Plant Physiology®

Plant Physiology, 2025, **199**, kiaf552
https://doi.org/10.1093/plphys/kiaf552
Advance access publication 30 October 2025
Research Article

Complete replacement of Arabidopsis oil-producing enzymes with heterologous diacylglycerol acyltransferases

Sean T. McGuire,[1] Jay Shockey,[2] Alexandra Richards,[1] Andrei Smertenko,[1] Philip D. Bates[1,*]

[1]Institute of Biological Chemistry, Washington State University, Pullman, WA 99164, USA
[2]United States Department of Agriculture, Agricultural Research Service, Southern Regional Research Service, 1100 Allen Toussaint Blvd, New Orleans, LA 70124, USA
*Author for correspondence: phil_bates@wsu.edu
The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instruction for Authors (https://academic.oup.com/plphys/pages/General-Instructions) is: phil_bates@wsu.edu.

Abstract

Acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1) and phospholipid:diacylglycerol acyltransferase 1 (PDAT1) share responsibility for triacylglycerol (TAG) biosynthesis, and their selectivities control TAG fatty acid (FA) compositions. For rational metabolic engineering of seed oils, replacing endogenous TAG biosynthesis with exogenous enzymes containing different substrate FA selectivities is desirable; however, the *dgat1-1/pdat1-2* double mutant is pollen lethal. Here, we evaluated the ability of 3 DGAT1s, from phylogenetically diverse plants with distinct TAG assembly processes, to completely replace endogenous TAG biosynthesis in Arabidopsis (*Arabidopsis thaliana*). We transformed *dgat1-1* mutant plants with expression constructs for *DGAT1*s from *Camelina sativa*, *Physaria fendleri*, and castor (*Ricinus communis*). Transgene expression was properly "contextualized" by using a previously determined minimum necessary expression unit containing the promoter/5′ UTR and first intron of native *AtDGAT1*; both of these DNA elements are essential for pollen expression. Next, we crossed homozygous lines with a *DGAT1/DGAT1/PDAT1/pdat1-2* parent. *C. sativa* and *P. fendleri* DGAT1s restored the FA compositions and transcriptional differences of *dgat1-1* to near wild-type and rescued the *dgat1-1/pdat1-2* pollen lethality. *R. communis* DGAT1 was active in *dgat1-1* seeds but produced unique oil profiles and alterations in the expression of lipid metabolic genes; it also failed to rescue *dgat1-1/pdat1-2* lethality. This study confirms that the promoter and first intron of *AtDGAT1* can modulate the expression of foreign *DGAT1* genes to fit the correct spatiotemporal profile necessary for completely replacing endogenous TAG biosynthesis. Furthermore, it demonstrates an additional layer of unexpected enzyme incompatibility between oilseed lineages, which may complicate bioengineering approaches that seek to replace essential genes with orthologs.

Introduction

Across the plant kingdom, many species accumulate "unusual" fatty acids (FAs) in their seed oils. These molecules have unique physicochemical properties and are useful for a plethora of industrial applications, ranging from lubricants and plastics to biofuels (Carlsson et al. 2011; Vanhercke et al. 2019; Ciastowicz et al. 2024). Most "unusual" FA accumulators have poor agronomic traits and are laborious to harvest. To circumvent these issues, a primary goal of the oilseed bioengineering community is to produce these oils in viable crops such as soybean, canola, or camelina. Key oil biosynthetic genes have been identified, but no definitive methodology has arisen that results in the desired compositions. One limitation is that most data have been interpreted from overexpression studies where transgene expression has been layered "on-top" of existing metabolic pathways; this can cause potentially deleterious pleiotropic effects (van Erp et al. 2015; Shockey et al. 2019; Regmi et al. 2020; Alkotami et al. 2021). Together, this underscores that although past research has defined many components of oil biosynthesis, our understanding of key interactions at the protein, transcriptional, and metabolite levels for successful metabolic regulation and bioengineering remains ambiguous. To avoid these issues, we require an understanding of how exogenous enzymes function when inserted into a non-native metabolic network, and without competition from the host species' orthologs.

Triacylglycerol (TAG) biosynthesis is essential during seed and pollen development (Zhang et al. 2009; McGuire et al. 2025). Two enzymes contribute to TAG production in Arabidopsis (*Arabidopsis thaliana*), acyl-CoA:diacylglycerol acyltransferase 1 (AtDGAT1, At2g19450) and phospholipid:diacylglycerol acyltransferase 1 (AtPDAT1, At5g13640). DGAT1 catalysis is acyl-CoA dependent, using a diacylglycerol (DAG) and acyl-CoA as substrates; while PDAT1 acts through an acyl-CoA-independent pathway, using DAG and an acyl group from the *sn*-2 position of phosphatidylcholine (PC) as substrates. These different modes of TAG assembly can determine oil compositions (Lager et al. 2020; Chen et al. 2022). In *Brassicaceae*, cell- and tissue-specific expression of both *DGAT1* and *PDAT1* is essential for pollen and seed

Received July 14, 2025. Accepted September 19, 2025.

development (the *dgat1-1/pdat1-2* double mutant is pollen lethal) as well as being indispensable during periods of stress (Zhang et al. 2009; Shomo et al. 2024; McGuire et al. 2025). Thus, due to the lethality of *dgat1-1/pdat1-2* in *Arabidopsis*, the capacity to completely remove and replace these 2 acyltransferases with exogenously supplied enzymes has not been investigated. We recently identified that both the promoter and first intron of *Arabidopsis* DGAT1 together provide proper tissue-specific transcription of *AtDGAT1*, allowing for complete rescue of the pollen lethal *dgat1-1/pdat1-2* double mutant with exogenously supplied *AtDGAT1* (McGuire et al. 2025).

It is well documented that reducing isozyme competition (by combining suppression techniques and genetic knockouts) increases target oil quantity (van Erp et al. 2015; Alkotami et al. 2024); however, these systems have relied solely on seed-specific overexpression of exogenous genes that does not match the endogenous profile of *DGAT1* expression throughout the plant and endogenous gene suppression that does not fully remove the endogenous enzymes. Therefore, these past studies do not properly "contextualize" expression of these essential genes, e.g. do not create the proper conditions for appropriate strength, timing, and tissue/organ specificity of transgene expression necessary to closely mimic that of the endogenous gene. Ultimately, this can lead to penalties in plant fitness, including delayed germination, reduction of seed oil content (van Erp et al. 2015), and impaired responses to abiotic stressors (Shomo et al. 2024) in plants accumulating target oils.

In this study, we utilized the endogenous regulatory components of *AtDGAT1* to produce native expression of foreign *DGAT1* genes in the absence of the native *Arabidopsis* acyltransferases, DGAT1 and PDAT1. This work represents a complete replacement of a committed oil biosynthetic reaction in an oilseed plant. Here we demonstrate that exogenous *Brassicaceae DGAT1*s from *Camelina sativa* and *Physaria fendleri* can wholly integrate into *Arabidopsis* lipid metabolism, allowing for recovery of *dgat1-1/pdat1-2* double mutants, where TAG is solely produced by the foreign enzymes. While expression of the more distantly evolved castor (*Ricinus communis*) *DGAT1* perturbs expression of various metabolic genes, with alterations in oil composition and amounts, such that it cannot rescue the *dgat1-1/pdat1-2* lethality. These results suggest that developmental coordination of TAG biosynthesis does not only depend on the capacity to produce TAG but also requires that the introduced *DGAT1* is incorporated into the metabolic organization of the host species to fully rescue the adverse metabolic changes produced in TAG biosynthetic loss-of-function mutants.

Results

Substrate specificities of foreign DGAT1s determine seed oil production and composition

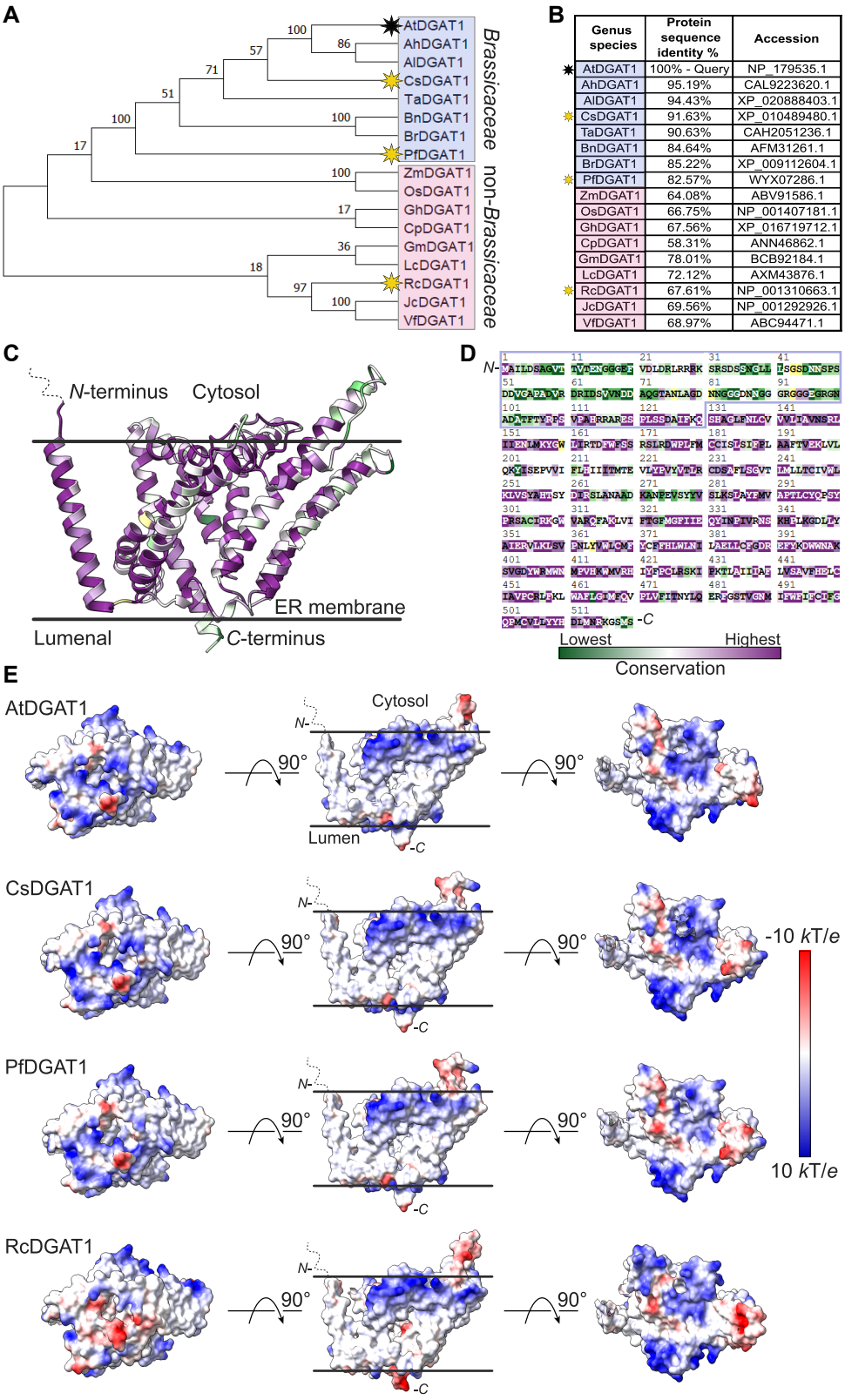
To accurately explore how expression of exogenous genes alters metabolism, we posited that expression of foreign *DGAT1*s would cause varied FA phenotypes based on inherent substrate specificities of each enzyme (Chen et al. 2022), even when supplied with *Arabidopsis* lipid substrates.

To test if we could use the regulatory components of *AtDGAT1* (both the promoter and first intron) to replace AtDGAT1 with closely related *Brassicaceae* as well as non-*Brassicaceae* DGAT1s, we generated a short list of DGAT1 candidates, by Neighbor-joining phylogenetic analysis of DGAT1 proteins from 8 *Brassicaceae* and 9 non-*Brassicaceae* oil crop species (Fig. 1A). We coupled this with structural conservation mapping to the AlphaFold3 predicted AtDGAT1 structure (UniProt: Q9SLD2) using ConSurf (https://consurf.tau.ac.il/consurf_index.php) and protein BLAST to determine % protein identity. This revealed that both protein sequence and structure between plant DGAT1s—apart from the *N*-terminus—are highly conserved, with residues near the predicted cytosolic or luminal leaflets being most varied (Fig. 1B to E). It should be stated that the topology depicted here still requires confirmation, as other experimental data suggest that both plant DGAT1 termini are cytosolically oriented (Shockey et al. 2006). Ultimately, we selected 3 DGAT1s from *C. sativa* (*CsDGAT1*, Svalof v1.1), *P. fendleri* (*PfDGAT1*, OVBV01015132.1), and *R. communis* (*RcDGAT1*, 29912.t000099), to encompass a range of plant lineages that have varying levels of protein conservation when compared to *Arabidopsis* DGAT1 (CsDGAT1 91.6% identity; PfDGAT1 82.6% identity; and RcDGAT1 67.6% identity (Fig. 1A, B (gold stars)). Further, these DGAT1s display electrostatic charge differences (Fig. 1E) that may reflect how they function or interact with nearby protein partners. In their native systems, the DGAT1s selected predominantly contribute to 3 distinct TAG biosynthetic pathways (Bates and Shockey 2025): (i) TAG synthesis from de novo DAG (Kennedy pathway, used by castor (Bafor et al. 1991)); (ii) TAG synthesis from PC-derived DAG (used by *Camelina* (Yang et al. 2017)); and (iii) TAG remodeling (used by *Physaria* (Bhandari and Bates 2021; Parchuri et al. 2024)). Further, both *Physaria* and castor accumulate high proportions of unusual hydroxy-fatty acids (HFAs, lesquerolic acid and ricinoleic acid, respectively). Native expression of foreign *DGAT1* cDNAs and the ability of each DGAT1 to use common FAs in a foreign plant system have not been demonstrated.

To accomplish native expression of the foreign *DGAT1*s in *Arabidopsis* tissues, we used the recently discovered full-length *AtDGAT1* promoter (*AtDGAT1Pro*) paired with the *AtDGAT1* first intron (McGuire et al. 2025) inserted into each foreign gene, thus providing the correct expression patterning for *CsDGAT1*, *PfDGAT1*, and *RcDGAT1* in *Arabidopsis* (Supplementary Fig. S1A). To test the functionality of each foreign DGAT1 in *Arabidopsis*, we transformed the *dgat1-1* mutant. In the absence of AtDGAT1, seeds have a unique FA phenotype, resulting in high polyunsaturated fatty acid (PUFA) and reduced oil content (Katavic et al. 1995). This phenotype is ascribed to PDAT1 and fatty acid desaturase 2 (FAD2) activity, resulting from various compensating metabolic adaptations to accumulate sufficient levels and compositions of TAG (Zhang et al. 2009; Aulakh and Durrett 2019; Neumann et al. 2024).

For each alternative DGAT1, a minimum of 10 T_1 primary transformants were grown, and the T_2 segregating seed was analyzed for FA composition and oil content (Fig. 2). Comparison between brown (untransformed) and *DsRed*-fluorescent (transformed) seed indicated that each foreign DGAT1 changed the FA composition and oil content of seeds in the *dgat1-1* background (Fig. 2A to F). Interestingly, transformants of the 2 *Brassicaceae DGAT1*s, *CsDGAT1* and *PfDGAT1*, restored the FA phenotype to near wild-type levels, while the *RcDGAT1* from the distantly related *Euphorbiaceae* had a unique FA composition. Individual transgenic lines were selected for further analysis based on the largest changes in FA compositions compared to *dgat1-1* seed.

Selected T_3 homozygous lines were grown alongside wild-type and *dgat1-1* plants to determine the effectiveness of the foreign DGAT1s to restore the *dgat1-1* FA phenotype and oil yield penalty (Fig. 3A, B). Each DGAT1 imposed unique compositional changes to the *Arabidopsis* seed oil. Consistent with the T_2 data, the CsDGAT1 protein recovered the *dgat1-1* phenotype, having only



Genus species	Protein sequence identity %	Accession
AtDGAT1	100% - Query	NP_179535.1
AhDGAT1	95.19%	CAL9223620.1
AlDGAT1	94.43%	XP_020888403.1
CsDGAT1	91.63%	XP_010489480.1
TaDGAT1	90.63%	CAH2051236.1
BnDGAT1	84.64%	AFM31261.1
BrDGAT1	85.22%	XP_009112604.1
PfDGAT1	82.57%	WYX07286.1
ZmDGAT1	64.08%	ABV91586.1
OsDGAT1	66.75%	NP_001407181.1
GhDGAT1	67.56%	XP_016719712.1
CpDGAT1	58.31%	ANN46862.1
GmDGAT1	78.01%	BCB92184.1
LcDGAT1	72.12%	AXM43876.1
RcDGAT1	67.61%	NP_001310663.1
JcDGAT1	69.56%	NP_001292926.1
VfDGAT1	68.97%	ABC94471.1

Figure 1. Phylogenetic analysis, protein sequence and structural conservation, and electrostatic potential of DGAT1s. **A**, **B)** *Brassicaceae* are indicated in blue and non-*Brassicaceae* in pink. The black star indicates wild-type AtDGAT1, while gold stars mark the foreign DGAT1s evaluated to rescue *Arabidopsis dgat1-1/pdat1-2* lethality. **A)** Neighbor-joining multiple sequence alignment. Bootstrap values (as percentages) from 1,000 replicates are indicated at each branch point. **B)** Protein sequence identity compared to AtDGAT1. **C**, **D)** Protein structural conservation analysis of monomeric DGAT1 sequences in **(A)** using the ConSurf server (Yariv et al. 2023). The dotted line at the *N*-terminal regions in **(C**, **E)** marks the disordered region with no predicted structural fold; the blue box in **(D)** is the region that was truncated for structural analysis. The orientation of DGAT1 is inferred from the cryoEM structure of human DGAT1 (Wang et al. 2020). **E)** Electrostatic surface representations of plant DGAT1s. Three orientations are shown. The electrostatic potential was calculated using the default settings in ChimeraX (Meng et al. 2023). The AtDGAT1 (UniProt: Q9SLD2) was used as a template for structural alignment from AlphaFold3 predicted structures for the other DGAT1s shown here.

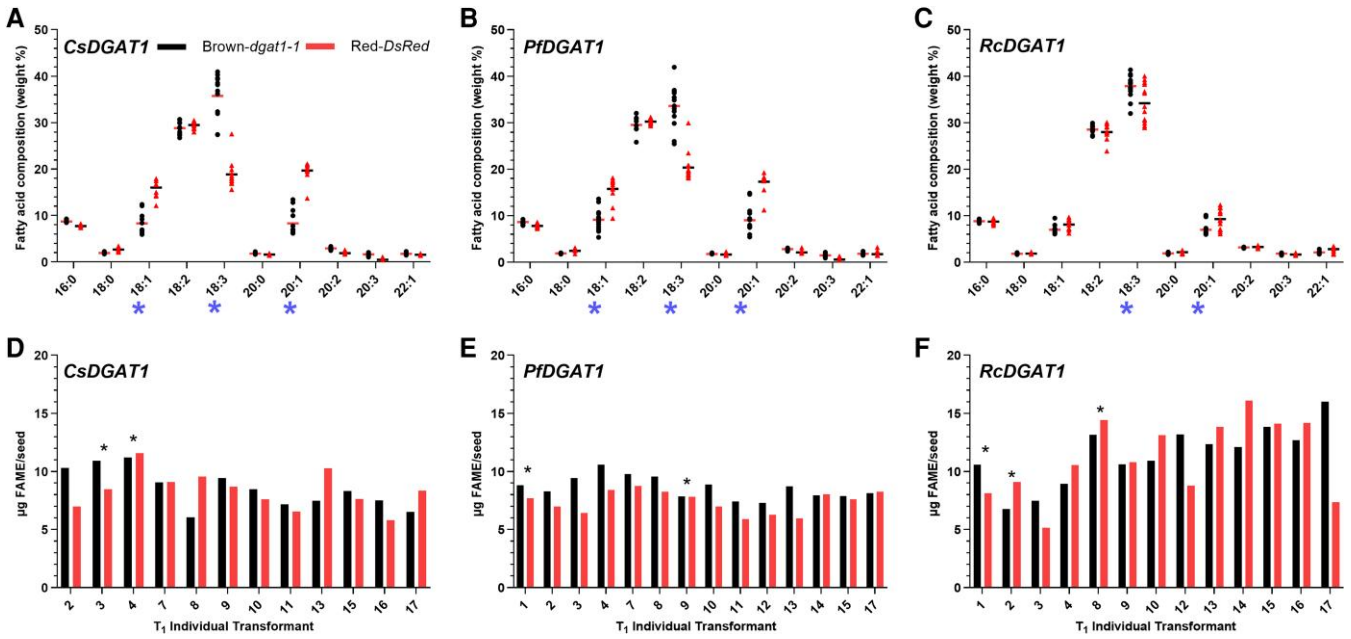


Figure 2. Oil phenotyping of T_2 segregating transgenic seed. **A** to **C)** Seed FA content of transgenics (red triangles) compared to null *dgat1-1* seed (black circles). Horizontal bars indicate the mean of each FA from all independent transgenic lines tested. **A)** *CsDGAT1* ($n = 12$); **B)** *PfDGAT1* ($n = 14$); and **C)** *RcDGAT1* ($n = 13$). Blue asterisks indicate statistical significance ($P \leq 0.05$, 2-way ANOVA analysis with Šidák correction). **D** to **F)** Oil content of transgenics (red) compared to null *dgat1-1* (black). **D)** *CsDGAT1*; **E)** *PfDGAT1*; and **F)** *RcDGAT1*. Black asterisks indicate lines selected for further analysis, based on a combination of changes in total oil and the largest changes in FA composition, that contain single gene insertions.

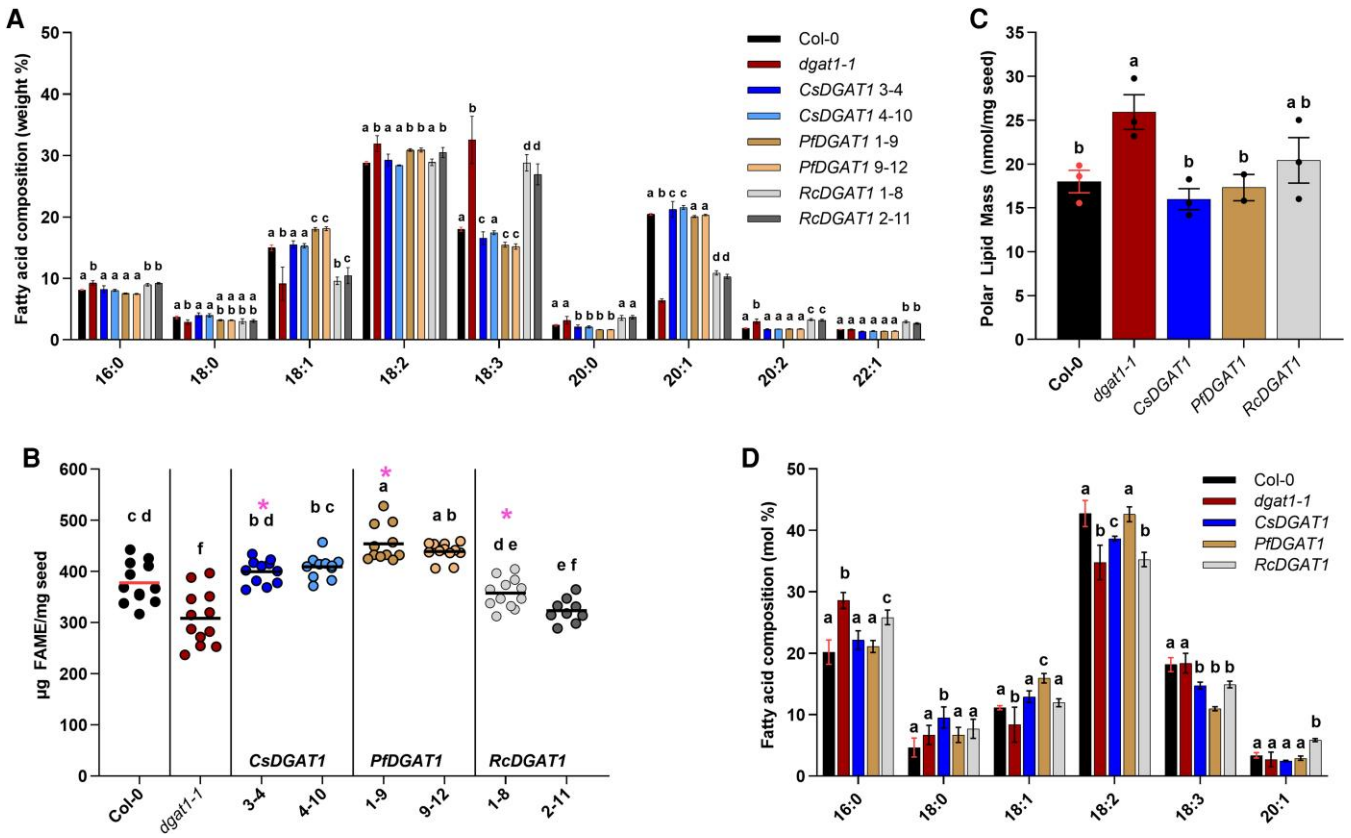


Figure 3. Seed oil analysis reveals inherent FA preferences of each exogenous DGAT1. **A)** Weight % FA composition from dry whole seed. Values are the mean ± SD harvested from $n \geq 9$ plants. Different letters in **(A, D)** indicate significant differences ($P \leq 0.05$, 2-way ANOVA, Dunnett's multiple comparison). **B)** Total seed FA content quantified as µg FAMEs per mg seed. Each data point represents an individual plant, and horizontal bars indicate the mean. Different letters in **(B, C)** indicate significant differences ($P \leq 0.05$, 1-way ANOVA, with Tukey's test for multiple comparisons). Magenta asterisks indicate lines that were selected for further analysis in **(C, D)**. **C)** Polar lipid mass isolated from mature seed from $n \geq 2$ extractions. **D)** Polar lipid mol% FA composition from mature seed $n \geq 3$ extractions. Mean ± SD is shown for **C** and **D**.

slight differences in 18:2, 18:3, and 20:1 content compared to wild-type (Fig. 3A, blue shades). The ability of CsDGAT1 to utilize many FA substrates is not unprecedented, as recent studies found that CsDGAT1 can use a variety of substrates compared to the selective CsDGAT2 (Lager et al. 2020; Lee et al. 2024). Equally, the PfDGAT1 rescued the *dgat1-1* mutant, showing decreased 18:3 and increased 18:1, 18:2, and 20:1 content (Fig. 3A, gold shades). The FA data presented agree with recent reports that PfDGAT1 may prefer 18:x-containing DAG and 18:x-CoA as substrates (Parchuri et al. 2024), and demonstrate the plasticity of PfDGAT1 for non-HFA substrates. Thus, the *Brassicaceae* family DGAT1s supplemented the *dgat1-1* FA phenotype to near wild-type levels, while RcDGAT1 lines showed increased 18:1/20:1 content but still retained higher 18:3 as seen in the *dgat1-1* parent. Moreover, in both RcDGAT1 lines analyzed, very-long chain fatty acids (VLCFAs) (20:2 and 22:1) were increased when compared to both *dgat1-1* and wild-type (Fig. 3A, gray shades). The modifications to the FA profile are consistent with data for *dgat1-1* plants overexpressing RcDGAT2 (Regmi et al. 2020), suggesting that although they are different isoforms, both castor DGATs appear to utilize various DAG and acyl-CoA substrates. These data indicate that RcDGAT1 may prefer VLCFAs and PUFAs when HFAs are absent. This aligns with the observation that yeast expressing RcDGAT1 utilized 18:3 FA and survived when 18:3 FAs were supplied at toxic levels (Trenz et al. 2022).

We also measured how each DGAT1 altered oil biosynthesis in the *dgat1-1* background (Fig. 3B). Both CsDGAT1 and PfDGAT1 restored oil content to near or above wild-type levels (Fig. 3B, blue and gold shades, respectively). In contrast, RcDGAT1 showed a partial restoration of the oil yield penalty of *dgat1-1* to levels between *dgat1-1* and wild-type (Fig. 3B, gray shades). The peak-performing lines of each DGAT1 (based on a combination of FA changes and ability to recover the oil penalty) were selected for further lipid analysis (designated by magenta asterisks). Additionally, we analyzed the total polar lipid content and composition of mature seeds (Fig. 3C, D). Both *Brassicaceae* DGAT1s restored the seed polar lipid mass to near wild-type (Fig. 3C) while having diverse effects on the polar lipid FA composition (Fig. 3D). Interestingly, RcDGAT1 polar lipid content was intermediate to wild-type and *dgat1-1*, such that it was not significantly different from either, and produced a unique FA profile. In the RcDGAT1 line, 20:1 content was increased; this FA is typically a very minor FA in phospholipids (Maatta et al. 2012; Zhou et al. 2014). This increase of 20:1 in the polar lipid fraction, but with a limited increase in 20:1 in TAG compared to other DGAT1s (Fig. 3A) may indicate that RcDGAT1 selects against PC-derived DAGs (Bates and Browse 2011) containing VLCFAs, which thus remain in the polar lipid fraction.

Due to the observed differences among the foreign DGAT1 lines, we performed *sn*-1,3-specific TAG digestions with *Aspergillus oryzae* lipase to evaluate the composition at the *sn*-1,3 and *sn*-2 positions (Supplementary Fig. S2). In evaluating the *sn*-2 position, we found that each transformant had distinct positional variations. CsDGAT1 had a near wild-type composition with only minor changes in the 18:3 content (Supplementary Fig. S2A, blue). PfDGAT1 showed significant increases in 18:2 and a decrease in 18:3 when compared to the wild-type (Supplementary Fig. S2A, gold). When combined with the overall increase in 18:1 in the total seed lipid (Fig. 3B), polar lipids (Fig. 2C), and the released FA fractions (indicative of the *sn*-1,3 composition (Supplementary Fig. S2B, C)) this may hint that PfDGAT1 prefers DAGs containing 18:1 at the *sn*-1 position or that it preferentially acylates the *sn*-3 position using 18:1- or 20:1-CoAs in *Arabidopsis*. Intriguingly, RcDGAT1 had a composition between *dgat1-1* and wild-type for each of the analyzed molecules (Supplementary Fig. S2). The in-depth seed lipid analysis of our transgenics demonstrates that plants containing the RcDGAT1 protein had an intermediate but wholly unique chemotype compared to both *dgat1-1* and wild-type.

The ability to mimic native *Arabidopsis DGAT1* expression for exogenous *DGAT1*s is a substantial contribution toward understanding how endogenously produced DGAT1 proteins function when supplied with *Arabidopsis*-specific DAG and acyl-CoA substrates, rather than those present in the native species.

RT-qPCR analysis reveals adaptations of lipid metabolic gene expression in plants expressing foreign *DGAT1*s

The lipid composition of *dgat1-1* seed is due to multiple metabolic adaptations, beyond the lack of AtDGAT1. Recently, a list of marker genes displaying dramatic fold-changes in expression has been identified in the *dgat1-1* mutant, including phosphatidylcholine:diacylglycerol cholinephosphotransferase (*PDCT*), stearoyl-acyl carrier protein Δ9-desaturase6 (*DES6*), mother of FT (*MFT*), and *FAD2* (Aulakh and Durrett 2019). As a proxy to determine how heterologous expression of each foreign *DGAT1* altered these genes (and others), we performed reverse transcription-quantitative PCR (RT-qPCR) on developing seeds from top-performing lines.

This suite of genes also likely contributes to the oil phenotypes when each foreign DGAT1 was expressed in *dgat1-1*. *PDCT* encodes a phosphatidylcholine:diacylglycerol cholinephosphotransferase that interconverts DAG and PC by transfer of the phosphocholine headgroup, this reaction is known to be important in determining flux of PUFAs into TAG by providing 18:1-DAGs for desaturation in the PC pool, as well as releasing 18:2- and 18:3-DAGs from PC for TAG production (Lu et al. 2009; Bates et al. 2012). *DES6* codes for a plastidial Δ9 stearoyl-ACP desaturase (SAD/AAD) that functions redundantly with other AAD enzymes to produce monoenes (Kazaz et al. 2020). The *MFT* gene product is a phosphatidylethanolamine-binding protein known to regulate oil accumulation during seed development or periods of stress. MFT modulates ABA insensitive 5 (*ABI5*), a transcription factor whose downstream targets include *DGAT1* and many other lipid biosynthetic genes (Xi et al. 2010; Kong et al. 2013). *FAD2* is the major FA desaturase responsible for the synthesis of extra-plastidial 18:2 FAs (Okuley et al. 1994), which are then the substrate for 18:3 production by FAD3 (Gishini et al. 2025). FA desaturation by FAD2 is a requirement for seed oil biosynthesis in the absence of DGAT1 (Neumann et al. 2024). Combined with the loss of DGAT1 activity, compensatory TAG production by PDAT1 and altered expression of many lipid biosynthetic genes, *dgat1-1* seeds are an excellent genetic resource to simplify interpretations of how native expression of foreign *DGAT1*s alters *Arabidopsis* oil biosynthetic gene expression.

We found that both CsDGAT1 (Fig. 4, blue shade) and PfDGAT1 (Fig. 4, gold shade) plants returned gene expression to near wild-type levels. The differences from wild-type likely reflect metabolic demands from the introduced DGAT. PfDGAT1 plants have greatly reduced *DES6* compared to the high levels in *dgat1-1*, yet it remains significantly higher than wild-type. Interestingly, Lysophosphatidylcholine acyltransferase 2 (*LPCAT2*) and Aminoalcoholphosphotransferase 2 (*AAPT2*) transcript levels were also increased in PfDGAT1 plants. AAPTs are responsible for both phosphatidylethanolamine (PE) and PC synthesis by

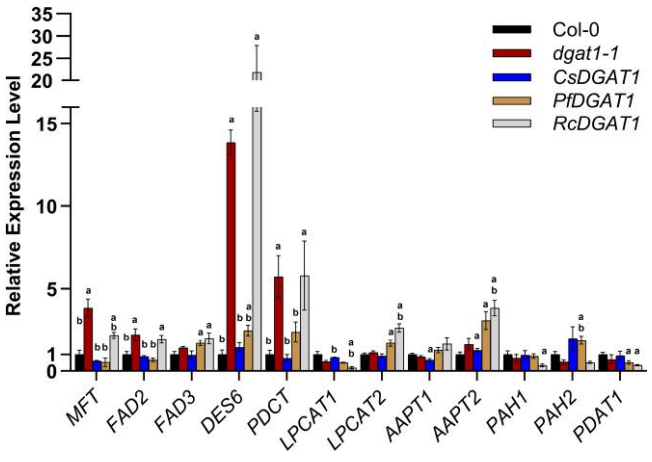


Figure 4. Expression of exogenous *DGAT1* alters expression of lipid biosynthetic genes in developing seeds in *dgat1-1*. RNA was isolated from developing seeds extracted mid-development (12 DAF) siliques. Transcript abundances were quantified by RT-qPCR. SECRETION-ASSOCIATED RAS SUPER FAMILY 2 (ASAR1; AT4G02080) was used as an internal control. Data represent the mean ± SEM of ≥3 biological replicates. Letters indicate significance with "a" being different from wild-type, and "b" significantly different than *dgat1-1* (Welch's *t*-test, $P \leq 0.05$).

adding the phosphobase moiety of CDP-choline/ethanolamine to the *sn*-3 position of DAG. When paired together, the residual increase of DES6 in PfDGAT1 plants may contribute to higher synthesis rates of 18:1 originating from the plastid, with LPCAT2 and AAPT2 allowing for 18:1 flux through PE/PC by acyl editing and PC-derived DAG utilization by PfDGAT1 (Parchuri et al. 2024; Bates and Shockey 2025) resulting in increased 18:1 content in the seed oil (Fig. 3A). Together, these results suggest that expression of *Brassicaceae DGAT1*s can almost fully restore the metabolic adaptations of mutant *dgat1-1* seed.

In contrast, RcDGAT1 plants contained high expression levels of *MFT*, *DES6*, *PDCT*, *FAD2*, and *FAD3* while having a reduction in *PDAT1* transcript compared to wild-type (Fig. 4, gray shade). Notably, RcDGAT1 plants also had increased expression of *LPCAT2* and *AAPT2*, with a concomitant reduction in *LPCAT1* when compared to both wild-type and *dgat1-1*. Beyond acyl editing, past studies identified that LPCAT2, rather than LPCAT1, plays a crucial role in supplying PC for PDAT1-catalyzed TAG synthesis in *dgat1-1*. In the *lpcat2/dgat1-1* double mutant, the oil penalty phenotype of *dgat1-1* is further exacerbated, resulting in a 40% reduction of total oil when compared to *dgat1-1* alone. However, the *lpcat1/dgat1-1* mutant exhibited only a 5% reduction in oil content when compared to *dgat1-1* (Xu et al. 2012). This is important to note because the RcDGAT1 plants had significant changes in both FA composition and oil content when compared to *dgat1-1* (Fig. 3A, B). Some of the changes in the RcDGAT1 plants may then be attributed to the increased expression of both *LPCAT2* and *PDCT* that in turn increase production of PC that can be (i) desaturated into molecular species preferred by RcDGAT1; or (ii) used by residual PDAT1 activity. Although PDCT is a key player in the production of PC, the AAPTs also produce the bulk of the phospholipids, PE and PC, while also catalyzing the reverse reaction to generate CDP-choline/ethanolamine and DAG (Goode and Dewey 1999). Thus, the increased expression of *AAPT2* in the RcDGAT1 transgenic seed implies enhanced demand for phospholipid synthesis in the developing seeds of this line. When combined with the heightened expression of *LPCAT2*, the increase in *AAPT2* may indicate a pathway for first introducing acyl groups into PC for desaturation (to 18:3) with subsequent release of PUFA-containing acyl-CoAs or DAGs by reverse action of each enzyme, respectively. Although the intricacies of the acyl-editing cycle (Bates et al. 2012; Karki et al. 2019; Bates and Shockey 2025) make it challenging to accurately interpret pathway alterations in the RcDGAT1 plants. PDAT1 activity may also contribute to the observed chemotypes, although the decrease in *PDAT1* expression in the RcDGAT1 line suggests that RcDGAT1 activity may be replacing PDAT1 for PUFA accumulation. We also assured accurate quantitation of *AtDGAT1* transcription versus that of the foreign *DGAT1*s by using specific primers to evaluate transcript abundance (Supplementary Fig. S3), and all foreign *DGAT1*s were expressed at similar levels. Nevertheless, the expression of *RcDGAT1* revealed an intermediate reduction in transcripts of many of the genes assayed, with equally unexpected changes in several key metabolic genes involved in phospholipid and oil biosynthesis, demonstrating a unique effect on plant metabolism absent from the *Brassicaceae DGAT1* plants.

Functional complementation of the lethal *dgat1-1/pdat1-2* mutant with foreign *DGAT1*s

Our main goal was to assess if we could completely replace endogenous TAG assembly with foreign DGAT1s. To examine this, we followed a similar segregation strategy to that published by McGuire et al. (2025). First, we crossed the top-performing lines (Fig. 3B, magenta asterisks), expressing *CsDGAT1*, *PfDGAT1*, and *RcDGAT1* in *dgat1-1* with plants that carried a *DGAT1/DGAT1//PDAT1/pdat1-2* background. If the cross was successful, and the properly contextualized transgenic DGAT1s (i.e. with the proper gene dosage, and accurate tissue targeting across the entire plant life cycle) continued to show evidence of functional restoration of the *dgat1*-1 mutation (as described above), recovery of wild-type, heterozygous mutant, and homozygous mutant alleles would be possible from the 2 unlinked genetic loci, *DGAT1/dgat1-1* and *PDAT1/pdat1-2*. After several generations of selection and segregation, we recovered plants homozygous for the transgene (*CsDGAT1*, *PfDGAT1* or *RcDGAT1*) that also carried either the *DGAT1/dgat1-1//pdat1-2/pdat1-2* or *dgat1-1/dgat1-1//PDAT1/pdat1-2* genotypes (Supplementary Fig. S4). Next, we tested if the foreign *DGAT1* in each of these lines could rescue the *dgat1-1/pdat1-2* double mutant lethality by assessing allele transmission frequencies of the *PDAT1/pdat1-2* locus. Interestingly, expression of *CsDGAT1* or *PfDGAT1* showed the expected 1:2:1 segregation of the *PDAT1/pdat1-2* alleles. In stark contrast, the plants expressing *RcDGAT1* were incapable of rescuing the *dgat1-1/pdat1-2* lethality, indicated by a 1:1:0 of the locus (Fig. 5A). Compared to the other *DGAT1*s, the inability of *RcDGAT1* to rescue the *dgat1-1/pdat1-2* double mutant was somewhat puzzling. To assess if pleotropic effects arose during *PDAT1/pdat1-2* segregation, we also tested the transmission of the *DGAT1/dgat1-1* alleles in the *DGAT1/dgat1-1/pdat1-2/pdat1-2* line expressing *PfDGAT1* or *RcDGAT1*. Unsurprisingly, we recovered double mutants when *PfDGAT1* was expressed (showing 1:2:1), but we were still unable to recover a functional double mutant in *RcDGAT1* expressing lines (showing a 1:1:0) (Fig. 5B). Seed oil content and composition were analyzed from all genotypes of plants segregating the *PDAT1/pdat1-2* locus (Supplementary Figs. S5, Fig. 6). Oil content and composition results of CsDGAT1 and PfDGAT1 with segregating *pdat1-2* are similar to the chemotypes of the transformants in the *dgat1-1* background alone (Fig. 3); thus, reinforcing that when a properly produced DGAT1 is present, PDAT1 has little effect on

A

tDGAT1/dgat1-1//PDAT1/pdat1-2 ⊗

Observed: tDGAT1	*tDGAT/dgat1-1/ PDAT1/PDAT1*	*tDGAT1/dgat1-1/ PDAT1/pdat1-2*	*tDGAT1/dgat1-1/ pdat1-2/pdat1-2*
CsDGAT1$^{\alpha}$	24 (23.25)	44 (46.5)	25 (23.25)
PfDGAT1$^{\alpha}$	29 (23.75)	38 (47.5)	28 (23.75)
RcDGAT1†	39 (19.25)	38 (38.5)	0 (19.25)

B

tDGAT1/pdat1-2//DGAT1/dgat1-1 ⊗

Observed: tDGAT1	*tDGAT/pdat1-2/ DGAT1/DGAT1*	*tDGAT1/pdat1-2/ DGAT1/dgat1-1*	*tDGAT1/pdat1-2/ dgat1-1/dgat1-1*
PfDGAT1$^{\alpha}$	17 (17.75)	36 (35.5)	18 (17.75)
RcDGAT1†	28 (14)	28 (28)	0 (14)

Figure 5. Contextualized expression of *Brassicaceae* DGAT1s can restore the loss of the endogenous *Arabidopsis* oil-producing acyltransferases, DGAT1 and PDAT1. Top performing transgenic lines expressing the heterologous *C. sativa* (Cs), *P. fendleri* (Pf), and *R. communis* (Rc) DGAT1 in the *dgat1-1/dgat1-1* background were crossed with a *DGAT1/DGAT1// PDAT1/pdat1-2* parent. **A)** Segregation analysis of the *PDAT1/pdat1-2* locus in plants homozygous for each *tDGAT1* and *dgat1-1*. **B)** Segregation of the *DGAT1/dgat1-1* locus in plants homozygous for each *tDGAT1* and *pdat1-2*. All data show the observed and expected (in parentheses) segregant count. Chi-square tests were performed for a 1:2:1 segregation. α indicates no statistical difference compared with expected segregation ratios; therefore, following laws of Mendelian inheritance. † denotes statistically significant differences compared to the null hypothesis with a $P \leq 0.05$.

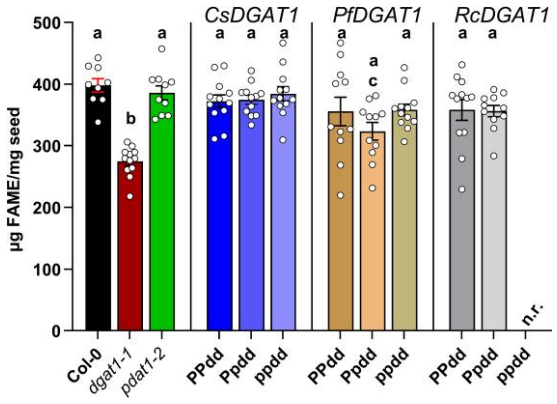


Figure 6. Seed oil content is restored in recovered plants segregating the *PDAT1/pdat1-2* locus for plants expressing exogenous DGAT1s. Total seed FA content quantified as µg FAMEs per mg seed. PPdd, *PDAT1/ PDAT1//dgat1-1/dgat1-1*; Ppdd, *PDAT1/pdat1-2//dgat1-1/dgat1-1*; ppdd, *pdat1-2/pdat1-2//dgat1-1/dgat1-1* seed. n.r. indicates not recovered. Values are the mean ± SD harvested from $n \geq 9$ plants. Different letters indicate significant differences ($P \leq 0.05$, Brown–Forsythe and Welch 1-way ANOVA).

seed oil content and composition (Zhang *et al.* 2009). These *RcDGAT1* experiments emphasize the evidence above that the castor enzyme cannot fully replace the function of AtDGAT1 and AtPDAT1 during pollen or seed development in *Arabidopsis*, even when expressed by the native *AtDGAT1* transcriptional unit.

The results with the 2 foreign *Brassicaceae* DGAT1s, originating from species with distinct oil compositions, indicate it is possible to use foreign enzymes with diverse substrate acyl selectivity to functionally complement a lethal *Arabidopsis* mutant defunct in oil production. However, another layer of "cross-species incompatibility" between *Brassicaceae* and *Euphorbiaceae* DGAT1s must be responsible for the observed phenotypes of RcDGAT1 plants, leading to its inability to rescue the lethality of the *dgat1-1/ pdat1-2* double mutant.

Assessment of DGAT1 protein:protein interactions with ER-localized lipid metabolic enzymes reveal possible diverse interactomes in diverged plant lineages

Physical protein interactions are important for modulating enzyme activity, subcellular/suborganellar localization, and possibly substrate channeling (Coleman 2019; Xu et al. 2019, 2020; Regmi et al. 2020; Parchuri et al. 2024; Bates and Shockey 2025). The clear differences in compatibility between the *Brassicaceae* (CsDGAT1, PfDGAT1) and *Euphorbiaceae* (RcDGAT1) DGAT1s when produced in *Arabidopsis* led us to postulate that interactions with available protein partners in seed or pollen tissues may be limiting. To test this hypothesis, we used a well-defined split-ubiquitin yeast 2-hybrid (Y2H) approach that has been used to evaluate many lipid biosynthetic protein interactions (Gidda et al. 2009; Shockey et al. 2016; Bates and Shockey 2025).

Here, each DGAT1 (AtDGAT1, dgat1-1, CsDGAT1, PfDGAT1, and RcDGAT1) was used as a protein bait against a suite of prey proteins known to interact directly with *Arabidopsis* DGAT1 (AtLPCAT2, AtPDAT1, AtPDCT) (Regmi et al. 2020). We included the dgat1-1 mutant because the mutant allele contains an exon 2 repeat insertion (an extra 81 bp = 27 residues) that produces a stable but nonfunctional protein (Lu et al. 2003). Our prey constructs also included a nonspecific phospholipase C (NPC6) (Ngo et al. 2018; Cai et al. 2020; Bose et al. 2021), which could be an interactor as its activity releases DAG that can be used for TAG synthesis by DGAT1. Further, we added the recently characterized *P. fendleri* TAG lipase like-1 (PfTAGL1) that localizes to lipid droplets and interacts with PfDGAT1 to induce TAG remodeling in *P. fendleri* (Parchuri et al. 2024). It should be noted that we tested if each DGAT1 could self- or hetero-oligomerize (with its partner DGAT1, or AtDGAT1, respectively).

Consistent with published data from Regmi et al. (2020), AtDGAT1 can interact with itself, LPCAT2, PDAT1, and PDCT (Supplementary Fig. S6A). Our screen revealed that AtDGAT1 interacts with the other DGAT1s, indicative of heterodimerization. Predictably, the other *Brassicaceae* DGAT1s, CsDGAT1 and PfDGAT1, interact with LPCAT2, PDAT1, and PDCT, while also interacting with themselves, respectively. Contrarily, the RcDGAT1 protein only interacted strongly with itself and PDAT1. Surprisingly, PfDGAT1 was the only DGAT1 capable of interacting with PfTAGL1, while none of DGAT1 enzyme fusions interacted with NPC6 (Fig. 7A, B; Supplementary Fig. S6). The prior outcome corroborates the finding that PfDGAT1 interacts with itself and PfTAGL1 (Parchuri et al. 2024). Most of the phenotypic data surrounding *dgat1-1* plants are ascribed to simple inactivation of DGAT1; however, lipid metabolic protein:protein interactions are critical for determining pathway flux. Therefore, we wanted to know whether this mutant protein could interact with the other proteins evaluated. Interestingly, the dgat1-1 protein interacted with itself, AtDGAT1 and CsDGAT1 while exhibiting a weak

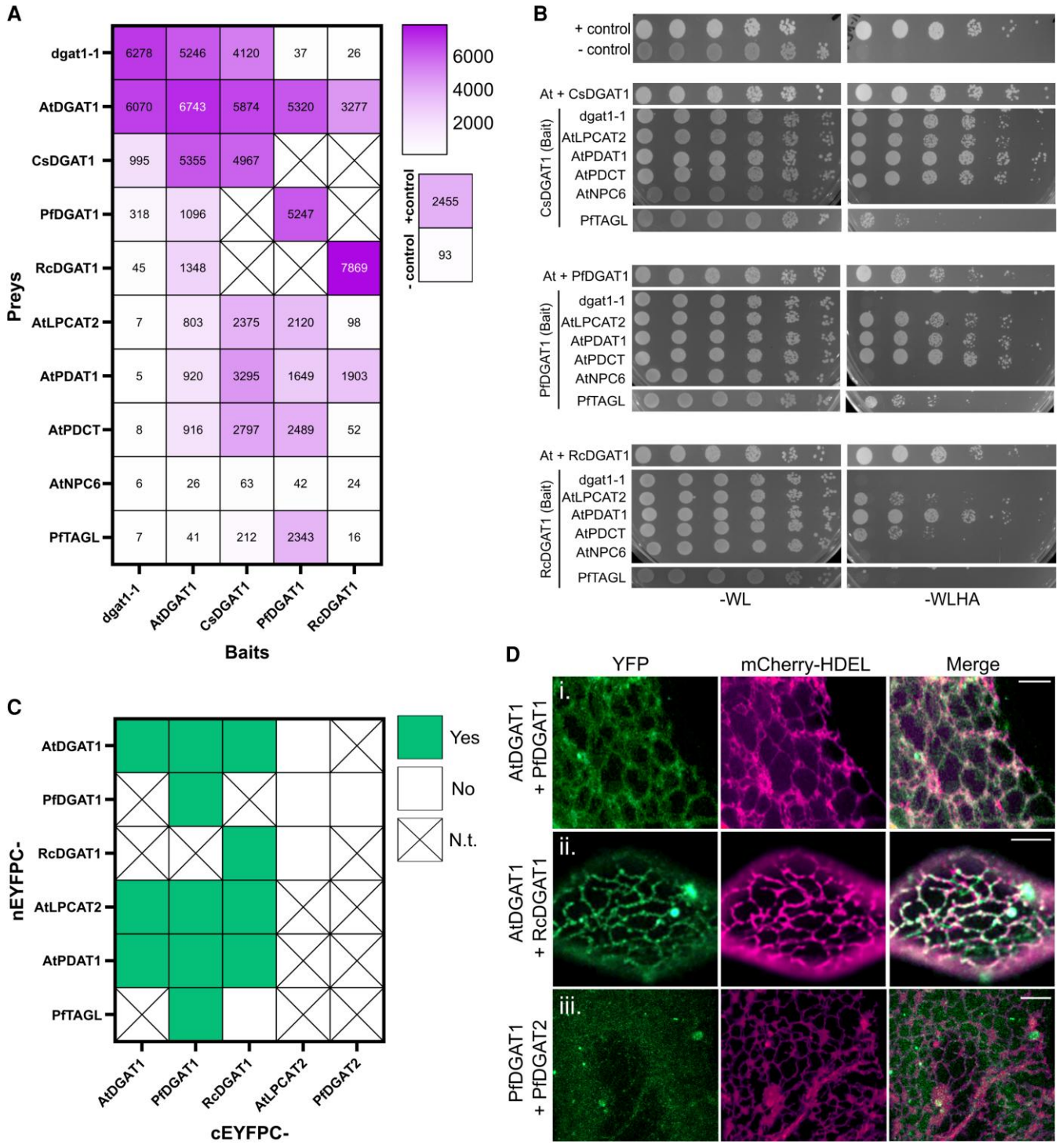


Figure 7. Protein:protein interactions of exogenous DGAT1s. **A)** Heat map indicating the presence or absence of protein:protein interactions derived from Y2H interaction tests from indicated bait and prey pairs. Semi-quantitative data are derived from β-galactosidase assays showing the relative strength of each interaction. **B)** Prototrophic growth of yeast containing the indicated baits with various prey on nonselective (SD-WL, left panel) and selective (SD-WLHA, right panel) media. Positive control consisted of bait pCCW-Alg5, while the prey was pAI-Alg5; negative controls contained pBT3-STE-AtDGAT1 with prey pDL2-Alg5. Only a subset of interaction plates is shown here; the rest are shown in Supplementary Fig. S6 **(C)** Heat map indicating *in planta* protein:protein interactions via YFP reconstitution in bimolecular fluorescence complementation assays in *N. benthamiana* leaf pavement cells. **D)** Confocal images of wild-type *N. benthamiana* leaves co-infiltrated with (i) nEYFPC-AtDGAT1 and cEYFPC-PfDGAT1; (ii) nEYFPC-AtDGAT1 and cEYFPC-RcDGAT1; (iii) the nEYFPC-PfDGAT1 and cEYFPC-PfDGAT2 pair was included as a negative control. Scale bars = 5 μm. All other confocal images are in Supplementary Figs. S7 and S8. Boxes marked with X indicate interactions not tested in **(A, C)**.

interaction with PfDGAT1 (in only one orientation). All other proteins tested (RcDGAT1, LPCAT2, PDAT1, PDCT, NPC6, PfTAGL1) failed to interact with dgat1-1 (Fig. 7A, B; Supplementary Fig. S6B).

Variation in topology, specific amino acids and structural folds likely cause alterations in the observed protein interactome, as predicted in Fig. 1. An excellent example is the observation that

PfTAGL1 only interacts strongly with PfDGAT1—even failing to interact with the closely related *Brassicaceae* DGAT1s. The predominant differences in DGAT1s can likely be attributed to the disordered *N*-termini (Fig. 1, C and D) and the varied charge distribution of each protein (Fig. 1E). Although minor when comparing the *Brassicaceae*, these differences are considerable enough to change their potential interactome. Additionally, we show that the dgat1-1 protein cannot interact with the endogenous interactome surrounding AtDGAT1 (Fig. 7A, B), and failure to interact with these endogenous oil biosynthetic components may add an additional layer of pleiotropic consequences when using the *dgat1-1* background.

As there were differences between the interactomes of the DGAT1 proteins tested here, we further evaluated some of the protein:protein interactions by bimolecular fluorescence complementation (BiFC) in *Nicotiana benthamiana* leaf pavement cells. We observed reconstituted YFP signal in the ER for AtDGAT1 co-infiltrated with itself, PfDGAT1, RcDGAT1, AtPDAT1, and AtLPCAT2; and for PfDGAT1 co-infiltrated with itself, AtPDAT1, PfTAGL1, and AtLPCAT2. Intriguingly, we observed reconstitution of YFP signal in the ER for RcDGAT1 co-infiltrated with itself, AtDGAT1, AtPDAT1, and AtLPCAT2 (Fig. 7C, D, Supplementary Figs. S7 and S8). Outcomes of the BiFC assays were consistent with the Y2H screen, apart from RcDGAT1-AtLPCAT2. However, it should be noted that these are 2 inherently different techniques, with one in yeast and the other from leaf samples collected ex vivo. It remains unclear if plant lipid metabolic protein:protein interactions are facilitated by other plant endogenous proteins not produced in the Y2H system. Altogether, we propose 3 models accounting for transcriptional, protein:protein interaction, and FA differences observed when each foreign *DGAT1* is expressed in *Arabidopsis* (Fig. 8).

Discussion

The loss of essential TAG biosynthetic enzymes can be restored by foreign enzymes in *Arabidopsis*

The present study demonstrates that it is possible to completely replace the final committed step of the TAG biosynthetic pathway, as driven by native DGAT1 or PDAT1, in an oilseed plant. This work validated the claim that the promoter and first intron of *Arabidopsis DGAT1* can be useful genetic tools for studying what occurs when endogenous TAG biosynthetic enzymes are removed and replaced by foreign enzymes with different substrate selectivities (McGuire et al. 2025). Additionally, as we demonstrate, when combined with genetic knockouts, proper expression may be a key bioengineering approach to modify seed oil FA compositions—by utilizing acyltransferases with unique FA selectivity. Thus, we hypothesize that a combination of alterations to transcripts and potential protein interactions is critical to completely rescue the *dgat1-1/pdat1-2* double mutant lethality. The changes of lipid biosynthetic genes (*AAPT2*, *DES6*, *LPCAT2*, *PDCT*, *PDAT1*) and protein interactions surrounding each foreign DGAT1 is summarized in Fig. 8. Firstly, the production of closest ortholog of AtDGAT1, CsDGAT1 (Fig. 1B), restores the FA compositions, transcriptional changes and protein:protein interactions to near wild-type allowing for complete recovery (Fig. 8A). Secondly, the further diverged PfDGAT1 (Fig. 1B) has minor FA and transcriptional modifications while maintaining protein interactions. Regardless, PfDGAT1 rescues the lethality (Fig. 8B). Finally, the highly diverged RcDGAT1 (Fig. 1B) significantly modifies the transcriptional landscape and uncouples some protein interactions. This leads to unique seed lipid compositions with increased PUFA relative to wild-type and increased VLCFA compared to *dgat1-1* seed (Fig. 3, Supplementary Fig. S5), and despite being able to produce TAG in seeds, it is incapable of rescuing the pollen lethality of the *dgat1-1/pdat1-2* double mutant (Fig. 8C). However, certain unknowns on how DGAT1s function within non-native environments remain. Mainly, which other factors affect the ability of different exogenous DGAT1s to restore the *dgat1-1/pdat1-2* lethal phenotype? It is not likely due to substrate FA selectivity alone, as all 3 foreign DGAT1s were clearly active when supplied with *Arabidopsis* substrates when produced in *dgat1-1* or in segregating populations of *dgat1-1/PDAT1/pdat1-2* (Figs. 2, 3, and 6, Supplementary Fig. S5).

First unknown: Does the host system transcriptional landscape need to be restored to phenotypically rescue double mutant lethality?

Recall that the *dgat1-1* mutant has a host of genetic adaptations that arise from loss of DGAT1 function (Aulakh and Durrett 2019). In the DGAT1 lines tested, both *Brassicaceae* proteins largely restored the genetic perturbations (at the transcriptional level), with CsDGAT1 conforming to wild-type (Fig. 4, blue shade) while PfDGAT1 had significant upregulation of *FAD3*, *DES6*, *LPCAT2*, *AAPT2*, and *PAH2*, and minor downregulation of *PDAT1* (Fig. 4, gold shade). However, even when regulated by the *Arabidopsis*-contextualized elements (promoter and first intron), the *Euphorbiaceae* RcDGAT1 failed to restore most genes to wild-type levels; in fact, these lines showed wholly different transcript levels for *MFT*, *FAD3*, *LPCAT2*, and *AAPT2* when compared to both wild-type and *dgat1-1* (Fig. 4, gray shade). Overall, we were able to ascertain that substrate utilization or metabolic bottlenecking (as driven by changes in lipid biosynthetic genes) is a possibility for describing how *RcDGAT1* is unable to restore the *dgat1-1* or *dgat1-1/pdat1-2* phenotypes. Therefore, the expression of *RcDGAT1* exerts pressure on the system that alters the transcriptional environment to better accommodate the biochemical and biophysical changes created by castor gene expression in *Arabidopsis*.

In vivo, native RcDGATs act predominantly as part of the Kennedy Pathway in developing castor seeds and immediately use de novo synthesized DAG to synthesize TAG (Bafor et al. 1991; Kroon et al. 2006). Thus, it is foreseeable that RcDGAT1 integrated into the *Arabidopsis* system (that uses mainly PC-derived DAG) relies on reverse LPCAT2 activity (Lager et al. 2013) to produce specific acyl-CoAs and DAG molecular species. Within the acyl editing cycle, the forward reaction catalyzed by LPCAT2 regenerates PC, which can be used by PDAT1, while the reverse action produces LPC and acyl-CoA (Bates and Shockey 2025). In our RcDGAT1 lines, *PDAT1* expression is significantly decreased while *LPCAT2* transcript is upregulated. This may indicate that in the RcDGAT1 expression lines, the reverse action of LPCAT2 is necessary to generate acyl-CoAs. Thus, altering the normal flux through the acyl-CoA independent pathway catalyzed by PDAT1, when DGAT1 is absent. The very high 18:3 content in RcDGAT1 lines (Fig. 3) along with the concomitant increase in *LPCAT2* transcript (Fig. 4) is consistent with reports that LPCAT2 has a stronger preference for PUFA-containing substrates relative to LPCAT1. LPCAT2 was also determined to be 5 times more active than LPCAT1; therefore, being the predominant catalytic isoform (Lager et al. 2013). Additionally, the ectopic expression of LPCAT1/2 in *Arabidopsis* enriched PUFA in seed oil (Wang et al. 2012). In yeast, the overexpression of both *LPCAT* and *DGAT1* leads to increased PUFA content in TAG (Pan et al. 2015). When combined with our lipid analysis from mature seed (Fig. 3, Supplementary Fig. S5), TAG digestions

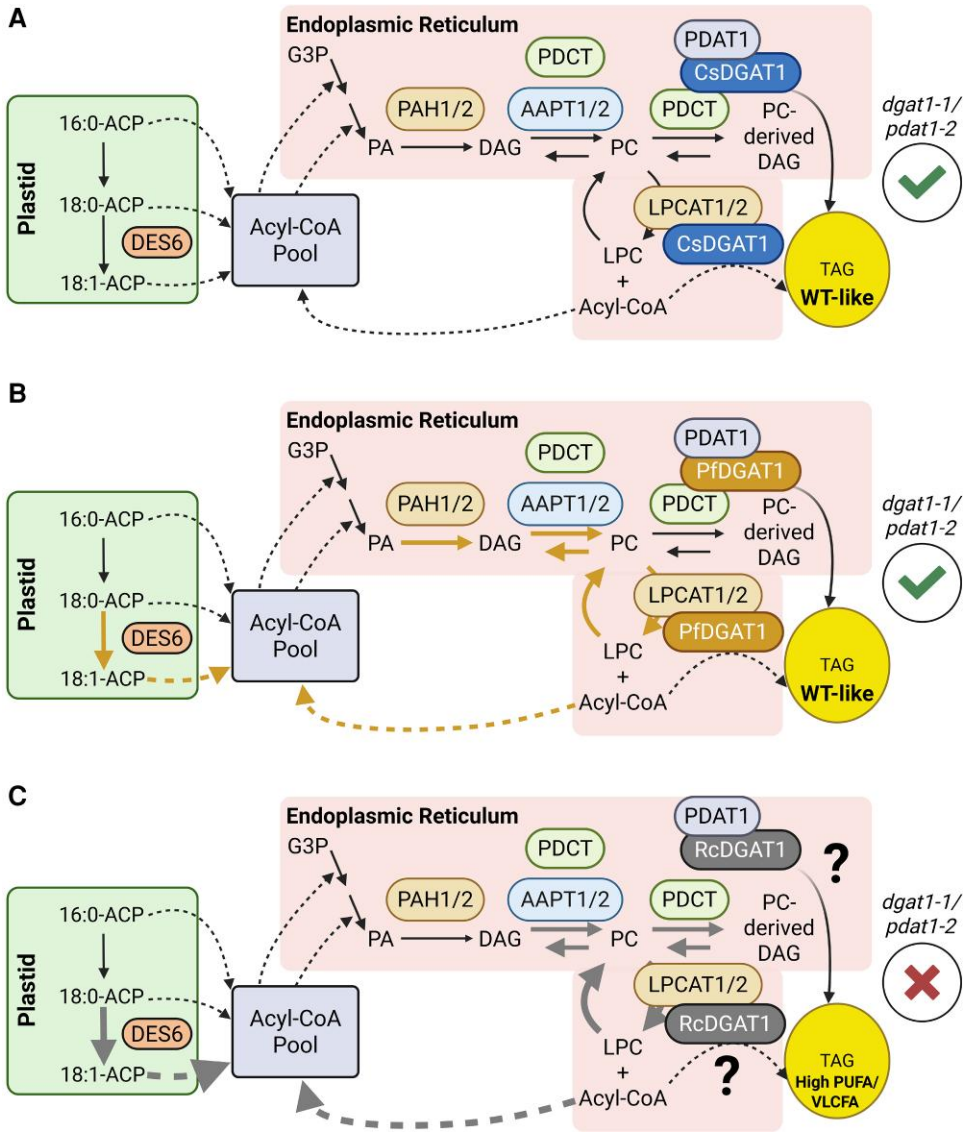


Figure 8. Model displaying the metabolic adaptations and interactions of each exogenous DGAT1. The model is constructed based on the transcriptional data, protein interaction, and oil composition results presented here and supplemented by findings from relevant past literature. All enzymes of note are colored and labeled. Arrows indicate major fluxes. Dotted lines indicate fluxes related to acyl-CoA substrates. The relative thickness and different color of arrows reflect the changes to the transcription levels of genes in relation to wild-type plants. **A)** *C. sativa* (Cs) *DGAT1* expression does not significantly alter transcription and rescues the *dgat1-1/pdat1-2* lethality. **B)** *P. fendleri* (Pf) *DGAT1* expression causes minor changes to the indicated genes (thickened arrows) but still enhances oil accumulation to allow for restoration of *dgat1-1/pdat1-2* lethality. **C)** *R. communis* (Rc) *DGAT1* expression significantly changes lipid metabolic gene expression (thickened arrows), modifies oil composition, and cannot rescue the *dgat1-1/pdat1-2* lethality. RcDGAT1 interacts with native PDAT1 and LPCAT2 but fails to interact with PDCT. Question marks indicate outstanding questions to whether RcDGAT1 utilizes PC-derived DAGs or the acyl-CoA generated by reverse LPCAT within the acyl editing cycle. Figure created with BioRender.

(Supplementary Fig. S2), and developing siliques (Supplementary Fig. S9), it is clear that DGAT1 serves as a central metabolic node and that metabolic adaptations may arise to accommodate introduced DGAT1 proteins and their substrate specificities, regardless of origin.

Second unknown: Is the functionality of exogenous DGAT1 dictated by protein:protein interactions or localization in the host system?

There is growing evidence that protein:protein interactions and possibly metabolons are important for efficient production of a diverse array of lipids in different plants (Busta et al. 2022). Most analyses of protein interactions in plant lipid biosynthetic pathways have focused on native-to-native interactions. A few of the species evaluated are Flax (*Linum usitatissimum*) (Xu et al. 2019, 2023), Avocado (*Persea americana*) (Behera et al. 2023), *P. fendleri* (Parchuri et al. 2024), and *A. thaliana* (Shockey et al. 2016; Regmi et al. 2020). However, the extent of exogenous proteins' ability to "puzzle-piece" into native systems remains largely unknown. When considering plant FA diversity (>450 plant FAs structures characterized to date (Ohlrogge et al. 2018)), it is plausible that chemotypic diversity allows for uncoupling of some protein interactions while essential evolutionary constraints cause others to remain intact. There is overwhelming evidence that stacking castor lipid biosynthetic genes alleviates growth penalties and enhances target oil accumulation (van Erp et al. 2011, 2015; Shockey et al. 2019; Lunn et al. 2021). Thus, our data add

to the literary evidence that different plant lineages may have disparate (or preferred) protein interactions—and different oil synthesis routes—that are evolutionarily linked to accumulate specialized seed oils, and by doing so fill an ecological niche. The inability of RcDGAT1 to interact with the same binding partners as the other *Brassicaceae* DGAT1s may indicate inefficient incorporation into the host that limits the ability of RcDGAT1 to fully restore the *dgat1-1/pdat1-2* mutant phenotypes.

We suggest that physical association of AtLPCAT2, AtPDCT, or AtPDAT1 with DGAT1 (exogenously or endogenously supplied) may help stabilize the protein in the endoplasmic reticulum for its physiological function—at least in *Arabidopsis*. In a *dgat1-1/pdat1-2* double mutant, AtDGAT1 or AtPDAT1 is unavailable to assist in stabilizing exogenous proteins at the sites of biochemical synthesis; thus, relying on other interactors to integrate correctly. It should be noted that although the dgat1-1 protein is nonfunctional, the modified topology of the mutant protein likely allows for the formation or dissociation of protein interactions. The addition of 27 residues near the first transmembrane domain possibly occludes the *N*-terminal residues required for interfacing with other MBOAT fold proteins, as suggested by Wang et al. (2020). Indeed, the dgat1-1 protein does not interact with the core metabolon units around DGAT1 (Supplementary Fig. S6B). As we suggest elsewhere (Regmi et al. 2020), there is a high probability that enzyme residency in a metabolon is necessary for efficient TAG biosynthesis. The evidence presented here shows the *dgat1-1* gene product ablates known protein interactions around the predicted TAG biosynthetic metabolon; thus, we advise researchers to carefully consider whether *dgat1-1* or other available mutants, such as *dgat1-2*, *dgat1-4*, or a CRISPR-based knockout, are more appropriate for their studies. The absence or alteration of these protein interactions may cause mislocalization or improper metabolon formation incompatible with proper contextualized function in *Arabidopsis*. Further, unique protein interactions with other components of lipid metabolism may occur when exogenous proteins are supplied; thus, the absence or presence of any single interaction may or may not determine if the foreign enzyme can fully restore a loss-of-function mutant. Future work will address this possibility.

The role of heterodimerization of exogenous DGAT1s with endogenous DGAT1 enzymes and the implications for function, substrate specificity, and stability of the complex have not been fully investigated. It has been suggested, by our lab and elsewhere, that DGAT1s and DGAT2 are biologically active as homodimers and homotetramers, and this active form is conserved across kingdoms (McFie et al. 2010; Caldo et al. 2017; Wang et al. 2020). While they catalyze similar acyl transfer reactions, DGAT1 and DGAT2 are dissimilar and share no sequence homology (Liu et al. 2012). Coupled with differences in structure, protein interactors, and enzyme substrate selectivity, these isoforms are known to occupy distinct suborganellar locations (Shockey et al. 2006; Regmi et al. 2020). Opposite to the observation by Regmi et al. (2020), where DGAT2s could not heterodimerize with AtDGAT1, our selected DGAT1s did heterodimerize with AtDGAT1 (Fig. 7). In fact, all the foreign DGAT1s tested could form homo- and hetero-dimers with themselves and AtDGAT1, respectively (Fig. 7, Supplementary Fig. S6C), indicating that a structural fold exists in the DGAT1 protein for higher order oligomerization. This has been attributed to a motif in the *N*-terminus (McFie et al. 2010; Caldo et al. 2017). It is also known that chimeric fusions of the *N*-terminus of one DGAT1 fused with the *C*-terminus of another can dramatically alter activity and dimerization state (Winichayakul et al. 2022). In the vein of plant biotechnology, it has been proposed that chimeras of both the acyl donor and acceptor sites in DGAT1 and DGAT2 may be modified to assist with accumulation of specialized oils (Jeppson et al. 2020). This strategy appears sound, but our data surrounding RcDGAT1 reveal that there may be insufficient protein:protein interactions, suggesting that there is a minimum number of native protein interactions that must also remain intact to remove overlapping or competing endogenous enzyme activities.

Thus, heterodimerization of acyltransferases (DGAT1 or otherwise) is not fully appreciated, and assembly of a heterogeneous complex (within a transgenic system where an ortholog is co-expressed with the native protein) may have unpredictable consequences and can alter activity, organellar localization, and/or complex stability. Altogether, to accumulate target oils, it is important to consider how a chimeric protein could be designed to (i) integrate with the endogenous protein interactome; and (ii) have broad specificity during normal plant development, but high specificity during seed oil deposition.

Utility of the promoter and first intron of *AtDGAT1* for bioengineering

The rationale to design discrete gene modules in a variety of organisms (plants, yeast, bacteria; Denby et al. 2018) to fine-tune metabolic outputs continues to gain traction and support. Here, we leveraged the sophisticated natural framework to validate a minimal engineerable unit to influence oil accumulation in *Arabidopsis*. The chimeric genetic constructs in our study demonstrate that the *Arabidopsis* promoter and first intron of *AtDGAT1* are useful tools for replacing the native TAG-producing acyltransferases with different *DGAT1*s. We propose that the spatial arrangement of these cis-regulatory elements may have evolved to target the expression of *DGAT1* to different tissues. Not only in *Arabidopsis* but also in other oilseed species, as some of the regulatory repeat elements in the first introns appear to be highly conserved across lineages (McGuire et al. 2025). Further, this work establishes that in designing DGAT1 chimeric proteins or expression cassettes a few important design considerations exist (i) phylogenetic proximity appears to play a role in determining how an enzyme can function in *Arabidopsis*; (ii) DAG-pool composition and/or localization may dictate utilization by foreign enzymes; and (iii) alternative placements of the intron remain to be investigated. All constructs tested have the exon 1–exon 2 junction split by the *Arabidopsis* first intron (84 bp). The exon 1 lengths and sequences relative to the first intron are slightly different between each construct (Supplementary Fig. S1A). In yeast, the 5′ splice site (5′ss) can be regulated by the combined influence of the last 3 nucleotides of the flanking exon and the first 6 nucleotides of the intronic region (Hümmer et al. 2021). Interestingly, the first exon 5′ss of *R. communis DGAT1* is 5′-CAA-3′ compared to the conserved 5′-CAG-3′ sequence found in 32 different plant *DGAT1*s (Supplementary Fig. S1B). *R. communis* is a member of the order *Malphighiales*, which also contains a highly conserved 5′-CAA-3′ sequence at the end of the first exon. Therefore, one possibility for the incompatibility between the *Brassicaceae* and castor is that the single nucleotide difference at the 5′ss alters transgene expression in different tissues, presumably with pollen being the most important, as corroborated by the 1:1:0 segregation ratios of both *PDAT1/pdat1-2* and *DGAT1/dgat1-1* loci in *RcDGAT1* expressing plants (Fig. 5).

The finding that exogenous *DGAT1* from *Brassicaceae* (*C. sativa* and *P. fendleri*) can rescue the *dgat1-1/pdat1-2* double mutant lethality when contextualized by the promoter and first intron of

AtDGAT1 not only confirms this minimal expression unit for proper expression but also demonstrates replacement of oil biosynthetic enzymes. The current study also reveals that distantly related oilseed lineages may be unable to completely restore the metabolic adaptations present in the double mutant. By elucidating how *Arabidopsis* processes exogenous *DGAT1*s and by thoroughly understanding the protein interactions, transcriptional regulation, and enzyme selectivities surrounding TAG biosynthesis (particularly in regard to unusual FA accumulators), it may be possible to engineer plants for enhanced oil production in the absence of endogenous TAG biosynthetic enzymes. Whether foreign DGAT1 enzymes will be able to wholly integrate into the system or require an unknown interactor, co-factor, or substrate remains an intriguing area of study for future investigations.

Materials and methods

Plant expression plasmid construction

Binary expression plasmids containing the recently described *AtDGAT1* promoter (McGuire et al. 2025) were used. Modified ORFs corresponding to *CsDGAT1* (CsSvalof.19G288700.1.p), *PfDGAT1* (PP588719.1), and *RcDGAT1* (29912.t000099) containing the first intron of *AtDGAT1* were designed as synthetic DNA blocks and purchased commercially (Integrated DNA Technologies, Coralville, Iowa, United States). In each case, the 84-bp *AtDGAT1* intron 1 sequence was placed in the exon 1–exon 2 splice site of the respective protein-coding sequences, thus mimicking the native *AtDGAT1* sequence. This location of intron 1 is well-conserved in the coding sequences of native *CsDGAT1*, *PfDGAT1*, and *RcDGAT1* (Supplementary Fig. S1), suggesting an important role for this element due to maintenance of this spatial arrangement over a substantial length of evolutionary time.

Foreign DGAT1 cloning

Synthetic gene products containing *AtDGAT1* intron 1 were digested and ligated into the *NcoI* and *SacII* sites of the AtDGAT1promoter/glycinin terminator plasmid vector described in McGuire et al. (2025) and sequence confirmed for accuracy. Promoter:gene:terminator cassettes were released with *AscI* and cloned into the *AscI* site of the DsRed-selectable plant binary vector pB110 (Shockey et al. 2015) and sequenced.

Transgenic plant production

Arabidopsis dgat1-1 plant lines were transformed with foreign DGAT1 binary plasmids carried by *Agrobacterium tumefaciens* strain GV3101, using the floral dip method (Clough and Bent 1998). T_1 seeds exhibiting DsRed fluorescence were selected and directly sown in soil. To obtain transgenic lines that only contained the foreign DGAT1 (CsDGAT1, PfDGAT1, RcDGAT1) without *AtDGAT1/AtPDAT1*, crosses between *dgat1-1/CsDGAT1*, *dgat1-1/PfDGAT1*, *dgat1-1/RcDGAT1* as the male donor anthers to *DGAT1/DGAT1//PDAT1/pdat1-2* female acceptor stigma were performed.

Genotyping complemented lines

Genomic DNA was isolated, and complementation assaying of a *dgat1-1/dgat1-1//pdat1-2/pdat1-2* double mutant was determined by amplification with allele-specific primers as described in Supplementary Table S1, Supplementary Fig. S4, and McGuire et al. (2025). Chi-squared (χ^2) tests were performed to determine significance between observed and expected allelic ratios.

Plant growth conditions

Seeds were cold-stratified at 4 °C for 48 h prior to being sown in soil in 3.5″ pots. Homozygous lines for lipid analysis were grown in a 20 to 23 °C growth chamber with a 24-h light regime (~120 to 130 µmol·photon·m^{-2}).

Lipid analysis

Segregating T_2 seeds was analyzed by separation under green light with a red filter, from which 20 red (transformed) and 20 brown (untransformed) seeds were selected. For subsequent analysis of homozygous lines (T_3 or later generations), 2 to 4 mg of seed was weighed. For oil analysis, the composition of seed TAG was analyzed by a gas chromatography method described previously (Garneau et al. 2025; McGuire et al. 2025).

From mature seed, lipids were separated by a single-development TLC system. Development solvent was hexane:diethyl ether:acetic acid (70:30:1.5, v/v/v). Visualization of bands was done under UV light after staining with 0.005% primuline in acetone:water (80:20, v/v). TAG and polar lipids were collected and eluted from the silica as described (Bates et al. 2009). TAG regiochemistry was determined by TAG lipase digestion, slightly modified from Cahoon et al. (2006), with *Aspergillus oryzae* lipase (Sigma, catalog no. L0777). Digestion products were resolved by TLC in a single-development system with hexane:diethyl ether:acetic acid (35:70:1.5, v/v/v). Lipase digestion products corresponding to the stereochemical positions of TAG: free fatty acid (FFA) (*sn*-1, 3), DAG (*sn*-1,2 and *sn*-2,3), and monoacylglycerol (MAG) (*sn*-2) bands were collected. FA analysis was performed as described above.

Neutral lipid accumulation time course, siliques aged 8, 12, 16 DAF were harvested and lipids were extracted (Hara and Radin 1978). Total lipids were quantified with the above GC method. To quantify TAG content across development, lipid classes were separated by a single-development TLC system as above. TAG was collected and derivatized into fatty acid methyl esters (FAMEs) directly from the silica and analyzed. All TLC systems contained 0.005% butylated hydroxytoluene antioxidant. Lipid classes were identified by separation alongside lipid standards.

RNA isolation and RT-qPCR

Total RNA was isolated from developing seeds using TriZol reagent, treated with RNase-free DNase (Zymo) and cleaned and concentrated by RNA Clean and Concentrator (Zymo). Complementary DNA was synthesized from 1 µg total RNA using the iScript cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's instructions. Relative mRNA levels were quantified with Power SYBR Green PCR Master Mix (Applied Biosystems) on a CFX-96 real-time PCR system (Bio-Rad) and normalized to wild-type expression using the $2^{-\Delta\Delta Ct}$ method with the reference genes *ASAR1* or *PP2AA3*. ≥3 biological replicates were analyzed for each plant line. Specific products were determined by melt-curve analysis and agarose gel electrophoresis. The primers used for RT-qPCR are described in Supplementary Table S1.

Bioinformatics

Multiple sequence alignments of DGAT1 proteins were generated by the MUSCLE algorithm (Edgar 2004) using default parameters. The Neighbor-joining phylogenetic tree was generated with Molecular Evolutionary Genetics Analysis (MEGA11) (Tamura et al. 2021) with default settings and 1,000 bootstrap replications. The AlphaFold3 (Abramson et al. 2024) predicted structure of a single AtDGAT1 protomer (UniProt: Q9SLD2), based on human

DGAT1 (UniProt: O75907), was used to map DGAT1 protein structure conservation with ConSurf (Yariv et al. 2023). Structural alignments, visualization, and electrostatic distribution mapping (with default constraints) were done in ChimeraX (Meng et al. 2023). WebLogo was used to create sequence logos of different DGAT1 exon 1 3′ ends (Crooks et al. 2004).

Cloning of split-ubiquitin Y2H plasmids and protein:protein interaction analyses

Y2H cloning and assays were performed with the DUALmembrane kit 3 (Dualsystems Biotech) following the manufacturer's instructions. Briefly, each plasmid was constructed by *Sfi*I restriction cloning of each gene into the vector pBT3-STE for AtDGAT1 and CsDGAT1, while PfDGAT1 and RcDGAT1 were added to the pBT3-C bait vector. All genes in bait constructs are *C*-terminal fusions of the GOI with a *C*-terminal half of ubiquitin/LexA transcription factor fusion (Cub-LexA). RcDGAT1 was codon optimized for enhanced protein expression in *Saccharomyces cerevisiae*. Prey plasmids were made in a similar manner (with cloning into pPR3-N), where each construct is an *N*-terminal fusion of the GOI with an *N*-terminal half of ubiquitin and an HA epitope.

For protein interaction testing, *S. cerevisiae* strain NMY51 was cotransformed with bait and prey combinations and plated on permissive synthetic dextrose media lacking tryptophan and leucine (SD-WL) and incubated at 30 °C for 2 to 3 d. Liquid overnight cultures in SD-WL were grown, normalized to an $OD_{660} = 0.5$, and subjected to serial 5-fold dilutions and plated on both permissive, SD-WL, and selective media lacking tryptophan, leucine, histidine, and adenine (SD-WLHA). Each protein interaction was also semi-quantitatively measured using cells normalized to $OD_{660} = 0.5$ in a β-galactosidase assay, measured in Miller Units using a 96-well plate system (Yeast beta-galactosidase assay kit, Thermo Scientific).

Vector construction for bimolecular fluorescence complementation

RcDGAT1, *AtLPCAT2*, and *AtPDAT1* were PCR amplified from existing constructs with specific primers to add *attB* sites (see Supplementary Table S1) for GATEWAY cloning into the pDONR207 entry vector. Next, destination vectors for BiFC were made by LR cloning into pSITE vectors. *AtDGAT1*, *RcDGAT1*, *AtLPCAT2*, and *AtPDAT1* were cloned into nYFPC1-pSITE vector containing the *N*-terminal part of YFP at the *N*-terminus of each protein and into cYFPC1-pSITE that has the *C*-terminal part of YFP tagged at the *N*-terminus of each protein. BiFC vectors containing *AtDGAT1*, *PfDGAT1*, *PfDGAT2*, and *PfTAGL1* were constructed previously in our lab (Parchuri et al. 2024).

Transient transfection assays and imaging

Wild-type *N. benthamiana* seedlings were grown in a growth chamber with ~100 µmol·m^{-2}·s^{-1} and 16 h day and 8 h night cycle. For BiFC assays, overnight culture of Agrobacterium harboring constructs with nYFPC-P1 (Protein 1), cYFPC-P2 (Protein 2), mCherry-HDEL, and viral silencing suppressor vectors p19 were resuspended in infiltration media [10 mM MES, pH 5.6, 10 mM $MgCl_2 \cdot 6H_2O$, 200 µM Acetosyringone] and incubated at room temperature, in the dark for at least 3 h. Bacterial cultures with the BiFC constructs were adjusted to $OD_{600} = 0.6$, while the density of cultures with mCherry-HDEL or p19 OD_{600} was unchanged. Bacterial cultures were mixed at 1:1:0.5:0.5 volume ratios. Leaves of 4 to 5-week-old plants were infiltrated with these mixtures and prepared for imaging after 3 to 5 d. Imaging was performed on a Zeiss LSM 980 Confocal (Zeiss) microscope with a 63×, NA1.4 oil immersion objective. YFP fluorescence was excited at 488 nm, and emitted light was collected with a 492 to 546 nm filter. To confirm ER localization, mCherry-HDEL was excited at 553 nm, and emitted light was collected with a 542 to 636 nm filter.

Accession numbers

Sequence data from this article can be found in the GenBank/EMBL data libraries under the accession numbers found in Supplementary Table S2 or in Fig. 1B.

Acknowledgments

We would like to thank Dr. Nicholas Neumann for supplying BiFC plasmid backbones for some of the genes used in this study.

Author contributions

P.D.B. and J.S. conceived the project and procured the funding. P.D.B. agrees to serve as the author responsible for contact and ensures communication. S.T.M., J.S., A.S., and P.D.B. designed experiments and analyzed the data. S.T.M., A.R., and J.S. performed the experiments. All authors contributed to writing the article and approved the submitted version.

Supplementary data

The following materials are available in the online version of this article.

Supplementary Figure S1. Transgenic construct designs and 5′ splice site among 32 plant DGAT1s.

Supplementary Figure S2. TAG regiochemistry of exogenous DGAT1 expressing dgat1-1 complemented lines.

Supplementary Figure S3. Native and exogenous DGAT1 gene expression.

Supplementary Figure S4. Representative genotyping of exogenous DGAT1 segregating populations of the DGAT1/dgat1-1 and PDAT1/pdat1-2 loci.

Supplementary Figure S5. Fatty acid compositions of exogenous DGAT1 plants segregating the PDAT1/pdat1-2 locus in the dgat1-1 background.

Supplementary Figure S6. Y2H serial dilution assays on permissive (-WL) and selective (-WLHA) media.

Supplementary Figures S7. Bimolecular fluorescence complementation assays of endoplasmic reticulum localized oil biosynthetic acyltransferases.

Supplementary Figures S8. Bimolecular fluorescence complementation assays of endoplasmic reticulum localized lipid biosynthetic proteins.

Supplementary Figure S9. Oil accumulation across silique development in exogenous DGAT1 expressing lines in the dgat1-1 background.

Supplementary Table S1. Primers used during this study. AscI sites are underlined.

Supplementary Table S2. Gene accession IDs.

Funding

This work is supported by the Agriculture and Food Research Initiative Competitive Grants Program Foundational and Applied Science Program, project award no. 2023-67013-39022, from the U.S. Department of Agriculture's National Institute of Food and

Agriculture. In addition, this work was supported by the Hatch Project #1015621 and Multi-State Project #1013013 from the U.S. Department of Agriculture. This material is based upon work supported by the National Science Foundation under Award No. NSF-MCB 2242822; and the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, under award number DE-SC0023142.

Conflict of interest statement. None declared.

Data availability

The data that support the findings of this study are available in the main text and the Supplementary material of this article. The transgenic lines produced for this manuscript can be made available after publication and upon request to the corresponding author.

References

Abramson J, Adler J, Dunger J, Evans R, Green T, Pritzel A, Ronneberger O, Willmore L, Ballard AJ, Bambrick J, *et al*. Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature*. 2024:630(8016):493–500. https://doi.org/10.1038/s41586-024-07487-w

Alkotami L, Kornacki C, Campbell S, McIntosh G, Wilson C, Tran TNT, Durrett TP. Expression of a high-activity diacylglycerol acetyltransferase results in enhanced synthesis of acetyl-TAG in camelina seed oil. *Plant J*. 2021:106(4):953–964. https://doi.org/10.1111/tpj.15210

Alkotami L, White DJ, Schuler KM, Esfahanian M, Jarvis BA, Paulson AE, Koley S, Kang J, Lu C, Allen DK, *et al*. Targeted engineering of camelina and pennycress seeds for ultrahigh accumulation of acetyl-TAG. *Proc Natl Acad Sci U S A*. 2024:121(47):e2412542121. https://doi.org/10.1073/pnas.2412542121

Aulakh K, Durrett TP. The plastid lipase PLIP1 is critical for seed viability in diacylglycerol acyltransferase1 mutant seed. *Plant Physiol*. 2019:180(4):1962–1974. https://doi.org/10.1104/pp.19.00600

Bafor M, Smith MA, Jonsson L, Stobart K, Stymne S. Ricinoleic acid biosynthesis and triacylglycerol assembly in microsomal preparations from developing castor-bean (Ricinus communis) endosperm. *Biochem J* 1991:280(Pt 2):507–514. https://doi.org/10.1042/bj2800507

Bates PD, Browse J. The pathway of triacylglycerol synthesis through phosphatidylcholine in Arabidopsis produces a bottleneck for the accumulation of unusual fatty acids in transgenic seeds. *Plant J*. 2011:68(3):387–399. https://doi.org/10.1111/j.1365-313X.2011.04693.x

Bates PD, Durrett TP, Ohlrogge JB, Pollard M. Analysis of acyl fluxes through multiple pathways of triacylglycerol synthesis in developing soybean embryos. *Plant Physiol*. 2009:150(1):55–72. https://doi.org/10.1104/pp.109.137737

Bates PD, Fatihi A, Snapp AR, Carlsson AS, Browse J, Lu C. Acyl editing and headgroup exchange are the major mechanisms that direct polyunsaturated fatty acid flux into triacylglycerols. *Plant Physiol*. 2012:160(3):1530–1539. https://doi.org/10.1104/pp.112.204438

Bates PD, Shockey J. Towards rational control of seed oil composition: dissecting cellular organization and flux control of lipid metabolism. *Plant Physiol*. 2025:197(2):kiae658. https://doi.org/10.1093/plphys/kiae658

Behera J, Rahman MM, Shockey J, Kilaru A. Acyl-CoA-dependent and acyl-CoA-independent avocado acyltransferases positively influence oleic acid content in nonseed triacylglycerols. *Front Plant Sci*. 2023:13:1056582. https://doi.org/10.3389/fpls.2022.1056582

Bhandari S, Bates PD. Triacylglycerol remodeling in Physaria fendleri indicates oil accumulation is dynamic and not a metabolic endpoint. *Plant Physiol*. 2021:187(2):799–815. https://doi.org/10.1093/plphys/kiab294

Bose D, Ngo AH, Nguyen VC, Nakamura Y. Non-specific phospholipases C2 and C6 redundantly function in pollen tube growth via triacylglycerol production in Arabidopsis. *Plant J*. 2021:106(2):409–418. https://doi.org/10.1111/tpj.15172

Busta L, Chapman KD, Cahoon EB. Better together: protein partnerships for lineage-specific oil accumulation. *Curr Opin Plant Biol*. 2022:66:102191. https://doi.org/10.1016/j.pbi.2022.102191

Cahoon EB, Dietrich CR, Meyer K, Damude HG, Dyer JM, Kinney AJ. Conjugated fatty acids accumulate to high levels in phospholipids of metabolically engineered soybean and Arabidopsis seeds. *Phytochemistry*. 2006:67(12):1166–1176. https://doi.org/10.1016/j.phytochem.2006.04.013

Cai G, Fan C, Liu S, Yang Q, Liu D, Wu J, Li J, Zhou Y, Guo L, Wang X. Nonspecific phospholipase C6 increases seed oil production in oilseed Brassicaceae plants. *New Phytol*. 2020:226(4):1055–1073. https://doi.org/10.1111/nph.16473

Caldo KMP, Acedo JZ, Panigrahi R, Vederas JC, Weselake RJ, Lemieux MJ. Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. *Plant Physiol*. 2017:175(2):667–680. https://doi.org/10.1104/pp.17.00934

Carlsson AS, Yilmaz JL, Green AG, Stymne S, Hofvander P. Replacing fossil oil with fresh oil—with what and for what? *Eur J Lipid Sci Technol*. 2011:113(7):812–831. https://doi.org/10.1002/ejlt.201100032

Chen G, Harwood JL, Lemieux MJ, Stone SJ, Weselake RJ. Acyl-CoA:diacylglycerol acyltransferase: properties, physiological roles, metabolic engineering and intentional control. *Prog Lipid Res*. 2022:88:101181. https://doi.org/10.1016/j.plipres.2022.101181

Ciastowicz Ż, Pamuła R, Białowiec A. Utilization of plant oils for sustainable polyurethane adhesives: a review. *Materials (Basel)*. 2024:17(8):1738. https://doi.org/10.3390/ma17081738

Clough SJ, Bent AF. Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. *Plant J*. 1998:16(6):735–743. https://doi.org/10.1046/j.1365-313x.1998.00343.x

Coleman RA. It takes a village: channeling fatty acid metabolism and triacylglycerol formation via protein interactomes. *J Lipid Res*. 2019:60(3):490–497. https://doi.org/10.1194/jlr.S091843

Crooks GE, Hon G, Chandonia JM, Brenner SE. WebLogo: a sequence logo generator. *Genome Res*. 2004:14(6):1188–1190. https://doi.org/10.1101/gr.849004

Denby CM, Li RA, Vu VT, Costello Z, Lin W, Chan LJG, Williams J, Donaldson B, Bamforth CW, Petzold CJ, *et al*. Industrial brewing yeast engineered for the production of primary flavor determinants in hopped beer. *Nat Commun*. 2018:9(1):965. https://doi.org/10.1038/s41467-018-03293-x

Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res*. 2004:32(5):1792–1797. https://doi.org/10.1093/nar/gkh340

Garneau MG, Parchuri P, Zander N, Bates PD. Rapid quantification of whole seed fatty acid amount, composition, and shape phenotypes from diverse oilseed species with large differences in seed size. *Plant Methods*. 2025:21(1):67. https://doi.org/10.1186/s13007-025-01388-3

Gidda SK, Shockey JM, Rothstein SJ, Dyer JM, Mullen RT. Arabidopsis thaliana GPAT8 and GPAT9 are localized to the ER and possess distinct ER retrieval signals: functional divergence of the dilysine

ER retrieval motif in plant cells. *Plant Physiol Biochem*. 2009:47(10):867–879. https://doi.org/10.1016/j.plaphy.2009.05.008

Gishini MFS, Kachroo P, Hildebrand D. Fatty acid desaturase 3-mediated α-linolenic acid biosynthesis in plants. *Plant Physiol*. 2025:197(2):kiaf012. https://doi.org/10.1093/plphys/kiaf012

Goode JH, Dewey RE. Characterization of aminoalcoholphosphotransferases from Arabidopsis thaliana and soybean. *Plant Physiol Biochem*. 1999:37(6):445–457. https://doi.org/10.1016/S0981-9428(99)80049-7

Hara A, Radin NS. Lipid extraction of tissues with a low-toxicity solvent. *Anal Biochem*. 1978:90(1):420–426. https://doi.org/10.1016/0003-2697(78)90046-5

Hümmer S, Borao S, Guerra-Moreno A, Cozzuto L, Hidalgo E, Ayté J. Cross talk between the upstream exon-intron junction and Prp2 facilitates splicing of non-consensus introns. *Cell Rep*. 2021:37(4):109893. https://doi.org/10.1016/j.celrep.2021.109893

Jeppson S, Mattisson H, Demski K, Lager I. A predicted transmembrane region in plant diacylglycerol acyltransferase 2 regulates specificity toward very-long-chain acyl-CoAs. *J Biol Chem*. 2020:295(45):15398–15406. https://doi.org/10.1074/jbc.RA120.013755

Karki N, Johnson BS, Bates PD. Metabolically distinct pools of phosphatidylcholine are involved in trafficking of fatty acids out of and into the chloroplast for membrane production. *Plant Cell*. 2019:31(11):2768–2788. https://doi.org/10.1105/tpc.19.00121

Katavic V, Reed DW, Taylor DC, Giblin EM, Barton DL, Zou J, Mackenzie SL, Covello PS, Kunst L. Alteration of seed fatty acid composition by an ethyl methanesulfonate-induced mutation in Arabidopsis thaliana affecting diacylglycerol acyltransferase activity. *Plant Physiol*. 1995:108(1):399–409. https://doi.org/10.1104/pp.108.1.399

Kazaz S, Barthole G, Domergue F, Ettaki H, To A, Vasselon D, De Vos D, Belcram K, Lepiniec L, Baud S. Differential activation of partially redundant Δ9 stearoyl-ACP desaturase genes is critical for Omega-9 monounsaturated fatty acid biosynthesis during seed development in Arabidopsis. *Plant Cell*. 2020:32(11):3613–3637. https://doi.org/10.1105/tpc.20.00554

Kong Y, Chen S, Yang Y, An C. ABA-insensitive (ABI) 4 and ABI5 synergistically regulate DGAT1 expression in Arabidopsis seedlings under stress. *FEBS Lett*. 2013:587(18):3076–3082. https://doi.org/10.1016/j.febslet.2013.07.045

Kroon JTM, Wei W, Simon WJ, Slabas AR. Identification and functional expression of a type 2 acyl-CoA:diacylglycerol acyltransferase (DGAT2) in developing castor bean seeds which has high homology to the major triglyceride biosynthetic enzyme of fungi and animals. *Phytochemistry*. 2006:67(23):2541–2549. https://doi.org/10.1016/j.phytochem.2006.09.020

Lager I, Jeppson S, Gippert A-L, Feussner I, Stymne S, Marmon S. Acyltransferases regulate oil quality in Camelina sativa through both acyl donor and acyl acceptor specificities. *Front Plant Sci*. 2020:11:1144. https://doi.org/10.3389/fpls.2020.01144

Lager I, Yilmaz JL, Zhou X-R, Jasieniecka K, Kazachkov M, Wang P, Zou J, Weselake R, Smith MA, Bayon S, *et al*. Plant acyl-CoA:lysophosphatidylcholine acyltransferases (LPCATs) have different specificities in their forward and reverse reactions. *J Biol Chem*. 2013:288(52):36902–36914. https://doi.org/10.1074/jbc.M113.521815

Lee K-R, Yeo Y, Lee J, Kim S, Im C, Kim I, Lee J, Lee S-K, Suh MC, Kim HU. Functional characterization of the effects of CsDGAT1 and CsDGAT2 on fatty acid composition in Camelina sativa. *Int J Mol Sci*. 2024:25(13):6944. https://doi.org/10.3390/ijms25136944

Liu Q, Siloto RMP, Lehner R, Stone SJ, Weselake RJ. Acyl-CoA:diacylglycerol acyltransferase: molecular biology, biochemistry and biotechnology. *Prog Lipid Res*. 2012:51(4):350–377. https://doi.org/10.1016/j.plipres.2012.06.001

Lu C, Xin Z, Ren Z, Miquel M, Browse J. An enzyme regulating triacylglycerol composition is encoded by the ROD1 gene of Arabidopsis. *Proc Natl Acad Sci U S A*. 2009:106(44):18837–18842. https://doi.org/10.1073/pnas.0908848106

Lu CL, de Noyer SB, Hobbs DH, Kang J, Wen Y, Krachtus D, Hills MJ. Expression pattern of diacylglycerol acyltransferase-1, an enzyme involved in triacylglycerol biosynthesis, in Arabidopsis thaliana. *Plant Mol Biol*. 2003:52(1):31–41. https://doi.org/10.1023/A:1023935605864

Lunn D, Wallis JG, Browse J. A multigene approach secures hydroxy fatty acid production in Arabidopsis. *J Exp Bot*. 2021:73(9):2875–2888. https://doi.org/10.1093/jxb/erab533

Maatta S, Scheu B, Roth MR, Tamura P, Li M, Williams TD, Wang X, Welti R. Levels of Arabidopsis thaliana leaf phosphatidic acids, phosphatidylserines, and most trienoate-containing polar lipid molecular Species increase during the dark period of the diurnal cycle. *Front Plant Sci*. 2012:3:49. https://doi.org/10.3389/fpls.2012.00049

McFie PJ, Stone SL, Banman SL, Stone SJ. Topological orientation of acyl-CoA:diacylglycerol acyltransferase-1 (DGAT1) and identification of a putative active site histidine and the role of the n terminus in dimer/tetramer formation. *J Biol Chem*. 2010:285(48):37377–37387. https://doi.org/10.1074/jbc.M110.163691

McGuire ST, Shockey J, Bates PD. The first intron and promoter of Arabidopsis DIACYLGLYCEROL ACYLTRANSFERASE 1 exert synergistic effects on pollen and embryo lipid accumulation. *New Phytol*. 2025:245(1):263–281. https://doi.org/10.1111/nph.20244

Meng EC, Goddard TD, Pettersen EF, Couch GS, Pearson ZJ, Morris JH, Ferrin TE. UCSF ChimeraX: tools for structure building and analysis. *Protein Sci*. 2023:32(11):e4792. https://doi.org/10.1002/pro.4792

Neumann N, Harman M, Kuhlman A, Durrett TP. Arabidopsis diacylglycerol acyltransferase1 mutants require fatty acid desaturation for normal seed development. *Plant J*. 2024:119(2):916–926. https://doi.org/10.1111/tpj.16805

Ngo AH, Lin YC, Liu YC, Gutbrod K, Peisker H, Dörmann P, Nakamura Y. A pair of nonspecific phospholipases C, NPC2 and NPC6, are involved in gametophyte development and glycerolipid metabolism in Arabidopsis. *New Phytol*. 2018:219(1):163–175. https://doi.org/10.1111/nph.15147

Ohlrogge J, Thrower N, Mhaske V, Stymne S, Baxter M, Yang W, Liu J, Shaw K, Shorrosh B, Zhang M, *et al*. PlantFAdb: a resource for exploring hundreds of plant fatty acid structures synthesized by thousands of plants and their phylogenetic relationships. *Plant J*. 2018:96(6):1299–1308. https://doi.org/10.1111/tpj.14102

Okuley J, Lightner J, Feldmann K, Yadav N, Lark E, Browse J. Arabidopsis FAD2 gene encodes the enzyme that is essential for polyunsaturated lipid synthesis. *Plant Cell*. 1994:6(1):147–158. https://doi.org/10.1105/tpc.6.1.147

Pan X, Chen G, Kazachkov M, Greer MS, Caldo KMP, Zou J, Weselake RJ. In vivo and in vitro evidence for biochemical coupling of reactions catalyzed by lysophosphatidylcholine acyltransferase and diacylglycerol acyltransferase. *J Biol Chem*. 2015:290(29):18068–18078. https://doi.org/10.1074/jbc.M115.654798

Parchuri P, Bhandari S, Azeez A, Chen G, Johnson K, Shockey J, Smertenko A, Bates PD. Identification of triacylglycerol remodeling mechanism to synthesize unusual fatty acid containing oils. *Nat Commun*. 2024:15(1):3547. https://doi.org/10.1038/s41467-024-47995-x

Regmi A, Shockey J, Kotapati HK, Bates PD. Oil-producing metabolons containing DGAT1 use separate substrate pools from those

containing DGAT2 or PDAT. *Plant Physiol.* 2020:184(2):720–737. https://doi.org/10.1104/pp.20.00461

Shockey J, Lager I, Stymne S, Kotapati HK, Sheffield J, Mason C, Bates PD. Specialized lysophosphatidic acid acyltransferases contribute to unusual fatty acid accumulation in exotic Euphorbiaceae seed oils. *Planta*. 2019:249(5):1285–1299. https://doi.org/10.1007/s00425-018-03086-y

Shockey J, Mason C, Gilbert M, Cao H, Li X, Cahoon E, Dyer J. Development and analysis of a highly flexible multi-gene expression system for metabolic engineering in Arabidopsis seeds and other plant tissues. *Plant Mol Biol.* 2015:89(1–2):113–126. https://doi.org/10.1007/s11103-015-0355-5

Shockey J, Regmi A, Cotton K, Adhikari N, Browse J, Bates PD. Identification of Arabidopsis GPAT9 (At5g60620) as an essential gene involved in triacylglycerol biosynthesis. *Plant Physiol.* 2016:170(1):163–179. https://doi.org/10.1104/pp.15.01563

Shockey JM, Gidda SK, Chapital DC, Kuan JC, Dhanoa PK, Bland JM, Rothstein SJ, Mullen RT, Dyer JM. Tung tree DGAT1 and DGAT2 have nonredundant functions in triacylglycerol biosynthesis and are localized to different subdomains of the endoplasmic reticulum. *Plant Cell*. 2006:18(9):2294–2313. https://doi.org/10.1105/tpc.106.043695

Shomo ZD, Mahboub S, Vanviratikul H, McCormick M, Tulyananda T, Roston RL, Warakanont J. All members of the Arabidopsis DGAT and PDAT acyltransferase families operate during high and low temperatures. *Plant Physiol.* 2024:195(1):685–697. https://doi.org/10.1093/plphys/kiae074

Tamura K, Stecher G, Kumar S. MEGA11: molecular evolutionary genetics analysis version 11. *Mol Biol Evol.* 2021:38(7):3022–3027. https://doi.org/10.1093/molbev/msab120

Trenz TS, Turchetto-Zolet AC, Margis R, Margis-Pinheiro M, Maraschin FDS. Functional analysis of alternative castor bean DGAT enzymes. *Genet Mol Biol.* 2022:46(1 Suppl 1):e20220097. https://doi.org/10.1590/1678-4685-gmb-2022-0097

van Erp H, Bates PD, Burgal J, Shockey J, Browse J. Castor phospholipid:diacylglycerol acyltransferase facilitates efficient metabolism of hydroxy fatty acids in transgenic Arabidopsis. *Plant Physiol.* 2011:155(2):683–693. https://doi.org/10.1104/pp.110.167239

van Erp H, Shockey J, Zhang M, Adhikari ND, Browse J. Reducing isozyme competition increases target fatty acid accumulation in seed triacylglycerols of transgenic Arabidopsis. *Plant Physiol.* 2015:168(1):36–46. https://doi.org/10.1104/pp.114.254110

Vanhercke T, Dyer JM, Mullen RT, Kilaru A, Rahman MM, Petrie JR, Green AG, Yurchenko O, Singh SP. Metabolic engineering for enhanced oil in biomass. *Prog Lipid Res.* 2019:74:103–129. https://doi.org/10.1016/j.plipres.2019.02.002

Wang L, Qian H, Nian Y, Han Y, Ren Z, Zhang H, Hu L, Prasad BVV, Laganowsky A, Yan N, *et al.* Structure and mechanism of human diacylglycerol O-acyltransferase 1. *Nature*. 2020:581(7808):329–332. https://doi.org/10.1038/s41586-020-2280-2

Wang L, Shen W, Kazachkov M, Chen G, Chen Q, Carlsson AS, Stymne S, Weselake RJ, Zou J. Metabolic interactions between the Lands cycle and the Kennedy pathway of glycerolipid synthesis in Arabidopsis developing seeds. *Plant Cell*. 2012:24(11):4652–4669. https://doi.org/10.1105/tpc.112.104604

Winichayakul S, Curran A, Moraga R, Cookson R, Xue H, Crowther T, Roldan M, Bryan G, Roberts N. An alternative angiosperm DGAT1 topology and potential motifs in the N-terminus. *Front Plant Sci.* 2022:13:951389. https://doi.org/10.3389/fpls.2022.951389

Xi W, Liu C, Hou X, Yu H. MOTHER OF FT AND TFL1 regulates seed germination through a negative feedback loop modulating ABA signaling in Arabidopsis. *Plant Cell*. 2010:22(6):1733–1748. https://doi.org/10.1105/tpc.109.073072

Xu J, Carlsson AS, Francis T, Zhang M, Hoffman T, Giblin ME, Taylor DC. Triacylglycerol synthesis by PDAT1 in the absence of DGAT1 activity is dependent on re-acylation of LPC by LPCAT2. *BMC Plant Biol.* 2012:12(1):4. https://doi.org/10.1186/1471-2229-12-4

Xu Y, Caldo KMP, Falarz L, Jayawardhane K, Chen G. Kinetic improvement of an algal diacylglycerol acyltransferase 1 via fusion with an acyl-CoA binding protein. *Plant J.* 2020:102(4):856–871. https://doi.org/10.1111/tpj.14708

Xu Y, Caldo KMP, Jayawardhane K, Ozga JA, Weselake RJ, Chen G. A transferase interactome that may facilitate channeling of polyunsaturated fatty acid moieties from phosphatidylcholine to triacylglycerol. *J Biol Chem.* 2019:294(41):14838–14844. https://doi.org/10.1074/jbc.AC119.010601

Xu Y, Singer SD, Chen G. Protein interactomes for plant lipid biosynthesis and their biotechnological applications. *Plant Biotechnol J.* 2023:21(9):1734–1744. https://doi.org/10.1111/pbi.14027

Yang W, Wang G, Li J, Bates PD, Wang X, Allen DK. Phospholipase Dζ enhances diacylglycerol flux into triacylglycerol. *Plant Physiol.* 2017:174(1):110–123. https://doi.org/10.1104/pp.17.00026

Yariv B, Yariv E, Kessel A, Masrati G, Chorin AB, Martz E, Mayrose I, Pupko T, Ben-Tal N. Using evolutionary data to make sense of macromolecules with a "face-lifted" ConSurf. *Protein Sci.* 2023:32(3):e4582. https://doi.org/10.1002/pro.4582

Zhang M, Fan J, Taylor DC, Ohlrogge JB. DGAT1 and PDAT1 acyltransferases have overlapping functions in Arabidopsis triacylglycerol biosynthesis and are essential for normal pollen and seed development. *Plant Cell*. 2009:21(12):3885–3901. https://doi.org/10.1105/tpc.109.071795

Zhou X-R, Callahan DL, Shrestha P, Liu Q, Petrie JR, Singh SP. Lipidomic analysis of Arabidopsis seed genetically engineered to contain DHA. *Front Plant Sci.* 2014:5:419. https://doi.org/10.3389/fpls.2014.00419