Lipidomic analysis of *Arabidopsis* seed genetically engineered to contain DHA

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**INTRODUCTION**

Metabolic engineering of omega-3 long-chain polyunsaturated fatty acids (ω3 LC-PUFA) in oilseeds has been one of the key targets in recent years. By expressing a transgenic pathway for enhancing the synthesis of the ω3 LC-PUFA docosahexaenoic acid (DHA) from endogenous α-linolenic acid (ALA), we obtained the production of fish oil-like proportions of DHA in *Arabidopsis* seed oil. Liquid chromatography-mass spectrometry (LC-MS) was used to characterize the triacylglycerol (TAG), diacylglycerol (DAG) and phospholipid (PL) lipid classes in the transgenic and wild type *Arabidopsis* seeds at both developing and mature stages. The analysis identified the appearance of several abundant DHA-containing phosphatidylcholine (PC), DAG and TAG molecular species in mature seeds. The relative abundances of PL, DAG, and TAG species showed a preferred combination of LC-PUFA with ALA in the transgenic seeds, where LC-PUFA were esterified in positions usually occupied by 20:1ω9. Trace amounts of di-DHA PC and tri-DHA TAG were identified and confirmed by high resolution MS/MS. Studying the lipidome in transgenic seeds provided insights into where DHA accumulated and combined with other fatty acids of neutral and phospholipids from the developing and mature seeds.

**Keywords:** Lipidomics, metabolic engineering, ω3 LC-PUFA, oilseed, LC-MS, triacylglycerol, *Arabidopsis*

Metabolic engineering of omega-3 long-chain (≥C20) polyunsaturated fatty acids (ω3 LC-PUFA) in oilseeds has been one of the key targets in recent years. By expressing a transgenic pathway for enhancing the synthesis of the ω3 LC-PUFA docosahexaenoic acid (DHA) from endogenous α-linolenic acid (ALA), we obtained the production of fish oil-like proportions of DHA in *Arabidopsis* seed oil. Liquid chromatography-mass spectrometry (LC-MS) was used to characterize the triacylglycerol (TAG), diacylglycerol (DAG) and phospholipid (PL) lipid classes in the transgenic and wild type *Arabidopsis* seeds at both developing and mature stages. The analysis identified the appearance of several abundant DHA-containing phosphatidylcholine (PC), DAG and TAG molecular species in mature seeds. The relative abundances of PL, DAG, and TAG species showed a preferred combination of LC-PUFA with ALA in the transgenic seeds, where LC-PUFA were esterified in positions usually occupied by 20:1ω9. Trace amounts of di-DHA PC and tri-DHA TAG were identified and confirmed by high resolution MS/MS. Studying the lipidome in transgenic seeds provided insights into where DHA accumulated and combined with other fatty acids of neutral and phospholipids from the developing and mature seeds.
The mobile phases were: A. 10 mM ammonium formate in water:methanol:tetrahydrofuran (50:20:30, v/v/v); B. 10 mM ammonium formate:methanol:water (65:25:4, v/v/v) as the first solvent system for total lipid extraction.

**TOTAL LIPID EXTRACTION**

Total lipids were extracted using methanol/chloroform from triplicate samples of the developing and the mature seeds (Zhou et al., 2013). Tri-17:0-TAG (Nuchek Prep, Elysian, MN, USA) was added based on the dry weight of developing or mature seeds, as an internal standard. Aliquots of the total lipid extracts from 1 mg of seeds (dry weight) were dried under N₂ followed by re-dissolving in 1 mL of butanol:methanol (1:1, v/v) containing 10 mM butyric acid as internal standard as described previously (Zhou et al., 2012). Developing seeds were harvested on dry ice at 10–12 days after flowering (DAF), frozen with liquid nitrogen and freeze dried prior to lipid analysis.

**TWO-DIMENSIONAL TLC**

The two-dimensional TLC analysis was carried out in one of the triplicate samples. Individual chloroplastidic and extra-chloroplastidic polar lipid classes were separated from the total lipids using two-dimensional TLC using chloroform:methanol:water (65:25:4, v/v/v) as the first solvent system and chloroform:methanol:NH₄OH:ethylpropylamine (65:35:5:0.5, v/v/v/v) as the second solvent system as described (Khozin et al., 1997). The lipid spots were visualized under UV after spraying 0.001% primuline in acetone/water (4:1, v/v) and were collected for GC analysis with a known amount of 17:0 free fatty acid as internal standard as described previously (Zhou et al., 2013).

**LIPID PROFILING WITH LC-MS**

The extracted total lipids were analyzed using an Agilent 1200 series LC coupled to an Agilent 6410B electrospray ionization QQQ-MS (Agilent, Palo Alto, California, USA). A 5 μL injection of each total lipid extract was chromatographically separated with an Ascentis Express RP-Amide 50 × 2.1 mm, 2.7 μm HPLC column (Sigma-Aldrich, Castle Hill, Australia) using a binary gradient with a flow rate of 0.2 mL/min. The mobile phases were: A. 10 mM ammonium formate in H₂O:methanol:tetrahydrofuran (50:20:30, v/v/v); B. 10 mM ammonium formate:methanol:tetrahydrofuran (35:45:20, v/v/v).
ammonium formate in H$_2$O:methanol:tetrahydrofuran (5:20:75, v/v/v). Selected neutral lipids (TAG and DAG) and phospholipids (PL, including PC, PE, PI, PS, PA, PG) were analyzed by multiple reaction monitoring (MRM) using a collision energy of 30 V and fragmentation energy of 60 V. Neutral lipids were targeted on the following major fatty acids: 16:0 (palmitic acid), 18:0 (stearic acid), 18:1ω9 (oleic acid, OA), 18:2ω6 (linoleic acid, LA), 18:3ω3 (α-linolenic acid, ALA), 18:4ω3 (stearidonic acid, SDA), 20:1, 20:2, 20:3, 20:4ω3, 20:5ω3, 22:4ω3, 22:5ω3, 22:6ω3, while phospholipids were scanned containing C$_{16}$, C$_{18}$, C$_{20}$, and C$_{22}$ species with double bonds of 0–3, 0–4, 0–5, 4–6 respectively. Individual MMRs for each TAG was based on ammoniated precursor ion and product ion from neutral loss of 20:1, SDA, EPA, and DHA. TAG and DAG were quantified using the 50 μM tristearin and diestearin injected in the same batch as external standards. Phospholipids were quantified with 10 μM of di-18:0-PC, di-17:0-PA, di-17:0-PE, 17:0–17:1-PI, and di-17:0-PS external standards (Avanti Polar Lipids, Alabaster, Alabama, USA) injected in the same batch, and presented as μM in the total lipid extract. All the LC-MS data were presented as average of triplicate samples with standard deviation, calculated with Microsoft Excel. Selected TAG, DAG, and PL species were further confirmed by Agilent 6520 Q-TOF MS/MS using the same chromatographic conditions as just described.

RESULTS AND DISCUSSION
MEMBRANE PHOSPHOLIPID CHARACTERIZATION: INCREASED PHOSPHOLIPIDS IN TRANSGENIC LINE

We previously showed that expressing seven enzymes for DHA biosynthesis pathway in Arabidopsis resulted in the efficient accumulation of DHA (up to 15.1%) in total seed lipids of transgenic line GA7 (Petrie et al., 2012). These enzymes included L. kluveri Δ12-desaturase, P. pastoris Δ15-ω3-desaturase, M. pusilla Δ6-desaturase, P. cordata Δ6- and Δ5-elongases and P. salina Δ5- and Δ4-desaturases. GA7 showed significantly increased ALA and a reduced proportion of 20:1 in total seed lipids. In addition, GA7 accumulated only very low amounts of C$_{20}$ intermediates. In the present study, we have further characterized the PL, DAG, and TAG species in detail using LC-MS analysis in MRM mode. Comparative LC-MS analysis was carried out on the developing and mature seeds of GA7 and wild type (WT) control in order to compare the lipid species.

An overall increase in PL was observed in GA7 when compared to the wild type seeds from the plants grown at the same time. The profiles and the amounts of the different PL classes were first analyzed by two-dimensional TLC followed by fatty acid analysis by GC, as the indicative result. The most abundant PL species, PC, in the Arabidopsis seeds, significantly increased (40%) in GA7 mature seeds compared to WT mature seeds as shown in Table 1, based on seed dry weight. Detailed lipidomic profiling was then carried out using LC-MS on triplicate samples. The LC-MS data showed even higher than 40% increase of PC from WT to GA7 (see below). It has been shown in multiple plants that fatty acid engineering can reduce total TAG production and thus reduce seed weight (Dauk et al., 2007; Li et al., 2012; Bates et al., 2014). The expression of DHA synthesis pathway in Columbia did reduce the mature seed weight from 2.05 ± 0.13 mg
in WT to 1.89 ± 0.03 mg in GA7 per 100 seeds (five replicates). In other words, GA7 resulted in about 8% reduction in seed weight compared to WT. Although we did not analyse the lipids concentration per individual seed, it is clear that the 8% reduction of seed weight compared with the 40% increase in PC from WT to GA7 still shows an increase in the levels of PC even on a per seed base. Both the WT and GA7 showed higher amounts of PC in the developing seeds (WT-d, GA7-d) than in their mature seeds (WT-m and GA7-m). Further, GA7 had increased total amounts of PC in the developing (2.3-fold higher) and mature seeds (1.4-fold higher) than the WT mature seeds. Again, the amount of PC was expressed based on seed dry weight. Nevertheless, ectopic expression of five membrane-associated fatty acid desaturases resulted in an altered unsaturated fatty acid profile of the PC fraction in GA7. The levels of oleic acid and linoleic acid in PC showed a significant reduction, with decreased of 10.5–0.4 and 47.4–1.4%, respectively. This is associated with a significant increase of newly synthesized ω3 and ω6 LC-PUFA. This result was confirmed by more detailed LC-MS analysis of PC species. Other PL classes followed a similar pattern of increase in GA7 compared to WT as described for PC, and altered the fatty acid profile similar to PC, i.e., a significant reduction of 18:2 and an associated increase of 18:3 and LC-PUFA based on seed dry weight (Table S1, and see detail LC-MS analysis below).

LC-MS analysis of the PL was focused on PA, PC, PE, PG, PI, and PS. The major fatty acids from palmitic acid to the end product ω3 DHA in the engineering pathway were analyzed. As shown in Figure 2, PC and PE were the major PL, followed by PA, with very low amounts of PG and PS. The LC-MS analysis confirmed that there were more total PLs in the developing seeds than in the mature seeds of GA7.

Both the developing and mature seeds of GA7 produced new LC-PUFA in phospholipids. PC precursor total ion scan showed that the WT seeds contained PC with total acyl chain lengths only up to C38 (PC 38:Y, in which a total of 38 carbons occurs

FIGURE 2 | Total phospholipids in developing and mature Arabidopsis seeds. Phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidyethanolamine (PE), phosphatidylglycerol (PG), phosphatidylserine (PS), and phosphatidylinositol (PI) were determined from triplicate samples with the mean and standard deviation shown. WT-m, Columbia wild type mature seeds; GA7-d and GA7-m, developing and mature seeds of transgenic GA7 line containing a DHA biosynthetic pathway (genes are described in the text). Data are shown as the mean of triplicate analysis with the error bars representing the standard deviations, concentrations (μM) have been determined in the extracts from the same amount of dry seed weight for both developing and mature seeds.

FIGURE 3 | Summed spectra from the precursor ion scan for phosphatidylcholine (PC) from the WT or GA7 Arabidopsis seeds. PC 36:Y indicates PC species with a total of 36 carbons with a total number of Y double bonds in the two acyl chains. Y axis represents the response of ion scan.
in the two acyl chains on PC with a total double bond number of Y). The most likely fatty acid combination of being C_{18} and C_{20} (Figure 3). The WT only had low amounts of C_{20} fatty acids in PC (Table 1). The developing GA7 seeds produced PC 40:Y, indicating the esterification of two C_{20} fatty acids. In the mature GA7 seeds, there were low yet detectable amounts of PC 42:Y and PC 44:Y, i.e., PC C_{20}/C_{22} or PC C_{22}/C_{22}, indicating the accumulation of LC-PUFA in the PC pool. Furthermore, precursor ion scan showed higher abundance of the earlier eluted part of the each PC chain length group in GA7 when compared to WT. The molecular species with more double bonds eluted slightly earlier than the molecular species with fewer double bonds in the group with same chain length. This indicated that the PC clusters in GA7 contain more double bonds. This was further supported by the quantification of the different PC species as shown in Figure 4A.

There was a large increase in PC 36:6 (PC 18:3/18:3 as the major species) in GA7 seeds, followed by PC 34:3 (PC 16:0/18:3 as the major species). PC species containing higher numbers of carbons and double bonds in their acyl chains such as PC 40:4 to PC 44:12 were only found in GA7, suggesting EPA or DHA also accumulated in the PC pool. There was a small amount of PC 44:12 (0.9 ± 0.01% out of total PC in mature GA7 seeds), which was likely PC 22:6/22:6. MS/MS analysis of this precursor (878.6 m/z) by Q-TOF confirmed the loss of PC head group (184.2 m/z) and the 22:6 fatty acid (568.3 m/z), as shown in Figure S1.
FIGURE 5 | Phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylglycerol (PG) pools in the developing and the mature *Arabidopsis* seeds. The phospholipids PE (A), PG (B) and PI (C) species are annotated similarly to PC. Data are shown as the mean of triplicate analysis with the error bars representing the standard deviations.
There was a much lower level of PA in the mature seeds than in the developing seeds based on dry seed weight (Figure 2). It should be noted that lipase activity during extraction could contribute to the higher amount of PA in developing seeds, through conversion of PC to PA, and therefore a reduction of the PC concentration would be expected. Two extraction methods were tested using either hot isopropanol quenching (either at 85 or 95°C) or with the procedure described above, and no significant difference in PA concentrations was found by these two methods. Our results showed both PA and PC (and even PE, PI) were higher in developing seeds than in mature seeds (Figure 2), suggesting the higher amount of PA was not an artifact from lipase activity. GA7 had significantly higher amounts of some PA species, especially PA 36:6, PA 36:5, PA 36:4, PA 34:3 and PA 40:8 than other species (Figure 4B). Similar to PC, PA species containing higher numbers of carbons and double bonds in their acyl chains, such as PA 40:6 to PA 40:9, were only found in GA7.

LC-PUFA in other PL groups were also analyzed (Figure 5). PE was the second largest pool of PL. However, only low levels of LC-PUFA appeared in the PE pool. LC-PUFA was much lower in the PI and PG pools. Interestingly, the most significant increase in these three pools of PL in GA7 was 34:3 (possibly composed of palmitic acid and ALA) when compared to the WT. Another interesting aspect was that the much lower levels of PG was found in the GA7 developing seeds, compared to PE and PI. The PS pool was very small, and the LC-MS responses to most of the PS species were close to the detection limit (see Figure S2).

The substantially increased concentration of membrane PL in GA7 seeds when compared to WT seeds, especially the higher levels of PL in developing seeds compared to mature seeds, could be due to the over-expression of the introduced membrane bound fatty acid desaturases and elongases. In higher plants, acyl groups esterified onto PC are the substrates of desaturation by ER membrane desaturases (Sperling et al., 1993). Acyl editing via a PC-deacylation and lysophosphatidylcholine (LPC)-reacylation cycle provided LC-PUFA in the acyl-CoA pool, for further desaturation and elongation by the introduced acyl-CoA dependent enzymes, and for TAG assembly via the Kennedy pathway. The PC-deacylation and LPC-reacylation cycle was catalyzed by acyl-CoA:LCPC acyltransferase (LPCAT) (Bates et al., 2012). Although the engineered pathway mainly targeted acyl-CoA desaturases and elongases, the substantially increased PC pool suggested the importance of the role of PC in accumulation of LC-PUFA in TAG. This suggestion is also supported by Bates and colleagues who showed that the DAG used for TAG synthesis was mostly derived from PC (Bates et al., 2009).

**ACCUMULATION OF LC-PUFA CONTAINING DAG AND TAG IN TRANSGENIC SEEDS**

Plants commonly have neutral lipid pools in seeds consisting of C16 to C22 fatty acids with zero to three double bonds. Arabidopsis seeds have low amounts of unsaturated C16 fatty acids and C22 fatty acids. However, due to the expression of the DHA biosynthesis pathway, there can be more than 20 individual fatty acids in the seed lipids. The total number of theoretical TAG species is 8000 (20 × 20 × 20). We therefore targeted only the neutral lipid DAG and TAG species that contain at least one acyl chain consisting of

![Figure 6](https://www.frontiersin.org) Abundance of different diacylglycerol (DAG) species in the WT and the GA7 Arabidopsis seeds. DAG species are labeled in format of DAG target/X:Y, where target will be 20:1 (A), SDA (B), EPA (C) or DHA (D), with another acyl containing X number of carbons and Y number of double bonds. Data are shown as the mean of triplicate analysis with the error bars representing the standard deviations.
20:1, SDA, EPA, or DHA by setting up MRM’s with neutral losses containing one of the four fatty acids of interest. These are shown in the format of TAG 20:1/X:Y, or TAG DHA/XY, where X and Y are the total numbers of carbons and double bonds of fatty acids on the remaining two positions (one position for DAG), respectively.

Analysis of DAG species containing one of the four targeted fatty acids showed that both the GA7 developing and mature seeds accumulated substantial amounts of DHA-containing DAG, with low amounts of DAG containing the intermediate SDA or EPA (Figure S3). In WT, the most abundant 20:1-containing DAG was DAG 20:1/18:2 (52.2% among all 20:1-containing DAG), followed by DAG 20:1/18:3. In contrast, the GA7 mature seeds showed about five times more DAG 20:1/18:3 than DAG 20:1/18:2 (65.0% vs. 12.2% among all 20:1-containing DAG). The major DAG species among all 4 analyzed DAG groups were the DAG molecules containing C18:3 (Figure 6). In GA7 mature seed lipids, a relatively high percentage of DAG SDA/16:0 and DAG EPA/16:0 was also found, making up 16.0% of total SDA-containing DAG and 23.0% of total EPA-containing DAG, although the overall amounts were low (Figures 6B,C). In contrast, DAG DHA/16:0 was found only at 10.7% of the total DHA-containing DAG, while the predominant DAG DHA/18:3 species was 52.0%.

DHA-containing TAG species were only found in the GA7 developing or mature seeds. The accumulation of LC-PUFA-containing TAG was at the expense of 20:1-containing TAG (Figure 7). This is in line with previous results reported for total lipids from the GA7 seeds showing that 20:1 was significantly reduced (Petrie et al., 2012). In GA7, most of the 20:1-containing TAG species were reduced when compared to WT, except for the TAG species that also contained ALA or LC-PUFA. For example, TAG 20:1/34:3 (TAG 20:1/16:0/18:3) and TAG 20:1/36:6 (TAG 20:1/18:3/18:3) were increased in GA7 compared to WT (Figure 8A). SDA-, EPA- and DHA-containing TAGs were only found in the GA7 developing or mature seeds. As expected, TAG species 20:1/40:5 to 20:1/40:10, consisting of 20:1 plus LC-PUFA beyond 20:3 in the pathway, were absent in WT. This is in contrast to their accumulation, although at low levels in GA7. The highest 20:1-containing TAG species in GA7 were TAG 20:1/36:2 and 20:1/36:6.

TAG species in the GA7 developing and mature seeds containing SDA, EPA or DHA that were absent from WT, are shown in Figures 8B–D. The levels of TAG SDA/34:3, TAG SDA/36:6, TAG SDA/36:7, TAG SDA/38:4 and TAG SDA/40:9 increased significantly from the developing seeds to the mature seeds. There was no significant change in profile of EPA- or DHA-containing TAG species in the developing or mature seeds. EPA accounted for only 1.8%, compared to 15.1% of DHA in total seed lipids.

MAJOR NOVEL ω3 LC-PUFA CONTAINING TAG SPECIES IN THE TRANSGENIC SEEDS

The most abundant TAG species among the four analyzed groups in mature GA7 seeds were the TAG species that also contained 18:3, i.e., TAG 20:1/36:6, TAG SDA/36:6, TAG EPA/36:6, TAG DHA/36:6. This was followed by TAG 20:1/34:3 and TAG 20:1/36:2, TAG SDA/34:3 and TAG SDA/38:4, TAG EPA/34:3 and TAG EPA/38:4, or TAG DHA/34:3 and TAG DHA/36:5. Very low amounts of tri-DHA TAG was also detected in the GA7 mature seeds. This highlights the utility of LC-MS based lipid profiling as this finding would be impossible with GC-MS based protocols. These identities were confirmed by MS/MS analysis (Figure S4) and summarized in Table 2. The results also suggested a likely preference of LC-PUFA combined with 18:3 in TAG. Based on the TAG fatty acid profile in the mature seed of GA7 which was dominated by 18:3, 22:6, 16:0, and 20:1 (Petrie et al., 2012), the TAG species distribution among all the DHA containing TAG species was calculated, if they were randomly associated. The TAG species that were significantly higher in measured association than the randomized prediction mainly contained 18:3 (Table S2). In contrast, the DHA containing TAG species combined with other dominant fatty acids like 16:0 and 20:1 were significantly lower than the randomized prediction. The predicted tri-DHA in all the DHA containing TAG species could be 0.9%, but only 0.1% was measured. These suggested that DHA distribution in TAG was not randomly. Non-random pattern of elongated acyl chains that are
FIGURE 8 | Abundance of different triacylglycerol (TAG) species in the control (WT) and the transgenic (GA7) Arabidopsis seeds. TAG species are labeled in the format of TAG containing target/X:Y, where target will be 20:1 (A), SDA (B), EPA (C) or DHA (D), with the other two acyl chains containing X number of total carbons and Y number of total double bonds. Data are shown as the mean of triplicate analysis with the error bars representing the standard deviations. Predominant TAG species within the subgroups are indicated.
incorporated into TAG in Arabidopsis seed is recently discovered by LC-MS (Li et al., 2014).

The positional distribution of the three fatty acids in the abundant TAG species could not be resolved by LC-MS analysis. Other studies using NMR analysis of DHA in Arabidopsis seed oil have shown that DHA is preferentially positioned at sn-1/3 (Petrie et al., 2012). The most abundant DHA-containing DAG or TAG were DAG DHA/18:3 or TAG DHA/18:3/18:3, implying (Petrie et al., 2012). The most abundant DHA-containing DAG species showed DAG 18:3/16:0 and DAG 18:3/18:3 had a significant increase in GA7 when compared to WT (data not shown). This observation was in agreement with that previously proposed for the PC-derived DAG contributing to synthesis of PUFA-containing TAG (Bates and Browse, 2012).

Recently, Ruiz-Lopez et al. (2013a) reported a maximum transgenic production of 14% DHA in C. sativa. By transgenically expressing a different set of seven enzymes, they found the DHA accumulation in PC was higher than DAG, with even lower levels in TAG, indicating the existence of an inefficient flux of DHA into TAG. This might reflect the different set of enzymes used in these two studies, or due to the different host background. In summary, the accumulation of high levels of DHA in transgenic Arabidopsis seed oil was accompanied with enhanced levels of PL especially PC, DHA-containing DAG and TAG species, as well as the decreased levels of 20:1-containing DAG and TAG. The work also showed evidence that there is a preference of engineered LC-PUFA, especially DHA, in neutral and phospholipid containing 18:3. This study demonstrates that LC-MS analysis of lipidome is an invaluable tool for gaining insights into how the engineered fatty acid combine with other fatty acids in lipids of transgenic seeds.

**AUTHOR CONTRIBUTIONS**

Conceived and designed the experiment: Xue-Rong Zhou, Surinder P. Singh. Performed the experiment: Xue-Rong Zhou, Damien L. Callahan, Pushkar Shrestha, Qing Liu, James R. Petrie. Analyzed the data: Xue-Rong Zhou, Damien L. Callahan, Pushkar Shrestha, Surinder P. Singh. Contributed reagents/materials/analysis tools: Damien L. Callahan, Pushkar Shrestha. Wrote the paper: Xue-Rong Zhou, Surinder P. Singh. All authors revised the draft and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fpls.2014.00419/abstract

Figure S1 | MS/MS analysis of phosphotidylcholine (PC) precursor at m/z 878.6 with a PC head group ion of m/z 184.2. Y axis represents the response of ion scan. The low abundant fragment at m/z 568.3 is due to the neutral loss of 22.6, thus confirming the identity as PC 22:6/22:6.

Figure S2 | Low abundance phosphatidylserine (PS) pool in the developing and the mature Arabidopsis seeds. The PS are annotated similarly to PC. Data are shown as the mean of triplicate analysis with the error bars representing the standard deviations.

Figure S3 | The abundances of long-chain polyunsaturated fatty acids (LC-PUFA) containing diacylglycerol (DAG) in the WT and GA7 Arabidopsis seeds. Data are shown as the mean of triplicate analysis with the error bars representing the standard deviations.

Figure S4 | Product ion spectra for identification of selected lipid species. (A) Fragmentation of the [M+NH4]+ ion at 940.75 m/z showing the neutral losses of 18.3 and 22.6, represents a triacylglycerol (TAG) composed of 18:3/18:3/20:1. (B) Fragmentation of the [M+NH4]+ ion at 922.79 m/z showing the neutral losses of 18.3 and 20.1, represents a TAG composed of 18:3/18:2/20:1. (C) Fragmentation of the [M+NH4]+ ion 1040.59 m/z showing the neutral loss of 22:6, represents a TAG composed with tri-DHA.

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