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Unraveling enhanced membrane lipid biosynthesis in *Chlamydomonas reinhardtii* starchless mutant *sta6* by using an electrospray ionization mass spectrometry-based lipidomics method*

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Abstract The unicellular green alga *Chlamydomonas reinhardtii*, a well-established model organism, has been widely used in dissecting glycerolipid metabolism in oxygenating photosynthetic organisms. In previous studies, it has been found that shunting carbon precursors from the starch synthesis pathway can lead to a 10-fold increase in TAG content as compared to the wild type, but it is unknown whether inactivation of AGPase may affect membrane lipids biosynthesis. The study aims to investigate global changes in lipid metabolism and homeostasis in the starchless mutant *C. reinhardtii sta6*. By utilizing an electrospray ionization/mass spectrometry (ESI/MS)-based lipidomics approach, a total of 105 membrane lipid molecules of *C. reinhardtii* were resolved, including 16 monogalactosyldiacylglycerol (MGDG), 16 digalactosyldiacylglycerol (DGDG), 11 phosphatidylglycerol (PG), 6 sulfoquinovosyldiacylglycerol (SQDG), 49 diacylglyceryl-*N*,*N*,*N*-trimethylhomoserine (DGTS), 2 phosphatidylethanolamine (PE), and 5 phosphatidylinositol (PI) molecules. The quantitative results indicated that the membrane lipid profiles were similar between the two *C. reinhardtii* strains grown under both low- and high-light conditions, but the cellular contents of a great number of lipids were altered in *sta6* due to the defect in starch biosynthesis. Under low-light conditions, *sta6* accumulated more PI, MGDG, DGDG but less amounts of DGTS as compared to WT. Under high light, s*ta6* cells contained higher content membrane lipids than cc-124, except for PG, which is more or less similar in both strains. Our results demonstrate that the cellular membrane lipid homeostasis underwent profound changes in the starchless mutant, and thereby its physiological impact remains to be explored.

Keyword: *Chlamydomonas reinhardtii*; chloroplast lipidomes; extraplastidic lipidomes; electrospray ionization mass spectrometry

1 INTRODUCTION

The unicellular green microalga *Chlamydomonas reinhardtii* has been widely used as a model organism for studying lipid metabolism in photosynthetic organisms (Harris, 2001; Siaut et al., 2011; Merchant

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et al., 2012; Li-Beisson et al., 2015). Lipids possess crucial functions in many biological processes in plants and microalgae, such as maintaining cellular membrane structures, storing energy and carbons, and mediating signaling pathways (Guschina et al., 2006; Hu et al., 2008; Barbaglia and Hoffmann-Benning, 2016). Lipid profiles of algal cells may be affected by various environment stresses, such as high light and nutrient deprivation conditions (Solovchenko et al., 2008).

The traditional methods for lipids analysis include thin layer chromatography (TLC), gas chromatography (GC) and GC-mass spectrometry (GC/MS) (Diehl et al., 1995; Xu et al., 2002; Rainville et al., 2007). However, there are many disadvantages for these methods. TLC, GC, and GC/MS involve time-consuming and laborious sample pre-treatment procedures. Moreover, these methods cannot be used to quantify the cellular content of a given lipid molecule, with limited applications in functional genomics studies (Rainville et al., 2007; Carrasco-Pancorbo et al., 2009).

In recent years, mass spectrometry (MS) based analytical methods have been used in analysis of lipidomes of biological samples with different genetic backgrounds or physiological interferences (Han and Gross, 2003, 2005; Welti et al., 2003; Rainville et al., 2007). Since microalgal glycerolipids have attracted great interest due to their potentials in energy and nutraceutical applications, lipidomes of a large number of microalgae including both membrane glycerolipids and triacylglycerols have been resolved (reviewed by De Costa et al., 2016). Lipidomics is a very effective biochemical analytical tool for precise identification of glycerollipids, which is not only able to resolve lipid classes and fatty acyl composition, but also can facilitate absolute quantification at molecular level, especially when coupled with ultra-high performance liquid chromatography that can essentially improve the sensitivity and reproducibility (Cutignano et al., 2016; Han et al., 2017; Řezanka et al., 2018).

For *C. reinhardtii*, the model species for dissecting lipid metabolism in microalgae, its lipidomes have been characterized in a few previous studies which showed monogalactosyldiacylglyceride (MGDG), digalactosydiacylglyceride (DGDG), sulfoquinovosyldiacylglycerol (SQDG), phosphatidylglycerol (PG), diacylglyceryl-*N*,*N*,*N*-trimethylhomoserine (DGTS), phosphatidylinositol (PI) and phosphatidyl ethanolamine (PE) are the major membrane lipids of *C. reinhardtii* (Yoon et al., 2012; Yang et al., 2015). MGDG, DGDG, SQDG, and PG constitute the chloroplast membranes in algal cells, playing important functional roles in photosynthesis (Harwood and Guschina, 2009; Kobayashi et al., 2016). In microalgae like *C. reinhardtii*, the extraplastidic membrane lipids are mainly composed of DGTS, PI and PE (Moellering et al., 2009), among which the betain lipid DGTS was the major class (Klug and Benning, 2001).

In this study, the analytical tool of ultra performance liquid chromatography (UPLC)-ESI-MS/MS was used to quantitatively analyze the lipidomes of *C. reinhardtii* wild type and starchless mutant *sta6* cells. The starchless mutant *sta6* was deficient of the gene encoding an ADP-glucose pyrophosphorylase subunit and hence incapable of synthesizing starch (Zabawinski et al., 2001). In previous study, shunting carbon precursors from the starch synthesis pathway in *C. reinhardtii* led to a 10-fold increase in triacylglycerol (TAG) content as compared to the wild type (Li et al., 2010a, b), but it is unknown whether inactivation of AGPase may have any effect on the cellular contents of membrane lipids. The study aims to investigate global changes in lipid metabolism and homeostasis in the starchless mutant. In addition, responses of such two *C. reinardtii* strains to the changing illumination conditions at the level of lipid molecules were investigated by using the lipidomics approach as well, which will underpin our understanding about the impact of blocking starch biosynthesis on cellular capabilities in adapting changing environmental conditions.

2 MATERIAL AND METHOD

2.1 Strains and growth conditions

Chlamydomonas reinhardtii cc-124 (WT) was purchased from *Chlamydomonas* resource center at the University of Minnesota (https://www.chlamycollection.org), and *sta6* was kindly provided by Arizona State University. Both strains were pre-cultured in the TAP growth medium (Gorman and Levine, 1965) at 25°C on an orbital shaker incubator (NBS Innova 44R, Eppendorf, Germany) at a constant rate of 100 r/min. The light intensity was kept at 50 μmol/(m^2·s), which was measured by using a photosynthetically active radiation (PAR) meter (Mastercycler pro S, Eppendorf, Germany). Algal cells from the logarithmic phase were inoculated into the 800 mL column photobioreactor (i.d., 5 cm)

containing 750 mL HSM medium (Sueoka, 1960), with an initial cell concentration of (2.0–2.5)×10^6 cells/mL. Algal cells were cultivated under 5 μmol/(m^2·s) (low light, LL) for 3 days and then transferred to 150 μmol/(m^2·s) (high light, HL) for 12 h. The algal samples from both illumination conditions were harvested by centrifugation at 3 000×*g* for 5 min at 4°C, and then freeze-dried.

2.2 Lipid extraction

Total lipids were extracted according to the method described previously (Wang and Benning, 2011) with minor modifications. The freeze-dried samples (about 10 mg, dry weight) were ground in liquid nitrogen and extracted with 6 mL solvents composed of methanol, chloroform and formic acid (20:10:1, v/v/v). The extracts were vigorously vortexed for 5 min followed by adding 3 mL of 0.2 mol/L H_3PO_4 and 1 mol/L KCl, and then vortexed briefly. After centrifuging at 1 000×*g* for 5 min, lipids dissolved in the lower chloroform phase were collected by using a glass pipette. Solvents were then evaporated under nitrogen stream. Lipid samples were stored at -80°C prior to use.

2.3 Lipidomes analysis and quantification with ESI/MS

Lipidomes analyses were performed on a triple quadrupole MS/MS (Xevo TQ-S, Waters, USA) with electrospray ionization (ESI) source coupled with an Acquity Ultra-Performance Liquid Chromatography (UPLC) system (Waters, USA). Lipid samples were separated on a BEH C_{18} column (length 50 mm, internal diameter 2.1 mm, particle size 1.7 μm; Waters) prior to the ESI/MS analysis. The column temperature was kept at 30°C and 35°C for the positive and negative ion mode, respectively. The ESI/MS analysis was carried out according to the method developed by Yoon et al previously (Yoon et al., 2012) with modifications. Samples were recovered in 1 mL chloroform/methanol (1:1, v/v). For absolute quantification, lipids samples were mixed with internal standards (ISTD), including MGDG 18:0/18:0, DGDG 18:0/18:0, DGTS 16:0/16:0 d9, PE 17:0/14:1, PG 17:0/20:4 and PI 17:0/20:4. Among these ISTD, PG 17:0/20:4 was used for both PG and SQDG quantification. The external standards (ESTD) for calibration included MGDG mixture standard containing MGDG 16:3/18:3, MGDG 16:3/18:2 and MGDG 16:1/18:3, DGDG mixture standard containing DGDG 16:3/18:3, DGDG 16:3/18:2, DGDG 16:1/18:3 and DGDG 16:0/18:3, DGTS 16:0/16:0, PG 16:0/18:1, PG 18:0/18:1, PE 18:0/18:1, PI 18:1/18:1 and SQDG 16:0/18:3 were used as ETSDs for the corresponding classes of membrane lipids. Lipid standards were all purchased from Avanti Polar Lipids Ltd. (USA) except that MGDG 18:0/18:0 and DGDG 18:0/18:0 were obtained from Matreya LLC (USA). For quantification, ESTD were titrated relative to a constant amount of ISTD to establish the correlation between the ratio of the analyte signal to the ISTD signal and the ratio of their concentrations (Wang et al., 2014). MGDG, DGDG, and DGTS were detected in the positive mode, while PG, SQDG, PE, and PI were analyzed in the negative mode. Multiple reaction monitoring (MRM) was employed for quantitative analysis.

2.4 Data processing

Raw files of the ESI/MS data were retrieved and analyzed with Masslynx v4.1 software (Waters). Student's *t*-test was used to compare the cellular contents of given lipids between WT and *sta6* (n=4, including two biological replicates and two technical replicates). If the test gives P value ≤0.05, the differences between two samples were interpreted as being significant.

3 RESULT

3.1 Growth of cc-124 and *sta6* under low light and high light

The biomass of cc-124 increased slightly in the first 2 h and then was fluctuated over 12 h under the LL conditions (Fig.1a). On the contrast, WT grew fast under the HL conditions, the biomass of which achieved 0.4 g/L after 12 h (Fig.1a). Distinct from cc-124, *sta6* showed slight growth under two conditions and no significant difference was observed (Fig.1b).

3.2 Lipidomes of cc-124 and *sta6* cells grown under low light

The lipidomics platform established in this study enabled absolute quantification of 7 classes of major membrane lipids of *C. reinhardtii* (Table 1). Under LL, the content of total membrane lipids was 180.57±14.75 and 283.63±11.90 nmol/mg (P<0.001) for WT and *sta6*, respectively. Under HL, the content of total membrane lipids was reduced by 11.7% to 159.13±7.14 nmol/mg in WT, while it was increased

Table 1 The contents of total membrane lipid classes and total membrane lipids in WT and *sta6* cultivated under low light (LL) and high light (HL) conditions

Lipid content (nmol/mg)	WT		*sta6*	
	LL	HL	LL	HL
MGDG	36.42±3.65	41.96±3.00	90.09±4.99	83.77±1.18
DGDG	11.94±0.92	28.07±1.78	35.93±2.06	124.09±2.90
SQDG	44.09±3.31	23.41±1.28	52.31±3.94	48.81±2.96
PG	42.42±0.80	38.32±2.13	39.58±2.85	40.06±2.64
PE	3.82±0.34	3.06±0.29	4.55±0.27	6.10±0.95
PI	4.48±0.23	2.46±0.21	37.26±2.34	49.99±1.74
DGTS	33.48±0.95	21.30±1.39	21.92±0.84	26.26±1.44
Total membrane lipids	180.57±14.75	159.13±7.14	283.63±11.90	386.82±34.52

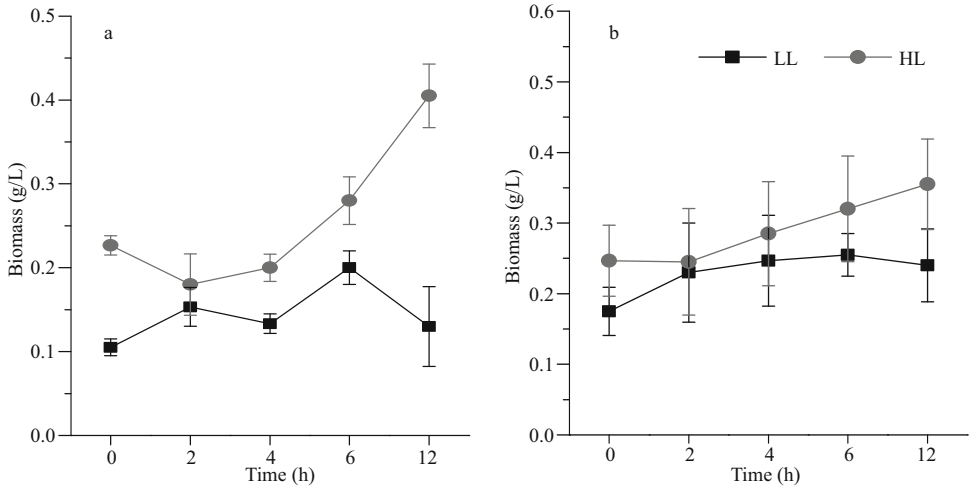

Fig.1 Biomass production of WT (a) and *sta6* (b) cultivated under low-light (LL) and high-light (HL) conditions

by 36.4% and reached 386.82±34.52 nmol/mg in *sta6*.

The galactolipids including MGDG and DGDG were the most abundant membrane lipids in WT and starchless mutant *sta6* grown under LL, accounting for 27.38% and 44.74% of the total membrane lipids in them, respectively (Fig.2, Table 1). In WT, the content of MGDG and DGDG was 36.42 nmol/mg DW and 11.94 nmol/mg DW, respectively. The content of MGDG and DGDG in *sta6* was greater than that of WT by 147.35% (P<0.001) and 200.78% (P<0.001), respectively.

Under LL, the most abundant MGDG molecular species was MGDG 18:3/16:4 in WT, of which the content was 20.59 nmol/mg DW (Fig.3a), accounting for 14.50% of the total MGDG. Different from WT, MGDG 18:1/16:1, 18:1/16:4, and 18:3/16:4 were the most abundant MGDG species in *sta6* cells grown under LL, accounting for 25.77% (corresponding to 23.11 nmol/mg DW), 23.27% (20.86 nmol/mg DW), and 23.90% (21.43 nmol/mg DW) of its total MGDG pool, respectively. The major DGDG species in WT and *sta6* under LL was both DGDG 18:3/16:1, which was 7.6 and 30.87 nmol/mg DW (Fig.3b), corresponding to 65.07% and 86.78% of the total DGDG, respectively.

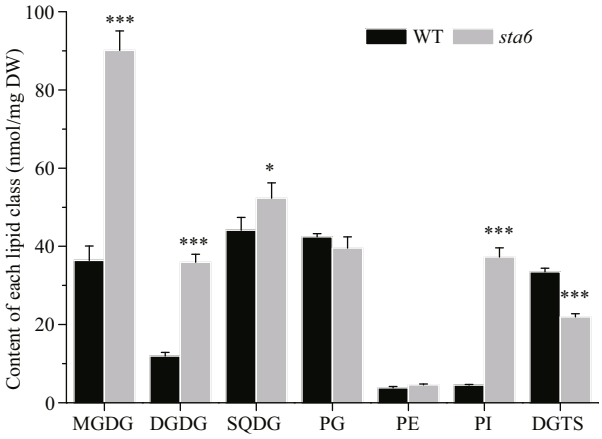

Fig.2 Contents of membrane lipids in *C. reinhardtii* WT and *sta6* cells under low light (LL)

Values represent the mean ± SD (n=4). * P<0.05, *** P<0.001.

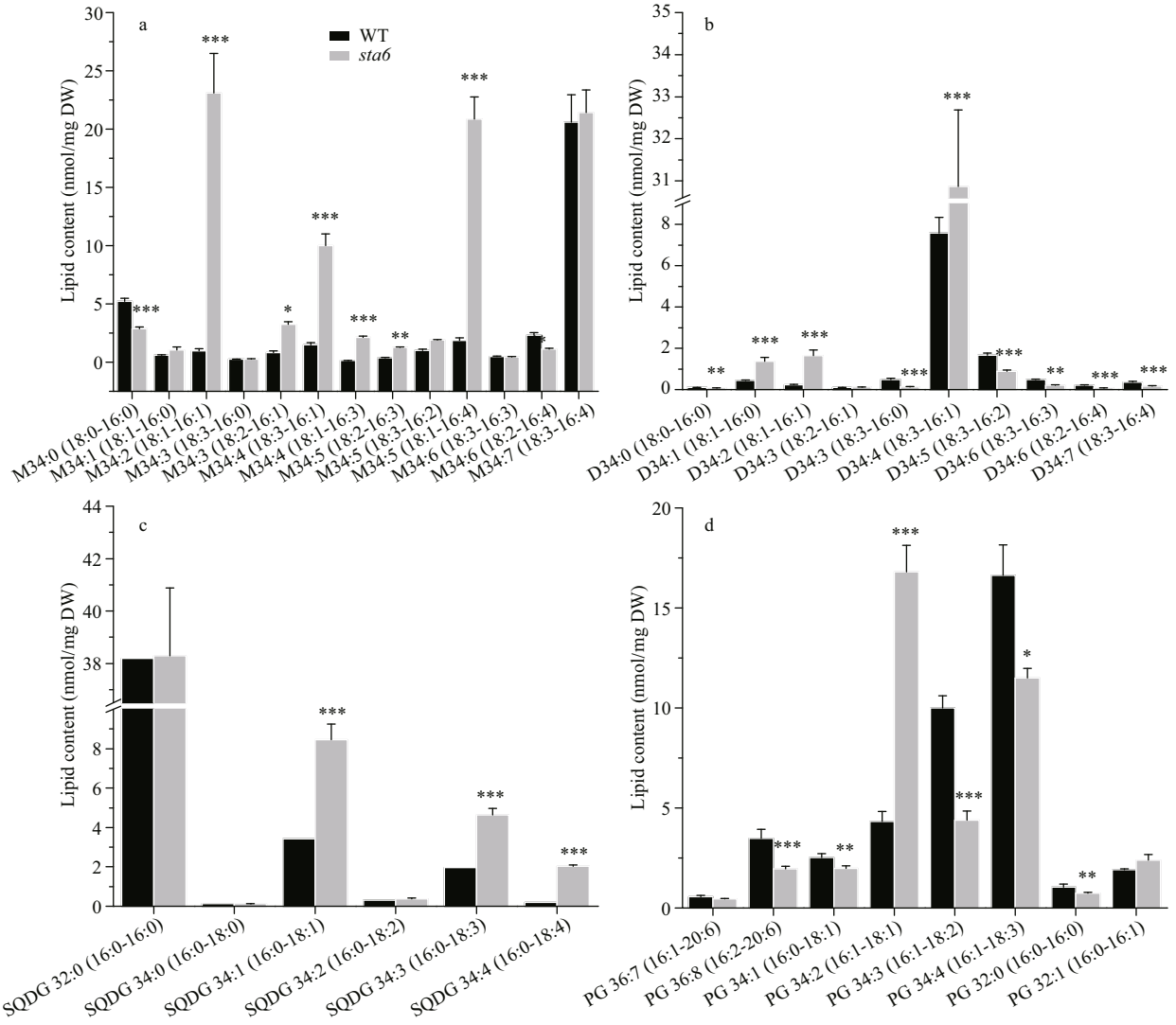

Fig.3 Contents of chloroplast membrane lipids molecular species in *C. reinhardtii* WT and *sta6* under low light (LL)

a. MGDG; b. DGDG; c. SQDG; d. PG. Values represent the mean±SD (n=4). *: P<0.05; **: P<0.01; ***: P<0.001.

SQDG and PG were two types of polar lipids present in the chloroplast membranes. The total SQDG content in *sta6* under LL was 53.92 nmol/mg DW, which was 18.64% higher than in WT (44.09 nmol/mg DW, P<0.05) (Fig.2). Among SQDG molecular species, the content of SQDG 16:0/16:0 was 38.20 and 38.29 nmol/mg DW in WT and *sta6*, respectively (Fig.3c), which was the major species in both WT and *sta6*. Among SQDG molecules, one of the highly unsaturated species SQDG 16:0/18:4 was the one enhanced to the most extent, which was increased by 975.84% (P<0.001) in *sta6* as compared with WT. As for PG, the only bulk phospholipid found in chloroplast (Wada and Murata, 2007), WT and *sta6* contained similar amounts of them, of which the cellular content was 42.42 and 39.58 nmol/mg DW, respectively (Fig.2). The most abundant PG species were PG 16:1/18:3 in WT, whereas it was replaced by PG 16:1/18:1 in *sta6* (Fig.3d), which accounted for 41.09% and 41.81% of their total PG, respectively. In addition, PG 16:1/18:1 showed the greatest change with an increase by 287.80% (P<0.001) in *sta6* compared to WT.

PI and PE were two types of phospholipids constituting the extraplastidic membranes in *C. reinhardtii*. Under LL, the total PI content was 4.36 nmol/mg DW in WT (Fig.2), among which PI 16:0/18:1 was the major species accounting for 94.24% of the total PI (Fig.4a). In *sta6*, the content of PI was increased dramatically by 731.58% (P<0.001) compared to that of WT. There was no significant change in total PE content between WT and *sta6*, which were 3.82 and 4.55 nmol/mg DW, respectively (Fig.2). Two PE species including 18:0/18:3 and

18:2/18:3 were identified in both strains, among which PE 18:0/18:3 was the major species (Fig.4a).

A large number of DGTS molecular species were identified in both strains. DGTS 16:0/18:3 was the most abundant species in WT (7.61 nmol/mg DW), whereas DGTS 16:0/16:1, 16:0/18:3 and 16:0/18:4 were three species with the highest contents in *sta6*, which was 3.08, 3.9, and 4.27 nmol/mg DW, respectively (Fig.4b). Among the membrane lipids, DGTS was the only group of lipid class that was reduced in *sta6* compared with WT (Fig.2, 33.48 vs 21.92 nmol/mg DW, P<0.001).

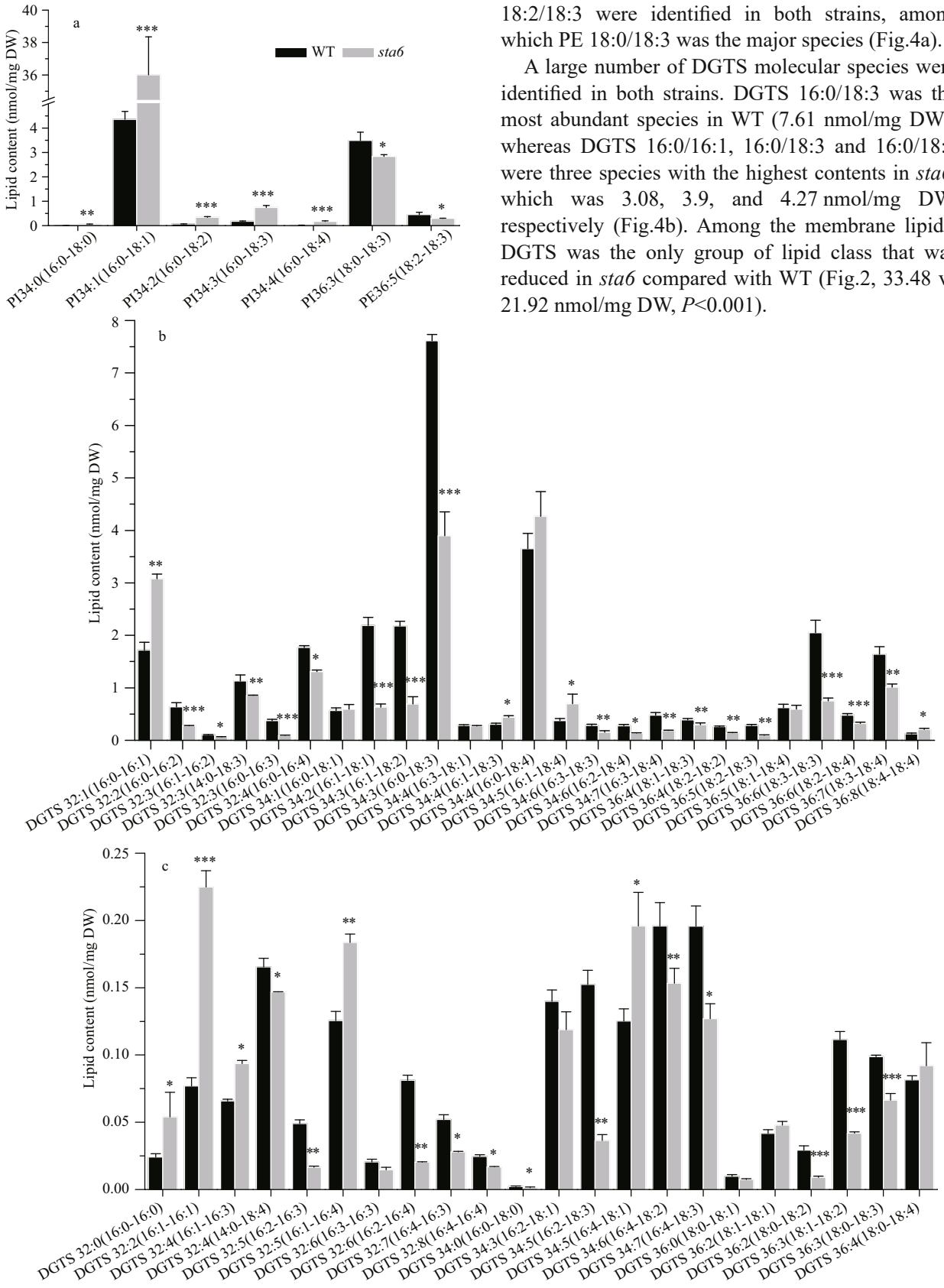

Fig.4 Contents of extraplastidic membrane lipids molecular species in *C. reinhardtii* WT and *sta6* under low light (LL)

a. PI and PE; b. DGTS major species; c. DGTS minor species. Values represent the mean±SD (n=4). *: P<0.05; **: P<0.01; ***: P<0.001.

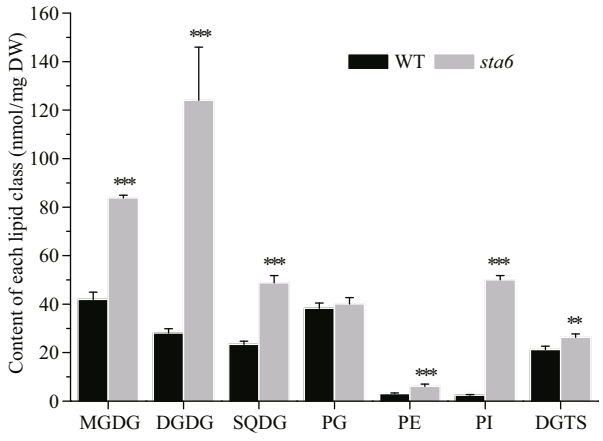

Fig.5 Contents of membrane lipids in *C. reinhardtii* WT and *sta6* cells under high light (HL)

Values represent the mean±SD (n=4). **: P<0.01; ***: P<0.001

3.3 Lipidomes of cc-124 and *sta6* cells grown under high light

After high light acclimation for 12 h, the contents of all the membrane lipids were significantly increased in *sta6* as compared to WT, except for PG. The chloroplast membrane lipid MGDG, DGDG, and SQDG was increased by 99.62% (P<0.001), 342.14% (P<0.001) and 108.50% (P<0.001), respectively, while the extraplastidic membrane lipids PI, PE, and DGTS was increased by 19-fold (P<0.001), 99.35% (P<0.001) and 23.32% (P<0.01), respectively (Fig.5).

The cellular contents of the chloroplast membrane lipid molecules were shown in Fig.6. For galactolipids, most MGDG and DGDG species in *sta6* were higher than that in WT (Fig.6a & b). The cellular content of MGDG 18:1/16:3 in WT was 0.14 nmol/mg DW, whereas it was 4.96 nmol/mg DW in *sta6*, increased by 33-fold (Fig.6a, P<0.001). The predominant DGDG 18:3/16:1 was 66.55 nmol/mg DW in *sta6*, upregulated by 392.51% compared to WT (Fig.6b, 13.51 nmol/mg DW, P<0.001). Similar to galactolipids, most SQDG species were up-regulated in *sta6* compared to in WT under HL. Among SQDG species, the most abundant SQDG 16:0/18:0 changed dramatically from 0.14 nmol/mg DW in WT to 0.63 nmol/mg DW in *sta6*, increased by 364.67% (Fig.6c, P<0.001). The total PG in two strains showed no significant change (Fig.5), because the cellular contents of two predominant species PG 16:1/18:2 and PG 16:1/18:3 were unchanged (Fig.6d). However, several minor PG species were increased in *sta6* compared to WT, including PG 16:1/18:1 increased by 138.63% (P<0.01) and PG 16:0/16:1 decreased by 41.41% (P<0.01) in *sta6* as compared with WT (Fig.6d).

Similar to the drastic changes in the chloroplast membrane lipids, the cellular content of extraplastidic membrane lipids including PI, PE, and DGTS showed drastic increase in *sta6* by 19-fold (P<0.001), 99.35% (P<0.001), and 23.32% (P<0.01), respectively, as compared to that in WT (Fig.7). Each PI molecular species in *sta6* was increased compared to that of WT under HL, among which the highly saturated PI 16:0/18:0 showed the largest increase by 22-fold (P<0.001) (Fig.7a). For the two PE species that identified in both WT and *sta6*, PE 18:0/18:3 rapidly increased by 46.84% in *sta6* as compared with that in WT (Fig.7a, 2.35 nmol/mg DW, P<0.05), whereas PE 18:2/18:3 remained unchanged. In numerous DGTS species, DGTS 16:0/16:1 increased to the most in *sta6* (Fig.7b, P<0.001), which was 182.70% higher than that of WT. However, a large number of DGTS species remained relatively stable in both strains, such as the dominant DGTS species, DGTS 16:0/18:3 (Fig.7b). By contrast, two DGTS species with highly unsaturated fatty acids, DGTS 18:3/18:4 and DGTS 18:4/18:4, were reduced by 31.33% (Fig.7b, P<0.01), and 47.93% (Fig.7c, P<0.001) in *sta6*, respectively, when compared to that of WT.

4 DISCUSSION

The starchless mutant *sta6* has been intensively investigated due to its capabilities in over-accumulating triacylglycerols under stress conditions (Krishnan et al., 2015; Fan et al., 2017; Tran et al., 2019). However, mechanisms underlying enhanced TAG biosynthesis in *sta6* remained elusive. There are emerging studies showing *sta6* exhibited comprehensive phenotypes, including retarded cellular growth under certain conditions, increased central carbon metabolism, and slowed NADPH reoxidation (Blaby et al., 2013; Krishnan et al., 2015). This study aimed at dissecting the alterations in the cellular structures with respect to the membrane lipids compositions, which will provide insights into the physiological consequences of blocking starch biosynthesis in microalgae.

By using the ESI/MS method, a total of 16 MGDG molecules, 16 DGDG molecules, 6 SQDG molecules, 11 PG molecules, 5 PI molecules, 2 PE molecules and 49 DGTS molecules were identified in both *C. reinhardtii* WT and *sta6*, indicative of that the membrane profiles are very similar between these two strains. On the other hand, quantitative analysis revealed that the cellular contents of a large number

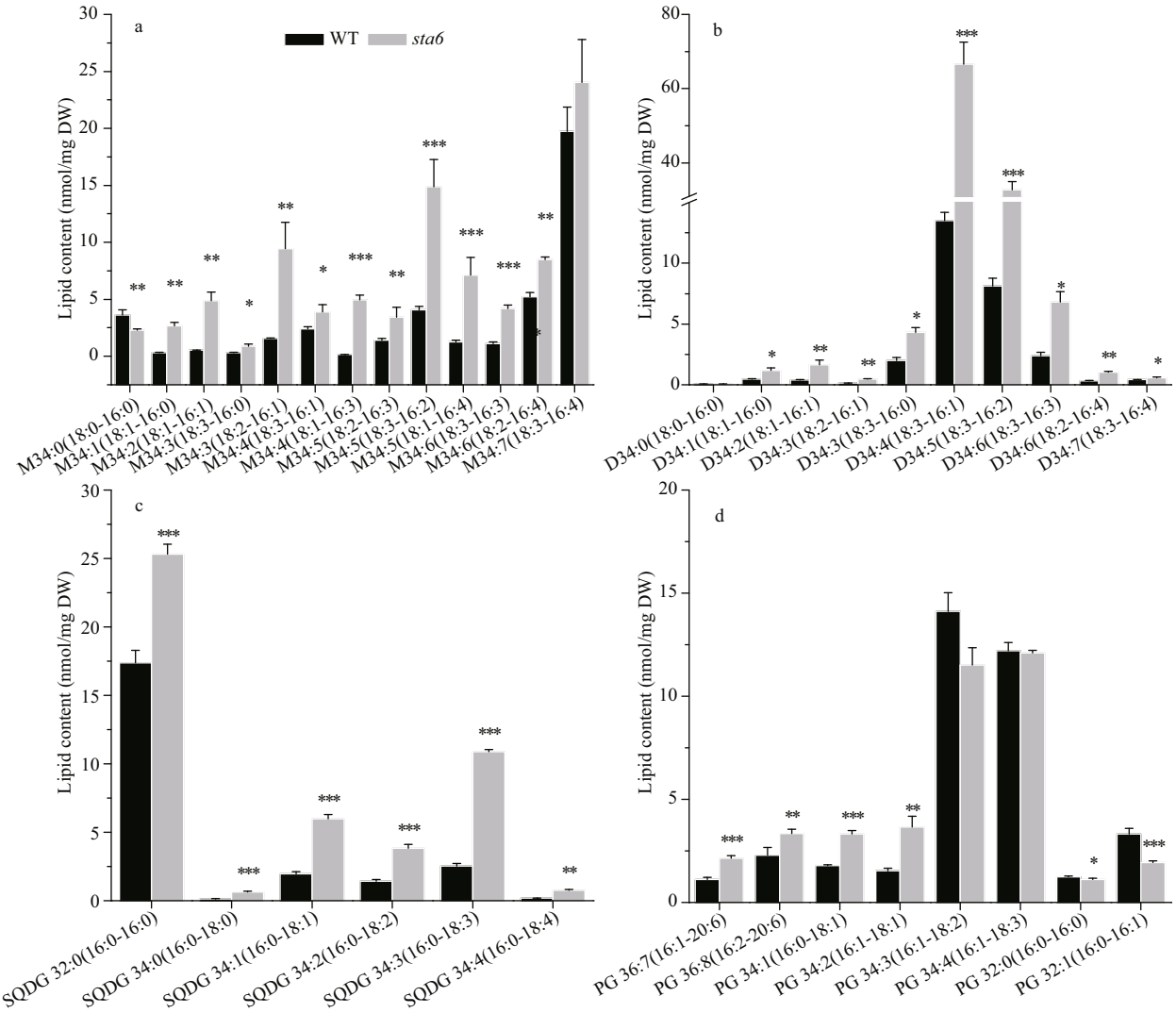

Fig.6 Contents of chloroplast membrane lipids molecular species in *C. reinhardtii* WT and *sta6* under high light (HL)

a. MGDG; b. DGDG; c. SQDG; d. PG. Values represent the mean±SD (n=4). *: P<0.05; **: P<0.01; ***: P<0.001.

of lipid molecules were changed in *sta6*, due to the deficiency in starch synthesis.

In general, the chloroplast membranes of *C. reinhardtii* are composed of the same lipid classes as the chloroplasts of plants and algae (Goss and Wilhelm, 2009). The chloroplast membranes of *C. reinhardtii* are enriched in uncharged galactolipids, MGDG and DGDG (Riekhof et al., 2003), corresponding to 60%–80% of total chloroplast lipids in oxygenic photosynthetic organisms and MGDG is the predominant one (Block et al., 1983; Riekhof et al., 2003; Sakurai et al., 2006). However, in this study, although galactolipids MGDG and DGDG are still the prevalent chloroplast lipids in both *C. reinhardtii* strains under LL and HL, DGDG became dominant in *sta6* under HL (Fig.5). This result suggests that the MGDG/DGDG ratio was somehow changed in starchless mutant, probably to maintain the structure and stability of the thylakoid membranes under HL (Murphy, 1982; Demé et al., 2014), because DGDG is a typical bilayer forming lipids while MGDG tended non-lamellar and hexagonal structures due to its cone-like molecular shape (Sen et al., 1981; Webb and Green, 1991).

Starch and lipids are two major energy storage forms in most unicellular green algae, especially under many stress conditions, such as high light. When the starch synthesis pathway was switched off, the metabolic shift of carbon flux away from starch synthesis into lipids synthesis pathways (Li et al., 2010b). It was found in this study that most chloroplast membrane lipids, except PG, showed higher concentrations in *sta6* than in WT under different light conditions, especially under HL. These results

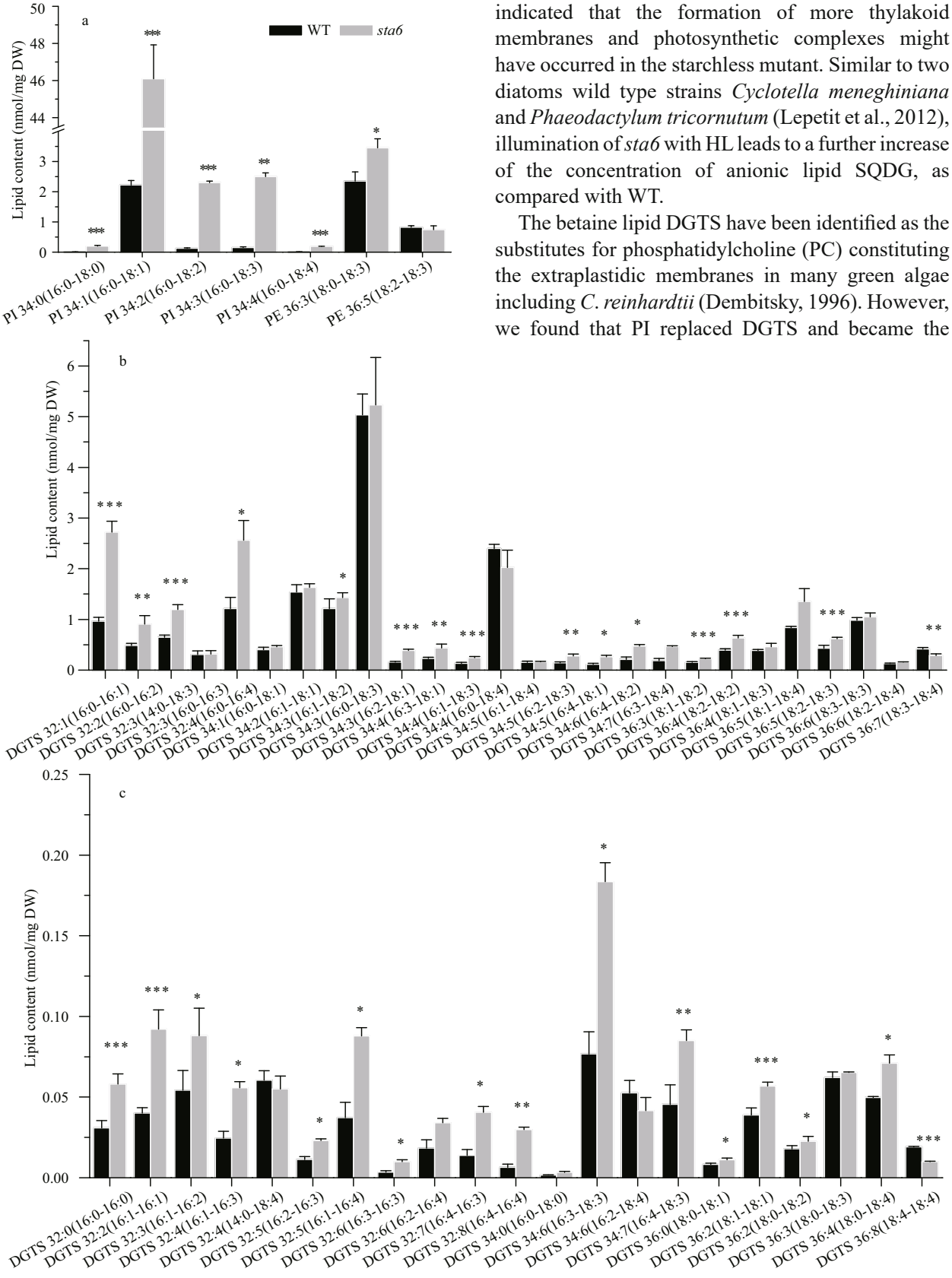

Fig.7 Contents of extraplastidic membrane lipids molecular species in *C. reinhardtii* WT and *sta6* under high light (HL)

a. PI and PE; b. DGTS major species; c. DGTS minor species. Values represent the mean±SD (n=4). *: P<0.05; **: P<0.01; ***: P<0.001.

indicated that the formation of more thylakoid membranes and photosynthetic complexes might have occurred in the starchless mutant. Similar to two diatoms wild type strains *Cyclotella meneghiniana* and *Phaeodactylum tricornutum* (Lepetit et al., 2012), illumination of *sta6* with HL leads to a further increase of the concentration of anionic lipid SQDG, as compared with WT.

The betaine lipid DGTS have been identified as the substitutes for phosphatidylcholine (PC) constituting the extraplastidic membranes in many green algae including *C. reinhardtii* (Dembitsky, 1996). However, we found that PI replaced DGTS and became the

major extraplastidic lipids in *sta6*. Since PI can be phosphorylated into phosphatidylinositol phosphates, such as phosphatidylinositol 4,5-bisphospate (PI(4,5)P_2), participating in signaling pathways (Boss and Im, 2012), we assumed the accumulated PI may play important role in signaling in *sta6* to cope with profoundly changed metabolism caused by blocked starch biosynthesis.

5 CONCLUSION

To better understand the impact of blocking starch synthesis on lipid metabolism and homeostasis of *C. reinhardtii*, we have employed a UPLC-ESI-MS/MS method to qualitatively and quantitatively analyze the membrane lipids of WT and starchless mutant *sta6*. The results indicated that the lipid profile of *sta6* is similar to that of WT, but cellular content of lipids are dramatically different between these two strains. In *sta6*, a large number of membrane lipids were up-regulated under both LL and HL conditions, which indicated a great amount of photosynthetically-fixed carbons were shunt into membrane lipid biosynthesis due to blockage of starch biosynthesis.

6 DATA AVAILABILITY STATEMENT

All data generated and/or analyzed during this study are included in this published article.

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