mixture of fatty alcohols (14OH, 16OH and 18OH) and their
729 lipids were extracted and analyzed as indicated in the Material and Method section. The
730 results combine three biological replicates. **(A)** Quantification of the amount of WEs
731 produced. **(B)** Wax ester compositions in mol%. Insert, fatty alcohol composition of WE
732 (in mol%).
733 Results are expressed as the mean \pm standard deviation with each value corresponding to
734 three to five biological replicates (*i.e.* yeast transformants). Statistical significance of
735 differences was determined by Student's t-test: * P < 0.1, ** P < 0.05, *** P < 0.01.

736

737

738 **Fig. 7. Wax esters produced by the mutant yeast H1246 expressing *TtFAR* alone or**
739 **together with *EgWS/DGAT_1*.** **(A)** TLC analysis of lipids from yeast cultures using
740 hexane/diethyl ether/acetic acid (90:7.5:1) solvent system. Lipids from 10 OD units of
741 each yeast culture were loaded on TLC plate. **TtFAR:** yeast strain H1246 expressing
742 TtFAR. **TtFAR + *EgWS/DGAT_1*:** yeast strain H1246 expressing TtFAR and
743 *EgWS/DGAT_1*. **Standard WE:** 10 μ g 17:0-17OH. **(B)** Quantification of the amount of
744 WEs produced. **(C)** Wax ester compositions in mol%.

745 Results are expressed as the mean \pm standard deviation with each value corresponding to
746 three to five biological replicates (*i.e.* yeast transformants). Statistical significance of
747 differences was determined by Student's t-test: * P < 0.1, ** P < 0.05, *** P < 0.01.

748

749

750 **Fig. 8 Comparative analysis of the total lipid and wax ester compositions of the**
751 **mutant yeast H1246 expressing *TtFAR* together with *EgWS/DGAT_1*.** **(A)** Total fatty

752 acid compositions; **(B)** Unsaturated fatty acid to saturated fatty acid ratios; **(C)** Fatty
753 alcohol compositions.

754 Results are expressed as the mean \pm standard deviation with each value corresponding to
755 three to five biological replicates (*i.e.* yeast transformants). Statistical significance of
756 differences was determined by Student's t-test: * P < 0.1, ** P < 0.05, *** P < 0.01.

757

758

1 **Table 1: Overview, annotation and correspondence of the different DGAT from palm oil reported in this and previous**
 2 **studies.**

3

Rosli et al., 2018			Bourgis et al., 2011	Dussert et al., 2013	Aymé et al., 2015	Jin et al., 2017	This study	Closest NCBI Homolog
ID	Gene model ID in PALMXplore oil palm gene database (http://palmxplore.mpob.gov.my)	Seq. length (aa)	Gene annotation	Gene annotation	Gene annotation	Gene annotation	Gene annotation	Reference
EgDGAT1_1	p5.00_sc00001_p0089	515		EgDGAT1_2				
EgDGAT1_2	p5.00_sc00018_p0155	512		EgDGAT1_1	EgDGAT1_1			
EgDGAT1_3	p5.00_sc00039_p0154	540	EgDGAT1				EgDGAT1_3 (569aa)	XP_010925472.1
EgDGAT2_1	p5.00_sc00004_p0063	332	EgDGAT2	EgDGAT2		EgDGAT2		
EgDGAT2_2	p5.00_sc00332_p0001	323					EgDGAT2-2 (325aa)	XP_010919928
EgDGAT3_1	p5.00_sc00148_p0024	360						
EgDGAT3_2	p5.00_sc00867_p0002	347						
EgWS/DGAT_1	p5.00_sc00116_p0044	519					EgWS/DGAT_1 (515aa)	XP_010924517.1
EgWS/DGAT_2	XP_010935670.1 (GenBank)	475						

4

Figure 1

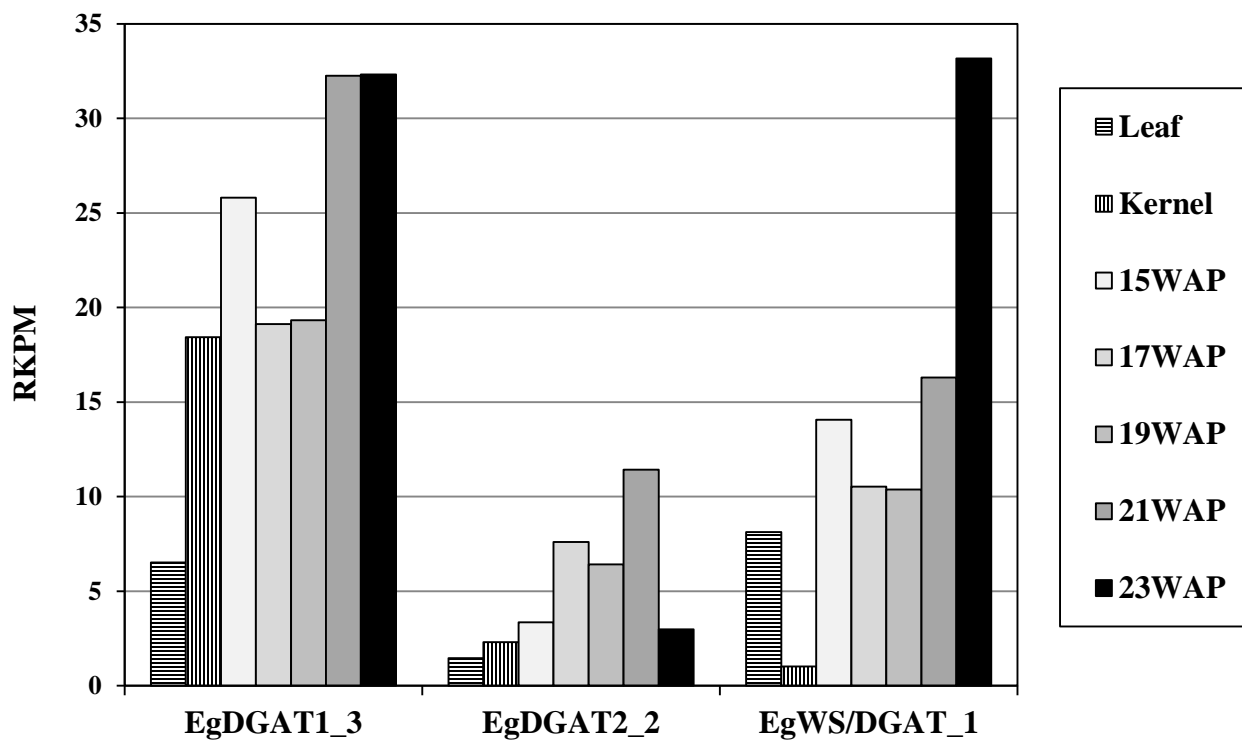
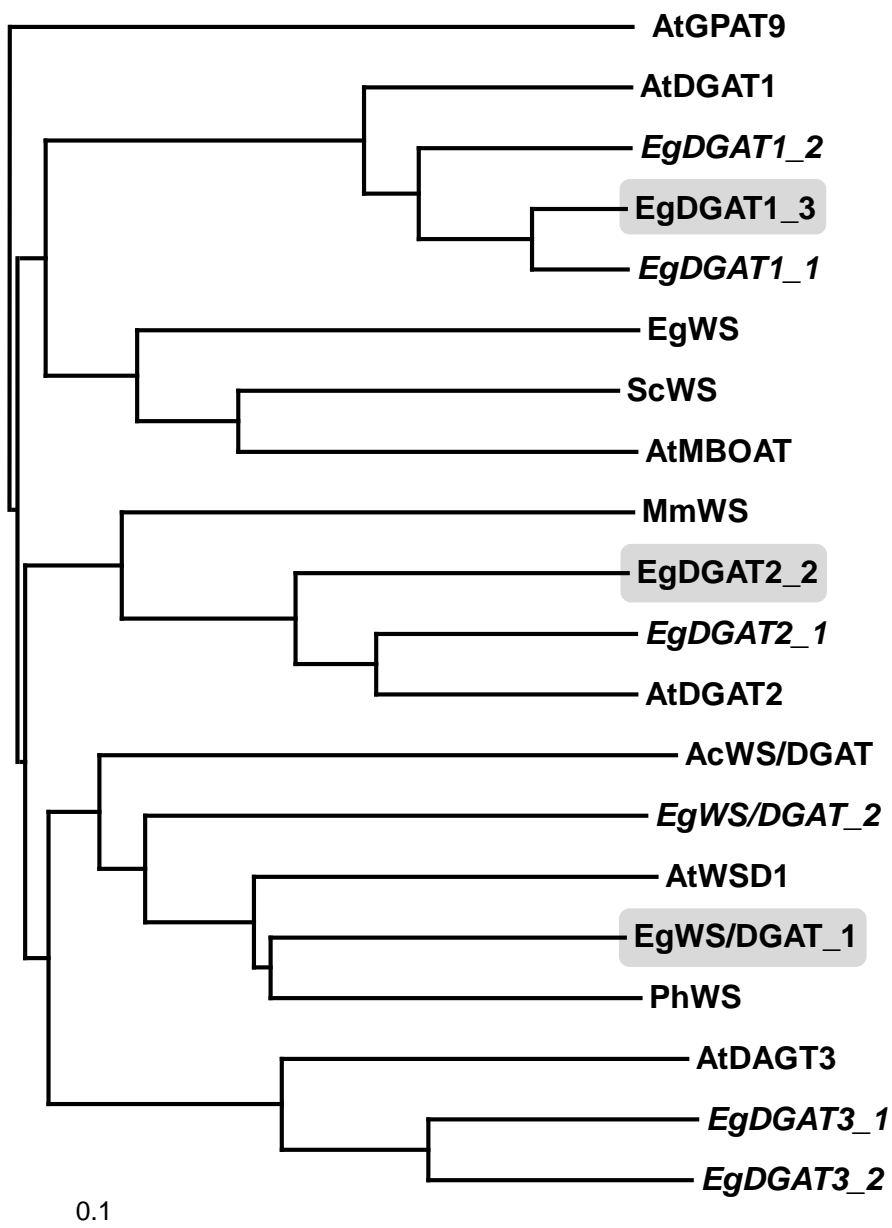
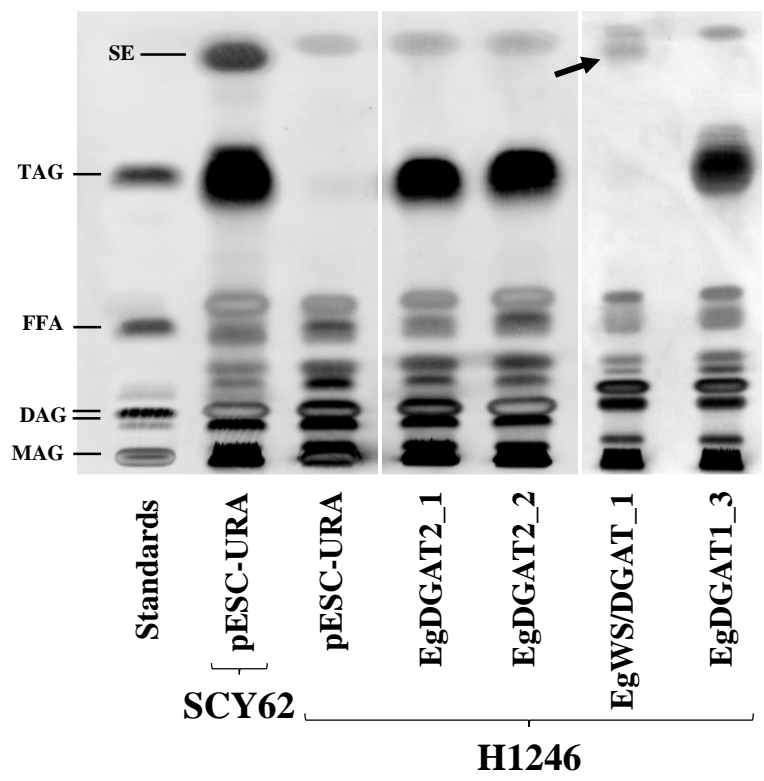
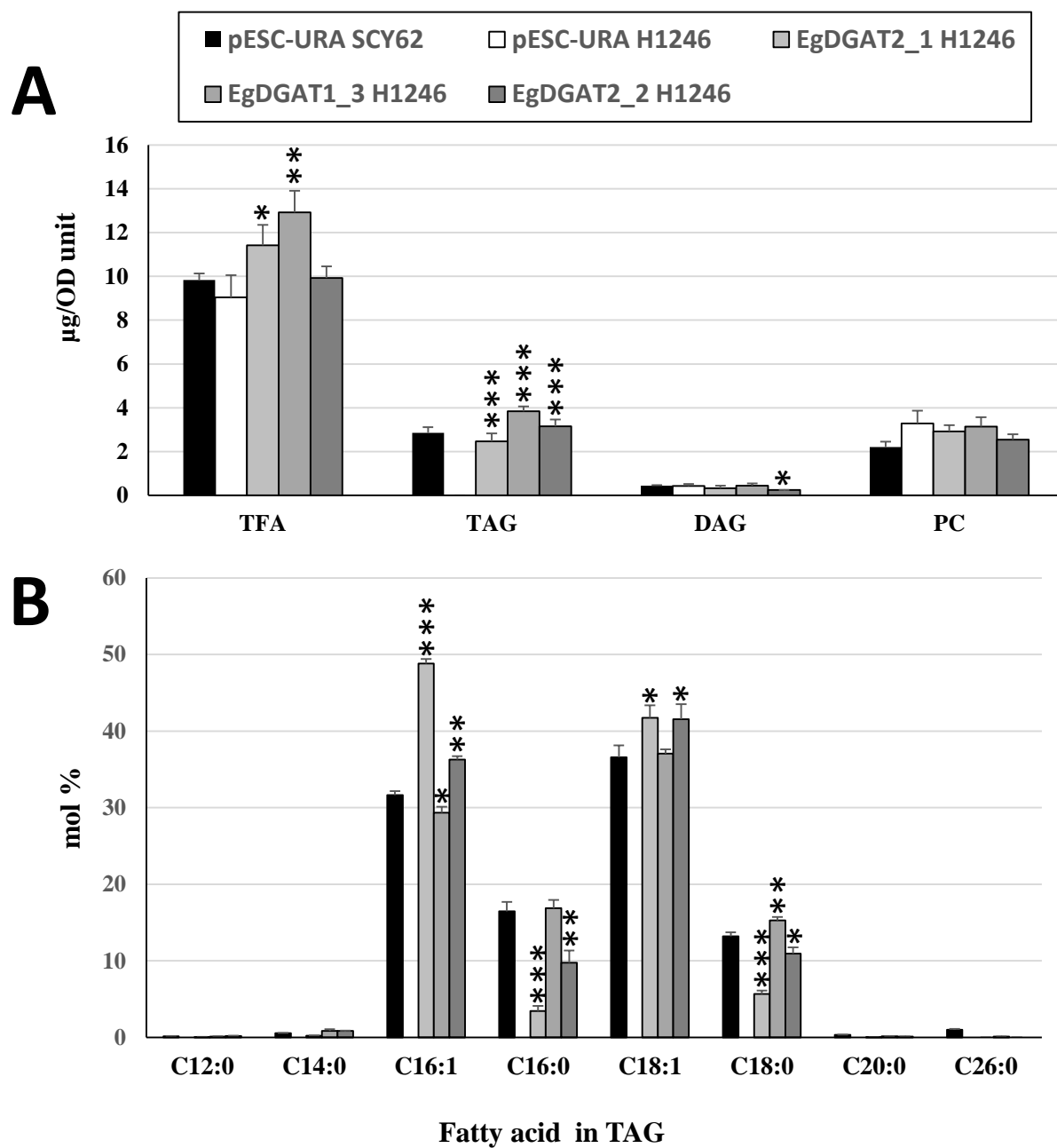
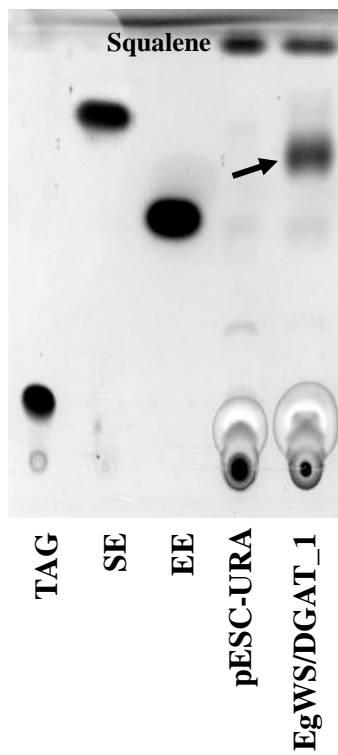


Figure 2







A**B**