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1 Postharvest lipidomics of lettuce leaves

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12 1 Abstract

13 Lipidomics is a rather new method in postharvest studies. A recent analysis of RNA-seq data on
14 lettuce leaves revealed important changes in gene expression linked to lipid metabolism. Here, we
15 report a global lipid analysis in lettuce leaves over 14 days of postharvest life, in dark cold conditions,
16 with or without an initial light treatment. Our LC-MS analyses cover 171 lipid compounds, among
17 which digalactosyldiacylglycerol, triacylglycerol, and phosphatidic acid, showing significant variations
18 in response to various postharvest conditions. Our results suggest the reallocation of phospholipid
19 fatty acids to galactolipids through storage time, which may be associated to lettuce capacities to go
20 through storage without rapid chlorophyll degradation contrary to other leafy vegetables. The
21 unsaturation level of fatty acids increased over storage time. Intermittent light treatment at the
22 beginning of storage stimulated phosphatidic acid accumulation, concomitantly with upregulation of
23 phospholipase D gene expression. We also observed an increase in triacylglycerol content after light
24 treatment. Usual indicators of membrane damage, electrolyte leakage, and malondialdehyde, were
25 unchanged over storage time. Results are discussed in the light of known changes in postharvest life
26 of lettuces and other leafy vegetables.

27 Keywords : *Lactuca sativa*; fatty acids; cold storage; galactolipids; triacylglycerol; phosphatidic acid

28 2 Introduction

29 Some multi-omics analyses such as genomics, transcriptomics, metabolomics, and proteomics have
30 become more and more present in the field of postharvest studies, allowing a better understanding
31 of the mechanisms involved in postharvest quality (Belay and Caleb, 2022; Habibi et al., 2024). We
32 recently analysed transcriptomic RNAseq data (Baron et al., 2022) to decipher the mechanisms
33 induced by an intermittent fluorescent light treatment (2h on/2h off) at the beginning of lettuce cold
34 storage, following initial studies by members of our team (Charles et al., 2018; Ripoll et al., 2019).
35 Baron et al. (2022) showed that carbon metabolism was strongly influenced by light and that parts of
36 lipid metabolisms, either anabolism or catabolism, are regulated by light during storage. We focused
37 on lipids and membrane metabolisms because they can be involved in membrane integrity, with the
38 objective to see if this phenomenon is delayed by light application. Indeed, membrane integrity is
39 known to be preserved by light application during romaine lettuce storage (Martínez-Sánchez et al.,
40 2011). Membrane degradation is one of the main issues in the study of the postharvest behaviour of
41 plant products (Marangoni et al., 1996) because these membranes ensure compartmentation within

42 cells and between organelles necessary for the continuation of biological and biochemical activity.
43 Measures of membrane integrity have shown that it is correlated to the overall quality of baby
44 lettuce (Wagstaff et al., 2007). In the postharvest senescence phase, the fate of the membrane is to
45 be degraded, even if disruption of the membrane is the latest event of senescence (Hopkins et al.,
46 2008), some early signs in membrane composition can indicate the beginning of membrane
47 degradation without observable phenotypes (Page et al., 2001). This last study showed that during
48 postharvest senescence, as in developmental senescence, there is a dismantling of lipids and the use
49 of fatty acids as energy substrates to reallocate carbon through other organs. This phenomenon has
50 been well described by Troncoso-Ponce et al. (2013) explaining the role of β -oxidation of fatty acids
51 during senescence and also emphasizing the role of upregulation of lipases during senescence, which
52 catalyses the first step of degradation of lipids during senescence. During postharvest storage, the
53 temperature is often regulated below 10 °C, this has also effects on membranes which can endure
54 some damage during acclimation to cold (Liang et al., 2020). Cold effects on membrane and lipid
55 metabolism have been extensively described, in particular the increase of unsaturation level leading
56 to a greater fluidity of membrane in cold condition (Nishida and Murata, 1996). Wounding is also a
57 stress for horticultural crops being harvested, this stress can be related to some lipid changes,
58 specifically enhanced unsaturation (Nishiuchi and Iba, 1998) and activity of phospholipases (Wang et
59 al., 2000). Lipidomic analysis is one of the omics that has not been widely used in postharvest studies
60 such as the others omics. Actually, several recent studies have focused on lipidome during the
61 postharvest behavior of cabbage, green bell pepper, and strawberries (Ma et al., 2023; Xu et al.,
62 2021; Zainal et al., 2023).

63 Thus, to follow the change of membranes composition during storage and throughout the
64 treatments, the focus will be on glycerolipids, which are the major components of membranes in
65 plants and in eukaryotic species globally. Fatty acids are produced in chloroplast and incorporated
66 into glycerolipids. The synthesis of glyceroglycolipids and phospholipids, mainly occurring in
67 chloroplasts and endoplasmic reticulum, are well explained in several reviews (Benning, 2008; Block
68 and Jouhet, 2015; Joyard et al., 1998). Phospholipids are the main components of extraplastidial
69 membranes and glycolipids, specifically galactolipids monogalactosyldiacylglycerol (MGDG) and
70 digalactosyldiacylglycerol (DGDG), are the main components of chloroplast membrane. Phospholipids
71 and glycolipids have common glycerolipid precursors: phosphatidic acid (PA) and diacylglycerol (DAG)
72 which are also degradation products of phospholipids by phospholipases: this makes them important
73 in the distribution of carbon resources between lipids classes. Lettuce is an 18:3 plant and has
74 probably lost the prokaryotic pathway for its galactolipid synthesis through the evolution (Mongrand
75 et al., 1998), making extraplastidial lipid an obligatory intermediate for galactolipid production. The
76 degradation of phospholipids is mainly attributed to phospholipases (Wang et al., 2012), degradation
77 of galactolipids is much more complex: some lipases, galactosidases, and acylation of MGDG are
78 responsible for its degradation (Hölzl and Dörmann, 2019). Fatty acids can also be used in another
79 glycerolipid form: triacylglycerol (TAG) which is synthesized from diacylglycerol by adding a third
80 fatty acid chain in the endoplasmic reticulum. TAG is then stored in lipid droplets waiting to be used
81 in anabolism or catabolism processes.

82 For a decade, light treatments have been studied on many agronomic species during the storage of
83 vegetables, trying to use it as a way to improve quality and length of storage. Some of them showed
84 beneficial effects on maintaining quality parameters during storage, like ascorbic acid (Toledo et al.,
85 2003), phenolic compounds (Zhan et al., 2013b), sugar (Noichinda et al., 2007), membrane integrity
86 (Martínez-Sánchez et al., 2011) or physiological parameters like photosynthesis or chlorophyll (Costa
87 et al., 2013). Lettuce postharvest responses to light treatment have also been studied and similar
88 observations were made on chlorophyll, ascorbic acid, sugars, and electrolyte leakage, showing the

89 beneficial aspects of using light in the postharvest life of lettuce (Kasim and Kasim, 2017; Liu et al.,
90 2015; Martínez-Sánchez et al., 2011; Zhan et al., 2013a). Additionally, studies of light treatment on
91 lettuce have highlighted an interesting issue because they notably decrease the browning and the
92 time of browning appearance (Charles et al., 2018; Zhan et al., 2012). However, light applications can
93 have deleterious effects depending on light intensity and photoperiods used to treat lettuce. Indeed,
94 high intensity (150 photosynthetic photon flux density - PPF) increases the water loss of lettuces
95 and therefore decreases quality (Charles et al., 2018). To solve this problem, methods have been
96 investigated to reduce water loss: intermittent light treatment has been proposed to limit this
97 phenomenon (Braidot et al., 2014; Charles et al., 2018), focusing on alternate light and dark periods
98 and therefore limiting water loss time period while preserving beneficial effects due to light.
99 Globally, applications of light during postharvest are based on the idea to counteract dark-induced
100 senescence (Sakuraba, 2021).

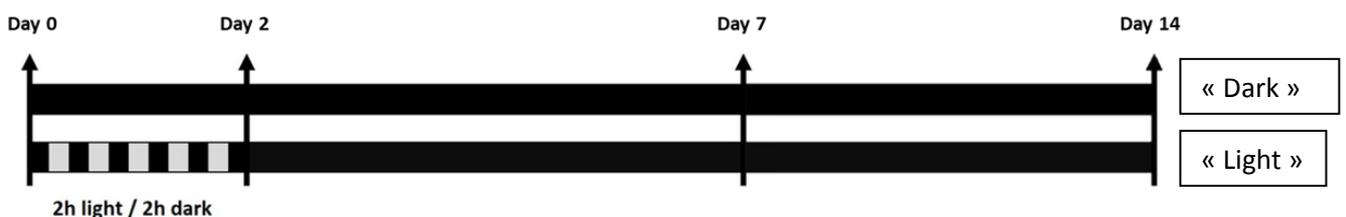
101 The aim of this study was mainly to characterize the changes of lipids during the postharvest life of
102 the lettuce leaves and the effect of light on these changes will be observed.

103 3 Materials and methods

104 3.1 Plant material and postharvest storage conditions

105 At day 0, butterhead lettuces (*Lactuca sativa* L. var Panoramis, SEMINIS®) obtained from a local
106 grower (Avignon, France), were immediately transported and cut to select inner leaves of uniform
107 size and color. Leaves were packaged (four whole leaves per bag) and stored according to Charles et
108 al. (2018). The control batch was stored in darkness for 14 d, while treated leaves were exposed to 2
109 d of intermittent light (2 h on / 2 h off) at $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ (cool-white fluorescent lamps, OSRAM 44ss
110 w/37, Mitsubishi Electric Corporation, Japan) followed by 12 d of storage in darkness, meaning 14 d
111 total storage (Fig. 1). The cold temperature of the two weeks of cold storage was $5.85 \pm 0.25 \text{ }^\circ\text{C}$.
112 Three bags of lettuce were done each day and for each modality, leading to three replicates. Three
113 replicates were done for all the measures, except for chlorophyll, electrolyte leakage and
114 malondialdehyde measurement parameters, which have four replicates. Sampling has been done on
115 the limbs of lettuce leaves.

116



117

118 **Figure 1:** Scheme of postharvest treatment of fresh-cut lettuce and sampling over cold storage (starting at Day
119 0)

120 3.2 Lipids and physiological analysis

121

122 3.2 .1 Lipid extraction

123 Lipid extraction and analysis were performed by Cellular and Plant Physiology Laboratory (LPCV) in
124 CEA Grenoble at the LIPANG platform. For each day (0, 2, and 7), leaves were frozen in liquid
125 nitrogen, and freeze-dried. Glycerolipids were extracted from these samples. Once freeze-dried, the
126 plants were crushed and suspended in 4 mL of boiling ethanol for 5 minutes to prevent lipid

127 degradation and lipids were extracted according to Simionato et al. (2013) by addition of 2 mL
128 methanol and 8 mL chloroform at room temperature. The mixture was then saturated with argon
129 and stirred for 1 hour at room temperature. After filtration through glass wool, cell remains were
130 rinsed with 3 mL chloroform/methanol 2:1 v/v, and 5 mL of NaCl 1 % in water (w/w) were then
131 added to the filtrate to initiate biphasic formation. The chloroform phase was dried under argon
132 before solubilizing the lipid extract in pure chloroform. The samples were then stored dry at -20 °C.

133 3.2.2 Fatty acid quantification

134 Total glycerolipids were quantified from their fatty acids: in an aliquot fraction, a known quantity of
135 15:0 was added and the fatty acids present were transformed as methyl esters (FAME) by a 1-hour
136 incubation in 3 mL 2.5 % H₂SO₄ in pure methanol at 100 °C (Jouhet et al., 2003). The reaction was
137 stopped by addition of 3 mL water and 3 mL hexane. The hexane phase was analyzed by gas
138 chromatography-flame ionization detector (GC-FID) (Perkin Elmer) on a BPX70 (SGE) column. FAME
139 were identified by comparison of their retention times with those of standards (Sigma) and
140 quantified by the surface peak method using 15:0 for calibration. Results are expressed on a dry
141 weight basis.

142 3.2.3 Lipid analysis by HPLC and MS/MS analysis

143 The lipid extracts corresponding to 25 nmol of total fatty acids were dissolved in 100 µL of
144 chloroform/methanol [2/1, (v/v)] containing 125 pmol of each internal standard. Internal standards
145 used were PE 18:0-18:0 and DAG 18:0-22:6 from Avanti Polar Lipid and SQDG 16:0-18:0 extracted
146 from spinach thylakoid (Demé et al., 2014) and hydrogenated as described in Buseman et al. (2006).
147 Lipids were then separated by HPLC and quantified by MS/MS.

148 The HPLC separation method was adapted from Rainteau et al. (2012). Lipid classes were separated
149 using an Agilent 1200 HPLC system using a 150 mm×3 mm (length × internal diameter) 5 µm diol
150 column (Macherey-Nagel), at 40 °C. The mobile phases consisted of
151 hexane/isopropanol/water/ammonium acetate 1M, pH5.3 [625/350/24/1, (v/v/v/v)] (A) and
152 isopropanol/water/ammonium acetate 1M, pH5.3 [850/149/1, (v/v/v)] (B). The injection volume was
153 20 µL. After 5 min, the percentage of B was increased linearly from 0 % to 100 % in 30 min and
154 stayed at 100 % for 15 min. This elution sequence was followed by a return to 100 % A in 5 min and
155 equilibration for 20 min with 100 % A before the next injection, leading to a total runtime of 70 min.
156 The flow rate of the mobile phase was 200 µL min⁻¹. The distinct glycerolipid classes were eluted
157 successively as a function of the polar head group.

158 Mass spectrometric analysis was done on a 6460 triple quadrupole mass spectrometer (Agilent)
159 equipped with a Jet stream electrospray ion source under following settings: Drying gas heater: 260
160 °C, Drying gas flow 13 L min⁻¹, Sheath gas heater: 300 °C, Sheath gas flow: 11 L min⁻¹, Nebulizer
161 pressure: 25 psi, Capillary voltage: ± 5000 V, Nozzle voltage ± 1000. Nitrogen was used as collision
162 gas. The quadrupoles Q1 and Q3 were operated at widest and unit resolution respectively. PC
163 analysis was carried out in positive ion mode by scanning for precursors of m/z 184 at a collision
164 energy (CE) of 34 eV. SQDG analysis was carried out in negative ion mode by scanning for precursors
165 of m/z -225 at a CE of -56eV. PE, PI, PS, PG, PA, MGDG, and DGDG measurements were performed in
166 positive ion mode by scanning for neutral losses of 141 Da, 277 Da, 185 Da, 189 Da, 115 Da, 179 Da,
167 and 341 Da at CEs of 20 eV, 12 eV, 20 eV, 16 eV, 16 eV, 8 eV and 8 eV, respectively. Quantification
168 was done by multiple reaction monitoring (MRM) with 30 ms dwell time. DAG and TAG species were
169 identified and quantified by MRM as singly charged ions [M+NH₄]⁺ at a CE of 16 and 22 eV
170 respectively with 30 ms dwell time. CL species were quantified by MRM as singly charged ions [M-H]⁻
171 at a CE of -45 eV with 50 ms dwell time. Mass spectra were processed by MassHunter Workstation
172 software (Agilent) for identification and quantification of lipids. Lipid amounts (pmol) were corrected

173 for response differences between internal standards and endogenous lipids and by comparison with
174 a quality control (QC). QC extract corresponds to a known lipid extract from *Arabidopsis* cell culture
175 qualified and quantified by TLC and GC-FID as described by Jouhet et al. (2017). Results are expressed
176 on a dry weight basis.

177 3.2.4 Chlorophyll, electrolyte leakage, and malondialdehyde (MDA) quantifications

178 Four replicated measurements were performed, on four individual leaves randomly chosen out of
179 the three bags. Approximately 100 mg of fresh lettuce were weighed, 3 mL of acetone was added
180 and then incubated for 15 minutes at 4 °C. The chlorophyll content was measured using the method
181 described by Lichtenthaler, (1987). Results are expressed on a dry weight basis.

182 Measures of electrolyte leakage (EL) were performed as: five leaf disks of 22 mm diameter were
183 taken from the four leaves of the bag and rinsed with deionized water. Then they were placed in 40
184 mL of deionized water and incubated at 30 °C for 3 hours with circular movement. Electrical
185 conductivity was measured with multiparameter HI9829 (Hanna Instrument Inc., Woonsocket, USA).
186 The total electrolyte level of the leaves was then measured after freezing the leaves for 24 hours and
187 2 hours recovery at ambient temperature. Electrolyte leakage was expressed as a percentage of total
188 electrolytes.

189 Around 200g of frozen powder was sampled and weighed and then mixed with 1 mL of 0.1 %
190 trichloroacetic acid (TCA) solution and placed in ice for 10 minutes. The solution was then
191 centrifugated for 10 minutes at 15 000g at 4 °C. Then the quantification was performed as previously
192 described in Hodges et al. (1999), with the following modifications: 250 µL of supernatant was used
193 and 500 µL of 20 % TCA solution containing 0.5 % TBA was added in the TBA series whereas 500 µL of
194 20 % TCA solution was added in the control series. A blank with 250 µL of 0.1 % TCA solution instead
195 of supernatant samples was also done for the TBA series to avoid the TBA absorbance effect. Results
196 are expressed on a fresh weight basis.

197 3.2.5 Statistical analysis

198 A total of three salad packs were analyzed for each day and each condition and all the analyses were
199 performed on each leaf (four leaves per bag). Thus, data represent mean values of three replicates (n
200 = 12). Error bars represent standard error. R software was used to perform statistical analysis,
201 Kruskal-Wallis test (package *stats*) was used to compare the two treatments for each day and also
202 day 14 darkness *versus* day 0 control for lipids measurement, this use of Kruskal-Wallis test on k=2
203 has been described in Breslow (1970) and Wei (1981). Multiple comparisons are detailed in Supp.
204 Table 1. Wilcoxon test (package *stats*) was used for chlorophyll measurements. For all the statistical
205 tests, the significative threshold has been set at 0.05.

206 3.3 RNA-seq reprocessing

207 RNA sequencing was performed previously on similar samples, the same cultivar, and the same
208 treatment conditions (Baron et al., 2022). The results were reanalyzed here focussing on some lipid
209 metabolisms, with a scope that was not exploited in the previous article.

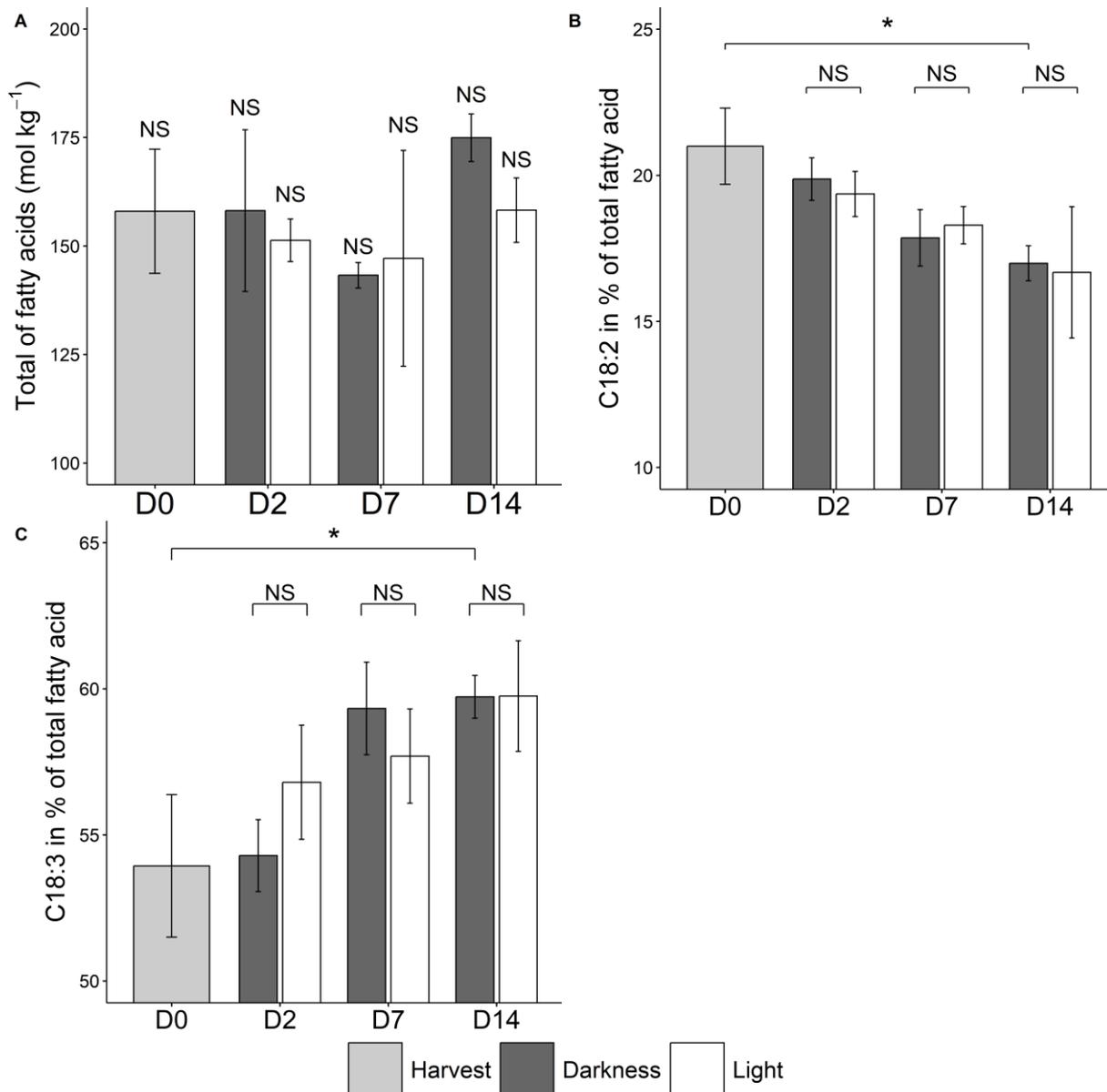
210 Log fold change (logFC) is a binary log that indicates the difference in the amount of RNA between
211 the means of two modalities, if it is 1 it means there is 2-fold higher RNA in one modality rather than
212 the other. False discovery rate gives information on the probability that the difference is
213 reproducible: the threshold was fixed here to 0.05 to consider a significative difference.

214

215 4 Results

216

217



218

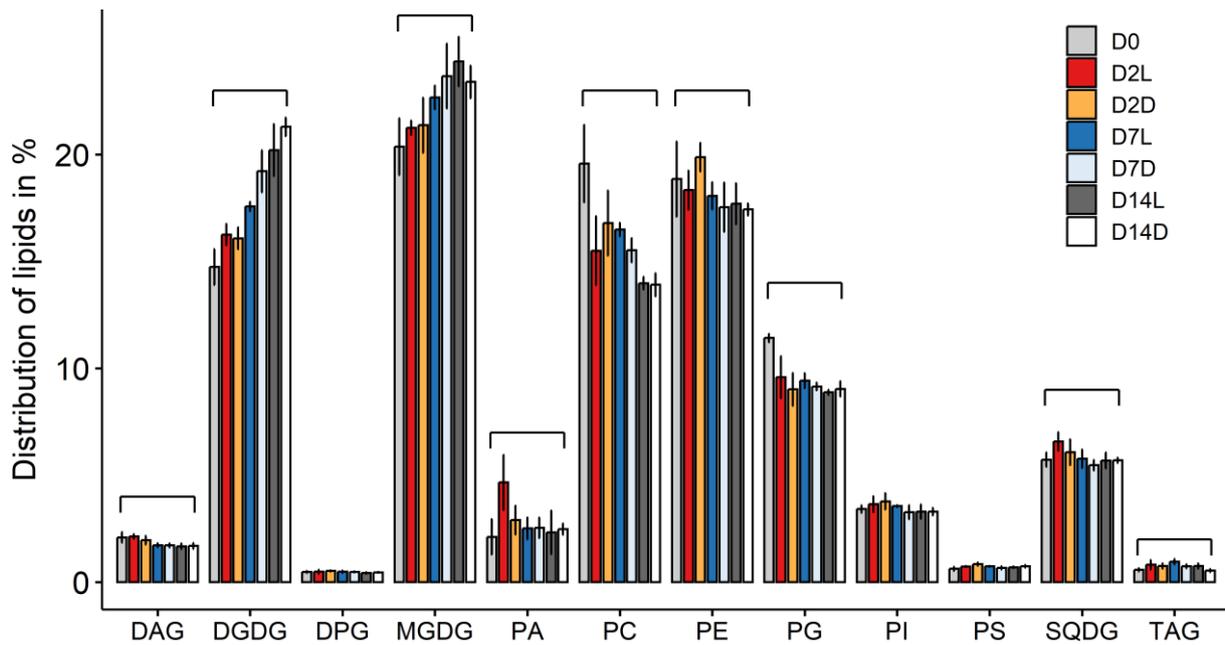
219 **Figure 2:** Evolution of C18:2 and C18:3 and total fatty acids. Percentage of [A] C18:2 and [B] C18:3 among all
 220 fatty acids and total fatty acid in absolute value [C] represented from harvest to day 14 of storage. D0: Harvest,
 221 D2: day 2 of storage, D7: day 7 of storage, D14: day 14 of storage. In legend, different modalities are
 222 represented Harvest (grey), Darkness (dark grey), and Light (white). For C18:2 and C18:3 Kruskal-Wallis test
 223 between the two treatments was done for each day of storage, and also between day 14 darkness and day 0
 224 Harvest. For total fatty acids, Kruskal-Wallis test was performed on all modalities. n = 3 replicates. NS stands for
 225 Non-Significant, * stands for a significant difference at 0.05.

226 No variation of total fatty acids content per leaf was observed between modalities (Fig. 2A). It
 227 allow us to consider the different lipids and fatty acids class in percent because there is no variation
 228 of fatty acid content during the treatment. Figure 2 also shows the evolution of 18:2 (Fig. 2B) and
 229 18:3 (Fig. 2C) among all fatty acids. For clarity, we show only comparisons between D0 and the last
 230 day of cold storage D14, and also pairwise comparison between light and dark cold storage days.

231 Details are given in Supp. Table 1 with all pairwise comparisons. No difference was found between
 232 both treatments on each day of analysis. Concerning 18:2, it significantly decreased during storage
 233 between day 0 control and day 14 darkness and also between day 0 control and day 7 darkness
 234 (Supp. Table 1). This decrease appears to be progressive during storage for both treatments. On the
 235 other hand, 18:3 increased progressively during storage and a significative difference was found
 236 between day 14 darkness and day 0 control.

237

238

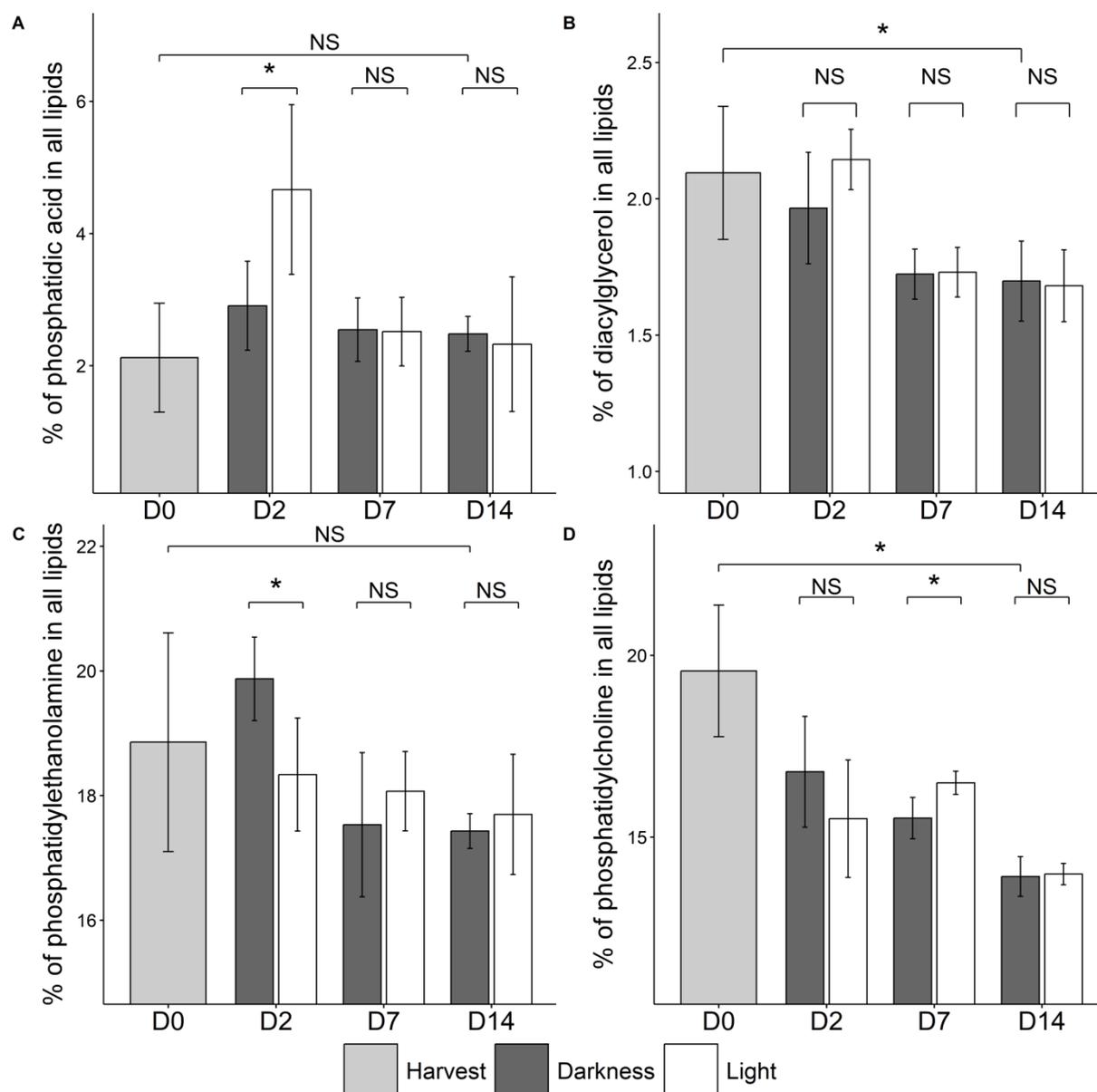


239

240

241 **Figure 3:** Distribution of lipids depending on the day of observation and the treatment, D0: Harvest, D2L: day 2
 242 of storage with light treatment, D2D: day 2 of storage with darkness treatment, D7L: day 7 of storage with light
 243 treatment, D7D: day 7 of storage with darkness treatment, D14L: day 14 of storage with light treatment, D14D:
 244 day 14 of storage with darkness treatment. The detailed statistics of data indicated by brackets are shown in
 245 other graphs focusing on each class in the following parts of the article. n = 3 replicates. Abbreviations:
 246 diacylglycerol (DAG), digalactosyldiacylglycerol (DGDG), diphosphoglycerol (DPG), monogalactosyldiacylglycerol
 247 (MGDG), phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphoglycerol
 248 (PG), phosphoinositol (PI), phosphatidylserine (PS), sulfoquinovosyl diacylglycerol (SQDG), triacylglycerol (TAG).

249 LC-MS analysis allows us to follow the variations of 171 compounds belonging to lipids (detailed in
 250 Supp. Table 2), these molecules were grouped in different lipid classes represented in Figure 3 in
 251 percentage of the class compared to all classes. We observed some variations that will be detailed in
 252 further graphs, like the increase of DGDG over storage and the increase of phosphatidic acid after the
 253 initial 2 d of intermittent light. Figure 3 shows the importance of some families, like MGDG, DGDG,
 254 PC et PE.

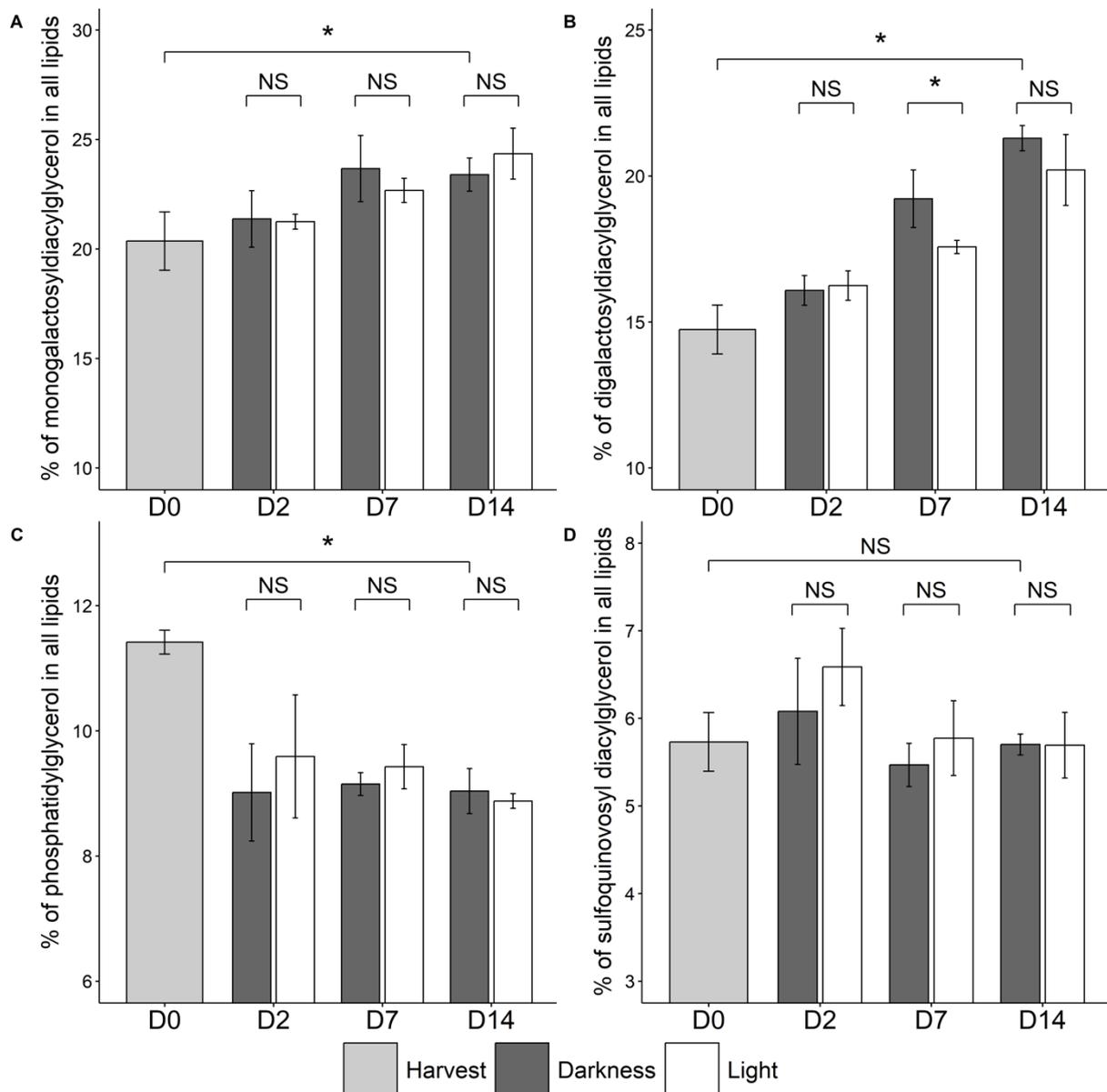


255

256 **Figure 4:** Distribution of [A] phosphatidic acid (PA), [B] diacylglycerol (DAG), [C] phosphatidylethanolamine (PE),
 257 and [D] phosphatidylcholine (PC) from harvest to day 14 of storage. D0: Harvest, D2: day 2 of storage, D7: day 7
 258 after storage, D14: day 14 after storage. In legend, different modalities are represented Harvest (grey),
 259 Darkness (dark grey), and Light (white). Kruskal-Wallis test between the two treatments was done for each day
 260 of storage, and also between day 14 darkness and day 0 Harvest. n = 3 replicates. NS stands for Non-Significant,
 261 * stands for a significant difference at 0.05.

262 In Figure 4, four classes were represented: phosphatidic acid (A), diacylglycerol (B),
 263 phosphatidylethanolamine (C) and phosphatidylcholine (D). The details of all compounds of each
 264 class are given in Supp. Table 2. Light treatment induced the accumulation of PA at day 2 which is the
 265 end of the light treatment. The amount of PA (Fig. 4A) was around 1.5-fold higher under light than in
 266 darkness. This effect was not permanent, and after 5 and 12 additional days in darkness, PA returned
 267 to the same level as in leaves never exposed to light. The PA level under darkness was constant
 268 during the storage, there was no difference between day 14 darkness and day 0. A few significant
 269 differences were observed between D0 and D2 and between D0 and D7, details are given in Supp.
 270 Table 1. For DAG (Fig. 4B), there was a lesser effect (not significant), with a slightly higher amount
 271 under light than in darkness on day 2. Additionally, DAG decreased during storage, the amount at day

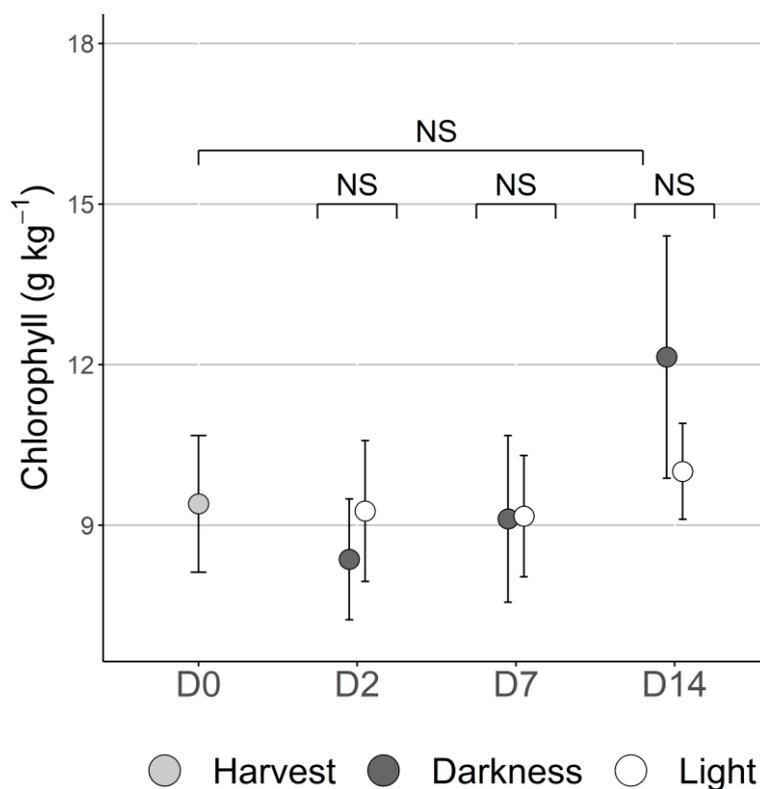
272 14 darkness was significantly less than the day 0 control. Phosphatidylethanolamine (PE) (Fig. 4C),
 273 was significantly less present in light samples compared to darkness samples at day 2. PE slightly
 274 decreased during storage but no significant difference was found between day 14 darkness and day
 275 0 control, which may be due to the great standard deviation of day 0 control. A clear decrease of
 276 phosphatidylcholine (PC) (Fig. 4D) was observed after day 0 for both light and darkness treatments,
 277 and a significant difference was observed between day 14 in darkness and day 0 in control. There
 278 also was an effect of light treatment at day 7: light treated leaves contained significantly more PC.
 279



280

281 **Figure 5:** Distribution of [A] monogalactosyldiacylglycerol (MGDG), [B] digalactosyldiacylglycerol (DGDG), [C]
 282 phosphatidylglycerol (PG), and [D] sulfoquinovosyl diacylglycerol (SQDG). from harvest to day 14 after storage.
 283 D0: Harvest, D2: day 2 of storage, D7: day 7 of storage, D14: day 14 of storage. In legend, different modalities
 284 are represented Harvest (grey), Darkness (dark grey), and Light (white). Kruskal-Wallis test between the two
 285 treatments was done for each day of storage, and also between day 14 darkness and day 0 Harvest. n = 3
 286 replicates. NS stands for Non Significant, * stands for a significant difference at 0.05.

287 In Figure 5, the four classes of plastidial glycerolipids are represented: [A], MGDG [B], DGDG, [C]
 288 phosphatidylglycerol (PG) and [D] sulfoquinovosyl diacylglycerol (SQDG). MGDG class is composed of
 289 14 compounds, DGDG of 15 compounds, PG of 13 compounds, and SQDG of 14 compounds (Supp
 290 Table 2). MGDG and DGDG (Fig. 5A and B) progressively increased during storage, with a significant
 291 difference between darkness day 14 and control day 0 for both classes. A few significant differences
 292 were observed between D0 and D2 and between D0 and D7, details are given in Supp Table 1. For
 293 these two classes, there was only one significant difference between dark and light treatments for
 294 DGDG on day 7 higher in darkness than in light. Concerning PG, there was a significant decrease after
 295 2 d of storage (see Supp. Table 1), then its content was constant to the end of storage, and a
 296 significant difference was observed between the control day 0 and the day 14 dark. Regarding PG
 297 (Fig. 5C), no significant difference was found between treatments for each day. SQDG was not
 298 affected either by time of storage (showing no difference between control day 0 and day 14 dark), or
 299 by light treatments (Fig. 5D).



300

301 **Figure 6:** Evolution of chlorophyll represented from harvest to day 14 of storage. D0: Harvest, D2: day 2 of
 302 storage, D7: day 7 of storage, D14: day 14 of storage. In legend, different modalities are represented Harvest
 303 (grey), Darkness (dark grey), and Light (white). Kruskal-Wallis test between the two treatments was done for
 304 each day of storage, and also between day 14 darkness and day 0 Harvest. n = 4 replicates. NS stands for Non
 305 Significant, * stands for a significant difference at 0.05 p-value.

306

307 The evolution of chlorophyll quantity was evaluated throughout the storage (Fig. 6), and no
 308 significant change was observed during the 14 d, even if it increased slightly at day 14. No difference
 309 was observed between light and darkness at each day of storage.

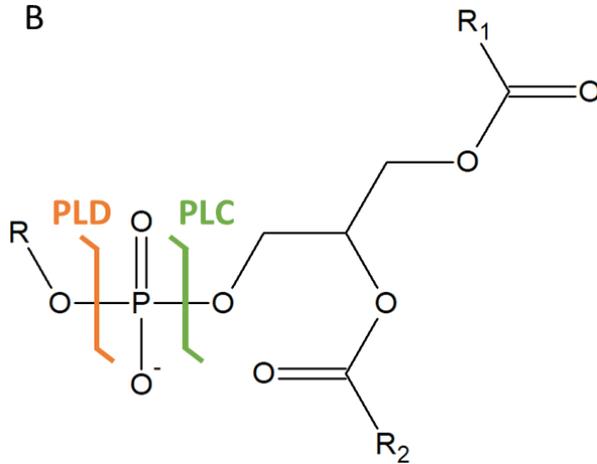
310

A

Lsat	Name	logFC	FDR
Lsat_1_v5_gn_5_171281	PLCD4_ARATH Phosphoinositide phospholipase C 4	0,839	1,52E-02
Lsat_1_v5_gn_9_61520	PLCD2_ARATH Phosphoinositide phospholipase C 2	0,615	1,70E-01
Lsat_1_v5_gn_4_41620	PHLC_MYCTU Phospholipase C 3	0,494	6,18E-01
Lsat_1_v5_gn_2_123380	PLCD6_ARATH Phosphoinositide phospholipase C 6	0,352	3,58E-01
Lsat_1_v5_gn_9_19301	PHLB_MYCTU Phospholipase C 2	-0,051	8,78E-01
Lsat_1_v5_gn_8_12041	PLCD2_ARATH Phosphoinositide phospholipase C 2	-0,103	7,56E-01
Lsat_1_v5_gn_8_70901	PLCD6_ARATH Phosphoinositide phospholipase C 6	-0,356	4,33E-01
Lsat_1_v5_gn_4_81261	PHLC_MYCTU Phospholipase C 3	-0,478	4,28E-01
Lsat_1_v5_gn_5_95800	PLDA1_RICCO Phospholipase D alpha 1	1,581	3,18E-05
Lsat_1_v5_gn_3_135001	PLDB1_ARATH Phospholipase D beta 1	1,064	1,12E-08
Lsat_1_v5_gn_1_44721	PLDA1_RICCO Phospholipase D alpha 1	0,621	3,44E-01
Lsat_1_v5_gn_3_4061	PLDG1_ARATH Phospholipase D gamma 1	0,477	3,37E-01
Lsat_1_v5_gn_2_75361	PLDD1_ARATH Phospholipase D delta	0,367	4,49E-01
Lsat_1_v5_gn_5_132081	PLDA1_TOBAC Phospholipase D alpha 1	0,353	4,71E-01
Lsat_1_v5_gn_8_82201	PLD3_MACFA Phospholipase D3	0,338	2,55E-02
Lsat_1_v5_gn_3_4321	PLDG1_ARATH Phospholipase D gamma 1	0,146	5,60E-01
Lsat_1_v5_gn_6_76021	PLDP1_ARATH Phospholipase D p1	0,088	7,36E-01
Lsat_1_v5_gn_7_42860	DDHD1_BOVIN Phospholipase DDHD1	-0,106	6,14E-01
Lsat_1_v5_gn_5_102100	PLDP2_ARATH Phospholipase D p2	-0,361	2,14E-01
Lsat_1_v5_gn_5_95680	PLDA1_RICCO Phospholipase D alpha 1	-0,425	5,90E-01
Lsat_1_v5_gn_1_33321	PLDA1_RICCO Phospholipase D alpha 1	-0,542	4,38E-03
Lsat_1_v5_gn_4_143760	PLDD1_ARATH Phospholipase D delta	-0,646	4,24E-04

311

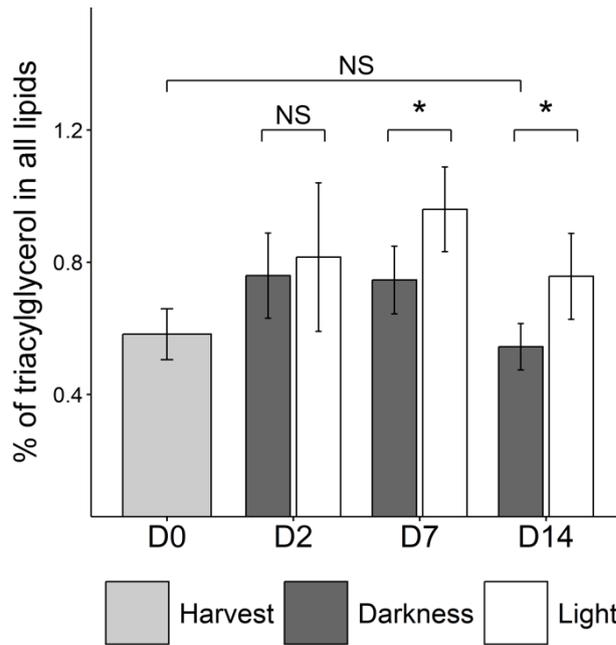
B



312

313 To understand the light effect on PA and DG quantities, RNA-seq results from a similar experiment
 314 were re-analyzed (Fig. 7A). Here we focused on phospholipases D and C. The comparison of
 315 expression between the darkness and the light treatment, at day 2, of phospholipase C and
 316 phospholipase D genes revealed a global overexpression at light of both enzyme classes. Four genes
 317 were significantly overexpressed at light: one phospholipase C4, one phospholipase D1 subunit
 318 alpha, one phospholipase D1 subunit beta, and one phospholipase D3, while two were under-
 319 expressed at light: one phospholipase D1 subunit alpha and one phospholipase D subunit delta.
 320 Globally, the logFC of the overexpressed genes was higher than the logFC of under-expressed ones.

Figure 7: [A] Differentially expressed genes after two days of cold storage, at 6 °C, comparing two light regimes, intermittent light versus dark for phospholipase C (upper part of the table) and phospholipase D (downer part of the table) genes. “logFC” stands for log Fold Change which is positive when there was more gene expression in the intermittent light modality. “FDR” stands for False Discovery Rate which is highlighted when it was inferior to 0.05. The “Lsat” annotation was done by homology with general genomic banks (Baron et al., 2022). [B] Phospholipid model showing where phospholipases C and D cut.

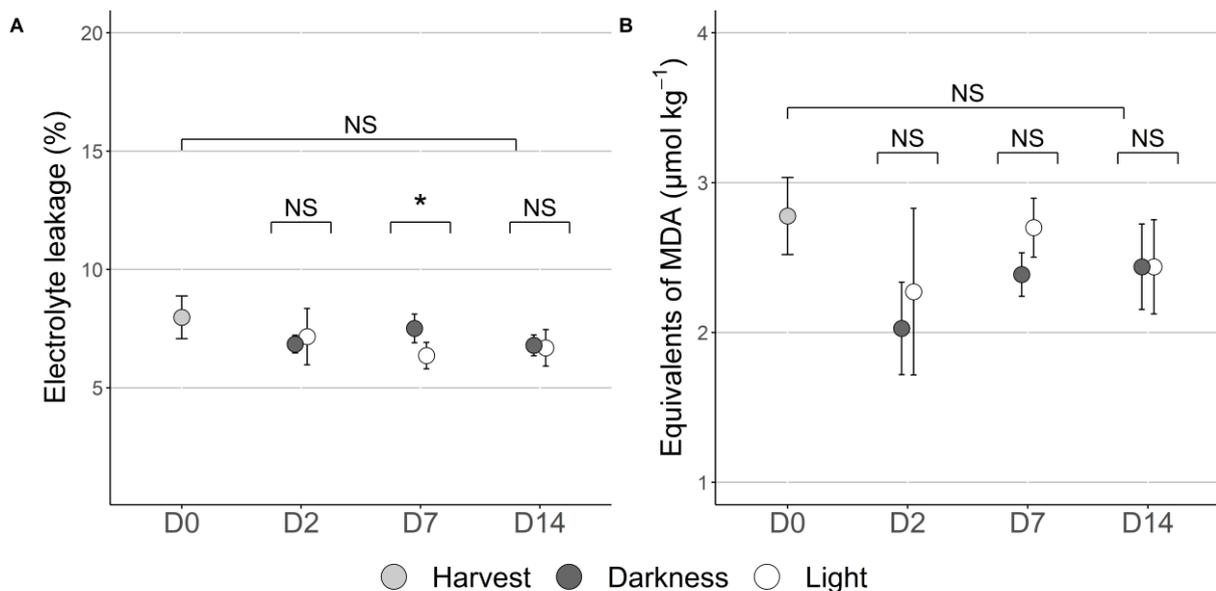


321

322 **Figure 8:** Distribution of triacylglycerol represented from harvest to day 14 of storage. D0: Harvest, D2: day 2 of
 323 storage, D7: day 7 of storage, D14: day 14 of storage. In legend, different modalities are represented Harvest
 324 (grey), Darkness (dark grey), and Light (white). Kruskal-Wallis test between the two treatments was done for
 325 each day of storage, and also between day 14 darkness and day 0 Harvest. n = 3 replicates. NS stands for Non
 326 Significant, * stands for a significant difference at 0.05 p-value.

327 The triacylglycerol class is composed of 28 compounds (Supp. Table 2). The amount of triacylglycerol
 328 was approximately 25 % greater in leaves treated by light than in the leaves kept dark at day 7 and
 329 day 14 (Fig. 8), but no difference was observed on day 2. There was a slight increase of TAG in both
 330 modalities on day 2 and day 7, compared to day 0. Then it appears to decrease in both treatments at
 331 day 14.

332



333

334 **Figure 9:** Evolution of electrolyte leakage [A], and equivalents of MDA (malondialdehyde) [B] represented from harvest to day 14 of storage. D0: Harvest, D2: day 2 of storage, D7: day 7 of storage, D14: day 14 of storage. In
 335

336 legend, different modalities are represented Harvest (grey), Darkness (dark grey), and Light (white). test
337 between the two treatments was done for each day of storage, and also between day 14 darkness and day 0
338 Harvest. n = 4 replicates. NS stands for Non Significant, * stands for a significant difference at 0.05 p-value.

339

340 Measurements of electrolyte leakage did not show evolution during the time (Fig. 9A), there is a
341 significative difference at day 7 between the two treatments but the difference is such slight that it is
342 not relevant because the evolution of electrolyte leakage appeared to increase beyond 20 % in
343 related study (Martínez-Sánchez et al., 2011). Thirdly, MDA levels were determined (Fig. 9B): no
344 evolution throughout the storage, and no difference between treatments was noticed.

345

346 5 Discussion

347 Lipids are crucial components of most plant membranes (chloroplastic and extraplastidial). Their
348 distinctive feature is that they are all composed of fatty acids. The analyses of total fatty acids gave
349 us information on the unsaturation status of lipids composing the leaves during the postharvest life
350 of lettuce. In this study the level of unsaturation in leaves increased progressively during storage as
351 shown by the decrease of C18:2 and the increase of C18:3 (Fig. 2), both representing up to 70 % of
352 total fatty acids together in the lettuce leaves. Several stresses that leaves have undergone can
353 explain this progressive unsaturation: firstly, it is known that wounding can induce desaturation by
354 induction of transcription of fatty acid desaturase (FAD), specifically FAD7, FAD8, and FAD3, as
355 reviewed in Nishiuchi and Iba (1998). Secondly, the cold is known to promote the level of
356 unsaturated fatty acids, which are required for low temperature survival (Iba, 2002), specifically to
357 maintain chloroplast function (Routaboul et al., 2000). Indeed, FADs are transcriptionally and
358 translationally regulated by cold stress (Matsuda et al., 2005; Matsuda and Iba, 2005). No trend of
359 overexpression of FADs was found in RNAseq, by comparing day 2 darkness to day 0 control and
360 there was an under expression at day 7 darkness to day 0 control (Supp. Fig. S1). A reason that could
361 explain this unsaturation without seeing more transcription of FADs is the fact that the level of
362 unsaturation is correlated to the age of cells (Mei et al., 2015) and that there is no more cell division
363 in postharvest lettuce, it is, therefore, logical to see accumulation of C18:3.

364 The distribution of each glycerolipid class (Fig. 3) analyzed in this study showed that treatments and
365 storage modified the accumulation of some glycerolipids, changes which will be discussed in the
366 following paragraphs. Only one study has indicated the quantity of all lipids found in lettuce and
367 showed that MGDG, DGDG, SQDG, PG, PC, and PE are the major lipids composing leaves (Novitskii et
368 al., 2015), the profile of lipids of this study is similar with our results.

369 5.1 Lipidome behavior over cold storage

370 PE and PC are the main components of extraplastidial membranes. Here, there was a clear decrease
371 of PC during storage (Fig. 4D) and a non-significant slight decrease of PE (Fig. 4C). The loss of PC and
372 PE is mainly due to the loss of 18:2 composed species and other saturated species between day 0 and
373 day 14 whereas 18:3 composed species increased during first days of storage until day 7 (Supp. Fig.
374 S2). The decrease of 18:2 composed phosphatidylcholine has already been observed in barley leaves
375 (Gut and Matile, 1989) and could be linked to storage effect. The increase of 18:3/18:3 species is
376 related to progressive unsaturation level of fatty acids. At day 14, this level of 18:3/18:3 is probably
377 counter-balanced by an effect of degradation of 18:3/18:3 species which begins to happen after 14 d
378 of storage. Here, this general decrease in phospholipids during storage does not seem linked to cold
379 acclimation because it is usually associated with an increase in phospholipids (Degenkolbe et al.,

380 2012). However, regarding PE composition, it appears that there is a transfer from C34 to C36 species
381 (Supp. Fig. S2) and it is coherent with cold acclimation that has been observed in *A. thaliana* over
382 acclimation to cold temperature (Li et al., 2015). Loss of phospholipids due to wounding has already
383 been observed in several leafy plants (Lee et al., 1997; Ryu and Wang, 1998) and in cucumber (Zhao
384 et al., 2010) and has been linked to phospholipase D activation after wounding (Wang et al., 2000).
385 Some studies have also shown that cold could promote phospholipase D and PA production (Wang et
386 al., 2000). But in these cases, the analyses were made a few minutes or a few hours after the
387 beginning of treatments, while here, the first analyses after treatments were made 2 d after, and no
388 overexpression between D2D and D0 were found in phospholipases (Supp. Fig. S3), furthermore, PA
389 shows no evolution during storage (Fig. 4A). So, it might be a transient effect of these treatments
390 that was not observed here. DAG decreased after wounding too (Fig. 4B), and then it was constant
391 up to day 14 of storage.

392 Four classes of lipids compose the chloroplast membrane: three types of glycolipids MGDG, DGDG,
393 SQDG, and PG which is the only phospholipid of chloroplast membrane. The level of MGDG and
394 DGDG increased during storage (Fig. 5), and the profile of these two classes showed an increase of
395 36:6 species (Supp. Fig. S4). Grouping this, with 18:3 accumulation during storage and with data
396 about PC and PE (mainly 18:2 composed, see in Supp. Fig. S2) decreasing during the same period: it
397 can be proposed that the decrease at the beginning of storage of endomembrane phospholipids,
398 specially 18:2 species, have delivered 18:2 forms of phosphatidic acid and diacylglycerol. The total
399 unsaturation level has increased during storage, therefore the pool of phosphatidic acid and
400 diacylglycerol have been distributed to MGDG and DGDG and were desaturated in 18:3 forms. This
401 increase in MGDG and DGDG is not coherent with some senescence phenotype that has been
402 observed where these compounds were degraded with chloroplast dismantling during this phase
403 (Gut and Matile, 1989). However, DGDG increase during cold acclimation was also reported in wheat
404 which is 18:3 species, with a particular increase in unsaturated DGDG (Li et al., 2015). Here, an
405 increase of DGDG corresponds to higher expression of DGDG synthase 1 and 2 (Supp. Fig. S5).
406 Decrease of MGDG was also observed during cold acclimation (Burgos et al., 2011) and that was not
407 the case here. Concerning phosphatidylglycerol, it is known that wounding induces a quick decrease
408 of this lipid class via phospholipase D activity (Zien et al., 2001), PG decrease was reproduced here.

409 Usually, dark-induced senescence has been specified as a rapid mechanism of chloroplast and
410 chlorophyll degradation (Bárcena et al., 2020; Braidot et al., 2014; Wada et al., 2009; Weaver and
411 Amasino, 2001) in *Brassicaceae* species, usually between 2 and 4 d after harvest. Regarding lettuce,
412 the degradation of chlorophyll in cold dark storage appears to be longer, taking more than 10 d
413 (Kasim and Kasim, 2007; Liu et al., 2015; Witkowska and Woltering, 2014). In this study there was no
414 chlorophyll degradation (Fig. 6), suggesting good maintenance of chloroplast integrity and thylakoid
415 membranes, well reflected in MGDG and DGDG levels. The difference in senescence between species
416 is highlighted here, *Brassicaceae* species seems to be highly sensitive to dark-induced senescence,
417 considering visual phenotype, including chlorophyll/chloroplast degradation. A typical issue in plant
418 senescence is the decrease of fatty acids entering the β -oxydation cycle in order to provide
419 glyoxylate cycle and remobilize carbon from lipids to sugar (Buchanan-Wollaston et al., 2003;
420 Troncoso-Ponce et al., 2013). In our study, it does not seem the case: after 14 d of storage, no
421 decrease in fatty acids was found (Fig. 2.C). Therefore, either the lettuces did not enter into the
422 senescence phase or the common behavior of dark induced senescence described in *Brassicacea*
423 species due to massive research in *A. thaliana* is not relevant for some other species. Indeed, it
424 seems that, after 14 d of storage, lettuce leaves are still looking fresh (Supp. Fig. S6). However,
425 senescence onset on chlorophyll, galactolipids, and fatty acids degradation after cut and dark
426 treatment begins quickly in *Brassicacea* so it is probable that the onset of senescence is not

427 regulated the same way between different species. To further apprehend the control of senescence
428 onset in lettuce, further investigations are needed.

429 5.2 Intermittent light effect on lipidome during storage

430 Phosphatidic acid (PA) was more present in light treated samples than in darkness on day 2 (Fig. 4A).
431 There was no significant difference in DAG when considering % (Fig. 4B), but regarding absolute
432 values of DAG, there was a significant effect of light treatment at day 2, maintaining DAG at higher
433 levels than in darkness (Supp. Fig. S7). PA and DAG are both precursors and degradation products of
434 phospholipids and galactolipids. It is possible that the increase of PA and DAG in lettuce exposed to
435 light was due to higher phospholipase C and D activities, which degrade phospholipids to
436 diacylglycerol and phosphatidic acid, respectively (Fig. 7B). Indeed, higher accumulation of
437 phospholipase C and D transcripts was observed in light treated leaves (Fig. 7A.), suggesting a
438 possible effect of light resulting in higher activities of these two phospholipases, at least by
439 transcriptional regulation. Strengthening this hypothesis, phosphatidylethanolamine (PE), which is
440 one of the substrate of phospholipase D, was less present in darkness modality on day 2 showing
441 probable greater degradation of this compound at light. Concerning phosphatidylcholine (PC), which
442 is also a substrate for phospholipases, no such phenom was observed, however, the levels of
443 phospholipids and PA are not on the same scale (Fig. 4). Furthermore, it is interesting to observe
444 different accumulation patterns between different molecules of each class: indeed 36:5 and 36:6 in
445 PC and PE decreased more under light than under darkness at day 2 (Supp. Fig. S2) and this was
446 probably the main contribution to the phosphatidic acid and diacylglycerol pools, knowing that the
447 increase of these classes is mainly due to 36:5, 36:6 for PA 18:2/18:3 and 18:3/18:3 for DAG (Supp.
448 Fig. S8). There are different types of phospholipase D (*alpha*, *beta*, *gamma*, *delta*) that can have
449 different substrate preferences between PC and PE and also different locations in the cell (Hong et
450 al., 2016). A few studies have shown a link between light regulation and activity/transcription of
451 phospholipase D and C. Herein our study, the phospholipases D that have been overexpressed at
452 light are mostly *alpha* which are located mostly in the plasma membrane. For phospholipase D, two
453 studies have observed an increase in phospholipase D activity and PA under light application
454 compared to a darkness modality in oat cells (Park et al., 1996) and sorghum leaf (Monreal et al.,
455 2010). A phosphoinositide-specific phospholipase characterized in peas has been shown to be down-
456 regulated by light during seedlings (Venkataraman et al., 2004), and another research group has
457 documented this topic and has highlighted a negative regulation by light of phospholipase D, in oat
458 seedlings, that was regulated by phytochrome (Kabachevskaya et al., 2007, 2004). Whatever the
459 case, these last three studies cited have not been performed after harvest, which may modulate
460 these metabolisms. We showed that intermittent light stimulated the transcription of some
461 phospholipase D and C and their products, further studies are necessary to understand whether this
462 stimulation depends on photosynthesis or phytochrome pathways. These two pathways have been
463 identified as the two main regulatory pathways of dark-induced leaf senescence (Liebsch and Keech,
464 2016).

465 Phosphatidic acid and diacylglycerol are “distribution hubs” of lipids, being both precursors and
466 degradation products of main classes of lipids, and they are involved in lipid signaling (Wang, 2004).
467 Phosphatidic acid is a mediator in stress response, membrane trafficking, and others specific
468 pathways, and it is a messenger involved in many regulations. Specifically, phospholipase D and
469 phosphatidic acid are involved in senescence as being enhancers of three senescence hormones:
470 abscisic acid, ethylene, and jasmonic acid (Fan et al., 1997; Jia and Li, 2015; Wang et al., 2000).
471 Further studies in lettuce should target the reactions to PA accumulation, for example, subcellular
472 signaling. Phospholipases have a main role in promoting senescence by degrading membrane lipids:
473 therefore, taking these results on phosphatidic acid and phospholipase and the current knowledge,

474 our results suggest that light application at the beginning of storage does not delay senescence of
475 extraplasmidial membranes.

476 TAGs are important during senescence because they act in the remobilization of fatty acids in other
477 parts of the plants. The level of TAG is higher in “light treatment” after day 2 whereas the level of
478 DAG and PA in light treated leaves returned to the same level as obscurity, suggesting that the high
479 levels of DAG and PA at day 2 in light treated leaves have been redistributed to TAG since there was
480 no more light in this modality. To confirm this observation, RNAseq analysis showed an upregulation
481 of DGAT genes in light treated lettuce compared to darkness on day 2 but not on day 7 (Supp. Fig.
482 S9). However TAG level of unsaturation at day 2 showed that the high amount of TAG in light treated
483 leaves is due to saturated species (Supp. Fig. S10). This suggests that photosynthesis in light treated
484 leaves, even if it was just above the light compensation point, allowed carbon accumulation that may
485 be allocated to fatty acid and stored in the TAG pool on day 2, and that pool was maintained until
486 day 14. Concerning the effect of cold storage on TAG amount, it seems that there was a little
487 accumulation in both light and darkness treated leaves (Fig. 8). Two studies have also observed this
488 trend of accumulation of TAG (Kaup et al., 2002; Lippold et al., 2012) during senescence and have
489 linked it to a particular trait of senescence with upregulation of diacylglycerol acyltransferase (DGAT)
490 (Kaup et al., 2002). Here, observation of the difference in transcription of DGAT revealed that there
491 was no upregulation of these genes along storage (Supp. Fig. S9). TAGs have also been observed to
492 increase during cold acclimation in many accessions of *A. thaliana* (Degenkolbe et al., 2012) during 2
493 weeks of adaptation to cold.

494 5.3 Membrane integrity indicators

495 Global measures to follow the membrane integrity do not indicate an expected degradation of the
496 membrane, either electrolyte leakage (EL) or measurement of malondialdehyde (MDA) (Fig 9A and
497 B). Ordinary, EL parameter is used for the determination of tissue quality during storage (Marangoni
498 et al., 1996) and it usually increases throughout the storage. In lettuces stored at low temperatures,
499 it seems that this increase in EL is observable only after more than 2 weeks of storage (Kim et al.,
500 2005; Salgado et al., 2014). Knowing that lettuces are commercialized and sold for a maximum of one
501 week after harvest, it seems that this parameter is not relevant for lettuce quality assessment.
502 However, several studies have found an increase of EL in the postharvest storage of vegetables and
503 showed that light application reduced the increase of EL (Glowacz et al., 2015; Liu et al., 2015;
504 Martínez-Sánchez et al., 2011). Confirming the weak variations of EL, the MDA level did not vary
505 during the storage (Fig 9B). MDA is a product of lipid peroxidation and is an indicator of degradation
506 of membrane components, usually, in lettuce it appears to increase during storage (Li et al., 2021;
507 Spinardi and Ferrante, 2012). In broccoli and in pak choi, light treatment permitted to reduce MDA
508 levels over the postharvest senescence (Jiang et al., 2019; Zhou et al., 2020). MDA is produced from
509 non-enzymatic pathway of lipid peroxidation and it seems that in senescence, it is the enzymatic
510 pathway that is more enhanced (Shimakawa et al., 2022). So, maybe MDA was not a good indicator
511 to observe a precocious effect of lipid peroxidation during postharvest senescence of lettuce.

512 6 Conclusion

513 We observed in this study the changes of a large spectrum of lipid compounds in lettuce leaves over
514 two weeks of postharvest storage: PC, PE, DAG, PA, TAG, MGDG, DGDG, PG, SQDG and also for fatty
515 acids (specifically C18:2 and C18:3). Storage appears to promote reallocation of fatty acids
516 composing phospholipids through PA and DAG intermediates to galactolipids, MGDG and DGDG.
517 There was a noticeable effect of intermittent light treatment enhancing phospholipase D and
518 phosphatidic acid and to a lesser extent diacylglycerol and SQDG. We are aware that this light

519 treatment is not easy to implement at a commercial scale, but because it stimulates phosphatidic
520 acid accumulation, this should encourage further studies to better adapt it to commercial practices,
521 e.g. LED flashes... Membrane integrity is preserved in butterhead lettuce during storage. That might
522 be a specific feature of the butterhead lettuce that has been chosen as a vegetable that is storing
523 well, when it is not too processed. Further studies are needed to better link lipidomic studies of leafy
524 vegetables to senescence and particularly the link of light phospholipase D regulation during
525 postharvest senescence.

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533 CRediT authorship contribution statement

534 **Thomas Baron:** Conceptualization, Formal analysis, Investigation, Writing – Original Draft,
535 Visualization. **Juliette Jouhet:** Conceptualization, Methodology, Investigation, Resources, Writing –
536 Review and Editing, Supervision. **Marion Schilling:** Data Curation, Investigation. **Mathilde Cussac:**
537 Investigation. **Florence Charles:** Conceptualization, Writing – Review and Editing. **Véronique Vidal:**
538 Methodology, Investigation, Resources. **Christian Chervin:** Conceptualization, Writing – Review and
539 Editing, Supervision, Project administration. **Huguette Sallanon :** Conceptualization, Resources,
540 Writing – Review and Editing, Supervision, Project administration, Funding acquisition.

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9 Supplemental data

Supplemental data, figure S.1: Differentially expressed genes of fatty acid desaturase for day 2 darkness vs day 0, “logFC” stands for log Fold Change which is positive when there was more expression of the gene in the day 2 darkness [A] and for day 7 darkness versus day 0, log fold change is positive when there was more expression of the gene in the day 7 darkness [B]. “FDR” stands for False Discovery Rate which is highlighted when it is inferior to 0.05. The “Lsat” annotation was done by homology with general genomic banks (Baron et al., 2022).

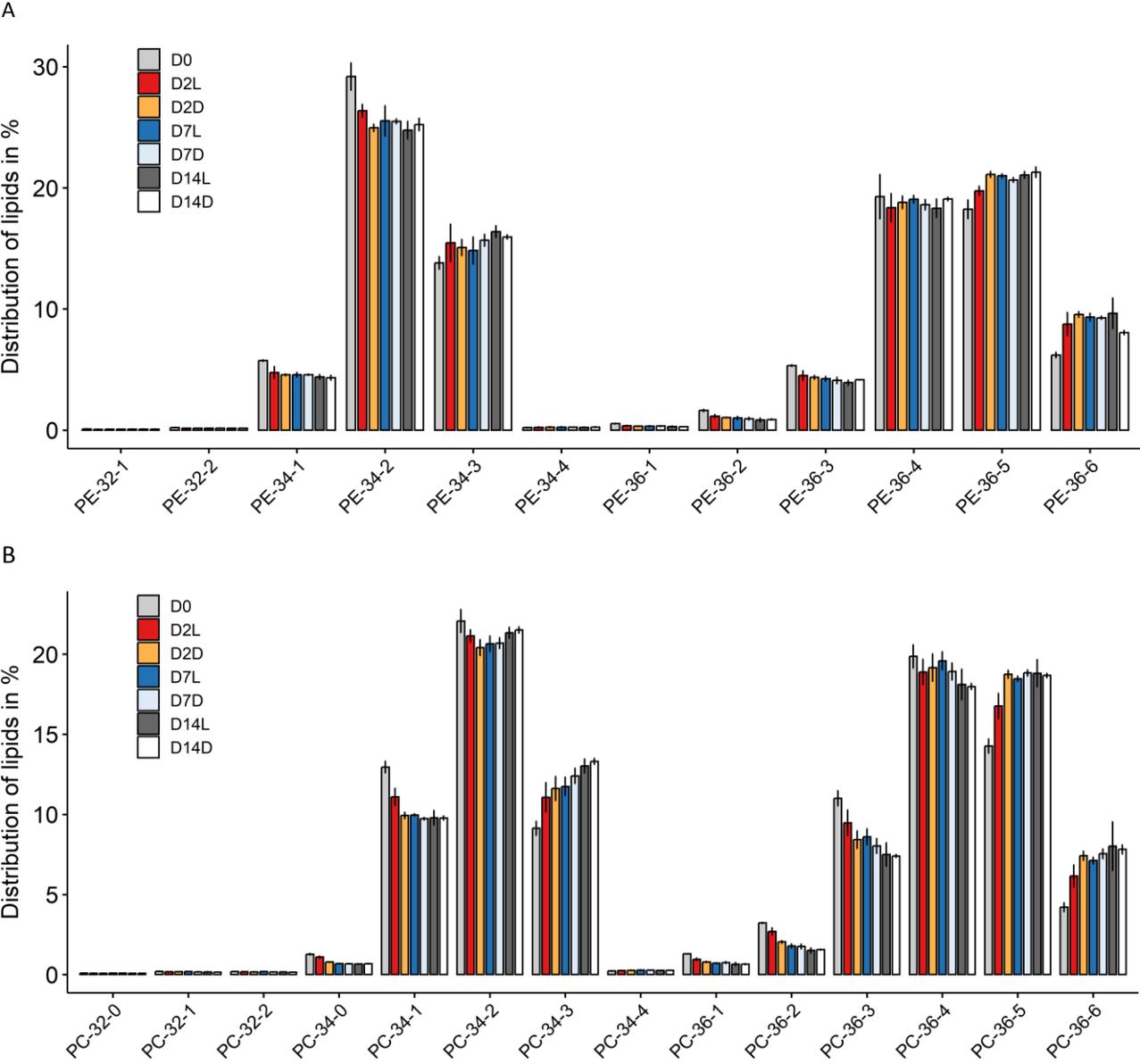
A

D2D vs D0			
Lsat	Name	logFC	FDR
Lsat_1_v5_gn_5_37300	ADS3_ARATH Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase, chloroplactic	2,288	2,10E-03
Lsat_1_v5_gn_9_35380	FAD3C_RICCO Omega-3 fatty acid desaturase, chloroplactic	1,582	8,48E-08
Lsat_1_v5_gn_8_74461	FAD6E_ARATH Omega-6 fatty acid desaturase, endoplasmic reticulum	1,429	4,74E-08
Lsat_1_v5_gn_8_150861	FD6E2_SOYBN Omega-6 fatty acid desaturase, endoplasmic reticulum isozyme 2	0,938	1,07E-01
Lsat_1_v5_gn_6_13521	FADS3_BOVIN Fatty acid desaturase 3	0,933	4,58E-05
Lsat_1_v5_gn_2_124580	FAD3C_SESIN Omega-3 fatty acid desaturase, chloroplactic	0,922	7,56E-02
Lsat_1_v5_gn_4_33880	FADS1_PAPAN Fatty acid desaturase 1	0,842	4,55E-02
Lsat_1_v5_gn_3_114881	FAD6E_BRAJU Omega-6 fatty acid desaturase, endoplasmic reticulum	0,554	4,68E-02
Lsat_1_v5_gn_5_91701	FAD6C_BRANA Omega-6 fatty acid desaturase, chloroplactic	0,383	3,49E-02
Lsat_1_v5_gn_8_75280	FAD6E_ARATH Omega-6 fatty acid desaturase, endoplasmic reticulum	0,334	2,52E-01
Lsat_1_v5_gn_5_131560	ADS3_ARATH Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase, chloroplactic	0,284	6,60E-01
Lsat_1_v5_gn_3_133981	FD6E2_SOYBN Omega-6 fatty acid desaturase, endoplasmic reticulum isozyme 2	0,053	9,16E-01
Lsat_1_v5_gn_5_163380	FAD6E_ARATH Omega-6 fatty acid desaturase, endoplasmic reticulum	0,024	9,25E-01
Lsat_1_v5_gn_7_2520	FADS2_MOUSE Fatty acid desaturase 2	-0,144	5,26E-01
Lsat_1_v5_gn_3_134181	FAD6E_BRAJU Omega-6 fatty acid desaturase, endoplasmic reticulum	-0,189	5,70E-01
Lsat_1_v5_gn_4_90280	ADS3_ARATH Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase, chloroplactic	-0,194	5,37E-01
Lsat_1_v5_gn_8_11680	FAD3C_SESIN Omega-3 fatty acid desaturase, chloroplactic	-0,257	6,41E-01
Lsat_1_v5_gn_1_74601	STAD_CARTI Acyl-[acyl-carrier-protein] desaturase, chloroplactic	-0,439	5,54E-03
Lsat_1_v5_gn_3_134300	FD6E1_SOYBN Omega-6 fatty acid desaturase, endoplasmic reticulum isozyme 1	-0,774	1,19E-01
Lsat_1_v5_gn_1_33441	ADS3_ARATH Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase, chloroplactic	-0,861	4,41E-01
Lsat_1_v5_gn_5_171481	FAD3C_RICCO Omega-3 fatty acid desaturase, chloroplactic	-1,361	5,33E-05
Lsat_1_v5_gn_2_58980	ADS3_ARATH Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase, chloroplactic	-1,811	7,33E-04
Lsat_1_v5_gn_1_34480	ADS3_ARATH Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase, chloroplactic	-2,965	4,48E-04
Lsat_1_v5_gn_6_109980	FD6E1_SOYBN Omega-6 fatty acid desaturase, endoplasmic reticulum isozyme 1	-2,986	1,56E-02

B

D7D vs D0			
Lsat	Name	logFC	FDR
Lsat_1_v5_gn_5_37300	ADS3_ARATH Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase, chloroplactic	2,670	0,00018796
Lsat_1_v5_gn_8_74461	FAD6E_ARATH Omega-6 fatty acid desaturase, endoplasmic reticulum	1,393	5,16E-08
Lsat_1_v5_gn_9_35380	FAD3C_RICCO Omega-3 fatty acid desaturase, chloroplactic	1,285	1,40E-05
Lsat_1_v5_gn_3_114881	FAD6E_BRAJU Omega-6 fatty acid desaturase, endoplasmic reticulum	0,681	0,00985676
Lsat_1_v5_gn_6_13521	FADS3_BOVIN Fatty acid desaturase 3	0,508	0,02939448
Lsat_1_v5_gn_8_150861	FD6E2_SOYBN Omega-6 fatty acid desaturase, endoplasmic reticulum isozyme 2	0,274	0,66690435
Lsat_1_v5_gn_8_75280	FAD6E_ARATH Omega-6 fatty acid desaturase, endoplasmic reticulum	0,043	0,89371188
Lsat_1_v5_gn_5_163380	FAD6E_ARATH Omega-6 fatty acid desaturase, endoplasmic reticulum	0,036	0,87485344
Lsat_1_v5_gn_7_2520	FADS2_MOUSE Fatty acid desaturase 2	-0,023	0,92406981
Lsat_1_v5_gn_5_91701	FAD6C_BRANA Omega-6 fatty acid desaturase, chloroplactic	-0,236	0,20079522
Lsat_1_v5_gn_8_11680	FAD3C_SESIN Omega-3 fatty acid desaturase, chloroplactic	-0,304	0,55138226
Lsat_1_v5_gn_3_133981	FD6E2_SOYBN Omega-6 fatty acid desaturase, endoplasmic reticulum isozyme 2	-0,352	0,38333711
Lsat_1_v5_gn_1_74601	STAD_CARTI Acyl-[acyl-carrier-protein] desaturase, chloroplactic	-0,423	0,00598912
Lsat_1_v5_gn_3_134181	FAD6E_BRAJU Omega-6 fatty acid desaturase, endoplasmic reticulum	-0,542	0,05505414
Lsat_1_v5_gn_4_33880	FADS1_PAPAN Fatty acid desaturase 1	-0,603	0,15034729
Lsat_1_v5_gn_4_90280	ADS3_ARATH Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase, chloroplactic	-0,608	0,02204722
Lsat_1_v5_gn_2_124580	FAD3C_SESIN Omega-3 fatty acid desaturase, chloroplactic	-0,871	0,0930538
Lsat_1_v5_gn_3_134300	FD6E1_SOYBN Omega-6 fatty acid desaturase, endoplasmic reticulum isozyme 1	-0,916	0,05046073
Lsat_1_v5_gn_2_58980	ADS3_ARATH Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase, chloroplactic	-1,374	0,00900594
Lsat_1_v5_gn_1_33441	ADS3_ARATH Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase, chloroplactic	-2,450	0,01591677
Lsat_1_v5_gn_6_109980	FD6E1_SOYBN Omega-6 fatty acid desaturase, endoplasmic reticulum isozyme 1	-2,539	0,03168613
Lsat_1_v5_gn_5_171481	FAD3C_RICCO Omega-3 fatty acid desaturase, chloroplactic	-2,622	2,38E-15
Lsat_1_v5_gn_5_131560	ADS3_ARATH Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase, chloroplactic	-3,413	1,33E-09
Lsat_1_v5_gn_1_34480	ADS3_ARATH Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase, chloroplactic	-10,724	7,10E-16

Supplemental data, figure S.2: Lipids profile of [A] phosphatidylcholine (PC) and [B] phosphatidylethanolamine (PE) for all modalities given in legend. Distribution is represented in % among their class.

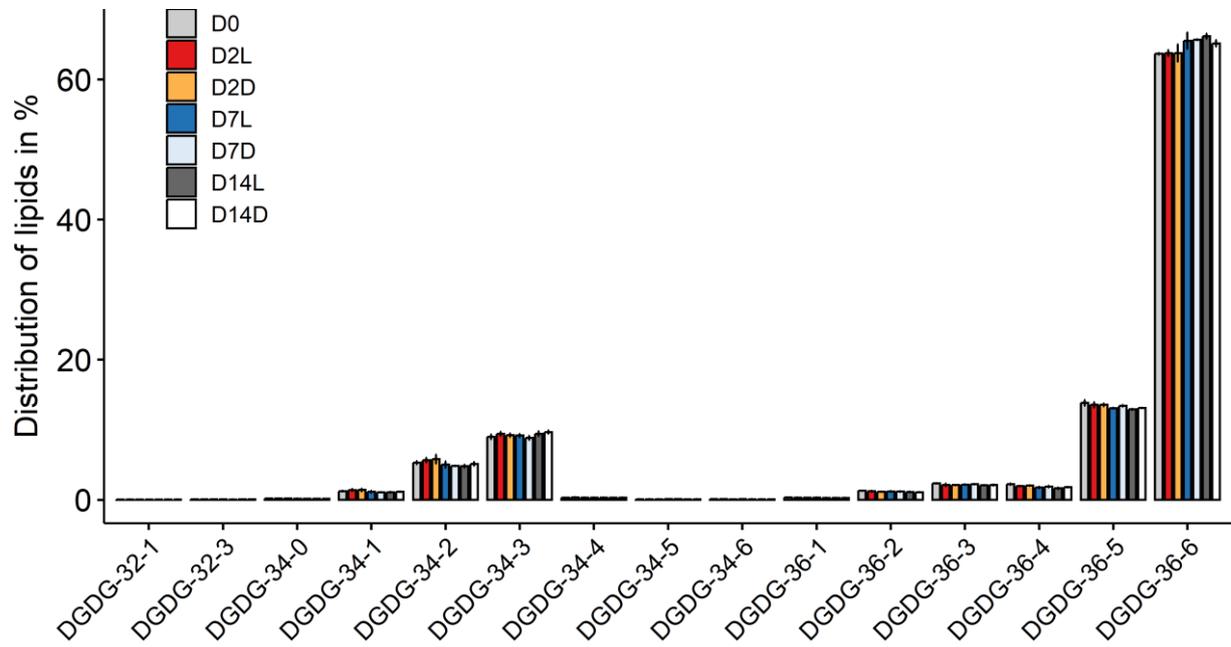


Supplemental data, figure S.3: Differentially expressed genes compared between 2 days of storage in dark and harvest (day 2 dark vs day 0), at 6 °C for phospholipase C (upper part of the table) and phospholipase D (downer part of the table) genes. “logFC” stands for log Fold Change which is positive when there was more gene expression in day 2 dark. “FDR” stands for False Discovery Rate which is also indicated and highlighted when it was inferior to 0.05. The “Lsat” annotation was done by homology with general genomic banks (Baron et al., 2022).

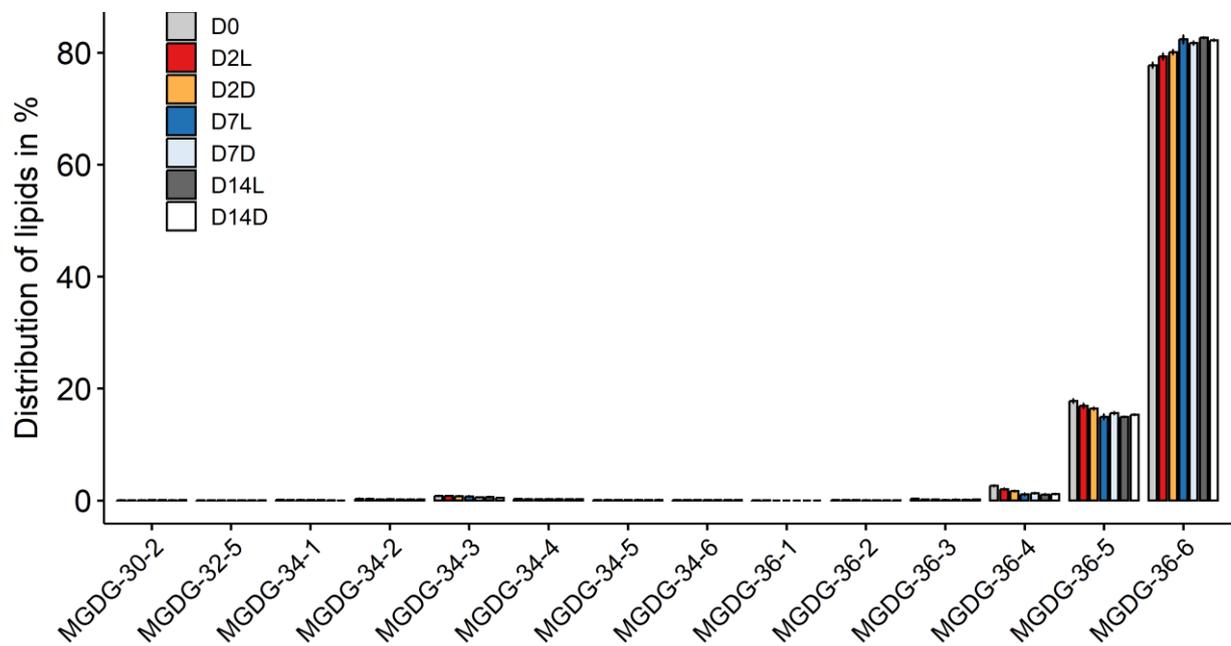
Lsat	Name	logFC	FDR
Lsat_1_v5_gn_9_19301	PHLB_MYCTU Phospholipase C 2 OS	0,472	3,73E-02
Lsat_1_v5_gn_9_61520	PLCD2_ARATH Phosphoinositide phospholipase C 2 OS	0,372	4,26E-01
Lsat_1_v5_gn_8_12041	PLCD2_ARATH Phosphoinositide phospholipase C 2 OS	0,272	2,98E-01
Lsat_1_v5_gn_2_123380	PLCD6_ARATH Phosphoinositide phospholipase C 6 OS	0,114	7,73E-01
Lsat_1_v5_gn_8_70901	PLCD6_ARATH Phosphoinositide phospholipase C 6 OS	0,068	8,87E-01
Lsat_1_v5_gn_4_41620	PHLC_MYCTU Phospholipase C 3 OS	-0,219	8,19E-01
Lsat_1_v5_gn_5_171281	PLCD4_ARATH Phosphoinositide phospholipase C 4 OS	-1,215	1,78E-04
Lsat_1_v5_gn_4_81261	PHLC_MYCTU Phospholipase C 3 OS	-2,064	1,73E-05
Lsat_1_v5_gn_5_102100	PLDP2_ARATH Phospholipase D p2 OS	0,777	1,84E-03
Lsat_1_v5_gn_3_135001	PLDB1_ARATH Phospholipase D beta 1 OS	0,469	2,43E-02
Lsat_1_v5_gn_3_4321	PLDG1_ARATH Phospholipase D gamma 1 OS	0,263	2,11E-01
Lsat_1_v5_gn_7_42860	DDHD1_BOVIN Phospholipase DDHD1 OS	0,093	6,26E-01
Lsat_1_v5_gn_8_82201	PLD3_MACFA Phospholipase D3 OS	-0,049	7,85E-01
Lsat_1_v5_gn_1_33321	PLDA1_RICCO Phospholipase D alpha 1 OS	-0,087	7,00E-01
Lsat_1_v5_gn_5_95680	PLDA1_RICCO Phospholipase D alpha 1 OS	-0,161	8,34E-01
Lsat_1_v5_gn_3_4061	PLDG1_ARATH Phospholipase D gamma 1 OS	-0,437	3,44E-01
Lsat_1_v5_gn_6_76021	PLDP1_ARATH Phospholipase D p1 OS	-0,518	5,73E-03
Lsat_1_v5_gn_4_143760	PLDD1_ARATH Phospholipase D delta OS	-0,555	2,31E-03
Lsat_1_v5_gn_2_75361	PLDD1_ARATH Phospholipase D delta OS	-0,717	6,88E-02
Lsat_1_v5_gn_5_132081	PLDA1_TOBAC Phospholipase D alpha 1 OS	-1,040	8,36E-03
Lsat_1_v5_gn_1_44721	PLDA1_RICCO Phospholipase D alpha 1 OS	-1,146	3,78E-02
Lsat_1_v5_gn_5_95800	PLDA1_RICCO Phospholipase D alpha 1 OS	-1,891	2,87E-07

Supplemental data, figure S.4: Lipids profile of [A] monogalactosyldiacylglycerol (MGDG) and [B] digalactosyldiacylglycerol (DGDG) for all modalities given in legend. Distribution is represented in % among their class.

A



B



Supplemental data, figure S.5: Differentially expressed genes of digalactosyldiacylglycerol synthase (DGDG) for day 2 darkness vs day 0, “logFC” stands for log Fold Change which is positive when there was more expression of the gene in the day 2 darkness [A], for day 7 darkness versus day 0, log fold change is positive when there was more expression of the gene in the day 7 darkness [B] and for day 2 light versus day 2 darkness, log fold change is positive when there was more expression of the gene in the day 2 light [C]. “FDR” stands for False Discovery Rate which is also indicated and highlighted when it was inferior to 0.05. The “Lsat” annotation was done by homology with general genomic banks (Baron et al., 2022).

A

D2D vs D0			
Lsat	Name	logFC	FDR
Lsat_1_v5_gn_7_5460	DGDG2_SOYBN Digalactosyldiacylglycerol synthase 2, chloroplastic OS	1,18587802	5,78E-09
Lsat_1_v5_gn_1_58180	DGDG1_LOTJA Digalactosyldiacylglycerol synthase 1, chloroplastic OS	0,69440128	0,00884238

B

D7D vs D0			
Lsat	Name	logFC	FDR
Lsat_1_v5_gn_1_58180	DGDG1_LOTJA Digalactosyldiacylglycerol synthase 1, chloroplastic OS	0,858	0,00070717
Lsat_1_v5_gn_7_5460	DGDG2_SOYBN Digalactosyldiacylglycerol synthase 2, chloroplastic OS	0,995	7,11E-07

Supplemental data, figure S.6: Photography of lettuce leaves at day 14 [A] in darkness modality leaves of replicate 1 and [B] in light modality leaves of replicate 1

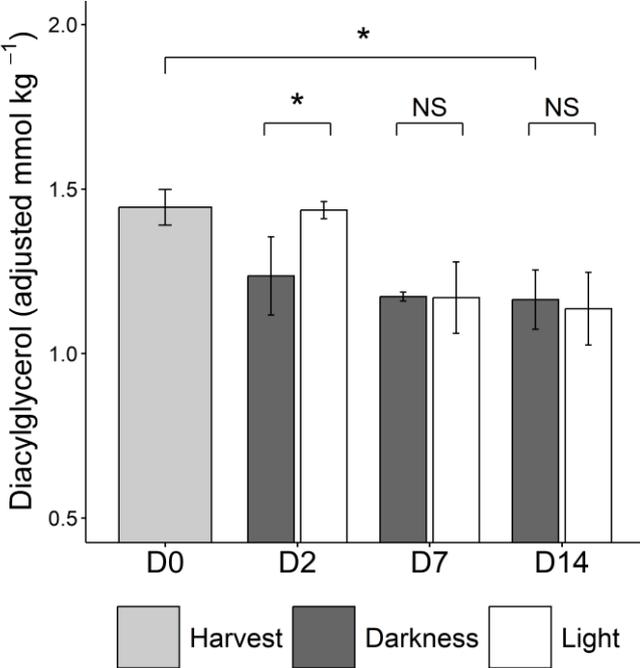
A



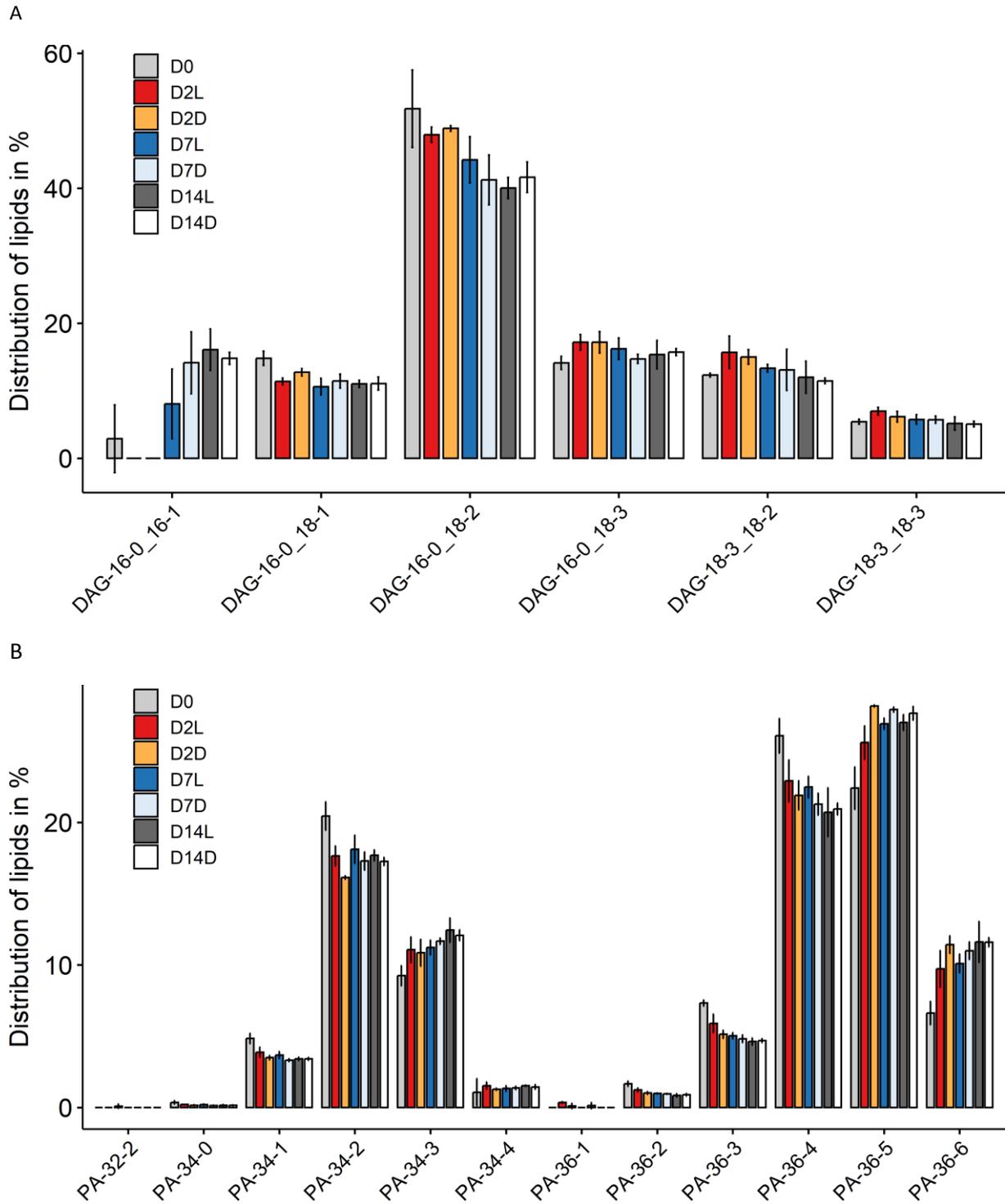
B



Supplemental data, figure S.7: Evolution of diacylglycerol represented from harvest to day 14 of storage. D0: Harvest, D2: day 2 of storage, D7: day 7 of storage, D14: day 14 of storage. In legend, different modalities are represented Harvest (grey), Darkness (dark grey), and Light (white). Kruskal-Wallis test between the two treatments was done for each day of storage, and also between day 14 darkness and day 0 Harvest.



Supplemental data, figure S.8: Lipids profile of [A] diacylglycerol (DAG) and [B] phosphatidic acid (PA) for all modalities given in legend. Distribution is represented in % among their class.



Supplemental data, figure S.9: Differentially expressed genes of diacylglycerol acyltransferase (DGAT) for day 2 darkness vs day 0, “logFC” stands for log Fold Change which is positive when there was more expression of the gene in the day 2 darkness [A], for day 7 darkness versus day 0, log fold change is positive when there was more expression of the gene in the day 7 darkness [B] and for day 2 light versus day 2 darkness, log fold change is positive when there was more expression of the gene in the day 2 light [C]. “FDR” stands for False Discovery Rate which is also indicated and highlighted when it was inferior to 0.05. The “Lsat” annotation was done by homology with general genomic banks (Baron et al., 2022).

A

D2D vs D0			
Lsat	Name	logFC	FDR
Lsat_1_v5_gn_8_113821	DGAT2_ARATH Diacylglycerol O-acyltransferase 2 OS	0,010	9,69E-01
Lsat_1_v5_gn_5_143061	DGAT1_ARATH Diacylglycerol O-acyltransferase 1 OS	-1,274	5,82E-10

B

D7D vs D0			
Lsat	Name	logFC	FDR
Lsat_1_v5_gn_5_143061	DGAT1_ARATH Diacylglycerol O-acyltransferase 1 OS	-0,536	1,04E-02
Lsat_1_v5_gn_8_113821	DGAT2_ARATH Diacylglycerol O-acyltransferase 2 OS	-0,091	6,72E-01

C

D2L vs D2D			
Lsat	Name	logFC	FDR
Lsat_1_v5_gn_5_143061	DGAT1_ARATH Diacylglycerol O-acyltransferase 1 OS	0,634	4,65E-03
Lsat_1_v5_gn_8_113821	DGAT2_ARATH Diacylglycerol O-acyltransferase 2 OS	0,165	4,88E-01

Supplemental data, figure S.10: Lipids profile of triacylglycerol (TAG) for all modalities given in legend. Distribution is represented in % among their class.

