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# Transcriptomic view of detached lettuce leaves during storage: A crosstalk between wounding, dehydration and senescence

Julie Ripoll<sup>1</sup>, Florence Charles<sup>1</sup>, Véronique Vidal<sup>1</sup>, Sandrine Laurent<sup>1</sup>, Christophe Klopp<sup>2</sup>, Félicie Lauri<sup>1</sup>, Huguette Sallanon<sup>1</sup>, David Roux<sup>1\*</sup>

## Affiliations

1. Avignon University, Qualisud UMR95, F-84 000 Avignon, France  
Qualisud, Univ Montpellier, CIRAD, Montpellier Sup'Agro, Université d'Avignon, Université de la Réunion, Montpellier, France
2. Plate-forme bio-informatique Genotoul, INRA, Biométrie et Intelligence Artificielle, BP 52627, 31326 Castanet-Tolosan Cedex, France.

\* Corresponding author: [david.roux@univ-avignon.fr](mailto:david.roux@univ-avignon.fr) +334.90.84.22.05

301 rue Baruch de Spinoza | BP 21239 | 84916 AVIGNON Cedex 9 | FRANCE

## 1 Abstract

2 Many methods of storage are currently used to delay the postharvest senescence and the ensuing  
3 quality losses of detached lettuce leaves. This transcriptome study explores the senescence mechanisms  
4 of packaged lettuce leaves over 7 d of cold storage in darkness. At the end of the storage, the detached  
5 lettuce leaves showed a 10 % water loss and 24 % cut-edge discoloration. No chlorophyll fluorescence  
6 variation was observed. A total of 1,048 and 1,846 genes were differentially expressed (DE) after 2 and 7  
7 d of storage, respectively. In terms of gene expression modulation, the data showed a global shutdown of  
8 primary-metabolism but no obvious chlorophyll breakdown. Although an early stress-response clearly  
9 takes place, the cold storage under darkness appeared to delay senescence by protecting chloroplasts  
10 from degradation and enhancing an antioxidative response. Then, a progressive global expression  
11 shutdown appeared, concomitantly with the dehydration stress. Among the transcripts that showed an  
12 early high expression, the data highlighted many stress-related genes (PIN, bzip TF, Heat-shock, stress A-  
13 like), 30 redox-regulation associated transcripts, the majority of the phenylpropanoid pathway markers  
14 and ethylene and auxin-related genes. Later (day 7), many dehydration-responsive markers showed an  
15 increased expression, as did some abscisic acid related markers. Phytohormones (cytokinins) and  
16 transcriptions factors (WRKYs) appeared to play complex roles as senescence progressed. No simple link  
17 between cut-edge discoloration and PPO-related genes expression was made. The main known process of  
18 senescence induction through the phaeophorbide *a* oxygenase and phospholipase D pathways were not

19 highlighted in this study. To the best of our knowledge, this study is the first to explore the transcriptional  
20 response of postharvest senescence in detached lettuce leaves. Deeper analysis of several markers will  
21 help to dissect the crosstalk between wounding, dehydration and senescence mechanisms. In addition,  
22 post-transcriptional studies are needed to conclude about the described patterns.

## 23 **Keywords**

24 *Lactuca sativa* L. – Postharvest – Storage – Wounding – Senescence – Transcriptome

## 25 **Introduction**

26 Plant senescence has long been an extensive research focus that could help to dissect how plant  
27 physiology integrates environmental stimuli. Depicting the plant senescence process was recently  
28 proposed as a significant help to solving current ecological and agricultural challenges (Thomas and  
29 Ougham 2016). Accordingly, when considering green vegetables, increasing our knowledge about the  
30 senescence of plant-detached organs could lead to new milestones in postharvest storage.

### 31 1/ Leaf senescence processes and regulation

32 Genetically programmed (natural) senescence, mainly induced by internal age-dependent factors,  
33 has been distinguished from stress-induced senescence that generally takes place in response to external  
34 factors such as nutritional deficiencies, pathogen attacks, various stresses or darkness. Although both  
35 types of senescence may lead to similar symptoms, stress-induced senescence is characterized by a faster  
36 process (a few days versus several weeks for genetically programmed senescence) (Springer et al. 2015).  
37 For example, a “dark-induced” senescence has been highly studied in *Arabidopsis* due to its precise and  
38 rapid implementation (Quirino et al. 2000). Genetically programmed senescence is characterized by a  
39 complex series of immutable cellular processes that aim to progressively deconstruct cells and remobilize  
40 nutrients: photosynthesis decrease, protein degradation, lipid lysis, etc. The mitochondria have been  
41 shown to manage many of the sequential steps while maintaining respiration. However, the chloroplast  
42 undergoes numerous catabolic events from the early start of senescence to the final chlorophyll  
43 degradation. Phytohormones play a complex regulatory role over this phenomenon that has not yet been  
44 completely elucidated (Lim et al. 2007; Khan et al. 2014). The most visual symptom of genetically  
45 programmed senescence in plants is due to the chlorophyll (Chl) degradation responsible for the autumnal  
46 colors due to the progressive loss in photosynthetic pigments (Chl *a*, Chl *b*, carotenoids and xanthophylls)  
47 (Hörtensteiner and Lee 2007). Several enzymes are involved such as chlorophyllases, Mg-dechelatas and  
48 phaephorbide *a* oxygenase (Hörtensteiner and Kräutler 2011). During this process, hydroxylation and  
49 glycosylation reactions share similarities with the detoxification of oxidative stress in cells (Quirino et al.  
50 2000). Chl breakdown also includes the progressive loss of chloroplastic proteins, mainly ribulose  
51 bisphosphate carboxylase (RuBisCO) and proteins from the light-harvesting complex (LHC) via the action  
52 of several endo- and exopeptidases. These products are the major sources of cellular nitrogen in senescing

53 leaves. Due to their light-absorbing properties, Chl subproducts (mainly produced by the action of the  
54 phaeophorbide *a* oxygenase) induce the release of reactive oxygen species (ROS), which in turn contribute  
55 to both stress and senescence signalization (Buchanan-Wollaston et al. 2003; Pružinská et al. 2005;  
56 Schippers et al. 2007). In Arabidopsis, the NAM/ATAF/CUC (NAC) transcription factor (TF) ANAC046 was  
57 found to control the expression of many senescence-related genes including Chl-catabolic genes such as  
58 phaeophorbide *a* oxygenase (Oda-Yamamizo et al. 2016). Consequently, during senescence, the chloroplast  
59 is the main source of ROS in contrast with the stress response where ROS are produced by the  
60 mitochondria (Schippers et al. 2007). ROS induce lipid peroxidation, protein modifications, purine  
61 oxidation and DNA damage. Their accumulation during senescence is paralleled by a decrease in  
62 antioxidant activity (Pastori and del Rio 1997; Luschin-Ebengreuth and Zechmann 2016); plants showing a  
63 high tolerance to oxidative stress appear to senesce slowly (Schippers et al. 2007). In this context, the  
64 antioxidant actors comprise the ascorbate-glutathione cycle (Zechmann 2014),  $\alpha$ -tocopherol, enzymatic  
65 scavengers and oxidase inhibitors (Leshem 1988). In addition, mitochondrial superoxide dismutase (SOD)  
66 participates in both the regulation and the signalization of leaf senescence (Zentgraf 2007).

67         The onset of senescence is reflected by the progressive disruption of membrane integrity and  
68 leakage of associated solutes. These alterations in membrane lipid composition are enzymatically  
69 mediated. The transcriptome of Arabidopsis leaves revealed senescence responses in lipases, acyl  
70 hydrolases, phospholipases (especially phospholipase D), lipoxygenases and  $\beta$ -oxidation enzymes (Guo et  
71 al. 2004). For example, the gene At2g30550 (lipase-like) shows a chloroplast-targeting sequence and de-  
72 esterifies fatty acids from complex lipids during senescence (Guo et al. 2004). These fatty acids in turn  
73 undergo  $\beta$ -oxidation in glyoxysomes, forming acetyl-coA for energy production via gluconeogenesis  
74 (Charlton et al. 2005)(De Bellis et al. 1990).

75         Genes whose expression was significantly modified (up- or downregulated), especially during  
76 senescence, have been clustered by many studies in a wide senescence-associated-genes (SAG) group  
77 (Buchanan-Wollaston 1997; Li et al. 2012). SAGs operate in, for example, lipid transfer, chelation and  
78 transport of metal ions, and oxidative-stress-response (anionic peroxidase, glutathione S-transferase).  
79 Many SAGs are themselves finely modulated by a number of TFs (Balazadeh et al. 2008; Kim et al. 2013)  
80 such as the NAC family (Kim et al. 2016), the basic region/leucine zipper (bZIP) family (Llorca et al. 2014)  
81 or the WRKY family (Jiang et al. 2016). These TFs frequently take roles in both the stress response and  
82 senescence, and NAC and bZIP families, which were recently characterized, are also involved in growth  
83 and development (Liu et al. 2014; Llorca et al. 2014). NAC TFs have been shown to modulate  
84 phytohormonal regulations of abiotic and biotic stresses and to play a key regulatory role in senescence  
85 by linking environmental signalization with development and cell death (Nuruzzaman et al. 2013; Kim et  
86 al. 2016). The WRKY TFs family has been widely documented and possesses an associated Arabidopsis

87 database (Eulgem et al. 2000). For example, AtWRKY57 is known to repress leaf senescence by interacting  
88 with the jasmonic acid (JA) pathway. AtWRKY54 and 70 have cooperative functions in negative regulation  
89 of senescence (Hu et al. 2012), whereas AtWRKY53, an early factor of drought response (Sun and Yu 2015),  
90 regulates senescence positively (Zentgraf et al. 2010). These three WRKYs then interact independently  
91 with AtWRKY30 (Besseau et al. 2012). AtWRKY22, 29, 33 and 53 are also implicated in the oxidative-stress  
92 response and regulate oxidation during senescence (Zentgraf 2007). Finally, some small RNAs have also  
93 been described as senescence-inducible and to set posttranscriptional controls during senescence  
94 (Thatcher et al. 2015).

95 Phytohormones have been shown to regulate leaf senescence in response to external factors such  
96 as modulation of the circadian rhythm or abiotic stresses. Auxin, cytokinins, gibberellins, and nitric oxide  
97 and Ca<sup>2+</sup> play roles in cell development and plant growth and appear to delay senescence (Miller et al.  
98 1999; Li et al. 2012). In contrast, abscisic acid (ABA), salicylic acid (SA), JA, ethylene and brassinosteroids  
99 are known to play a regulatory role in plant responses to stresses, and might also act as positive regulators  
100 of leaf senescence (Sarwat et al. 2013).

101 Among the posttranslational modifications that can occur during leaf senescence  
102 (phosphorylation, glycosylation, methylation and acetylation), ubiquitylation takes an important place  
103 (Woo et al. 2013). The ubiquitin-proteasome pathway is a central feature of autolysis in programmed cell  
104 death. The Skp1/Cul1/F-box (SCF) protein complex (an ubiquitin ligase) has been described to be positively  
105 regulated in senescing leaves by controlling negative regulators of senescence, especially the SCF complex  
106 showing an ORE9 F-box domain (Woo et al. 2001). Each F-box domain has a specific role in the stress  
107 response and/or senescence (Stefanowicz et al. 2015).

## 108 2/ Postharvest senescence

109 Packaged salads represent the most important sales of the fresh-cut fruits and vegetables  
110 sector (Martín-Belloso and Soliva-Fortuny 2011). The shelf life of fresh-cut lettuce is generally  
111 approximately 5-7 d in wholesale or retail markets, but it can vary from 5 to 18 d depending on treatments  
112 and storage temperature (Zhou et al. 2004; Kang et al. 2007). The two main causes of quality deterioration  
113 are the loss of the “crisp green” appearance related to a 3-5 % water loss and the development of cut-  
114 edge discoloration which can be the consequence of bleaching, pinking and browning (Sinha et al. 2010;  
115 Hunter et al. 2017). Wounding and discoloration of fruits and vegetables contribute significantly to food  
116 waste due to consumer rejection, despite not reflecting the overall quality of the fresh product.  
117 Vegetables, mainly harvested at a young stage, endure an important wound-stress that provokes response  
118 mechanisms that dominate the cell metabolism and subsequently initiate/accelerate the senescence  
119 process. The majority of studies have focused on the genetically programmed and dark-induced leaf  
120 senescence. Postharvest-induced and natural senescence have been shown to share common features,

121 particularly a decrease in fatty acids, lysophospholipids and sesquiterpene lactones (Garcia et al. 2016),  
122 but the current literature does not propose an exhaustive molecular view of postharvest senescence and  
123 to what extent it diverges from natural senescence.

124 Fresh-cuts have been shown to express a wound response proportional to the severity of the  
125 physical injury driven by the postharvest process. The breaking of the cellular compartments at the  
126 wounding site triggers the stress response through the whole detached organs and accelerates many  
127 metabolisms, particularly respiration (Martín-Belloso and Soliva-Fortuny 2011). As O<sub>2</sub> reaches the open  
128 cellular matrix, **discoloration occurs. Color (pinkening and/or browning) can result from an increased activity**  
129 **of both phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO) enzymes. However, levels of**  
130 **PPO and subsequent development of discoloration have not always been correlated. In addition, pigments**  
131 **resulting from PPO oxidation of polyphenols are also dependent on the initial substrates of PAL. Brown**  
132 **color could also be associated with peroxidase (POD) activity on lignins in presence of H<sub>2</sub>O<sub>2</sub> (Hunter et al.**  
133 **2017).** PAL catalyzes the deamination of phenylalanine which is the first step in phenylpropanoids  
134 metabolism. This leads to the subsequent formation of hydroxycinnamic acids and different classes of  
135 phenolic compounds including flavonoids, which are also PPO substrates (Llorach et al. 2008; Hisaminato  
136 et al. 2001; Ke and Saltveit 1988). In Arabidopsis, the genes from the PAL family (PAL1-PAL4) have also  
137 shown a modulated expression over time and according to the type of plant tissues (Huang et al. 2010). In  
138 the context of packaged lettuce, leaf cutting is typically associated with a rapid increase in polyphenol  
139 content, and PAL has been used as an efficient lifetime marker (Sinha et al. 2010). In lettuce, PAL activity  
140 was described to increase after 3 and 7 d after harvest, whereas PPO activity was more cyclic (Chen et al.  
141 2017; Landi et al. 2013a; Degl'Innocenti et al. 2007) with daily variations. PPOs allow hydroxylation of  
142 monophenols to *o*-diphenol and the oxidation of *o*-diphenol to *o*-quinone using O<sub>2</sub>. In many plants and  
143 fruits, the subsequent nonenzymatic polymerization of quinones with other phenolics and/or amino acids  
144 produces the pigments responsible for discoloration (Sinha et al. 2010). The PPO family appears to be  
145 extremely variable (in terms of number of genes and introns) among the plant species and is totally absent  
146 from Arabidopsis (Tran et al. 2012).

147 Several storage methods have been developed to enhance the shelf life of fresh products. For  
148 lettuce leaves, the most used protocol is storage in darkness under cold temperatures (4-6 °C) mainly to  
149 limit water losses (Martín-Belloso and Soliva-Fortuny 2011; Liu et al. 2015). Nevertheless, under these  
150 conditions, Chl breakdown is observed after 6 d (Hisaminato et al. 2001). It was hypothesized that the  
151 absence of light led to a delayed senescence via reduced RuBisCO levels (Lers 2007). However, we have  
152 recently proposed that a 2 d treatment of fresh-cut lettuce by intermittent (2 hours cycles) moderate level  
153 light (50 μmol m<sup>-2</sup> s<sup>-1</sup>) could maintain product quality by reducing discoloration, minimizing weight loss  
154 and respiration and maintaining a high level of photosynthetic capacity (Charles et al. 2017). The packaging

155 of fresh-cut lettuce has allowed the use of modified atmospheres for shelf life enhancement (Kim et al.  
156 2005). Among numerous sets, low O<sub>2</sub> combined with high CO<sub>2</sub> atmospheres are very efficient for both  
157 limiting phenols accumulation (PAL inhibition) and respiration (Sinha et al. 2010). Recently, it was observed  
158 that at ambient temperature, yellow sesquiterpenes called lettucenins (assembled as chromophores) can  
159 cause discoloration (Mai and Glomb 2013). These molecules are specific to the response to mechanical  
160 injury in lettuces and may represent 25 to 40 % of the colored compounds (Mai and Glomb 2014).

161 Many of the postharvest processes developed in lettuce have raised extended shelf-life or  
162 increased access to accurate biochemical markers, but little progress has been made in terms of  
163 knowledge of the underlying physiology of detached organs. Literature remains sparse when considering  
164 gene expression modulation associated with the observed disorders. New high throughput molecular  
165 approaches have been highlighted as promising postharvest tools (Hertog et al. 2011), since they could  
166 help to: i) elucidate the cellular process ii) enhance the efficiency of storage technologies iii) reveal new  
167 molecular markers reflecting both product quality and the physiological state. Lettuce is a shade plant,  
168 and its leaves, which are often quite large, are naturally adapted to low irradiation. Consequently, it is  
169 expected that the senescence-like symptoms that occur in lettuce during storage might highly differ from  
170 the phenomena described in other plant models and particularly from Arabidopsis under dark-induced  
171 senescence. To date, recent transcriptome studies have focused on lettuce responses to a pathogen  
172 infection (De Cremer et al. 2013) or on genome mapping (Truco et al. 2013). In this work, transcriptome  
173 next-generation-sequencing (RNA-seq) was exploited to monitor packaged lettuce leaves during 7 d of  
174 storage under darkness at 6 °C. A focus was made on five themes, according to the current literature  
175 regarding senescence-related genes: i) photosynthesis and energy-related pathways, ii) stress response  
176 pathways, iii) the phenylpropanoid pathway (antioxidant metabolism), iv) hormonal regulations, v) well-  
177 known markers of senescence induced-phenomena (TFs, cell cycle development, Chl breakdown, lipid  
178 metabolism, phospholipase D pathway, and regulators of protein degradation (*i.e.*, ubiquitination)).

## 179 **Methods**

### 180 1- Plant material and postharvest storage conditions

181 At day 0, butterhead lettuces (*Lactuca sativa* L.) were purchased at a local grower (from a single  
182 seedling) in Avignon, France and were immediately transported to the laboratory. The outer leaves, the  
183 core and the upper part of lettuce were removed. Leaves from the intermediate crown showing uniform  
184 size and color were selected and washed in chlorinated water (80 mg L<sup>-1</sup>, pH =7.4) for 10 min, rinsed with  
185 tap water for 5 min and then rapidly drained on blotting paper. Rib edges were properly cut with a sharp  
186 knife. These minimally processed leaves were kept in their entirety. Four leaves were packaged in OPP film  
187 (Oriented polypropylene film, CFS, France) of 40 µm thickness, and with an oxygen permeability up to 14 L  
188 m<sup>-2</sup> 24 h<sup>-1</sup>. Three bags were used per time point. Packaged lettuce leaves were stored at 6 °C for 7 d in

189 standard climatic chambers (Sanyo MLR-351-H, Panasonic, USA) in darkness. The study focuses at 0, 2 and  
190 7 d.

#### 191 2- Discoloration and physiological response measurements

192 From day 0 to day 7, the global weight loss of lettuce samples was monitored by a simple package  
193 weighing. The first 5 mm slice of each leaf midrib was scanned (600 dpi color Epson scanner) to quantify  
194 the cut-edge discoloration (resulting from mixed bleaching, browning and pinking) using the software FIJI  
195 (Schindelin et al. 2012). Fluorescence of chlorophyll *a* was measured on dark-adapted leaves (30 min) using  
196 a fluorimeter (Handy-PEA, Hansatech, King's Lynn, UK). A focus was made on the maximum photochemical  
197 efficiency of light harvesting of PSII ( $F_v/F_m$ ) and the performance index (PI) of Strasser et al. (2004). O<sub>2</sub>  
198 content inside each lettuce package was measured by a gas analyzer (Checkmate 9900, PBI Dansensor,  
199 France).

#### 200 3- RNA extraction and sequencing

201 From day 0 to day 7, leaves were frozen in liquid nitrogen. Total RNA was extracted using mini  
202 columns (RNeasy Plant Mini Kit, QIAGEN, France) according to the protocol supplied. RNA quality was  
203 checked with a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, USA). Next generation sequencing of  
204 RNA (RNA-seq) was performed at the GeT-PlaGe core facility (INRA, Toulouse). RNA-seq libraries were  
205 prepared according to Illumina's protocols using the TruSeq Stranded mRNA sample prep kit (Illumina, San  
206 Diego, USA) to analyze mRNA. Briefly, mRNA were selected using poly T beads. Then, RNA were  
207 fragmented to generate double stranded cDNA and adaptors were ligated to be sequenced. 10 cycles of  
208 PCR were applied to amplify libraries. Library quality was assessed using a Fragment Analyzer (AATI,  
209 Ankeny, USA), and libraries were quantified by QPCR using the Kapa Library Quantification Kit (Kapa  
210 Biosystems, Wilmington, USA). RNA-seq experiments were performed on an Illumina HiSeq3000 using a  
211 paired-end read length of 2 x 150 bp with Illumina HiSeq3000 sequencing kits.

#### 212 4- RNA-seq data alignment and quantification

213 The RNA-seq library read qualities were assessed using FastQC (Babraham Institute, Cambridge,  
214 UK)]. The remaining sequencing adapters were removed using trim\_galore (Babraham Institute,  
215 Cambridge, UK). The reads were splice-aligned on the genome using STAR (Dobin et al. 2013) (version  
216 2.4.0i using standard parameters) on the lettuce genome version 5 (Reyes-Chin-Wo et al. 2017). The  
217 expression was quantified using featureCounts (Liao et al. 2014) (version V1.4.5-P1 using standard  
218 parameters).

#### 219 5- Statistical analysis

220 One sample (packaged lettuce) consisted of 4 leaves collected from 4 lettuces. The whole  
221 experiment was repeated 3 times (Data reflect 3 biological replicates per time point (day 0, day 2 and  
222 day 7). Differential gene expression analyses were performed in R 3.3.2 (R core Team 2016). The



223 Bioconductor package EdgeR was used for DE analyses with the trimmed mean of m values of TMM  
224 normalization between each pair of samples (Robinson and Oshlack 2010; Robinson et al. 2010; McCarthy  
225 et al. 2012). Gene enrichment analysis was performed with the Gene Ontology package topGO (Alexa and  
226 Rahnenfuhrer 2016) and pathway related analyses were performed with the Kyoto Encyclopedia of Genes  
227 and Genomes (KEGG) pathway (Kanehisa Laboratories, Japan). DE gene annotations were verified with  
228 SwissProt, GO enrichment, and the KEGG pathways according to conserved domains in the sequence.  
229 Numbers of DE genes for day 2 and day 7 in comparison to day 0 were visualized with a Venn diagram  
230 (Hanbo 2016). Heatmaps of DE genes by selected family were plotted according to each day (gplots  
231 package, (Warnes et al. 2016)).

232 Cut discoloration, weight loss and physiological measures were analyzed with a Kruskal-Wallis  
233 multiple comparisons test (pgirmess package, (Giraudoux 2016)).

## 234 **Results and discussion**

### 235 1- Physiological data

236 In packaged lettuce leaves, the first visual markers of senescence are withering due to water losses  
237 and cut-edge discoloration (Martín-Belloso and Soliva-Fortuny 2011; Hunter et al. 2017). Storage under  
238 cold darkness is often used to keep the water losses below 5 %, the limit value for consumer rejection  
239 (Zhou et al. 2004; Kang et al. 2007; Sinha et al. 2010). Here, water losses were close to 5 % at day 2 and  
240 10 % at day 7 (*Table 1*). The cut-edge discoloration surface was  $\approx$  22 to 24 % from day 2 to day 7.

241 Dehydration **during senescence may** generally leads to a decrease in chlorophyll *a* fluorescence  
242 indices  $F_V/F_M$  and performance index (PI) due to the sensitivity of photosystems II (Maxwell and Johnson  
243 2000). **The  $F_V/F_M$  parameter can be used as a marker of severe drought stress, as it starting to decline only**  
244 **at lethal dehydration level and was described as an interesting parameter to monitor leaf senescence**  
245 **(Kotakis et al., 2014; Kalaji et al., 2017). PI, a global index of performance, is composed of three**  
246 **components: the force due to the concentration of active reaction centers, the force of the light reactions**  
247 **related to the quantum yield of primary photochemistry and the force related to the dark reactions (Živčák**  
248 **et al., 2008).** PI has been described as a “drought factor index” during dehydration (Goltsev et al. 2012).  
249 Here, the observed water loss was not severe enough to induce changes in  $F_V/F_M$  and PI values that  
250 remained stable (*Table 1*), **as well as the others OJIP flux parameters (data not shown, see Ripoll et al.**  
251 **(2016) for a user-friendly approach of these parameters).** Respiration is known to increase during  
252 senescence and may limit the shelf life of lettuce (Del Nobile et al. 2006). The monitoring of headspace  $O_2$   
253 showed no variation here, according to the package permeability.

### 254 2- RNA-seq quality

255 67,989 distinct contigs were identified, and among them 19,107 genes were conserved after the  
256 edgeR filtering and normalization procedure (Robinson et al. 2010). The sequenced data aligned well

257 ( $\approx 95\%$ ) to the reference genome (supplementary data 1A). Intersample global expression range displayed  
258 a strong homogeneity (supplementary data 1B). The sample correlation table (supplementary data 1C)  
259 showed that the biological replicates clustered together well. The averaged biological coefficient of  
260 variation was 22.38 % (supplementary data 2A, red line). As expected, highly expressed genes displayed a  
261 lower variation (Marioni et al. 2008).

### 262 3- RNA-seq differential expression analysis

263 A total of 1,048 and 1,846 genes were differentially expressed (DE,  $|\log_2$  fold change (FC)|  $> 1.5$   
264 and false discovery rate (FDR)  $< 0.01$ ) respectively after 2 and 7 d of storage under cold darkness  
265 (supplementary data 2B and C, red spots). At day 2, 511 genes were upregulated and 537 were  
266 downregulated. At day 7, 749 and 1,097 genes were respectively up- and downregulated. Day 2 and day 7  
267 shared 695 modulated genes while 353 genes were specific to day 2 and 1,151 to day 7 (Figure 1). For the  
268 selected genes, 60 % were well-described through the TopGO enrichment and 25 % had a known KEGG  
269 pathway. As a comparison, it has been initially shown on *Arabidopsis thaliana* that the dark-induced and  
270 the developmental senescence respectively raised 385 and 827 differentially expressed genes (Schippers  
271 et al. 2007). However, these data have been supplemented progressively and now raise more than 3,500  
272 SAGs through the leaf senescence database (Li et al. 2017).

### 273 4- Chloroplast and primary metabolism related genes

274 Among the cellular compartments, the chloroplast is the most affected by abiotic stress and  
275 senescence (Pintó-Marijuan and Munné-Bosch 2014). Free Chl breakdown products, especially  
276 pheophorbide *a*, are strong light-absorbing molecules that can produce reactive oxygen species that in  
277 turn induce oxidative damage and accelerate senescence (Pružinská et al. 2005). In addition,  
278 hydroxylations of chlorophyllide *a* in *b* were hypothesized to be catalyzed by cytochrome P450-type  
279 enzymes, also known to take a role in detoxification processes (Hörtensteiner and Lee 2007). During the  
280 storage of lettuce leaves, the RNA-seq data highlighted the modulation of many Chl hemostasis-related  
281 genes: a chlorophyllide *b* reductase, a protochlorophyllide reductase and two chlorophyllases (Table 2).  
282 Other Chl components and key enzymes were globally downregulated: 5 palmitoyl-  
283 monogalactosyldiacylglycerol delta-7 desaturase (except for one that was upregulated during the whole  
284 storage period), a chorismate mutase, a geranylgeranyl diphosphate reductase (which provides phytol for  
285 both chlorophyll and tocopherol biosynthesis) and some palmitoyl-monogalactosyldiacylglycerol delta-7  
286 desaturases (required for chloroplast biogenesis at low temperatures (Hugly and Somerville 1992)). These  
287 fatty acid desaturases are also involved in thylakoid membrane management and in response to wounding  
288 (Heilmann et al. 2004). Concomitantly, no pheophorbide-related genes were significantly modulated  
289 during the storage of lettuce leaves. All of these data suggest the absence or low level of chlorophyll  
290 dismantling in terms of transcriptomic regulation.

291 Our data also highlighted 26 DE cytochrome P450-related genes during storage: 15 were upregulated and  
292 11 were downregulated at day 2 and/or day 7 (supplementary data 3). Further studies are needed to  
293 understand the roles of P450s in the context of postharvest storage and senescence due to their numerous  
294 functions.

295 During natural senescence, photosynthetic performance decreases due to alterations of some  
296 chloroplast structures and functions (Panda and Sarkar 2013). The chronology of these phenomena that  
297 mainly target the thylakoids through the dismantling of PSI and PSII, vary strongly from one species to  
298 another (Kotakis et al. 2014; Wang et al. 2014). During the storage of lettuce leaves, our RNA-seq data  
299 showed that many photosystem subcomponent-related genes were affected. Three chlorophyll *a-b*  
300 binding protein (CAB) genes were downregulated at day 2 and/or day 7 (*Table 2*). Two PsbP related genes  
301 (PSII subunit) were downregulated at day 7, as were a gene coding for the PSI reaction center subunit XI  
302 and a gene related to the cytochrome *b6-f* complex. A gene related to the PSII reaction center W protein  
303 was induced during the 7 d of storage while two genes related to oxygen-evolving enhancer proteins  
304 required for PSII stability (Yi et al. 2006) were affected; one was upregulated at day 2 and one was  
305 downregulated during the whole storage period. Globally, the overall expression of these photosystem-  
306 related genes agree with the progressive loss of photosynthetic activity that takes place during storage in  
307 darkness and the initiation of the senescence. However, the Chl fluorescence measurements indicated no  
308 significant loss of photosynthetic function during the 7 d of storage (*Table 1*).

309 When considering the primary metabolism, *i.e.*, energy-related genes (Calvin cycle, glycolysis,  
310 starch metabolism, fermentation, ATP synthesis, photorespiration), that were significantly affected by the  
311 storage period, 21 markers among 24 were downregulated (*Table 3*). This is highly coherent with the  
312 absence of light during storage. Only one enolase, an alcohol dehydrogenase and a probable adenylate  
313 kinase were upregulated at day 2 and/or day 7. Enolase converts phosphoglycerate to  
314 phosphoenolpyruvate which then contributes to the shikimic acid pathway and phenylpropanoids  
315 biosynthesis (Voll et al. 2009). Alcohol dehydrogenases are enzymes related to fermentation processes.  
316 Here, two alcohol dehydrogenases were downregulated at day 7. Adenylate kinases are responsible for  
317 the transfer of phosphate between ATP and AMP, and here one was downregulated at day 7 (*Table 3*).  
318 Among the downregulated markers, there were two genes related to ATP production, two genes with  
319 related photorespiratory activity and two genes related to RuBisCO activation. Three enzymes involved in  
320 the Calvin cycle were concomitantly downregulated at day 7 (*Table 3*). One of these enzymes was  
321 proposed to be a chloroplastic fructose-1,6-bisphosphatase, which is mainly involved in starch  
322 biosynthesis (Cho and Yoo 2011). Three other starch biosynthesis related genes were downregulated (at  
323 day 2 and/or 7), as was a gene related to glucose homeostasis (day 7). With respect to glycolysis, three  
324 genes related to the fructose and xylulose pathways were downregulated. Two chloroplastic phosphate

325 translocators were negatively affected (*Table 3*). These genes play a role in phosphoenolpyruvate  
326 transport and are also involved in the shikimic acid pathway (Flügge 1999).

327 To summarize, during storage under darkness at cold temperatures, lettuce leaves showed an  
328 expected global shutdown of primary-metabolism (photosynthesis) related genes. In terms of genes  
329 regulation, they also displayed no obvious Chl breakdown (we can note the absence of phaephorbide *a*  
330 oxygenase regulation) and a slight expression decrease in genes coding for photosystem substructures.  
331 This indicated no global transcriptomic regulation of photosystem dismantling as confirmed by the stable  
332 photosynthesis functionality (Chl *a* fluorescence measurements). Finally, we can note that an enolase  
333 (glycolysis pathway) was upregulated and appeared to be linked to the phenylpropanoids pathway, which  
334 is involved in discoloration symptoms.

#### 335 5- Wound and antioxidant response

336 Postharvest mechanical wounding results in the activation of defensive genes similarly to  
337 herbivore attacks, including proteinase inhibitors (Farmer and Ryan 1990) and lipoxygenase (JA and ABA  
338 signaling) (Bell et al. 1995) associated with potential deleterious effects on membrane functions (Baysal  
339 and Demirdöven 2007). Accordingly, our data highlighted here the induction of trypsin inhibitor genes and  
340 a chloroplastic lipoxygenase 2 at day 7 (Figure 2).

341 We can also note the increased expression of a PERK1-like gene (receptor kinase) at day 2 which  
342 has been described as an early-induced wound-response gene (Silva and Goring 2002).

343 Keeping fresh products in the dark at cold temperatures with a high humidity level in packages has  
344 been shown to best maintain their fresh weight (Martín-Belloso and Soliva-Fortuny 2011; Liu et al. 2015).  
345 However, once lettuce leaves are packaged, they undergo dehydration during the overall postharvest  
346 storage as confirmed in *Table 1*. The dehydrins belong to the late embryogenesis abundant (LEA) group  
347 and have a protective role mainly in response to abiotic stress, although their complex roles are not fully  
348 understood (Graether and Boddington 2014). Promoters of dehydrin genes can display ABA-responsive  
349 elements, C-repeat / drought-responsive / low temperature-responsive elements and bZIP TFs interactions  
350 (Hanin et al. 2011). Our data showed an increased expression of many dehydration-responsive genes  
351 mainly at day 7 (Dehydrin family, Dehydrin LEA, Lea5, and Dehydration-responsive element-binding  
352 protein 3) (Figure 2). Interestingly, some of these markers have also been shown to be involved in  
353 senescence (Weaver et al. 1998) and the abiotic stress response (Nakashima et al. 2014; Franco-Zorrilla et  
354 al. 2014). Four bZIP TFs were differentially expressed in lettuce leaves during storage (3 up- and 1  
355 downregulated, supplementary data 3); bZIP TFs have been shown to take important roles during abiotic  
356 stress responses, notably during drought (Llorca et al. 2014)

357 During genetically programmed senescence, the decrease in antioxidant capacity leads to an  
358 oxidative stress that accelerates the overall phenomenon (Zentgraf 2007). However, postharvest

359 procedures, and, to a larger extent, plant wounding, have been linked to an increased respiration rate, the  
360 early production of reactive oxygen species (which can participate in a defensive strategy and signaling  
361 through the plant tissues, including the cut-edge discoloration phenomenon observed in packaged  
362 lettuces), and the consequent increased antioxidant activity (Toivonen 2004; Sinha et al. 2010; Savatin et  
363 al. 2014). In the present work, 30 genes associated with redox regulation were affected by the postharvest  
364 storage, among which 20 showed a high expression at day 0 and a progressive downregulation during  
365 storage (Figure 2).

366 Proline has a fundamental role in the stress response by promoting cellular apoptosis or survival  
367 through its interactions with redox proteins and glutathione (Liang et al. 2013). During storage here, two  
368 genes involved in proline biosynthesis were induced, while two proline dehydrogenases were  
369 downregulated. Two genes related to glutathione metabolism were respectively down- and upregulated  
370 at day 7. In parallel, 24 DE genes were assigned to an oxidoreductase activity: two catalases showed  
371 opposite profiles, two superoxide dismutases were downregulated during storage, two quinone-  
372 oxidoreductases were downregulated, five thioredoxins were affected (2 downregulated and 3  
373 upregulated) and 10 genes coding for a peroxidase activity were modulated (3 upregulated and 7  
374 downregulated mainly at day 7, Figure 2), among which were two L-ascorbate peroxidases and two lignin-  
375 forming anionic peroxidase. The downregulation of catalase and superoxide dismutase related genes  
376 during storage appeared coherent with their decreased activities observed in tobacco during senescence  
377 and the consequent increase in chloroplast lipid peroxidation (Dhindsa et al. 1981). However, one catalase  
378 gene appeared to be only upregulated from the second day of storage. Among the five thioredoxin related  
379 genes, the two downregulated genes showed an F-type activity which has been shown to activate  
380 numerous enzymes of the photosynthetic carbon cycle (Serrato et al. 2008). Concerning the three  
381 upregulated genes, they take roles in the redox homeostasis through their disulfide reductase and  
382 isomerase activities (Serrato et al. 2008).

383 Concerning stress-related genes, most of them were upregulated late during storage (Figure 2)  
384 such as two universal stress A-like protein, an S-(+)-linalool synthase (upregulated at day 7), a zeaxanthin  
385 epoxidase (decreased at day 2) and a soluble inorganic chloroplastic pyrophosphatase (decreased at  
386 day 7). Universal stress protein A has a potential role in redox state regulation through its chaperone  
387 function (Jung et al. 2015). Zeaxanthin epoxidase is known to play an important role in the xanthophyll  
388 cycle and abscisic acid biosynthesis. It also participates in nonphotochemical quenching processes and  
389 redox state homeostasis (Zhao et al. 2016). In addition, during storage, two heat shock proteins were  
390 positively regulated at day 7, while another one was negatively regulated at day 2. Three heat stress-  
391 related TFs were also upregulated at day 7. Mutants lacking such TFs have been described to show  
392 increased senescence symptoms (Breeze et al. 2008).

393 To conclude, in terms of gene expression regulation, the senescence process observed during  
394 storage of lettuce leaves appeared to be widely characterized by a chronological sequence that starts with  
395 an early antioxidant response (day 0) and ends with both dehydration and stress responses (day 7).

#### 396 6- Cut-edge discoloration and the phenylpropanoids pathway

397 Enzymatic discoloration is commonly reported as a consequence of both the oxidation of phenolic  
398 compounds by PPO and an increase in PAL activity. Peroxidases activity on lignins in presence of H<sub>2</sub>O<sub>2</sub> has  
399 also been associated to the production of brown pigments (Landi et al. 2013; Hunter et al. 2017). Recent  
400 studies on lettuce also suggested that some specific sesquiterpenes syntheses may contribute to tissue  
401 discoloration at ambient temperature (Mai and Glomb 2013; Mai and Glomb 2014). The present data  
402 identified 4 PAL- and 6 PPO-related genes in lettuce leaves (supplementary data 3). No PAL gene was  
403 significantly affected by storage (Figure 3), and 3 PPO genes were downregulated at day 2 while one was  
404 upregulated. Measurements of PPO activities are required to understand their role in the cut-edge  
405 discoloration, but we can hypothesize that a poststress pool of enzymes (expressed between day 0 and  
406 day 2) may explain the observed phenomenon as described earlier (Landi et al. 2013). In addition, the  
407 recent literature highlighted no systematic correlation between discoloration and PPO levels (Hunter et  
408 al. 2017).

409 Globally, the majority of genes involved in the phenylpropanoids pathway showed a high  
410 expression immediately after cutting and were downregulated during storage (Figure 3) except for a PPO,  
411 a 4-coumarate-CoA-ligase-like and a flavonol synthase / flavanone-3-hydroxylase. This enzyme is very  
412 sensitive to abiotic stress and is known to be activated during dehydration (Ma et al. 2014). Other DE genes  
413 downregulated during storage were two 4-coumarate-CoA-ligases, a chalcone synthase, 2 chalcone  
414 isomerases and a trans-cinnamate 4-monooxygenase (CYP73A). Finally, a probable cinnamyl alcohol  
415 dehydrogenase involved in the latest steps of lignin biosynthesis was only upregulated at day 0, suggesting  
416 an adjustment of the cinnamyl alcohols pathway at the onset of postharvest senescence.

#### 417 7- Phytohormones

418 Auxins, gibberellins and cytokinins may delay the process of senescence, whereas ethylene, SA,  
419 JA, brassinosteroids and ABA promote it (Sarwat et al. 2013). Our experiment on storage of cut lettuce  
420 leaves raised more than 80 DE hormone-related genes (Figure 4). The data highlighted early-responding  
421 (day 0) and late-responding (day 7) genes. Day 2 appeared as an intermediate physiological state. When  
422 focusing on ethylene- and auxin-related genes, 55 to 60 % of them were upregulated at day 0, while 26 %  
423 were upregulated at day 7. These data suggest a strong mobilization of auxin- and ethylene-responding  
424 pathways at the onset of the storage (*i.e.*, immediately after the cut stress). However, ABA-related genes  
425 showed a different profile and were only upregulated from day 2 to day 7. Finally, cytokinins-related  
426 markers did not display such an asymmetrical pattern between early- and late-responsive genes. Eleven

427 genes associated with the NAC TF family, known to play a role in the hormonal response to abiotic stress  
428 and senescence (Nakashima et al. 2012; Takasaki et al. 2015), were upregulated during postharvest  
429 storage (supplementary data 3).

430 The auxin signaling pathway involves a large group of responsive genes such as auxin-responsive  
431 and induced proteins, auxin response factors (ARF), small auxin upregulated RNA (SAUR), auxin-responsive  
432 Gretchen Hagen3 (GH3) and YUCCA genes (Mueller-Roeber and Balazadeh 2014). In dark-induced and  
433 developmental senescence, IAA levels were found to decrease while RNAs of the GH3 genes family were  
434 increased; GH3 genes aim to conjugate IAA into amino acids, contributing to auxin homeostasis (Mueller-  
435 Roeber and Balazadeh 2014). The main hypothesis on the auxin delaying effect on senescence was linked  
436 to an increase in free auxin levels (Mueller-Roeber and Balazadeh 2014). Although it is not simple to  
437 interpret, some aspects of our data are consistent with this statement (Figure 4). The majority of IAA  
438 related genes were highly expressed at day 0, and a GH3 gene was progressively upregulated during the  
439 storage progression. Expressions of Auxin transport and efflux carrier genes were also modulated,  
440 suggesting an adjustment of the auxin-signaling pathway.

441 Cytokinin phytohormones have an important role in both cell division and proliferation at early  
442 plant developmental stages, and they also maintain existing cells at late stages by limiting senescence  
443 (Dani et al. 2016). They have been shown to maintain chloroplast integrity via an upregulation of the  
444 antioxidant response during dark-induced senescence (Zavaleta-Mancera et al. 2007). They also contribute  
445 to maintaining the chlorophyll cycle and the LHC (Talla et al. 2016). A few actors of the senescence-  
446 delaying role of cytokinins have been elucidated such as a receptor (AHK3), a response regulator (ARR2)  
447 and a response factor (CRF6) (Zwack and Rashotte 2013). However, enzymes such as cytokinin-O-  
448 glucosyltransferase and phosphoribosyltransferase have been shown to downregulate the cytokinin signal  
449 (Allen et al. 2002; Sakakibara 2006). Our data showed that 17 genes linked to these enzymes were  
450 affected (8 up- and 9 down-regulated), suggesting a complex modulation of cytokinins homeostasis and  
451 the subsequent senescence process (Figure 4).

452 Ethylene has an important role in the senescence of leaves and the ripening of fruits. The  
453 biosynthesis of ethylene, although complex, is catalyzed by a 1-aminocyclopropane-1-carboxylic acid  
454 synthase, ACC (Xu and Zhang 2014). ACC downregulation has been shown to delay the senescence process,  
455 suggesting a role for ethylene in promoting senescence (Khan et al. 2014). Our results showed that the  
456 lettuce storage did not directly affect ethylene biosynthesis but rather impacted 3 receptors/sensors and  
457 28 ethylene responsive TFs mainly at the onset of the storage period (Figure 4). A deeper analysis of the  
458 TFs functions is necessary to draw a precise conclusion of the role of ethylene in this context.

459 Abscisic acid is known to induce senescence (Khan et al. 2014). Lettuce storage led to the  
460 upregulation of 4 members of the ABA signaling pathway, mainly from the middle to the end of the storage

461 period (Figure 4), including one hydroxylase and 3 ABA-insensitive 5-like proteins. These genes are  
462 involved in the response to water deprivation conditions (Kang et al. 2002; Kushiro et al. 2004), suggesting  
463 a higher effect of dehydration than senescence on ABA signaling in the present work. Our data also  
464 highlighted a probable glutamate carboxypeptidase (upregulated at day 7), which is involved in ABA  
465 homeostasis and the stress response (Shi et al. 2013) and could play a role in the implementation of  
466 senescence.

467 Salicylic acid level is known to increase in senescing leaves (Khan et al. 2014), but SA appeared not  
468 to be involved in dark- or starvation-induced senescence (Buchanan-Wollaston et al. 2005). The absence  
469 of SA-related genes from our DE analyses appeared consistent.

470 Jasmonate compounds are involved in signaling during plant responses to abiotic and biotic  
471 stresses and play a positive role in leaf senescence (Wasternack 2014). Only one gene related to the  
472 complex JA biosynthesis, the allene oxide synthase enzyme, was upregulated, mainly at the end of the  
473 storage period (Figure 4).

474 Brassinosteroid hormones were suggested as regulators of senescence mechanisms via the  
475 alteration of the cell lipid composition (Fedina et al. 2017), but the literature remains scarce. Only one  
476 related gene was upregulated at day 0, a serine carboxypeptidase 24 (Figure 4).

477 To conclude, although cytokinin and ethylene treatments generally promote senescence (Gan and  
478 Amasino 1997), here the regulations of these pathways were clearly affected by the crosstalk between  
479 wound, dehydration and dark-induced senescence. The small number of SA, JA and brassinosteroid  
480 response genes found in our results, and the complex regulations of auxin, ethylene and cytokinin  
481 pathways may suggest a delay in the involvement of senescence mechanisms.

## 482 8- Markers of senescence

483 Regulation of senescence involves a large and complex panel of molecular actors among which are  
484 many TFs related to SAG regulation such as the WRKY family. Here, we identified 12 DE WRKY genes: 5  
485 were upregulated and 7 were downregulated (supplementary data 3). Further studies are needed to  
486 determine their specific roles in the context of lettuce leaf storage.

### 487 Cell cycle shutdown

488 The cell cycle shutdown has been widely described during senescence (Sablowski and Carnier  
489 Dornelas 2014). Here, 8 genes related to cell division processes, mainly highly expressed at the onset of  
490 storage, were DE (7 downregulated at day 7, Table 4). They include 4 cyclin-D and 3 cyclin-dependent  
491 kinase (CDK). One of the CDK genes was the only upregulated marker during storage. These actors are  
492 fundamental regulators of cell cycle homeostasis and are linked to cytokinins (Lee et al. 2006; Sablowski  
493 and Carnier Dornelas 2014). Further, a phospholipid hydroperoxide glutathione peroxidase was



494 downregulated at day 7. This has been described as showing a cell-protecting role against heat stress  
495 induced cell death (Chen et al. 2004).

#### 496 Cell wall dismantling

497         The plant cell wall, composed of a cellulose/xyloglucan network, continually changes in  
498 composition and structure throughout plant development and can be modified in stress conditions. Our  
499 data highlighted a global shutdown of cell-wall-associated markers that were mainly upregulated at the  
500 onset of the experiment (day 0). Among 25 genes, only 4 were upregulated during storage (Table 5). This  
501 trend indicates a possible shutdown of cell wall maintenance and even cell wall loosening. Lettuce mutants  
502 showing higher xyloglucan endotransglucosylase / hydrolase activity can have a higher shelf life (Wagstaff  
503 et al. 2010). Our data highlighted 11 of these markers in lettuce leaves during storage (all were  
504 downregulated during storage, except one). A probable glycerophosphoryl diester phosphodiesterase 2  
505 was downregulated at day 7. This type of protein was suggested to be involved in cell wall organization  
506 (Hayashi et al. 2008). In addition, one repetitive proline-rich cell wall protein was downregulated at day 7,  
507 and a wall-associated receptor kinase 2 was upregulated at day 2 only. Repetitive proline-rich cell wall  
508 proteins constitute a large family of cell wall proteins inducible by environmental stimuli (Marcus et al.  
509 1991) with specific organ expression patterns, such as the wall-associated receptor kinase (He et al. 1999).  
510 Six genes related to cell wall extension (Expansin-A family) were downregulated at day 7 except for one.  
511 These proteins mediate cell wall loosening and contributes to the strength of the cell (Marowa et al. 2016).  
512 Four genes involved in demethylesterification of cell wall proteins (probable pectinesterases) were  
513 downregulated at day 7 and one was upregulated at day 2.

#### 514 Lipid homeostasis

515         During senescence, alteration of lipids occurs in all compartments. In chloroplasts it is mainly due  
516 to an increase in ROS. Figure 5 details the 65 lipid-related genes that were affected during the storage of  
517 lettuce leaves. A very clear pattern emerges: ≈82 % of the markers highly expressed at the start of the  
518 experiment were downregulated during storage. Day 2 appeared as an intermediate state but showed 7  
519 markers specifically upregulated, while only 4 genes were upregulated at day 7. This indicates both an  
520 overall shutdown of lipid biosynthesis and no clear senescence initiation. Fatty acids are known to  
521 decrease with the age of the leaf while glycerolipid synthesis is expected to increase (Troncoso-Ponce et  
522 al. 2013). Regarding chloroplastic lipids, two genes related to probable plastid-lipid-associated proteins  
523 were downregulated at day 7, whereas one was upregulated. Genes involved in fatty acid biosynthesis  
524 were progressively decreased from 6 at day 2 to 13 genes at day 7. Only two were upregulated at day 7.  
525 Phospholipid biosynthesis was upregulated for 3 genes at day 2, while 2 others were downregulated.  
526 Degradation of lipids during senescence mainly serves to provide substrates to sink tissues generating an  
527 important traffic and changes in lipid metabolism (Troncoso-Ponce et al. 2013). In senescing membranes,

528 alterations in lipid composition are enzymatically mediated. The transcriptome of Arabidopsis leaves  
529 revealed senescence responses such as lipases, acyl hydrolases, phospholipases (especially the  
530 phospholipase D pathway), lipoxygenases and  $\beta$ -oxidation enzymes (Guo et al. 2004). Here, 5 genes that  
531 show a lipolytic activity were upregulated while 26 were downregulated. One lipase was upregulated at  
532 day 7. The GDSL esterase/lipase family is a relatively new subclass of lipolytic enzymes that can hydrolyze  
533 acyl-CoA, esters or amino acid derivatives substrates (Chepyshko et al. 2012). Sixteen GDSL esterase/lipase  
534 were downregulated at day 7, and three were upregulated at day 2. GDSL esterase/lipase are subjected  
535 to regulations in response to stressful conditions (Chepyshko et al. 2012). The phospholipase D family is  
536 known to display increased expression in senescent Arabidopsis leaves (Fan et al. 1997), and its silencing  
537 led to blocked lipid degradation and delayed ABA-promoted senescence (Jia et al. 2013). Here, 4 genes  
538 with phospholipase D activity were downregulated, one at day 2 and three at day 7. Other genes with a  
539 phospholipase activity were also downregulated such as the phospholipase A1 Igama 3 gene sharing  
540 similarities with an Arabidopsis enzyme (At2g30550) known to handle the de-esterification of fatty acids  
541 from complex lipids during senescence (Guo et al. 2004). Concerning  $\beta$ -oxidation enzymes, only a long-  
542 chain-alcohol oxidase was upregulated at day 2. A long-chain-fatty-acid--CoA ligase 1 was downregulated  
543 at day 7, as well as a peroxisomal 3-ketoacyl-CoA thiolase 2 at day 2. Patatin-like proteins are also lipases  
544 involved in galactolipid turnover and are known to be upregulated during leaf senescence (Troncoso-Ponce  
545 et al. 2013). Four genes in the patatin-like family were downregulated at day 7, except for one. Lipids,  
546 phospholipids and sterol transport were affected as illustrated by the two nonspecific lipid-transfer  
547 protein-like protein At5g64080 and 6 nonspecific lipid-transfer proteins downregulated at day 2 and three  
548 at day 7. One oxysterol-binding protein-related protein 2A involved in sterols transport was  
549 downregulated during storage. Annexin proteins interact with membrane lipids in response to stress and  
550 are involved in interconnections between  $\text{Ca}^{2+}$ , ROS, actin and lipid signaling (Laohavisit and Davies 2011).  
551 The annexin multigene family is known to be regulated by abiotic stress, such as dehydration or wounding  
552 (Cantero et al. 2006). Here, two annexins were downregulated.

### 553 Protein catabolism

554 During natural senescence, protein breakdown is one of the most important contributing  
555 processes to nitrogen remobilization (Woo et al. 2013; Poret et al. 2016). Eight genes showing an aspartic  
556 proteinase activity were downregulated at day 7, comprising 6 aspartic proteinase nepenthesin genes  
557 (Table 6) and two basic 7S globulin. Aspartic proteases take part in environmental stress responses such  
558 as wounding or dehydration (Chen et al. 2015). The ubiquitination process corresponds to the tagging of  
559 proteins by ubiquitins (monomers or polyubiquitins) mainly to induce the degradation of the targeted  
560 protein but also to modify its activity or cellular location. It can also take a role in cell signaling (Li and Ye  
561 2008). For that purpose, E1 (activation), E2 (conjugation) and E3 (ligation) enzyme types are required

562 (Hershko and Ciechanover 1998). E3 ubiquitin-protein ligase and the U-box domain confer the specificity  
563 of substrate for ubiquitination processes. E3 are involved in the transfer of ubiquitin to E2 ligases, while  
564 U-box proteins participate in stress signaling (Lee and Kim 2011). Nineteen genes catalyzing the  
565 ubiquitination process were involved: 15 E3 ubiquitin-protein ligases (Table 6), 3 U-box domain-containing  
566 proteins upregulated at day 7, and 1 anaphase-promoting complex APC10 downregulated at day 2 and 1  
567 ubiquitin carboxyl-terminal hydrolase downregulated at day 7. Among the E3 ubiquitin-protein ligases,  
568 seven of them can be associated with negative regulation of water stress response (Cho et al. 2008), and  
569 they were downregulated at day 7 except for one. The anaphase-promoting complex APC10 is a subunit  
570 of the APC/C complex (an E3 ubiquitination complex), and this subunit is implied in leaf growth regulation  
571 in Arabidopsis (Eloy et al. 2011). Ubiquitin carboxyl-terminal hydrolase are deubiquitinating enzymes of  
572 the autophagic pathway. They ensure a pool of reusable ubiquitin (Ingvarsdén and Veierskov 2001). The  
573 SCF protein complex participates in the E3 ubiquitination process and is required in senescing leaves (Woo  
574 et al. 2001; Hopkins et al. 2007). This complex is composed of F-box domains, each one of which has a  
575 specific role in the stress response or senescence (Stefanowicz et al. 2015). Many components of the SCF  
576 complex were affected by the storage period: one SKP1-like protein was induced at day 7, a cullin-1 was  
577 downregulated at day 7 and 10 F-box genes were affected (6 upregulated and 4 downregulated, Table 6).  
578 The affected F-box in this experiment were not all characterized, and further studies are needed. Only one  
579 was downregulated and has been involved in the regulation of the ethylene signaling pathway (Qiu et al.  
580 2015). A zinc finger A20 and AN1 domain-containing stress-associated protein was upregulated at day 7.  
581 It is involved in the environmental stress response (Vij and Tyagi 2006) and is required for RING finger-  
582 containing protein stability.

583 Globally, the downregulation of genes involved in the cell cycle suggests a cell cycle arrest in stored  
584 lettuce leaves. The metabolisms around the cell wall were also downregulated, as well as lipid  
585 biosynthesis, degradation and transport. The absence of phospholipase D activity or oxidation of fatty  
586 acids contradicts the expected effects of senescence or wounding (Vu et al. 2014) and suggests a delay in  
587 the onset of senescence. Concomitantly, the postharvest storage induced regulations of ubiquitination  
588 processes mainly related to dehydration while links to senescence cannot be made without  
589 complementary studies on the various highlighted F-box containing genes.

## 590 **Conclusion**

591 As expected, dark and cold storage did not limit discoloration symptoms and reduced activities of  
592 glycolysis and the Calvin cycle. However, transcriptome data highlighted a complex pattern of PPO-related  
593 gene expression that challenges the usual link made between PPO and discoloration. The postharvest  
594 senescence appeared here as a mixture between an early stress response to wound followed by a late  
595 dehydration and dark-induced senescence. The early antioxidant activity observed may delay the

596 senescence implementation. In chloroplasts, the diminution of CAB biosynthesis at day 7 suggested that  
597 senescence may begin only 7 d after harvest. Despite the downregulation of phospholipase D biosynthesis  
598 here, lipid metabolism can be proposed as a good target or indicator of postharvest senescence since an  
599 overall decrease in biosynthesis, degradation and transport were suggested. The cold and dark postharvest  
600 storage also created favorable conditions to maintain chlorophylls. It induced modifications of hormonal  
601 signalization mainly in response to stresses rather than senescence and appeared to down-regulate cell  
602 cycle maintenance. Many questions remain, due to the numerous TFs and unannotated genes highlighted.  
603 The present work has focused mainly on transcriptomic modulations. Although regulation models appear,  
604 translome, proteome and epigenetic studies are needed to confirm and specify the phenomena  
605 described.

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630 **Abbreviations**

- 631 ABA: abscissic acid
- 632 ARF: auxin response factor
- 633 bZIP: the basic region/leucine zipper (transcription factor)
- 634 CAB: chlorophyll *a/b* binding protein
- 635 CDK: cyclin-dependent kinase
- 636 Chl: chlorophyll
- 637 DE: differentially expressed
- 638 FC: fold change
- 639 FDR: false discovery rate
- 640  $F_V/F_M$ : maximum photochemical efficiency of light harvesting of PSII
- 641 GH3: auxin-responsive *Gretchen Hagen 3*
- 642 IAA: indole acetic acid
- 643 JA: jasmonic acid
- 644 KEGG: Kyoto Encyclopedia of Genes and Genomes
- 645 LHC: light harvesting complex
- 646 NAC: NAM/ATAF/CUC (transcription factor)
- 647 PAL: phenylalanine ammonia-lyase
- 648 PI: performance index
- 649 PPO: polyphenol oxidase
- 650 PS: photosystems
- 651 RCs: reaction centers
- 652 ROS: reactive oxygen species
- 653 RuBisCO: ribulose biphosphate carboxylase
- 654 SA: salicylic acid
- 655 SAG: senescence associated-genes
- 656 SAUR: small auxin upregulated RNA
- 657 SCF: Skp1/Cul1/F-box (complex)
- 658 SOD: superoxide dismutase
- 659 TF: transcription factor

660 **Declaration**

661 **Availability of data and material**

662           The datasets used and/or analyzed during the current study are available from the corresponding  
663 author on reasonable request.

664 **Competing interests**

665           The authors declare that they have no competing interests.

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668 **Authors' contributions**

669           The original idea of this project was developed by FC, VV, HS, FL and DR. DR, SL and VV performed  
670 the experiments. CK performed RNA-seq quality analyses. JR compiled data and performed data analyses.  
671 JR and DR were the major contributors in writing the manuscript. All authors read and approved the final  
672 manuscript.

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1108 **Figures and tables legends**

1109 **Tables**

1110 *Table 1: Lettuce leaves physiological parameters. Letters indicate significant differences at  $p < 0.05$*   
1111 *(Kruskal-Wallis test); s.e.: standard error; a.u.: arbitrary unit.*

1112  
1113 *Table 2: Chloroplastic genes differentially expressed in lettuce leaves after 2 and 7 d of storage in darkness*  
1114 *at 6 °C. DE genes were filtered with a cut-off  $|\log_2\text{-FC}| > 1.5$  and  $\text{FDR} < 0.01$ . NDE: non-differentially*  
1115 *expressed.*

1116  
1117 *Table 3: Energy-related genes (Calvin cycle, glycolysis, starch metabolism, fermentation, ATP synthesis,*  
1118 *photorespiration) differentially expressed in lettuce leaves after 2 and 7 d of storage in darkness at 6 °C.*  
1119 *DE genes were filtered with a cut-off  $|\log_2\text{-FC}| > 1.5$  and  $\text{FDR} < 0.01$ . NDE: non-differentially expressed.*

1120 *Table 4: Cell-cycle-regulation related genes differentially expressed in lettuce leaves after 2 and 7 d of*  
1121 *storage in darkness at 6 °C. DE genes were filtered with a cut-off  $|\log_2\text{-FC}| > 1.5$  and  $\text{FDR} < 0.01$ . NDE: non-*  
1122 *differentially expressed.*

1123  
1124 *Table 5: Cell-wall related genes differentially expressed in lettuce leaves after 2 and 7 d of storage in*  
1125 *darkness at 6 °C. DE genes were filtered with a cut-off  $|\log_2\text{-FC}| > 1.5$  and  $\text{FDR} < 0.01$ . NDE: non-*  
1126 *differentially expressed.*

1127  
1128 *Table 6: Protein catabolism related genes differentially expressed in lettuce leaves after 2 and 7 d of storage*  
1129 *in darkness at 6 °C. DE genes were filtered with a cut-off  $|\log_2\text{-FC}| > 1.5$  and  $\text{FDR} < 0.01$ . NDE: non-*  
1130 *differentially expressed.*

1131  
1132 **Figures**

1133 *Figure 1: Number of differentially expressed genes in lettuce leaves after 2 and 7 d of storage in darkness*  
1134 *at 6 °C ( $|\log_2$  fold change (FC)|  $> 1.5$  and  $\text{FDR} < 0.01$ ).*

1135  
1136 *Figure 2: Relative expression of stress and anti-oxidant response related genes DE in lettuce leaves stored*  
1137 *in darkness at 6 °C during 7 d. Coloration is based on mean CPM after normalization (TMM method) and*

1138 screening ( $|\log_2\text{-FC}| > 1.5$  and  $\text{FDR} < 0.01$ ). Red and green colors indicate respectively down- and up-  
1139 regulated genes.

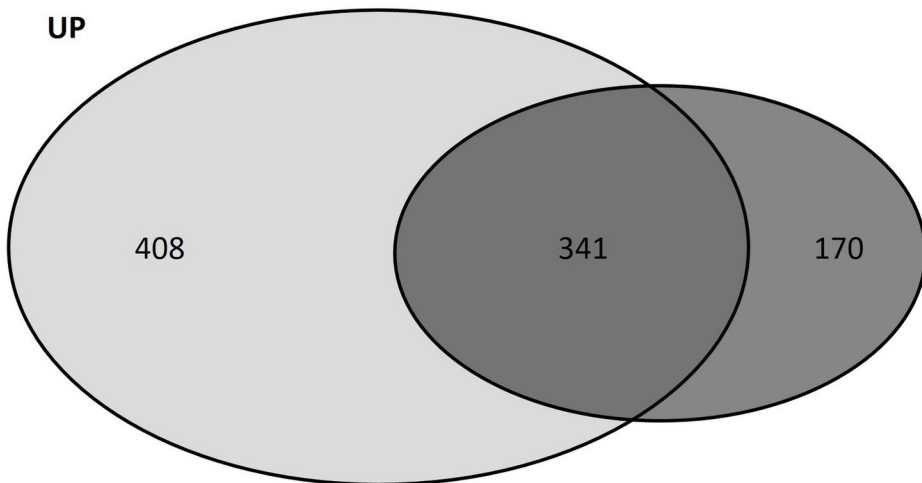
1140  
1141 *Figure 3: Phenylpropanoid pathway related genes DE in lettuce leaves stored in darkness at 6 °C during 7 d.*  
1142 *Coloration is based on mean CPM after normalization (TMM method) and screening ( $|\log_2\text{-FC}| > 1.5$  and*  
1143 *FDR < 0.01) and indicates relative expression of selected markers over time. Enzymes catalyzing reactions*  
1144 *are expressed in italics. PAL: phenylalanine ammonia-lyase, CYP73A: trans-cinnamate 4-monooxygenase,*  
1145 *4CL: 4-coumarate—CoA ligase, HCT: shikimate O-hydroxycinnamoyltransferase, C3'H:*  
1146 *coumaroylquininate(coumaroylshikimate) 3'-monooxygenase, PPO: polyphenol oxidase, CSE:*  
1147 *caffeoylshikimate esterase, CHS: Chalcone synthase, CHI: Chalcone isomerase, F3H: flavanone,2-*  
1148 *oxoglutarate:oxygen oxidoreductase (3-hydroxylating), FLS: flavonol synthase, PCAH: Probable cinnamyl*  
1149 *alcohol dehydrogenase.*

1150  
1151 *Figure 4: Relative expression of phytohormone related genes DE in lettuce leaves stored in darkness at 6 °C*  
1152 *during 7 d. Coloration is based on mean CPM after normalization (TMM method) and screening ( $|\log_2\text{-FC}|$*   
1153 *> 1.5 and FDR < 0.01). Red and green colors indicate respectively down- and up-regulated genes.*

1154  
1155 *Figure 5: Relative expression of lipid metabolism related genes DE in lettuce leaves stored in darkness at*  
1156 *6 °C during 7 d. Coloration is based on mean CPM after normalization (TMM method) and screening ( $|\log_2\text{-}$*   
1157 *FC| > 1.5 and FDR < 0.01). Red and green colors indicate respectively down- and up-regulated genes.*

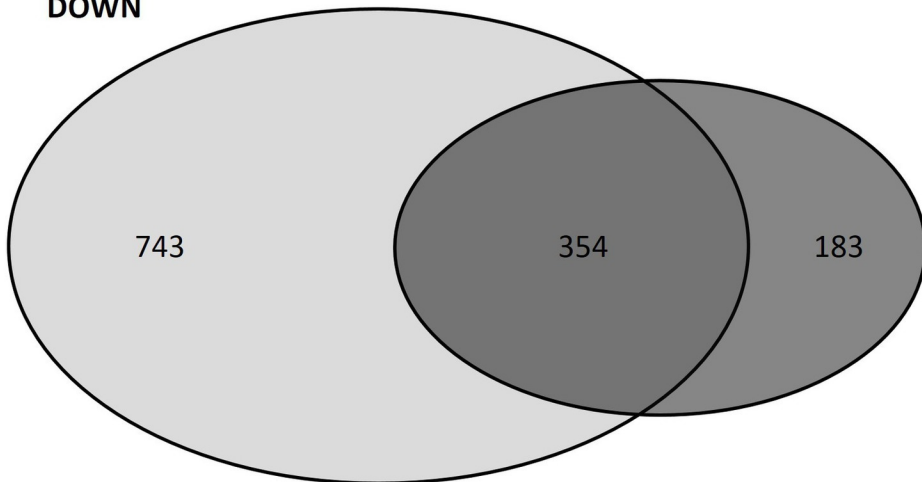
1158  
1159 **(Supplementary data (1, 2 and 3) joined aside.)**

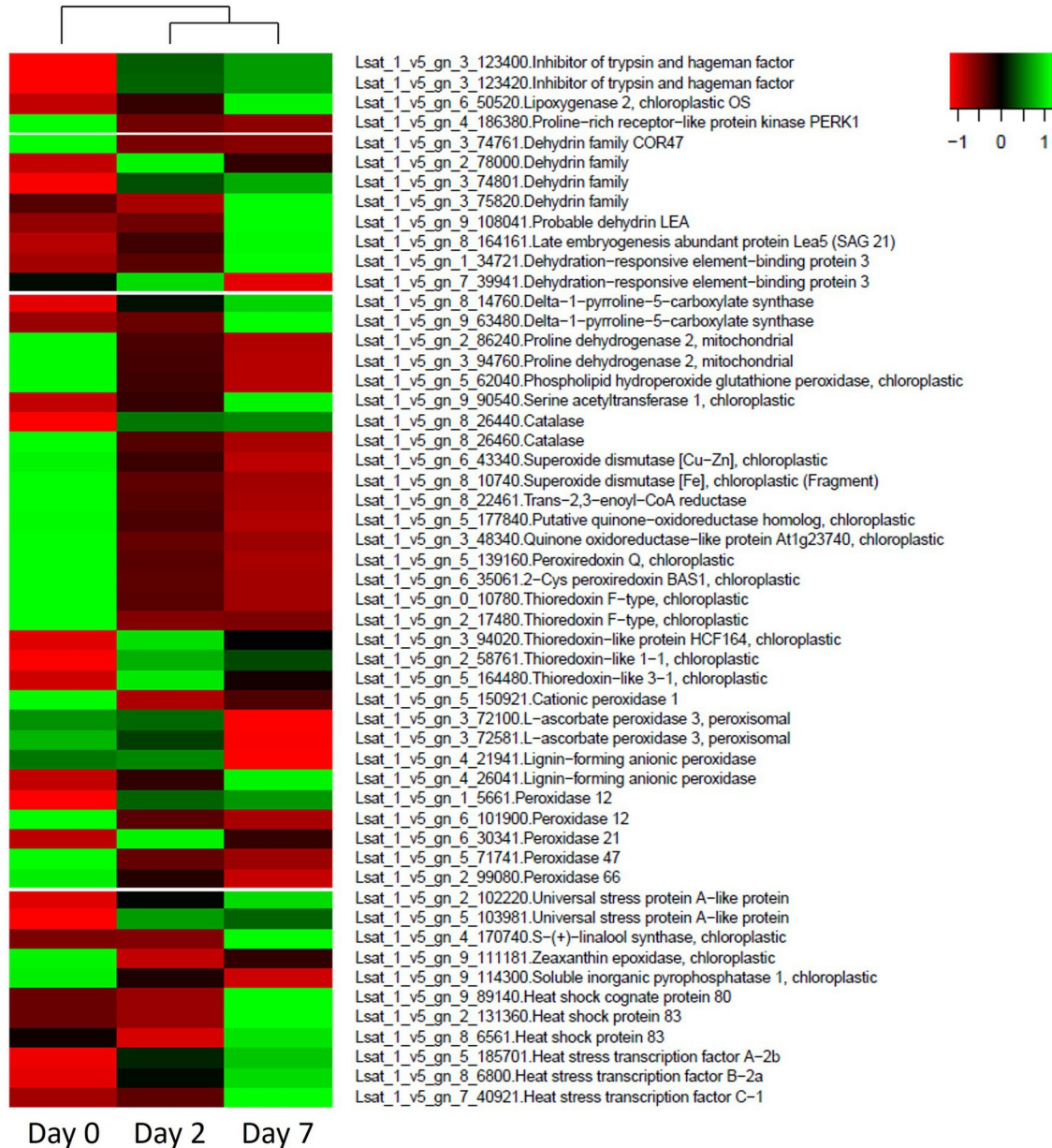
**UP**



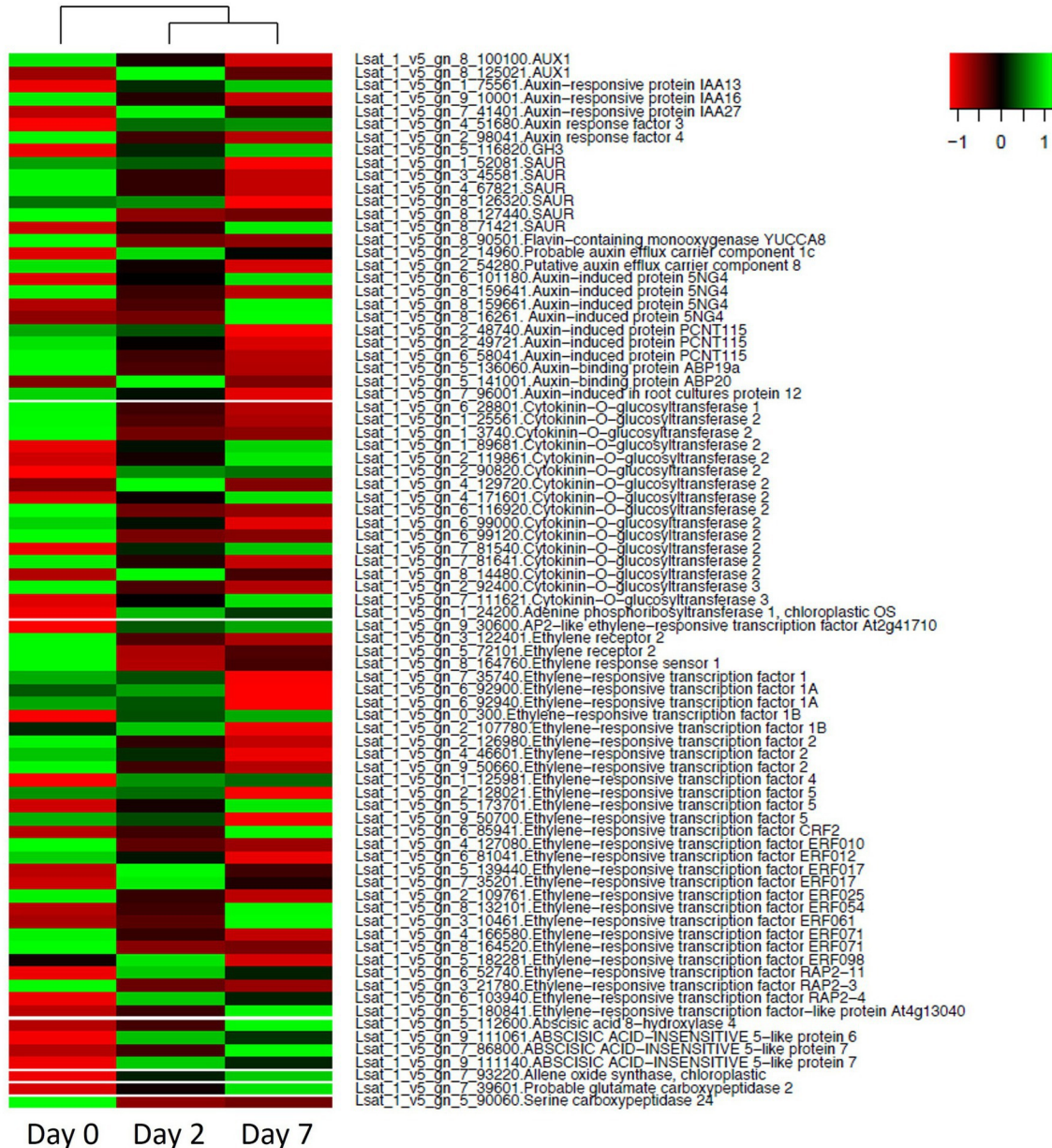
■ Day 2  
■ Day 7

**DOWN**

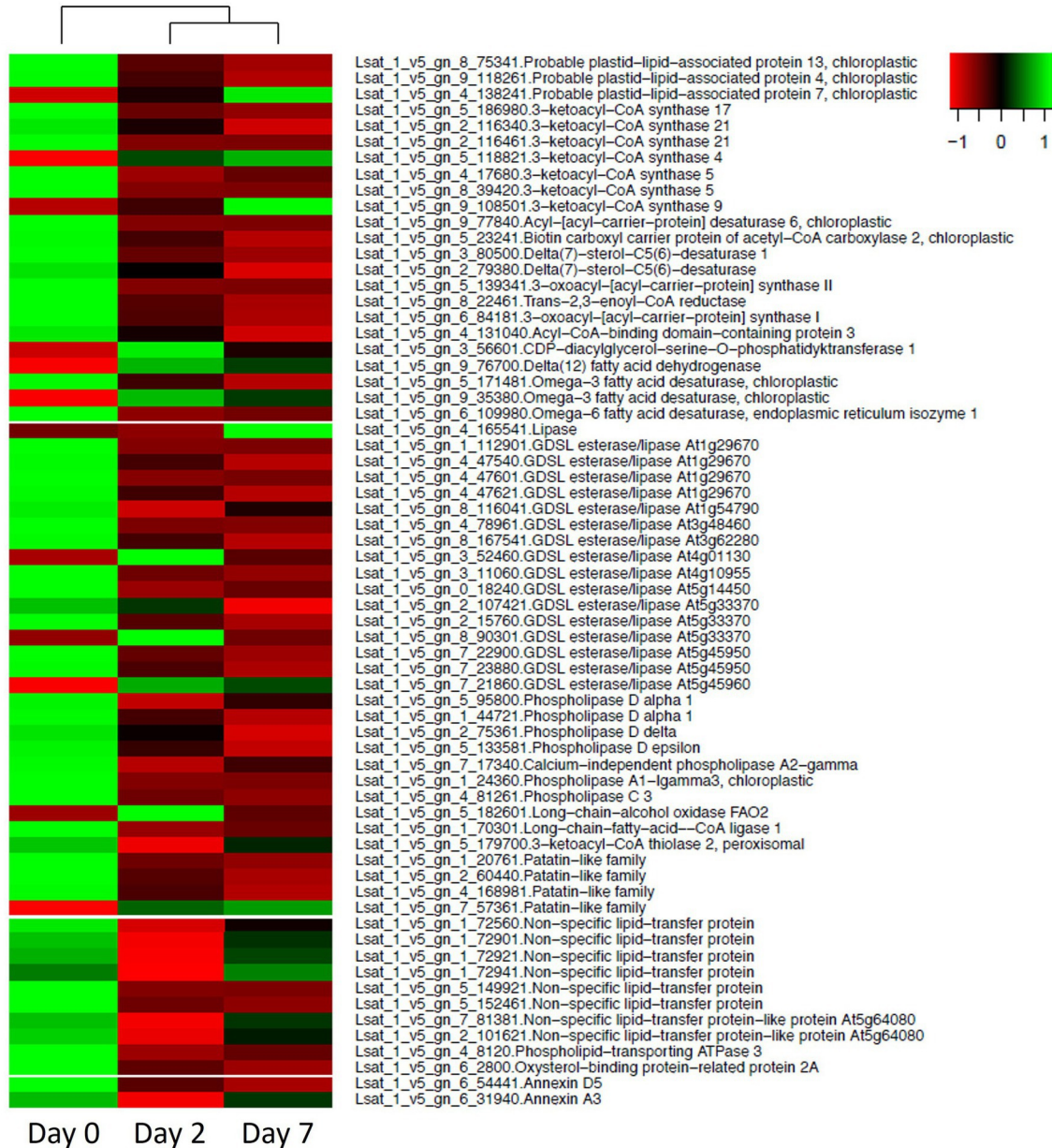












Day 0 Day 2 Day 7

	DAY 0		DAY 2		DAY 7	
	mean	s.e.	mean	s.e.	mean	s.e.
<b>Cut-edge discoloration (%)</b>	0.00 a	0.00	22.40 b	2.50	23.70 b	6.90
<b>Weight loss (%)</b>	0.00 a	0.00	5.42 ab	1.64	10.43 b	1.72
<b>F<sub>v</sub>/F<sub>M</sub> (a.u.)</b>	0.85 a	0.01	0.86 a	0.01	0.85 a	0.01
<b>PI (a.u.)</b>	1.98 a	0.46	2.30 a	0.60	2.40 a	0.39
<b>O<sub>2</sub> (%)</b>	20.77 a	0.06	20.57 a	0.21	20.83 a	0.06

Lsat number	Orthologue	Gene_name	log <sub>2</sub> FC Day 2	log <sub>2</sub> FC Day 7	FDR Day 2	FDR Day 7
Lsat_1_v5_gn_5_103641	NYC1_ARATH	Probable chlorophyll(ide) b reductase NYC1	4.38	3.90	3.04E-12	1.31E-10
Lsat_1_v5_gn_5_6580	POR_DAUCA	Protochlorophyllide reductase	2.29	1.53	1.74E-10	1.49E-05
Lsat_1_v5_gn_8_66120	CLH2_ARATH	Chlorophyllase-2	2.88	2.81	7.05E-20	1.99E-19
Lsat_1_v5_gn_8_66180	CLH2_ARATH	Chlorophyllase-2	-2.93	NDE	7.97E-04	NDE
Lsat_1_v5_gn_2_88180	CHMU_ARATH	Chorismate mutase	-1.56	-1.59	8.19E-13	8.58E-14
Lsat_1_v5_gn_4_108620	CHLP_TOBAC	Geranylgeranyl diphosphate reductase	1.56	NDE	1.52E-03	NDE
Lsat_1_v5_gn_1_33240	ADS3_ARATH	Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase	-3.53	-3.94	1.96E-07	2.88E-09
Lsat_1_v5_gn_1_33441	ADS3_ARATH	Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase	NDE	-2.46	NDE	6.95E-03
Lsat_1_v5_gn_1_34480	ADS3_ARATH	Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase	-2.98	-10.73	2.28E-05	1.08E-20
Lsat_1_v5_gn_2_58980	ADS3_ARATH	Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase	-1.82	NDE	1.03E-04	NDE
Lsat_1_v5_gn_5_131560	ADS3_ARATH	Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase	NDE	-3.42	NDE	2.45E-11
Lsat_1_v5_gn_5_37300	ADS3_ARATH	Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase	2.27	2.66	8.83E-04	4.20E-05
Lsat_1_v5_gn_2_102941	CB21_TOBAC	Chlorophyll a-b binding protein 16	NDE	1.93	NDE	2.92E-07
Lsat_1_v5_gn_9_104780	CB23_TOBAC	Chlorophyll a-b binding protein 36	NDE	-1.87	NDE	4.66E-04
Lsat_1_v5_gn_4_28820	CB2G_SOLLC	Chlorophyll a-b binding protein 3C	-2.06	-1.53	6.36E-08	4.15E-05
Lsat_1_v5_gn_9_16861	PSAL_ARATH	Photosystem I reaction center subunit XI	NDE	-1.78	NDE	2.91E-13
Lsat_1_v5_gn_9_9761	PSBW_ARATH	Photosystem II reaction center W protein	4.58	2.79	2.75E-75	6.97E-31
Lsat_1_v5_gn_9_48361	PPD3_ARATH	PsbP domain-containing protein 3	NDE	-3.37	NDE	2.56E-39
Lsat_1_v5_gn_5_23660	PPL1_ARATH	PsbP-like protein 1	NDE	-2.42	NDE	1.82E-40
Lsat_1_v5_gn_4_110701	PSBQ2_ARATH	Oxygen-evolving enhancer protein 3-2	1.63	NDE	2.66E-15	NDE
Lsat_1_v5_gn_5_4581	PSBQ2_ARATH	Oxygen-evolving enhancer protein 3-2	-1.57	-2.68	1.12E-06	4.56E-17
Lsat_1_v5_gn_0_5321	UCRIA_PEA	Cytochrome b6-f complex iron-sulfur subunit	NDE	-1.79	NDE	4.35E-11

Lsat number	Orthologue	Gene_name	log <sub>2</sub> FC Day 2	log <sub>2</sub> FC Day 7	FDR Day 2	FDR Day 7
Lsat_1_v5_gn_5_153800	RCA1_LARTR	Ribulose biphosphate carboxylase/oxygenase activase 1, chloroplastic	-3.24	-1.54	3.61E-14	4.67E-05
Lsat_1_v5_gn_2_118741	RCA2_LARTR	Ribulose biphosphate carboxylase/oxygenase activase 2, chloroplastic	-1.51	-2.33	9.14E-08	2.38E-17
Lsat_1_v5_gn_0_40540	G3PB_PEA	Glyceraldehyde-3-phosphate dehydrogenase B, chloroplastic	NDE	-2.53	NDE	1.15E-18
Lsat_1_v5_gn_8_80661	F16P1_PEA	Fructose-1,6-bisphosphatase, chloroplastic	NDE	-1.75	NDE	1.12E-13
Lsat_1_v5_gn_7_69401	PGKH_TOBAC	Phosphoglycerate kinase, chloroplastic	NDE	-2.51	NDE	3.26E-21
Lsat_1_v5_gn_2_91600	TPT2_BRAOB	Phosphate translocator, chloroplastic	-3.17	-5.75	1.46E-20	5.83E-50
Lsat_1_v5_gn_5_14941	TPT2_BRAOB	Phosphate translocator, chloroplastic	NDE	-1.77	NDE	4.01E-19
Lsat_1_v5_gn_7_65521	GLGL1_ARATH	Glucose-1-phosphate adenyltransferase large subunit 1, chloroplastic	NDE	-2.45	NDE	3.87E-30
Lsat_1_v5_gn_5_27340	GLGL1_BETVU	Glucose-1-phosphate adenyltransferase large subunit, chl/amyloplastic	-3.47	NDE	1.45E-06	NDE
Lsat_1_v5_gn_2_73301	SSG1_SOLTU	Granule-bound starch synthase 1, chloroplastic/amyloplastic	-3.20	-3.81	1.14E-33	3.00E-45
Lsat_1_v5_gn_9_52960	PGMP_SOLTU	Phosphoglucomutase, chloroplastic /cytoplasmic	NDE	-1.68	NDE	4.52E-16
Lsat_1_v5_gn_4_61761	PFPB_SOLTU	Pyrophosphate--fructose 6-phosphate 1-phosphotransferase subunit beta	-2.03	-2.44	1.48E-13	1.99E-19
Lsat_1_v5_gn_2_129481	ENO_ORYSJ	Enolase	2.41	3.65	2.73E-13	3.78E-28
Lsat_1_v5_gn_7_51781	DXS_ORYSJ	Probable 1-deoxy-D-xylulose-5-phosphate synthase, chloroplastic	NDE	-1.58	NDE	6.38E-06
Lsat_1_v5_gn_8_41941	ALFC3_ARATH	Probable fructose-bisphosphate aldolase 3, chloroplastic	-1.74	NDE	1.97E-12	NDE
Lsat_1_v5_gn_6_96701	ADH1_PETHY	Alcohol dehydrogenase	NDE	2.35	NDE	6.43E-05
Lsat_1_v5_gn_3_14780	ADHL3_ARATH	Alcohol dehydrogenase-like	NDE	-1.68	NDE	2.36E-05
Lsat_1_v5_gn_8_28340	ADHL7_ARATH	Alcohol dehydrogenase-like	NDE	-2.08	NDE	5.52E-14
Lsat_1_v5_gn_2_109940	KADC1_ARATH	Probable adenylate kinase, chloroplastic	2.95	3.42	1.23E-27	8.82E-37
Lsat_1_v5_gn_5_90580	KADC2_ARATH	Probable adenylate kinase, chloroplastic	NDE	-1.80	NDE	1.11E-16
Lsat_1_v5_gn_7_16100	ATPG_TOBAC	ATP synthase gamma chain, chloroplastic	NDE	-1.53	NDE	2.25E-09
Lsat_1_v5_gn_6_21280	ATPX_SPIOL	ATP synthase subunit b, chloroplastic	NDE	-1.80	NDE	2.78E-13
Lsat_1_v5_gn_4_156361	GLYM_FLAPR	Serine hydroxymethyltransferase 1, mitochondrial	NDE	-2.91	NDE	1.86E-19
Lsat_1_v5_gn_5_77601	GLNA2_DAUCA	Glutamine synthetase, chloroplastic	NDE	-2.95	NDE	2.42E-31

Lsat number	Orthologue	Gene_NDEme	log <sub>2</sub> FC Day 2	log <sub>2</sub> FC Day 7	FDR Day 2	FDR Day 7
Lsat_1_v5_gn_5_116960	CCD11_ARATH	Cyclin-D1-1	NDE	-2.38	NDE	1.14E-04
Lsat_1_v5_gn_3_88380	CCD32_ARATH	Cyclin-D3-2	NDE	-1.86	NDE	2.11E-09
Lsat_1_v5_gn_1_124201	CCD61_ARATH	Putative cyclin-D6-1	NDE	-1.65	NDE	7.21E-06
Lsat_1_v5_gn_1_84401	CCU41_ARATH	Cyclin-U4-1	-1.88	-1.69	1.54E-09	2.12E-08
Lsat_1_v5_gn_3_91021	KRP3_ARATH	Cyclin-dependent kiNDEse inhibitor 3	NDE	-2.36	NDE	1.89E-07
Lsat_1_v5_gn_5_179460	KRP3_ARATH	Cyclin-dependent kiNDEse inhibitor 3	1.70	1.84	8.68E-03	2.95E-03
Lsat_1_v5_gn_7_87161	KRP7_ARATH	Cyclin-dependent kiNDEse inhibitor 7	-2.62	NDE	1.50E-05	NDE
Lsat_1_v5_gn_5_62040	GPX1_PEA	Phospholipid hydroperoxide glutathione peroxidase, chloroplastic	NDE	-1.97	NDE	1.64E-22

Lsat number	Orthologue	Gene_name	log <sub>2</sub> FC Day 2	log <sub>2</sub> FC Day 7	FDR Day 2	FDR Day 7
Lsat_1_v5_gn_7_83821	XTH15_ARATH	Probable xyloglucan endotransglucosylase/hydrolase protein 15	NDE	-5.68	NDE	2.69E-08
Lsat_1_v5_gn_7_83841	XTH15_ARATH	Probable xyloglucan endotransglucosylase/hydrolase protein 15	NDE	-4.31	NDE	1.35E-10
Lsat_1_v5_gn_8_67581	XTH23_ARATH	Probable xyloglucan endotransglucosylase/hydrolase protein 23	-1.90	-1.55	8.39E-06	1.81E-04
Lsat_1_v5_gn_8_67381	XTH23_ARATH	Probable xyloglucan endotransglucosylase/hydrolase protein 23	NDE	-1.83	NDE	3.99E-05
Lsat_1_v5_gn_8_67401	XTH23_ARATH	Probable xyloglucan endotransglucosylase/hydrolase protein 23	NDE	-1.55	NDE	3.01E-05
Lsat_1_v5_gn_6_24740	XTH32_ARATH	Probable xyloglucan endotransglucosylase/hydrolase protein 32	-2.31	-3.54	4.69E-03	2.03E-05
Lsat_1_v5_gn_6_44180	XTH32_ARATH	Probable xyloglucan endotransglucosylase/hydrolase protein 32	3.31	5.00	1.71E-09	1.51E-22
Lsat_1_v5_gn_3_47880	XTH33_ARATH	Probable xyloglucan endotransglucosylase/hydrolase protein 33	-2.39	-4.13	1.29E-04	6.22E-10
Lsat_1_v5_gn_4_6921	XTH33_ARATH	Probable xyloglucan endotransglucosylase/hydrolase protein 33	NDE	-4.85	NDE	1.48E-11
Lsat_1_v5_gn_3_84840	XTH6_ARATH	Probable xyloglucan endotransglucosylase/hydrolase protein 6	-3.91	-6.31	1.37E-24	2.97E-50
Lsat_1_v5_gn_4_159341	XTH7_ARATH	Probable xyloglucan endotransglucosylase/hydrolase protein 7	NDE	-2.47	NDE	2.17E-08
Lsat_1_v5_gn_5_3340	GLPQ2_ARATH	Probable glycerophosphoryl diester phosphodiesterase 2	NDE	-1.71	NDE	3.20E-06
Lsat_1_v5_gn_3_45680	PRP2_MEDTR	Repetitive proline-rich cell wall protein 2	NA	-2.81	NA	2.20E-11
Lsat_1_v5_gn_8_33001	WAK2_ARATH	Wall-associated receptor kinase 2	2.65	NA	1.36E-04	NA
Lsat_1_v5_gn_4_178600	EXPA1_ARATH	Expansin-A1	-2.09	-2.10	5.58E-04	3.10E-04
Lsat_1_v5_gn_4_109320	EXP15_ARATH	Expansin-A15	NDE	2.23	NDE	2.29E-03
Lsat_1_v5_gn_8_73960	EXPA4_ARATH	Expansin-A4	-2.22	-2.00	6.42E-06	1.98E-05
Lsat_1_v5_gn_5_170161	EXPA4_ARATH	Expansin-A4	NDE	-2.38	NDE	6.79E-06
Lsat_1_v5_gn_2_124880	EXPA8_ARATH	Expansin-A8	-1.71	-2.23	8.73E-15	1.56E-24
Lsat_1_v5_gn_9_59761	EXPA8_ARATH	Expansin-A8	NDE	-1.60	NDE	3.03E-06
Lsat_1_v5_gn_5_137740	PME3_CITSI	Pectinesterase 3	1.98	NDE	2.83E-11	NDE
Lsat_1_v5_gn_1_52601	PME15_ARATH	Probable pectinesterase 15	-2.11	-1.71	1.38E-04	8.46E-04
Lsat_1_v5_gn_5_138221	PME12_ARATH	Probable pectinesterase/pectinesterase inhibitor 12	NDE	-1.73	NDE	1.12E-07
Lsat_1_v5_gn_9_2560	PME40_ARATH	Probable pectinesterase/pectinesterase inhibitor 40	-2.17	-2.96	3.88E-06	2.17E-10
Lsat_1_v5_gn_8_144820	PME51_ARATH	Probable pectinesterase/pectinesterase inhibitor 51	-2.12	-1.77	6.99E-04	3.45E-03

Lsat number	Orthologue	Gene_name	log <sub>2</sub> FC Day 2	log <sub>2</sub> FC Day 7	FDR Day 2	FDR Day 7
Lsat_1_v5_gn_1_115840	NEP1_NEPGR	Aspartic proteinase nepenthesin-1	NDE	-2.04	NDE	6.13E-11
Lsat_1_v5_gn_2_25300	NEP1_NEPGR	Aspartic proteinase nepenthesin-1	-1.62	NDE	4.31E-03	NDE
Lsat_1_v5_gn_2_37681	NEP1_NEPGR	Aspartic proteinase nepenthesin-1	NDE	-1.56	NDE	1.45E-03
Lsat_1_v5_gn_8_38360	NEP1_NEPGR	Aspartic proteinase nepenthesin-1	NDE	-3.76	NDE	2.89E-07
Lsat_1_v5_gn_2_55601	NEP2_NEPGR	Aspartic proteinase nepenthesin-2	NDE	-1.95	NDE	1.06E-13
Lsat_1_v5_gn_3_61320	NEP2_NEPGR	Aspartic proteinase nepenthesin-2	-1.81	-3.65	6.01E-04	3.73E-12
Lsat_1_v5_gn_7_68021	7SB1_SOYBN	Basic 7S globulin	NDE	-1.65	NDE	4.02E-09
Lsat_1_v5_gn_9_4161	7SB1_SOYBN	Basic 7S globulin	NDE	-1.90	NDE	2.41E-08
Lsat_1_v5_gn_1_64121	UBL5_ARATH	Ubiquitin-like protein 5	NDE	1.87	NDE	8.23E-11
Lsat_1_v5_gn_1_127601	UBI2P_PETCR	Polyubiquitin	-1.51	NDE	1.37E-10	NDE
Lsat_1_v5_gn_7_16760	UBI2P_PETCR	Polyubiquitin	-1.52	NDE	1.97E-10	NDE
Lsat_1_v5_gn_2_53840	PUB22_ARATH	E3 ubiquitin-protein ligase PUB22	1.74	NDE	7.85E-11	NDE
Lsat_1_v5_gn_1_58201	PUB23_ARATH	E3 ubiquitin-protein ligase PUB23	NDE	-2.30	NDE	1.05E-07
Lsat_1_v5_gn_1_58280	PUB23_ARATH	E3 ubiquitin-protein ligase PUB23	NDE	-2.01	NDE	1.31E-08
Lsat_1_v5_gn_2_34501	PUB23_ARATH	E3 ubiquitin-protein ligase PUB23	NDE	-1.74	NDE	5.75E-04
Lsat_1_v5_gn_2_35740	PUB23_ARATH	E3 ubiquitin-protein ligase PUB23	NDE	-2.45	NDE	9.54E-05
Lsat_1_v5_gn_6_33780	PUB23_ARATH	E3 ubiquitin-protein ligase PUB23	NDE	-1.94	NDE	2.90E-08
Lsat_1_v5_gn_7_10960	PUB23_ARATH	E3 ubiquitin-protein ligase PUB23	NDE	-2.61	NDE	8.87E-08
Lsat_1_v5_gn_8_133561	RMA1_CAPAN	E3 ubiquitin-protein ligase RMA1H1	NDE	1.51	NDE	4.04E-05
Lsat_1_v5_gn_9_25941	SINA2_ARATH	E3 ubiquitin-protein ligase SINAT2	NDE	-1.71	NDE	6.62E-07
Lsat_1_v5_gn_4_3921	RGLG2_ARATH	E3 ubiquitin-protein ligase RGLG2	NDE	1.82	NDE	2.02E-07
Lsat_1_v5_gn_4_33741	SINA5_ARATH	E3 ubiquitin-protein ligase SINAT5	1.71	NDE	6.80E-05	NDE
Lsat_1_v5_gn_2_127400	ARATH	E3 ubiquitin-protein ligase	2.18	1.97	1.75E-12	1.46E-10
Lsat_1_v5_gn_7_97260	RHA1B_ARATH	E3 ubiquitin-protein ligase RHA1B	NDE	-1.59	NDE	1.27E-05
Lsat_1_v5_gn_4_17160	ATL6_ARATH	E3 ubiquitin-protein ligase ATL6	NDE	-2.37	NDE	1.46E-03
Lsat_1_v5_gn_8_52720	RNG1L_ARATH	E3 ubiquitin-protein ligase RING1-like	NDE	1.64	NDE	9.47E-09
Lsat_1_v5_gn_0_34660	PUB11_ARATH	U-box domain-containing protein 11	NDE	3.48	NDE	3.03E-15
Lsat_1_v5_gn_5_17060	PUB19_ARATH	U-box domain-containing protein 19	NDE	2.66	NDE	5.29E-19
Lsat_1_v5_gn_9_17860	PUB9_ARATH	U-box domain-containing protein 9	NDE	1.55	NDE	1.53E-12
Lsat_1_v5_gn_4_151201	APC10-ARATH	APC10	-1.54	NDE	4.75E-07	NDE
Lsat_1_v5_gn_2_35221	UBP8_ARATH	Ubiquitin carboxyl-terminal hydrolase 8	NDE	-1.50	NDE	2.27E-07

Lsat_1_v5_gn_3_47680	SKP1B_ARATH	SKP1-like protein 1B	NDE	1.93	NDE	3.19E-05
Lsat_1_v5_gn_3_24140	CUL1_ARATH	Cullin-1	NDE	-1.68	NDE	3.83E-11
Lsat_1_v5_gn_5_157480	FB118_ARATH	F-box protein At2g26850	NDE	2.67	NDE	2.48E-08
Lsat_1_v5_gn_9_103981	FB135_ARATH	F-box protein At3g07870	1.58	1.65	5.06E-05	1.91E-05
Lsat_1_v5_gn_9_104220	FB135_ARATH	F-box protein At3g07870	-1.79	NDE	3.37E-10	NDE
Lsat_1_v5_gn_9_104300	FB135_ARATH	F-box protein At3g07870	NDE	-2.33	NDE	1.67E-04
Lsat_1_v5_gn_9_51281	FB253_ARATH	F-box protein At5g07610	NDE	3.59	NDE	3.07E-06
Lsat_1_v5_gn_9_125181	P2B15_ARATH	F-box protein PP2-B15	NDE	-1.80	NDE	2.60E-09
Lsat_1_v5_gn_9_65340	FB20_ARATH	F-box protein At1g30790	NDE	-2.13	NDE	4.44E-09
Lsat_1_v5_gn_6_97240	SKP2A_ARATH	F-box protein SKP2A	2.60	2.88	1.84E-03	6.06E-04
Lsat_1_v5_gn_0_29401	FBK67_ARATH	F-box/kelch-repeat protein At3g23880	2.93	NDE	1.75E-03	NDE
Lsat_1_v5_gn_7_12620	FDL7_ARATH	Putative F-box/FBD/LRR-repeat protein At1g66290	1.55	1.60	4.22E-05	2.03E-05
Lsat_1_v5_gn_5_95460	EBF2_ARATH	EIN3-binding F-box protein 2	-3.24	-1.89	4.56E-25	1.78E-10
Lsat_1_v5_gn_1_66321	SAP5_ARATH	Zinc finger A20 and AN1 domain stress-associated protein 5	NDE	2.02	NDE	3.15E-10