Increased Expression of Fatty Acid and ABC Transporters Enhances Seed Oil Production in Camelina

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Research

Keywords: Camelina, lipid metabolism, oil production, seed weight, transporters

Posted Date: November 5th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-100742/v1

Version of Record: A version of this preprint was published on February 27th, 2021. See the published version at https://doi.org/10.1186/s13068-021-01899-w.

Abstract

Background: Lipid transporters play an essential role in lipid delivery and distribution, but their influence on seed oil production in oilseed crops is not well studied.

Results: Here we examined the effect of two lipid transporters, *FAX1* (*fatty acid export1*) and *ABCA9* (*ATP-binding cassette transporter subfamily A9*) on oil production and lipid metabolism in the oilseed plant *Camelina sativa*. Overexpression (OE) of *FAX1* and *ABCA9* increased seed weight and size, with *FAX1*-OEs and *ABCA9*-OEs increasing seed length and width, respectively, whereas *FAX1*/*ABCA9*-OEs increasing both. *FAX1*-OE and *ABCA9*-OE displayed additive effects on seed oil content and seed yield. Also, OE of *FAX1* and *ABCA9* affected membrane lipid composition in developing siliques, especially on phosphatidylcholine, phosphatidylethanolamine, and phosphatidylglycerol. The expression of some genes involved in seed oil synthesis was increased in developing seeds of *FAX1*- and/or *ABCA9*-OEs.

Conclusions: These results indicate that increased expression of *FAX1* and *ABCA9* can potentially be applied to improving camelina oil production.

Background

Fatty acids (FAs) are the major and essential component of membrane lipids and important energy stores for metabolism and cellular energy homeostasis [1, 2]. In addition, FAs participate in many regulatory processes in organismal growth, development, and stress responses. In plants, FAs are synthesized in plastids and exported out of plastids and to endoplasmic reticulum (ER) for elongation, desaturation, and other embellishments [3, 4]. One transporter, FAX1 (fatty acid export 1), a membrane protein in chloroplast inner envelopes, was identified to transport FAs out of chloroplasts in *Arabidopsis thaliana* [5]. Overexpression (OE) of *FAX1* led to an increase in ER-derived lipids and a decrease in several plastid-produced lipids in flowers and leaves [5]. *FAX1* overexpression also increased the biomass production and seed oil content in Arabidopsis [5, 6].

FA associated with ER is involved in the biosynthesis of various complex lipids, including triacylglycerol (TAG). An ER-localized ATP-binding cassette (ABC) transporter subfamily A, ABCA9, was described to transport FA/acyl-CoA to the ER in Arabidopsis [7]. Developing seeds of *ABCA9*-knockout mutant (*abca9*) incorporated less ^{14}C-oleoyl-CoA into TAG compared with WT seeds. OE of *ABCA9* enhanced TAG deposition by up to 40%, with enlarged seeds, larger embryo, more densely packed with oil bodies [7]. When *ABCA9* was overexpressed, the seed size and seed oil content were increased, and *abca9* had opposite effects [7]. However, the function of FAX1 and ABCA9 and influence on seed oil accumulation in oilseed crops remain to be tested.

Camelina sativa, an archaic oilseed crop, has been cultivated more than 3,000 years [8, 9]. Camelina was an important oilseed crop in Europe and Asia for centuries [10, 11], but its cultivation declined in the past century and was replaced by higher-yielding crops such as rapeseed (*Brassica napus*) [8, 12]. Camelina has several advantages as an oilseed crop. Camelina seed oil contains a high level of unsaturated FAs (>

90%), with the level of polyunsaturated α-linolenic acid being 30–40% of the total oil [13–15]. In addition, camelina seed meals contain a low level of glucosinolates, toxic for feed use [8, 15]. Furthermore, camelina is a low-input crop with a low requirement for water and nutrients, and it is resistant to common Brassicaceae pests and pathogens and adaptable to hostile environmental conditions [10, 16, 17]. Moreover, camelina has a short life cycle with 85–100 days from seeds to seeds, and it is easy to transform genetically [18, 19]. However, low seed and oil yield relative to other oil crop such as canola are the major concerns for camelina production. In this study, we overexpressed the two Arabidopsis transporters, *FAX1* and *ABCA9*, in camelina to test their functions on oil accumulation, yield and other agronomic traits, and lipid metabolism in developing seeds.

Results

Overexpression of FAX1 and ABCA9 in camelina

To investigate the function of *FAX1* and *ABCA9* in camelina, we overexpressed each of Arabidopsis *FAX1* and *ABCA9* genomic DNAs under the control of the cauliflower mosaic virus (CaMV)-35S promoter (Fig. 1a). FAX1 and ABCA9 were fused with a HA-tag and a Flag-tag at the C-terminus, respectively. The production of FAX1-HA and ABCA9-Flag in camelina was confirmed by immunoblotting using anti-HA and anti-Flag antibodies, respectively (Fig. 1b, *upper* panel). The homozygous OE plants producing FAX1-HA or ABCA9-Flag were identified. In addition, we generated camelina lines overexpressing both *FAX1* and *ABCA9* by transforming the *FAX1*-HA construct into homozygous *ABCA9*-OE plants. The production of FAX1-HA and ABCA9-Flag in *FAX1*/*ABCA9*- single and double OEs were confirmed by immunoblotting (Fig. 1b, *lower* panel). Camelina has 3 homologues of each of *FAX1* and *ABCA9* (Fig. 1c), and in WT camelina the transcript levels of the three *FAX1s* were relatively high in seedlings and developing seeds, but low in leaves, roots, and stems (Fig. 1c, *left* panel). The transcript levels of the three *ABCA9* were relatively high in 4-week-old developing seeds (Fig. 1c, *right* panel).

FAX1 and ABCA9 increase seed and oil yield

FAX1-OEs, *ABCA9*-OEs, *FAX1*/*ABCA9*-OEs, and WT plants were grown side by side to determine the effect of *FAX1* and *ABCA9* on camelina growth and production. The OE lines had similar flowering time, plant height, and branch number as WT (Additional file 1: Fig. S1). However, the thousand-seed weight (TSW) of *FAX1*-OEs and *ABCA9*-OEs were 21% and 22% higher on average than that of WT, respectively, and *FAX1*/*ABCA9*-OEs were 40% higher on average than that of WT (Fig. 2a). Interestingly, *FAX1*-OEs and *ABCA9*-OEs had distinctive effect on seed length and seed width. *FAX1*-OEs increased seed length by 13.6%, whereas *ABCA9*-OEs increased seed width by 25.3%, while the seed length and width of *FAX1*/*ABCA9*-OEs were increase by 13.2% and 31.7%, respectively (Fig. 2a and 2b). The seed width of *FAX1*-OEs and seed length of *ABCA9*-OEs were similar with those of WT (Fig. 2b). The above results indicate that *FAX1* and *ABCA9* increase seed length and width, respectively, to increase seed weight.

To determine the effect of *FAX1* and *ABCA9* on oil production in camelina, we grew the single and double OE plants together with WT side by side with multiple replicates (n = 18). Compared to WT, the seed yield

per plant on average was 13.6% higher for *FAX1*-OE, 37.6% higher for *ABCA9*-OE, and 44.6% higher for *FAX1*/*ABCA9*-OEs (Fig. 2c, top). Moreover, the average oil content of WT seeds was 28.4%, whereas that *FAX1*-OE and *ABCA9*-OE seeds was 29.4% and 30.1%, respectively, and that of *FAX1*/*ABCA9*-OE seeds was 32.2% (Fig. 2c, middle). The average increase of seed oil content over WT was 3.6% for *FAX1*-OE, 5.8% for *ABCA9*-OE, and 13.3% for *FAX1*/*ABCA9*-OE seeds. The results indicate that *FAX1* and *ABCA9* increase seed oil content and seed yield simultaneously and these two genes had an additive effect on seed oil content and seed yield, leading a substantial improvement on overall oil yield. Combining the increases in seed oil content and seed yield, the oil production per plant of *FAX1*-OEs and *ABCA9*-OEs was 21.5% and 55.3% higher than that of WT, respectively, whereas that of *FAX1*/*ABCA9*-OEs was 75.1% higher than that of WT (Fig. 2c, bottom).

The *FAX1*-OE and *ABCA9*-OE seeds displayed altered FA composition from WT seeds. Compared with that of WT seeds, the level of C16:0 of *FAX1*-OEs and *ABCA9*-OEs was decreased by 6.2% and 4.3%, respectively, whereas that of *FAX1*/*ABCA9*-OEs was decreased by 13.9% (Additional file 2: Fig. S2). Conversely, the level of C18:0 was increased by 4.8% and 5.1% for *FAX1*-OEs and *ABCA9*-OEs, respectively, and 11.6% for *FAX1*/*ABCA9*-OEs. The level of C18:1 was increased by 12.5% and 16.1% for *FAX1*-OEs and *ABCA9*-OEs, respectively, and 28.6% for *FAX1*/*ABCA9*-OEs. The level of C18:2 was increased by 5.7% and 6.6% for *FAX1*-OEs and *ABCA9*-OEs, respectively, and 10% for *FAX1*/*ABCA9*-OEs whereas that of C18:3 was decreased by 4% for *FAX1*-OEs and *ABCA9*-OEs and 10% for *FAX1*/*ABCA9*-OEs comparing to that of WT. In addition, the level of C20:1 was decreased by 4.3% and 11.1% in *FAX1*-OE and *ABCA9*-OE seeds, and 10.4% in *FAX1*/*ABCA9*-OE seeds (Additional file 2: Fig. S2). The above results indicate that increased *FAX1* and *ABCA9* expression positively affect the level of C18:0, C18:1 and C18:2, but negatively affect the level of C16:0, C18:3 and C20:1.

FAX1 and ABCA9 affect membrane glycerolipid composition

We further examined the effect of *FAX1*-OE and *ABCA9*-OE on membrane glycerolipid composition. Total lipids were extracted from developing siliques of 2- and 4-weeks after flowering from WT, and *FAX1*-1, *ABCA9*-1, and *FAX1*/*ABCA9*-1 OE lines, and analyzed using electrospray ionization tandem mass spectrometry (ESI-MS/MS). In 2-week-old siliques (WOS), which was considered as the outburst stage of lipid synthesis [20], compared to WT, the amount of phosphatidylcholine (PC) was increased 13%, 28%, and 33% in *FAX1*-1, *ABCA9*-1, and *FAX1*/*ABCA9*-1, respectively (Fig. 3). The amount of phosphatidylethanolamine (PE) was increased 60%, 104%, and 127% in *FAX1*-1, *ABCA9*-1, and *FAX1*/*ABCA9*-1. The total membrane glycerolipid level was 9%, 24%, and 31% higher in *FAX1*-1, *ABCA9*-1, and *FAX1*/*ABCA9*-1 than WT at the early stage of developing siliques. However, the amount of phosphatidylglycerol (PG) was decreased approximately 45% in all three OE lines, and the level of monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) had no obvious changes (Fig. 3). When the lipid data were calculated as mol% of total lipids analyzed, PE in *FAX1*-1, *ABCA9*-1, and *FAX1*/*ABCA9*-1 was 6, 12, and 13 mol% higher, respectively, than that of WT. However, the major plastidic lipids, PG, MGDG, and DGDG were all lower in three OE lines than WT (Additional file 3: Fig. S3). Phosphatidic acid (PA) constituted less than 0.2 mol% in the developing siliques of all lines tested and

the PA mol% in *FAX1*-OE, *ABCA9*-OE, and *FAX1*/*ABCA9*-OE lines was all lower than that of WT (Additional file 3: Fig. S3).

In 4-WOS, which was considered as the plateau stage of lipid synthesis [20], the level of PC in *FAX1*-1, *ABCA9*-1, and *FAX1*/*ABCA9*-1 was 14%, 14%, and 17% higher than that of WT (Fig. 3); The level of PE in *FAX1*-1, *ABCA9*-1, and *FAX1*/*ABCA9*-1 was 12%, 19%, and 25% higher than that of WT. The total membrane glycerolipid content in *FAX1*-1, *ABCA9*-1, and *FAX1*/*ABCA9*-1 was 3%, 5%, and 8% higher than that of WT (Fig. 3). However, the level of PG and DGDG was decreased in *FAX1*-1 whereas was comparable among WT, *ABCA9*-1 and *FAX1*/*ABCA9*-1 at this stage (Fig. 3). PC in *FAX1*-1, *ABCA9*-1, and *FAX1*/*ABCA9*-1 was 1.9, 3, and 3.5 mol% higher than WT whereas mol% of MGDG and DGDG in the three OE lines was slightly lower than that of WT. The mol% of PE and PG was comparable between WT and OE lines at this stage (Additional file 3: Fig. S3). Also, the PA mol% was lower all OE lines than WT (Additional file 3: Fig. S3).

The major PC species (34:3, 34:2, 36:5, and 36:4 PC) in *FAX1*-1, *ABCA9*-1, and *FAX1*/*ABCA9*-1 were increased in 2-WOS, and 36:5, 36:4, and 36:3 PC were increased in 4-WOS compared with those of WT (Fig. 4). Similarly, the major PE species (34:3, 34:2, 36:6, 36:5, 36:4, and 36:3 PE) of *FAX1*-1, *ABCA9*-1, and *FAX1*/*ABCA9*-1 were all increased in 2-WOS, and only 34:3, 34:2, 36:5, and 36:4 PE were increased in 4-WOS compared to those of WT (Fig. 4). Also, the effect of *FAX1* and *ABCA9* exhibited additive effect, especially on 34:2 and 36:4 PC, and 34:3, 34:2, 36:5, and 36:4 PE (Fig. 4). In contrast, the major PG species (32:0, 34:4, 34:3, 34:2, and 34:1 PG) of *FAX1*-1, *ABCA9*-1, and *FAX1*/*ABCA9*-1 were all decreased compared to those of WT in 2-WOS, while only 34:3 PG was decreased in *FAX1*-1, and 34:4 PG decreased in *ABCA9*-1, and *FAX1*/*ABCA9*-1 in 4-WOS (Fig. 4). Although the total amount of MGDG was similar between WT and OE lines (Fig. 3), the level of some MGDG species, especially in *FAX1*-1, was different from that of WT. For instance, two major MGDG spices, 34:6 and 36:6 MGDG, in *FAX1*-1 was decreased both in 2- and 4-WOS compared with WT (Fig. 4). For DGDG, only 36:5 DGDG in 2-WOS, and 36:6 and 36:5 DGDG in 4-WOS of *FAX1*-1 were lower than WT (Fig. 4). Similarly, the mol% of 34:2 PC of *ABCA9*-1 and *FAX1*/*ABCA9*-1 in 2-WOS, and 36:5 PC of *ABCA9*-1 and *FAX1*/*ABCA9*-1 in 4-WOS were higher than that of WT (Additional file 4: Fig. S4); the mol% of 34:2 PE of *ABCA9*-1 and *FAX1*/*ABCA9*-1 was higher than WT both in 2- and 4-WOS; whereas the mol% of 34:2 PG of *FAX1*-1 was lower than WT; the major MGDG species 36:6 was lower in *FAX1*-1 and *FAX1*/*ABCA9*-1; and the major DGDG species 36:6 was lower in *FAX1*-1 in 4-WOS (Additional file 4: Fig. S4). The above lipid data indicate that overexpression of *FAX1* and *ABCA9* also affects membrane lipid composition in developing siliques, and the effect differs at different developmental stage.

FAX1 and ABCA9 alter the expression of genes in oil production in seeds

To gain insights into the enhancing effect of *FAX1*- and *ABCA9*-OE on oil production, we compared the transcript level of genes that were reported to be involved in oil accumulation in developing seeds between the OE lines and WT. First, we checked the expression level of *AtFAX1* and *AtABCA9* in 2- and 4-week-old developing seeds (WODS). The transcript level of *AtFAX1* was high in *FAX1*-OE and

FAX1/*ABCA9*-OE lines, but was not detected in *ABCA9*-OE lines, as expected. Similarly, the level of *AtABCA9* was high in *ABCA9*-OE and *FAX1*/*ABCA9*-OE lines, but was not detected in *FAX1*-OE lines (Additional file 5: Fig. S5). The expression level of three copies of *DGAT1* (*Acyl-CoA: diacylglycerol acyltransferase 1*) was higher in *ABCA9*-1, and slightly lower in *FAX1*-1 in 2-WODS, and was comparable among these 4 lines in 4-WODS (Fig. 5). The expression level of two *DGAT2s* was higher in 2-WODS, and all three copies of *DGAT2* were higher in 4-WODS in all three single and double OE lines than WT. The expression level of only expressed copy of *PDAT1* (*phospholipid: diacylglycerol acyltransferase 1*) was also higher both in 2- and 4-WODS in all three single and double OE lines than WT. The expression level of three copies of *PDAT2* was all higher in *ABCA9*-1 and *FAX1*/*ABCA9*-1 in 2-WODS, and two copies were all higher in three OE lines whereas the other copy was lower in *ABCA9*-1 and *FAX1*/*ABCA9*-1 in 4-WODS. The expression of two of three *WRI1* (*wrinkled 1*) copies was detected in developing seeds. The expression level of one *WRI1* was lower in *FAX1*-1 and *ABCA9*-1 whereas, the other *WRI1* was higher in *FAX1*/*ABCA9*-1 in 2-WODS (Fig. 5). At the later 4-WODS, the expression level of two detected copies was lower in *ABCA9*-1 than WT. The expression level of all the homologues of *LEC1* (*leaf cotyledon 1*) was higher both in 2- and 4-WODS in all three single and double OE lines than WT. For *NPC6* (*nonspecific phospholipase C 6*), only one copy displayed a higher expression level in *FAX1*- and *ABCA9*-single and double OEs in 2- and 4-WODS, and another homeologue had a higher expression level in *FAX1*-1 and *ABCA9*-1 in 2-WODS (Fig. 5). The above results indicate that increased *FAX1* and *ABCA9* expression also lead to an increase in the expression of some of genes involved in oil synthesis and accumulation, such as *DGAT2*, *PDAT1*, and *LEC1*.

Discussion

In this study, we show that co-OE of two Arabidopsis transporters, *FAX1* and *ABCA9*, significantly improve camelina seed and oil production. Each of the two transporter enhances seed oil production and their effects are additive. The glycerolipid species data show that *FAX1*-1 was lower than WT and *ABCA9*-1 in both the amount (nmol per silique) and mol% of plastidic lipids 34:3- and 34:2-PG, 34:6- and 36:6-MGDG in 2- and 4-WOS, and 36:5-, 36:4-, and 36:3-DGDG in 2-WOS, and 36:6- and 36:5-DGDG in 4-WOS. In comparison, *ABCA9*-1 was higher than WT and *FAX1*-1 in the amount and mol% of extra-plastidic lipids 34:2-PC, and 34:2-PE in 2-WOS, and 34:2- and 36:5-PC, and 34:2-, 36:5-, and 36:4-PE in 4-WOS. In addition, the amount of plastidic lipids 34:6- and 36:6-MGDG, and 36:5-DGDG, as well as 34:3- and 34:2-PG, in *FAX1*-1 was decreased compared with that in *ABCA9*-1. The decrease in *FAX1*-1 may result from an enhanced transport of FAs from plastids to cytosol/ER by FAX1. In contrast, the amount of the most extra-plastidic lipids, 34:2-PC, and 34:2-, 36:5-, and 36:4-PE in *ABCA9*-1 was increased compared to *FAX1*-1. The increase in *ABCA9*-1 may result from enhanced FA transport to ER by ABCA9 for glycerolipid synthesis. FAX1 was localized at the inner envelope of the chloroplasts transporting FAs out of chloroplasts [5], whereas ABCA9 was associated with ER and proposed to transport FAs to ER [7]. Thus, the additive effect of *FAX1* and *ABCA9* could result from their distinctive functions in FA transport and lipid metabolism.

In addition, *FAX1*- and *ABCA9*-OE developing camelina seeds also displayed increased level of expression of specific genes related to TAG production, such as *DGAT2*, *PDAT1*, and *LEC1*. Those increases may result from an increase in metabolic demand as the overall activity for lipid production and/or more substrates are available for those enzymes. However, how the increased expression of *FAX1* and *ABCA9* leads to the increased expression of those genes needs further investigation. In addition, *ABCA9*-OE and *FAX1*-OE exhibited different impacts on the expression of these genes. The expression level of *DGAT1* and *PDAT2* in 2-WODS was increased in *ABCA9*-1 and/or *FAX1*/*ABCA9*-1 but not in *FAX1*-1 compared to that in WT, which may explain the reason of the higher seed oil content in *ABCA9*-OEs than that in *FAX1*-OEs. Moreover, a combination of increased *FAX1* and *ABCA9* expression increased significantly seed weight, seed oil content, seed yield, and overall seed oil production, while exhibited no apparent adverse effect on major agronomic traits, like plant height, flowering time, and branch number. The results indicate that the manipulation of *FAX1* and *ABCA9* has a great application potential to improving overall seed and oil production in oilseed crops.

Conclusion

Here, we co-overexpressed two lipid transporters, *FAX1* and *ABCA9*, in camelina to investigate the transporters function on lipid accumulation in oilseed crop. The results show that OE of *FAX1* and *ABCA9* increased seed weight and size. Interestingly, *FAX1*-OEs and *ABCA9*-OEs had distinctive effect on seed length and seed width. *FAX1*-OEs increased seed length only, whereas *ABCA9*-OEs increased seed width only, while the seed length and width of *FAX1*/*ABCA9*-OEs were increased simultaneously, to increase seed weight. Moreover, OE of *FAX1* and *ABCA9* can increase seed oil content and seed yield simultaneously and these two genes had an additive effect on seed oil content and seed yield, leading to a substantial improvement on overall oil yield. Combining the increases in seed oil content and seed yield, the oil production per plant of *FAX1*/*ABCA9*-OEs was 75.1% higher than that of WT. The lipid data also indicate that overexpression of *FAX1* and *ABCA9* affects membrane lipid composition in developing siliques, and the effect differs at different developmental stage. The real-time PCR data show that increased *FAX1* and *ABCA9* expression also lead to an increase in the expression of some of genes involved in oil synthesis and accumulation, such as *DGAT2*, *PDAT1*, and *LEC1*. These results indicate that increased expression of *FAX1* and *ABCA9* can potentially be applied to improving camelina oil production.

Methods

Plant materials and growth conditions

To overexpress Arabidopsis *ABCA9* transporter in camelina, the genomic sequence of *ABCA9* (AT5G61730) was amplified by PCR using Col-0 Arabidopsis genomic DNA as a template and by forward primer with *KpnI* site and reverse primer with *PacI* site. The C-terminal Flag tag was fused upstream of the terminator manually by adding its coding sequence to the reverse primer. To overexpress Arabidopsis *FAX1* transporter in camelina, the genomic sequence of *FAX1* (AT3G57280) was amplified by PCR using

Col-0 Arabidopsis genomic DNA as a template and by forward primer with *EcoRI* site and reverse primer with *SmaI* site. The C-terminal HA tag was fused upstream of the terminator manually by adding its coding sequence to the reverse primer. The details for gene cloning, plant transformation, putative transgenic plants identification were performed as described previously [21, 22]. Camelina plants were grown in greenhouse at 21 °C with approximately 16 hr light.

To compare plant growth and yield traits among these OE lines and WT, we used 2.5-gallon pots with BM7-35% soil (Berger) and each pot had 4 plants with one as WT as control in greenhouse. There were 18 biological replicates with a completely randomized block design for each transgenic line. The greenhouse condition and plants managements were as described previously [21, 22]. Measurements of flowering time, plant height, branch number, thousand-seed weight, and plant yield per plant were performed as described [23].

Phylogenetic analysis

The coding sequences of *AtFAX1* and *AtABCA9* were used as queries to search for homologous genes in the camelina reference genome [11] using BLASTn program with an E-value of 1E-50 and an identity of 50% set as thresholds. The phylogenetic tree was drawn by Phylodendron (http://iubio.bio.indiana.edu/treeapp/treeprint-sample1.html).

Immunoblotting and transcript analysis

Total protein extraction and immunoblotting were performed as described previously [22]. Protein concentrations were measured using the Bradford assay (Bio-Rad, 500 – 0205). RNA extraction, real-time PCR analysis, and semi-quantitative RT (reverse transcription)-PCR of transcript levels were performed as described previously [24, 25]. Total RNA was extracted from 2- and 4-week old developing seeds. Camelina *ACT2* (*Csa19g026200.1*) were used as internal standard and for cDNA input adjustment. All primers used in RT-PCR and real-time PCR are listed in Additional file 6: Table S1.

Seed oil content and fatty acid composition analyses

Seed oil content and FA composition were determined as described previously [21, 22]. Fatty acid methyl esters (FAMEs) from TAG were identified by comparing their retention times with known standards. The FA composition was calculated as mol %.

Lipid extraction and profiling

Polar lipids were extracted and analyzed by ESI-MS/MS based on a method described previously [22, 26]. The mass spectrometry data for lipids were processed using the software Analyst 1.5.1.

Accession numbers

Sequence data from this article can be found in the following database under the accession numbers: Arabidopsis Genome Initiative database: *FAX1*, AT3G57280; and *ABCA9*, AT5G61730. *Camelina sativa* Genome Resources (http://www.camelinadb.ca/): *CsACT2*, Csa19g026200.1; *CsFAX1*: Csa09g069740.1,

Csa06g033010.1, Csa04g043700.1; *CsABCA9*: Csa02g069700.1, Csa18g035110.1, Csa11g097540.1; *CsDGAT1*: Csa01g042590.1, Csa19g056370.1, Csa15g084220.1; *CsDGAT2*: Csa04g037310.1, Csa09g058550.1, Csa06g025650.1; *CsPDAT1*: Csa13g016300.1, Csa08g005560.1, Csa20g019000.1; *CsPDAT2*: Csa04g024660.1, Csa06g018480.1, Csa09g035780.1; *CsWRI1*: Csa06g028810.1, Csa09g064030.1, Csa04g040400.1; *CsLEC1*: Csa17g028800.1, Csa03g025850.1, Csa14g027200.1; and *CsNPC6*: Csa09g050690.1, Csa06g022410.1, Csa04g033750.1.

Abbreviations

FA: fatty acid; ABC: ATP-binding cassette; FAX: fatty acid export; TAG: triacylglycerol; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PG: phosphatidylglycerol; PA: phosphatidic acid; MGDG: monogalactosyldiacylglycerol; DGDG: digalactosyldiacylglycerol.

Declarations

Acknowledgements

Not applicable.

Author Contributions

G.C. designed and performed most of the experiments, and wrote and revised the manuscript. G.W. performed *ABCA9* cloning and transformation. S.K. did ABCA9 protein analysis and helped edit the manuscript. J.L. helped grow plants and edit manuscript. Y.Z. provided partial support to the project and revised the manuscript. X.W. proposed, designed, and supervised the study and revised the manuscript. All authors discussed the results and commented on the manuscript.

Funding

The work is supported by the National Natural Science Foundation of China (31801029), National Key Research and Development Program of China (2016YFD0100506, 2017YFE0104800), the US Department of Energy (DE-AR0000202), the U.S. Department of Energy (DOE), Office of Basic Energy Sciences (BES), Materials Sciences and Engineering Division under Award #DE-SC0001295, and the International Postdoctoral Exchange Fellowship Program 2016 by the Office of China Postdoctoral Council (20160034).

Availability of data and materials

All data generated or analyzed during this study are included in the article and its additional files.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Supplementary Information

Additional file 1: Figure S1. Effect of *AtFAX1*- and *AtABCA9*-OEs on other major agronomic traits.

Additional file 2: Figure S2. Effect of *AtFAX1*- and *AtABCA9*-OEs on seed fatty acid composition.

Additional file 3: Figure S3. Alterations of membrane glycerolipid levels (mol%) in developing siliques of *AtFAX1*- and *AtABCA9*-OEs.

Additional file 4: Figure S4. Alterations of PC, PE, PG, MGDG, and DGDG species (mol%) in developing siliques of *AtFAX1*- and *AtABCA9*-OEs.

Additional file 5: Figure S5. Gene expression level of *AtFAX1* and *AtABCA9* in developing seeds of camelina OE lines.

Additional file 6: Table S1. Primers (5’ to 3’) used for cloning and RT-PCR.

Figures

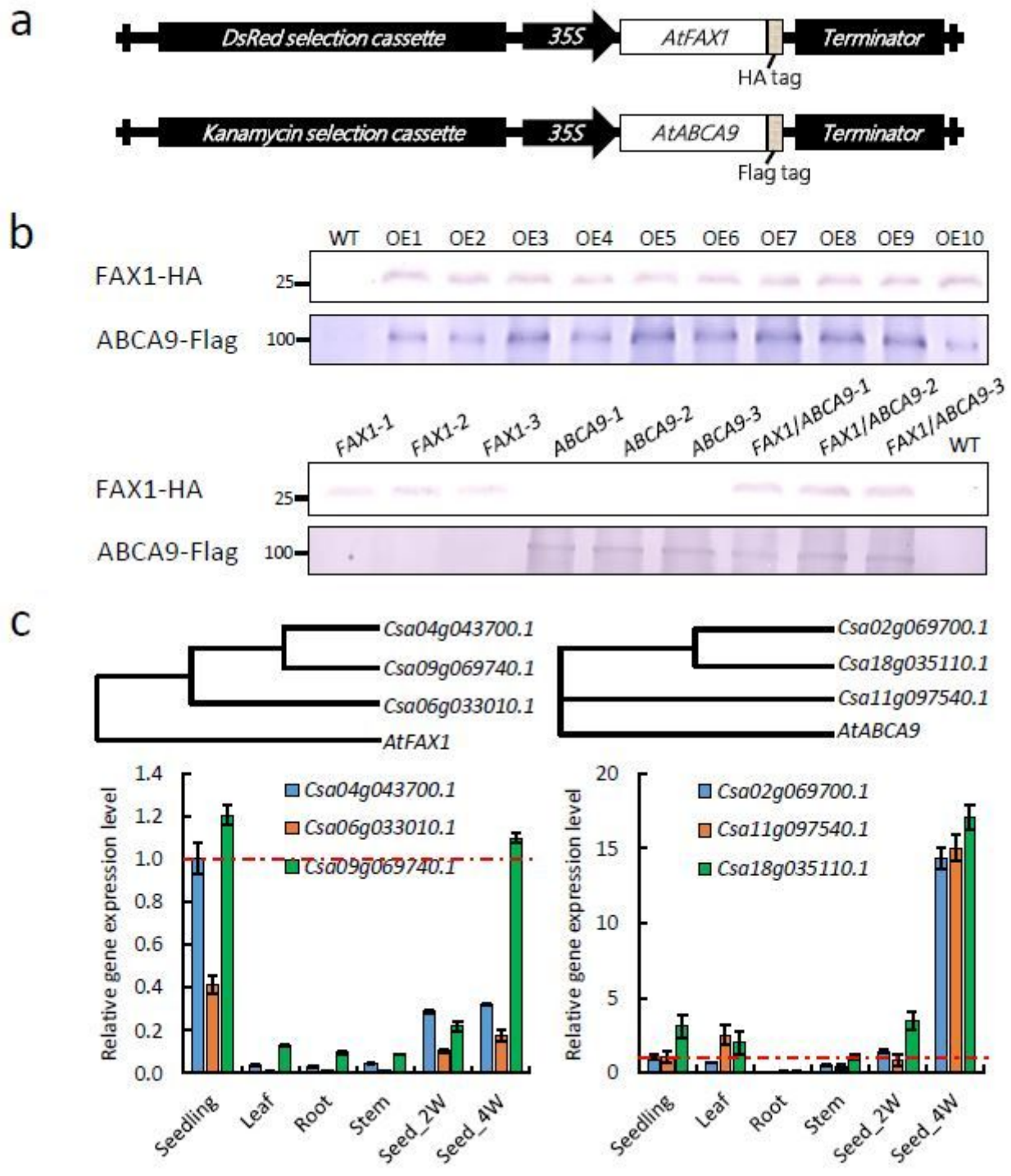

Figure 1

Overexpression of AtFAX1 and AtABCA9 in camelina. a The overexpression (OE) constructs of AtFAX1 and AtABCA9. b Immunoblotting of HA-tagged FAX1 and Flag-tagged ABCA9 in AtFAX1-OE, AtABCA9-OE, and AtFAX1/ABCA9-OE camelina leaves. Total proteins (10 μg/lane) were extracted from leaves of 3-week-old plants, separated by 10% SDS-PAGE, and transferred to a polyvinylidene difluoride membrane. The membrane was blotted with anti-HA or anti-Flag antibody conjugated with alkaline phosphatase.

Lanes OE1 through OE10 represent different transgenic lines harboring the FAX1-HA or ABCA9-Flag OE construct. Numbers on the left of each panel mark protein molecular mass standards in kilodaltons. c The phylogenetic tree of FAX1 (left) and ABCA9 (right) in camelina, and their expression pattern in seedlings, leaves, roots, stems, and 2- and 4-week (W) old developing seeds. ACT2 (actin2) was used as the internal standard for cDNA input adjustment. The expression level is relative to the value of Csa04g043700.1 for FAX1 and Csa02g069700.1 for ABCA9 in seedlings (red dashed line). Values are means ± SE (n=3).

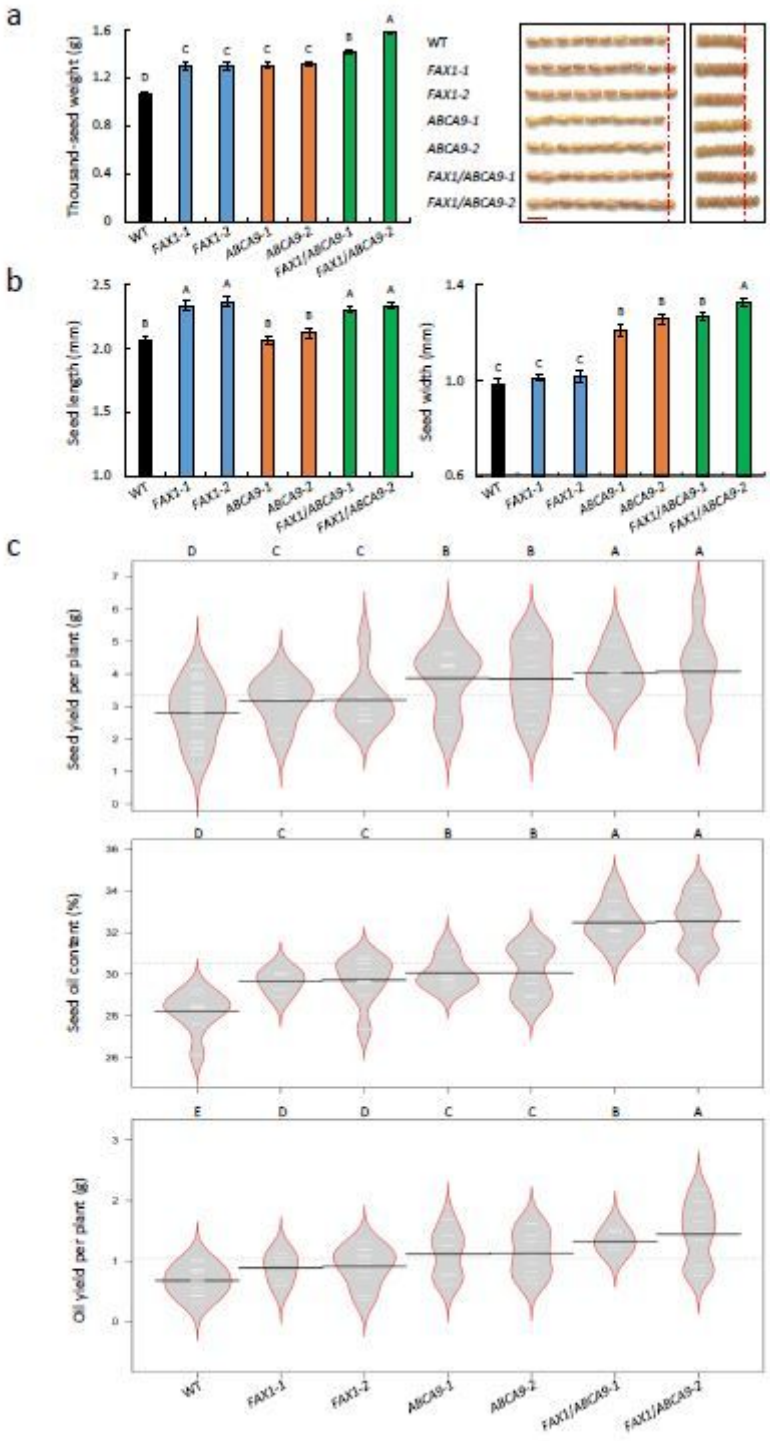

Figure 2

Effect of AtFAX1- and AtABCA9-OEs on seed size and oil yield. a Thousand-seed weight and seed morphology of camelina dry seeds. Values are means ± SE (n=12). Bar=3 mm. b Seed length and width of camelina dry seeds. Values are means ± SE (n=30). c Seed yield per plant (g), Seed oil content (w/w), and oil yield per plant (g) of different camelina lines. AtFAX1- and AtABCA9-single and double OEs were grown side by side together with WT. Seeds from whole plant were harvested and dried at room temperature for at least one month before measurement. Seed oil content was measured by gas chromatography, and calculated based on the internal standard (C17:0) peak area. Seeds from each plant were measured with 3 technical replicates, and the mean values are represented here as the seed oil content of each plant. Black lines show the averages, white lines represent individual data points (n=18), and polygons represent the estimated density of the data. Capital letters indicate a significant difference ($P < 0.01$) based on Duncan-test.

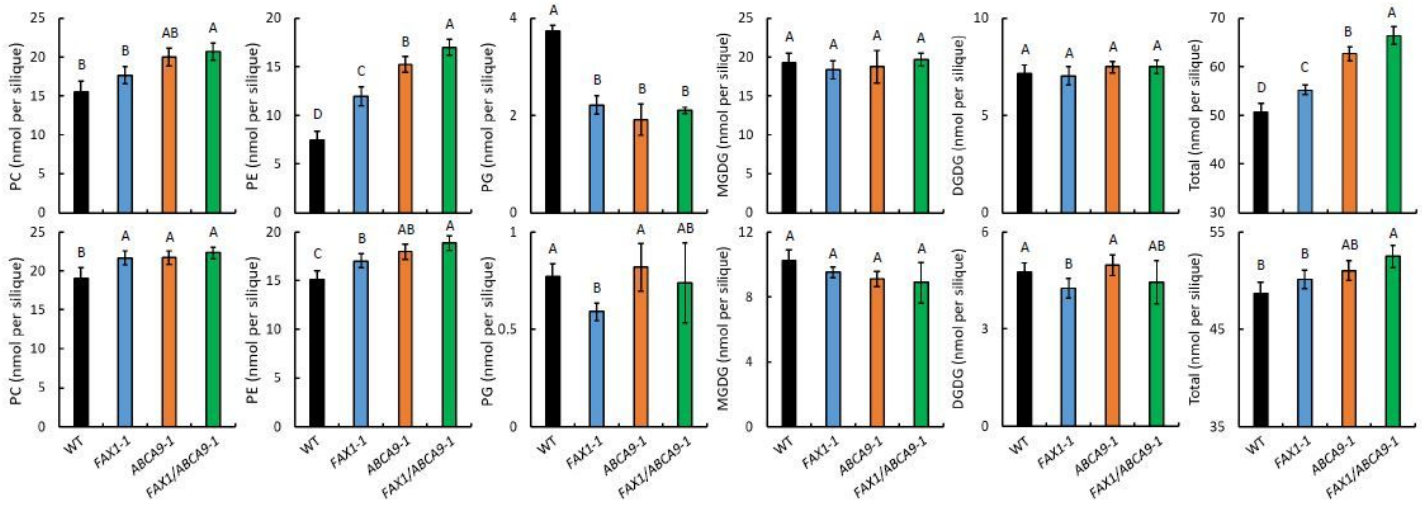

Figure 3

Alterations of membrane glycerolipid levels (nmol per silique) in developing siliques of AtFAX1- and AtABCA9-OEs. 2-week (upper panels) and 4-week (lower panels) old developing siliques were sampled for lipid profiling by ESI-MS/MS. The total lipid levels referred to the total amount of major phospholipids (PC, PE, and PG) and galactolipids (MGDG and DGDG) measured. Values are means ± SE with 5 biological replicates. Capital letters on the top of each panel indicate a significant difference ($P < 0.01$) based on Duncan-test. PC: phosphatidylcholine; PE: phosphatidylethanolamine; PG: phosphatidylglycerol; MGDG: monogalactosyldiacylglycerol; and DGDG: digalactosyldiacylglycerol.

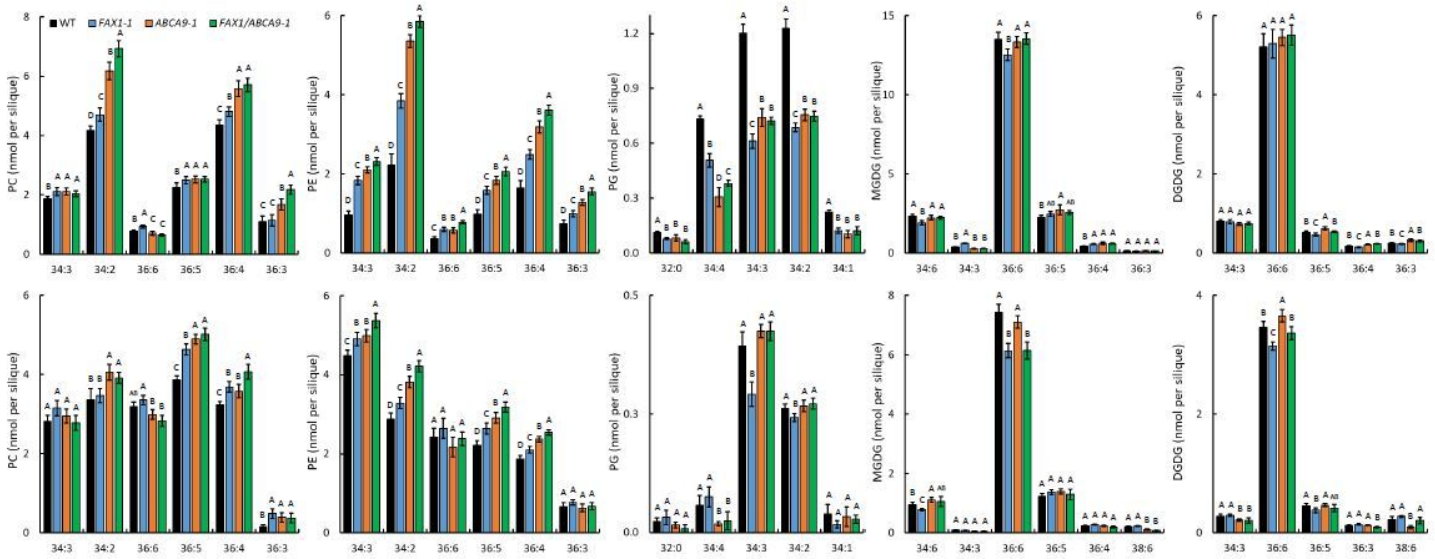

Figure 4

Alterations of PC, PE, PG, MGDG, and DGDG species (nmol per silique) in developing siliques of AtFAX1- and AtABCA9-OEs. 2-week (upper panels) and 4-week (lower panels) old developing siliques were sampled for lipid profiling by ESI-MS/MS. The species levels were measured by internal standards. Values are means ± SE with 5 biological replicates. Capital letters on the top of each panel indicate a significant difference ($P < 0.01$) based on Duncan-test.

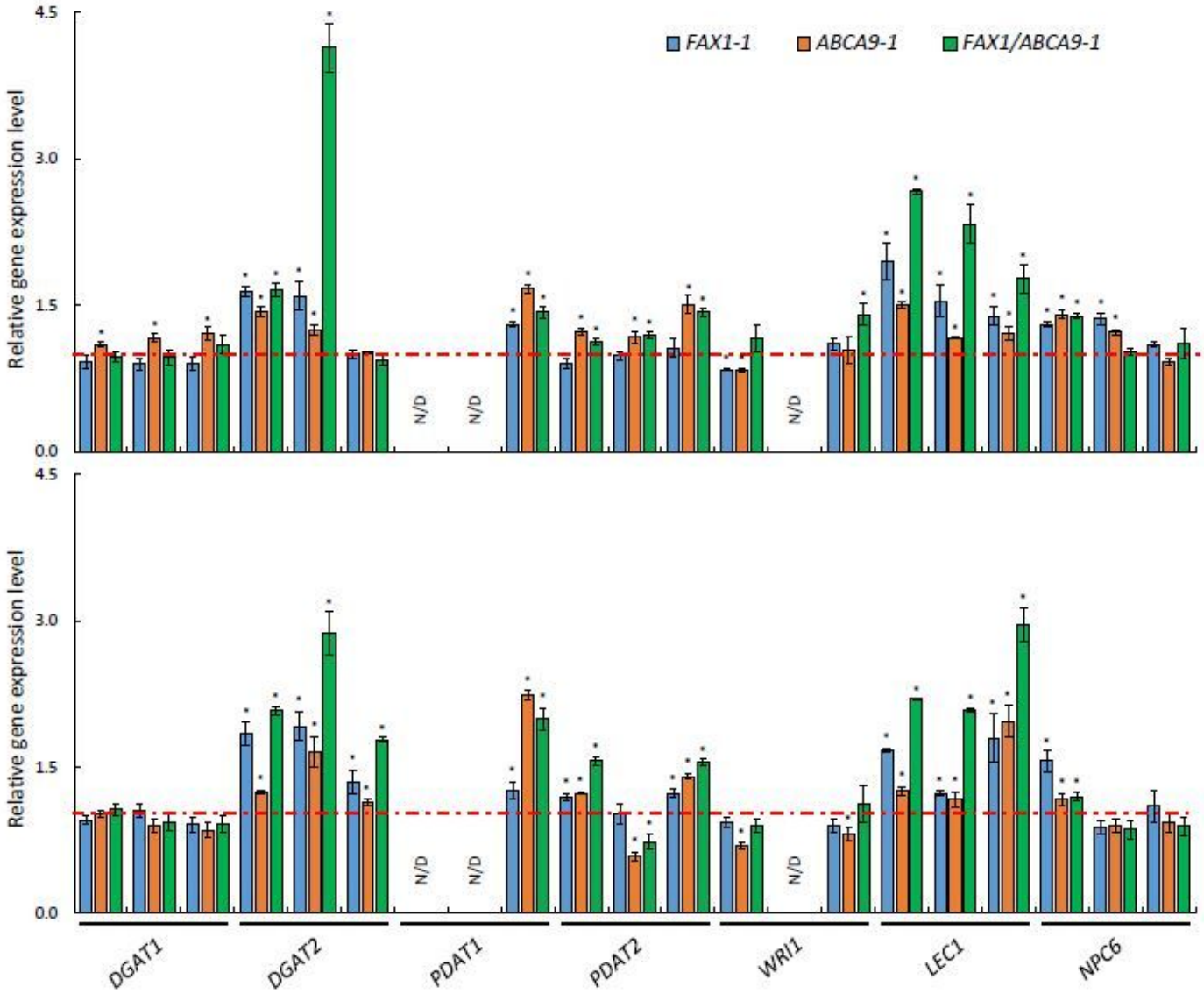

Figure 5

Effect of AtFAX1- and AtABCA9-OEs on the expression of genes involved in oil accumulation. Samples were collected with 2-week (upper panel) and 4-week (lower panel) old developing seeds for RNA extraction and real-time PCR. ACT2 (actin2) was used as the internal standard for cDNA input adjustment. Values are the relative expression level compared with WT (red dashed line) and are means ± SE (n=3). * Significant difference ($P < 0.05$) based on Student's t test compared with WT. N/D: not detected. DGAT: Acyl-CoA: diacylglycerol acyltransferase; PDAT: phospholipid: diacylglycerol acyltransferase; WRI1: wrinkled 1; LEC1: leaf cotyledon 1; and NPC6: nonspecific phospholipase C 6.

Supplementary Files

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- Transporterssuppfigures10122020.pptx
- TableS112062019.xlsx