Characterisation of transcriptional and chromatin events in relation to floral transition and identification of nuclear organisation determinants

del Prete, S.

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Chapter 3

Molecular and Morphological Characterisation of the Floral Transition in *Arabidopsis thaliana* Induced by Photoperiodic Switch

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*This chapter is part of a paper in preparation.*
Abstract

The switch from vegetative to reproductive growth is a crucial decision in the life cycle of plants. It is influenced by both endogenous and environmental cues and affects the ability to produce offspring. In *Arabidopsis thaliana* the floral transition can be rapidly induced by a shift from short-day (SD) to long-day (LD) conditions and is associated with dramatic changes at the morphological, physiological and molecular levels. We characterised the events associated with the SD-LD shift using different parameters: bolting time, leaf number and expression of key flowering genes. The bolting time, as well as the leaf number, was dependent on the duration of the initial growth in SD. From the analysis of the expression profile we defined the temporal window of the floral transition as the time restricted by the early expression of *CONSTANS (CO)* and the floral meristem marker *APETALA1 (AP1)*. In this paper we focused on the leaves, as a tissue system to investigate the flowering gene network. Based on the changes occurring in leaves at the morphological and ploidy levels, we propose the 3rd and 4th leaf as optimal material to investigate the transcriptional changes related to the flowering time.
Introduction

The transition to flowering corresponds to the switch from a vegetative to a reproductive developmental program, and is one of the key phase transitions in the plant life cycle, due to its crucial impact on reproductive success and survival. Higher plants have developed diverse strategies to initiate and tightly control successful flowering in response to different environmental cues, such as photoperiod, light quality/intensity/quantity, nutrient resources or temperature, depending on their growth habitats and in response to the seasonal changes. Flowering time thus results from complex interactions between both external and endogenous factors (McClung et al., 2016; Bratzel and Turck, 2015; Li et al., 2016). In the past 30 years, genetic dissection of the flowering time control in the plant model *A. thaliana* led to the identification of five major pathways: photoperiod, vernalization, gibberellin hormonal, ambient temperature and autonomous (Bernier and Périlleux, 2005; Amasino, 2010; Srikanth and Schmid, 2011). In *A. thaliana*, the photoperiod pathway is one of the most important to control flowering time. In the mid-1930s, it was proposed that a florigen signal is transmitted to the shoot apical meristem (SAM) to initiate the meristem identity switch (Chailakhyan, 1936). Much later, the FLOWERING LOCUS T (FT) protein was identified as a florigenic signalling molecule (Corbesier et al., 2007; Jaeger and Wigge, 2007; Mathieu et al., 2007). In *A. thaliana*, the florigen is produced by the phloem companion cells of the rosette leaves in response to inductive long days. FT gene expression is controlled by the transcription factor CONSTANS (CO). FT moves systemically in the phloem sap towards the SAM, where it activates flowering genes and the cascade of morphological and molecular changes required to initiate floral primordia (Song et al., 2015; Liu et al., 2013). The control of floral transition is highly complex since all flowering pathways merge together at the floral integrator gene FT and SUPPRESSOR OF OVEREXPRESSION OF CO1 (SOC1). In addition, different levels of chromatin organization are involved in controlling flowering. Chromatin-based mechanisms play a critical role in the control of the transcriptional state of key flowering genes (He, 2012; Bratzel and Turck, 2015). Recently, it has been highlighted that other molecules such as sugars or cytokinins can also promote the floral switch and participate in florigen signalling (Ortiz-Marchena et al., 2014, 2015; Bernier and Périlleux, 2005; Bernier, 2013; Wahl et al., 2013). A tremendous number of
studies identified more than 300 genes involved in these processes, which converge to the key floral integrators (Bouché et al., 2015). All these key flowering genes have been gathered in the FLOR-ID interactive database. Most studies focused on leaf-to-meristem signalling or meristem identity switches. Yet, much remains to be unravelled in mature leaves and in particular the response to the developmental switch considering the whole plant as an integrated system.

In *Arabidopsis thaliana* a photoperiod change can trigger the flowering time. A transient transfer of plants from short-day (SD) to long-day (LD) (SD-LD-SD) conditions or displaced SD conditions have been used to induce the floral transition in *A. thaliana* and in another Brassicaceae, *Sinapis alba* (Corbesier et al., 1996; King et al., 2008; D’Aloia et al., 2009). This experimental photoperiod system allows a controlled and synchronized induction of flowering (Corbesier et al., 1996) and gives the possibility to better analyse the early events associated with the floral transition in the whole plant. In *A. thaliana*, a simple SD-LD transfer system has been exploited to investigate genome-wide transcription profiles in shoot meristem (Torti et al., 2012), and in roots (Bouché et al., 2016), during floral commitment. However, changes occurring in mature leaves, besides the classical changes in *CO* and *FT* expression were not deeply investigated.

Here we aim to quantify, analyse and characterise the morphological and molecular changes in mature leaves in *Arabidopsis thaliana* that mark a single inductive SD-LD shift. We examined the effect of LD treatment on the floral transition taking into consideration the duration of the initial growth in SD. We recorded morphometric flowering indicators, and by checking the sequential expression of the main flowering genes, we defined the floral transition window. We then studied the associated changes occurring in leaves taking into consideration their growth and ploidy levels. We highlighted that the 3\textsuperscript{rd} and 4\textsuperscript{th} leaf, due to their morphological characteristics, are most suitable for the investigation of the floral transition events in the rosette leaves. The system established in this work will be used to further investigate the transcription of the flowering gene network in mature leaves. Further comparisons between transcriptome data acquired in shoot, root and leaf using this inductive LD system, will give a complete picture and highlight the specificity of different organs in the integrated floral transition process.
Results

Plant age at the time of the photoperiodic switch is critical for bolting

To set up the photoperiodic switch for floral induction, we cultured Col-0 plants for 3, 4 or 5 weeks in SD and then transferred them in LD conditions. Compared to previous studies, plants were older at the time of transfer and after transfer were kept in LD. We recorded morphometric indicators of flowering time, such as leaf number and bolting time (Table 1). Consistent with previous works (Corbesier et al., 1996; Pouteau et al., 2006), the total leaf number was related to the photoperiod length in SD. The earlier the switch occurred, the earlier the plant flowered and the smaller the total and rosette leaf numbers. Plants continuously grown in SD conditions presented a much higher number of total and rosette leaves (81.3 ± 7.0 and 71.6 ± 7.3, respectively; our previous study (Latrasse et al., 2011)). Furthermore, the days to bolt from transfer were inversely correlated to the exposure time in SD, meaning that the older the plants were at the photoperiodic switch, the faster they responded. There was a positive correlation between either the age of the plants or the quantity of photosynthetically active light perceived in SD and the bolting time (counting the days from the sowing), which is in agreement with previous studies (Pouteau et al., 2006).

The bolting time is defined as the time from sowing to the elongation of the first internode while the floral transition is defined as the initiation of the first flower bud; both characterise the reproductive phase change (Pouteau and Albertini, 2009). Bolting and floral transition can be characterised by the number of days to bolt and the total leaf number, respectively. The relationship between the two transitions can be appreciated by the percentage of cauline leaf with respect to the total leaf numbers, which in turn depends on the photoperiod (Pouteau and Albertini, 2009). The percentage of cauline leaves was 11.9% and 17.6% for plants in SD and LD, respectively (our previous study (Latrasse et al., 2011)). With the SD-LD switch, it was relatively constant, (18.1 ± 1.1)%, (16.3 ± 1.6)% and (17.7 ± 1.2)% for Col-0 plants grown 3, 4 and 5 weeks in SD,
Molecular and morphological characterisation of the floral transition in *Arabidopsis thaliana* induced by photoperiodic switch

respectively, and similar to the percentage observed for plants grown in LD. The SD-LD switch seems to mimic LD conditions in terms of relationship between bolting time and floral transition.

To better refine the developmental window in which the floral transition occurred, we monitored the expression of an early marker of the floral meristem, *APETALA 1* (*AP1*) on the *AP1::GUS* transgenic line (Hempel and Feldman, 1994; Hempel et al., 1997). Col-0 and *AP1::GUS* plants showed similar flowering time and morphometric indicators in the different growing conditions (Supplemental table 1). *AP1* expression was detected 8 days after transfer (dat) in the apical shoots of 60% of *AP1::GUS* plants cultured 3 weeks in SD, and 5 dat in 50% of *AP1::GUS* shoots, grown 4 and 5 weeks in SD (Figure 1). No difference was observed in these last two conditions, suggesting that at 4 weeks, the plants reached an age-dependent optimum to respond to the LD stimulus to switch the meristem identity. These data showed that the completion of the floral transition, corresponding to the formation of floral meristems occurred between 5 and 8 dat according to the SD period.

<table>
<thead>
<tr>
<th>Number of weeks in SD</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light hours in SD</td>
<td>168</td>
<td>224</td>
<td>280</td>
</tr>
<tr>
<td>Light hours in LD</td>
<td>240</td>
<td>224</td>
<td>176</td>
</tr>
<tr>
<td>Total light hours</td>
<td>408</td>
<td>432</td>
<td>456</td>
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<tr>
<td>Rosette leaves</td>
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<td>41.2±1.5</td>
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<td>Cauline leaves</td>
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<td>6.5±0.1</td>
<td>8.8±0.6</td>
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<tr>
<td>Total leaves</td>
<td>32.1±0.2</td>
<td>40.2±1.5</td>
<td>50.1±1.6</td>
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<tr>
<td>% Cauline leaves</td>
<td>18.1±1.1</td>
<td>16.3±1.6</td>
<td>17.7±1.2</td>
</tr>
<tr>
<td>Number of days to bolt from sowing</td>
<td>35±1</td>
<td>41±1</td>
<td>47±1</td>
</tr>
<tr>
<td>Number of days to bolt from transfer</td>
<td>14±1</td>
<td>13±1</td>
<td>12±1</td>
</tr>
</tbody>
</table>

**Table 1: Flowering time of Col-0 plants.**
Rosette, cauline and total leaf numbers were recorded on bolted plants when first flower appeared. The bolting time was recorded when the stem was 0.5 cm high. Three biological replicates were performed with 12 plants, each.
Key flowering gene expression peaks at 3 days after the transfer

To further characterise the floral transition events in our SD-LD switch, we examined the expression patterns of five key flowering genes: CO and FT, which promote flowering time in LD; SHORT VEGETATIVE PHASE (SVP), a repressor of floral transition; SOC1, a floral integrator involved in inflorescence meristem formation; and the floral meristem marker AP1 (Figure 2). Since FT and CO have a circadian rhythmic expression with a peak of CO expression and an FT increased mRNA level before the dusk in LD (Suárez-López et al., 2001; Turck et al., 2008), we carried out our analyses on material collected just before dusk (Zeitgeber time 15).

The expression profiles of the different genes were globally similar with 3 or 4 weeks of initial growth in SD. The expression level of CO and FT increased after the switch in LD (Figure 2). CO expression increased at 1 day after the LD shift for the plants grown 3 weeks in SD, whereas the activation of FT was slightly delayed, consistently with an activating role of CO.
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A high level of expression for *CO* and *FT* occurred at 3 dat. The expression of *SVP* and *SOC1* fluctuated during the time course, with a transient decrease after the shift. *AP1* mRNAs were detected 7 dat in plants cultured 4 weeks in SD. The discrepancy between *AP1* mRNA level and the GUS assay may be due to the detection limits of the two techniques. The GUS staining allows the observation of the *AP1* expression in only few cells, whereas mRNAs were analysed in the whole rosette. Thus, our data allowed to better refine the timing of the events induced by the LD shift: *CO* expression is rapidly induced, followed by *FT* expression, and, at 5 dat, the floral transition is completed with the production of the first floral meristems and the *AP1::GUS* expression.

**The SD-LD switch stimulates growth in the youngest leaves**

To better characterise the timing of the morphological changes in leaves associated with the photoperiodic switch, we recorded the growth of the first six leaves of plants grown 4 weeks in SD and transferred in LD, and we compared...
with the leaves growth in SD. At the time of the LD shift (day 0), plants had 7 leaves, mostly under developed and with a round-elliptic shape (Figure 3). Recording the size of the entire rosette plants we observed that during the first days the whole-rosette area did not differ significantly between the two growing conditions. A difference in the rosette size, between the SD and SD-LD conditions, was found after 5 days (Figure 3). When we focused on the area of individual leaves, we observed that the size of the first pair of leaves was already established 2 days before transfer (dbt), independently of the photoperiodic conditions, and no growth was detected over time (Figure 4). The 3\textsuperscript{rd} and 4\textsuperscript{th} leaf (second pair) showed a slow growth rate, with only 1.6 times increase in size from 0 to 5 dat and with no significant differences between SD and SD-LD conditions. A difference in growth was observed only after 9 days in SD-LD switch compared to SD conditions. However, the sizes of the second pair of leaves at 15 days were similar, showing that the change in the light regime impacted the growth rate with a delay from the switch of at least 5 days, without exceeding an apparent growth size limit (Figure 4). The third pair of leaves presented a higher growth rate compared to the second pair. For instance, the leaf area doubled from day 0 to day 2, on both conditions.

\textbf{Figure 3: Growth of Col-0 rosettes in response to SD or SD-LD conditions.}
\begin{itemize}
\item a) Rosette plants before transfer (dbt), without transfer (dwt) and after transfer (dat). Scale bar, 5 mm.
\item b) Rosette leaf area measurement. Col-0 plants were grown in SD for 4 weeks, then kept in SD (continuous line) or transferred in LD (dash line). Two biological replicates were performed with 10 plants, each. Experimental values are mean ± SEM.
\end{itemize}
An accelerated growth rate was observed in the 5th and 6th leaf in response to the SD-LD switch, starting after day 3 (Figure 4). Interestingly, no major morphological changes were recorded in the 3rd and 4th leaf during the 0-to-5-dat window. Because of their slow growth rate, the 3rd and 4th leaf are suitable to investigate the molecular changes associated with floral transition in mature leaves independently of developmental events that occur in the leaves.
The SD-LD switch increases the DNA content of the leaf cells

The growth of organs can be achieved by an increase in cell number, cell size, or both. Endoreduplication, the increase in the DNA content in the cell nucleus without cell division, plays also a critical role in the control of growth processes (Kondorosi et al., 2000; Massonnet et al., 2011). We thus characterised the ploidy levels of the second pair of leaves and compared with the one of the first pair of leaves, which had a stunted growth. We recorded the ploidy levels after the SD-LD switch, and under continuous SD conditions, for comparison. Whatever the leaf position, its age and the growth conditions, the percentage of nuclei with a 2C ploidy level was rather constant (Figure 5), suggesting a low cell division rate in agreement with the growth analysis. The other ploidy levels
Molecular and morphological characterisation of the floral transition in *Arabidopsis thaliana* induced by photoperiodic switch (from 4C to 32C) evolved over time and their dynamics were dependent on the leaf position and the growth conditions. The numbers of 4C and 8C nuclei decreased in the 3rd and 4th leaf in both conditions, whereas the numbers of 16C and 32C nuclei increased, suggesting that endoreduplication occurred, which was also observed in the first pair of leaves. The decrease in 8C nuclei and the increase in 16C nuclei were more pronounced in response to the LD shift. In the two groups of leaves a population of 32C nuclei appeared after 15 days. This population was more numerous in the SD-LD plants compared to SD ones, and even more in the second pair of leaves. We also recorded the endoreduplication factor, which was in agreement with the increase of 16C and 32C nuclei and the difference between the growing conditions and the leaf position (Supplemental table 2). Thus, our data showed that the photoperiodic shift was accompanied by a significant change in endoreduplication in mature leaves. Interestingly, previous results showed that the increase in light intensity increases the ploidy level (Cookson et al., 2006) and more recently, a correlation between endoreduplication and the response to UV-B radiation was demonstrated, which might participate to an adaptation to high solar irradiation (Gegas et al., 2014). Whether the rapid observed LD shift-dependent changes in ploidy, already initiated at 2 dat, participates to the developmental process of the floral transition, or whether it is an adaptation to environmental changes induced by the switch (i.e. light duration), will be interesting to further question.

**Discussion**

**An inductive SD-LD switch to study the floral transition in mature leaves**

The transition to flowering mainly concerns the commitment and conversion of the shoot apical meristem. However, the plant is a complex system and the SAM is not isolated from the other organs. For instance, the leaves perceive stimuli that are translated and transferred towards the SAM via a complex and still elusive systemic signalling pathway. A major difficulty in studying these signalling mechanisms is that the floral transition is tightly and intricately associated with other developmental processes that occur in parallel, such as
organ growth and cell differentiation. Therefore, it is not completely understood how the whole plant contributes to this developmental transition. Here, we used the LD exposure system to induce flowering (Corbesier et al., 1996) and investigate changes in a pair of mature rosette leaves, independently of growth. A similar system has been used in apical shoots and roots to study the global transcriptome changes. In these studies the transition was induced when 2-week-old plants were exposed transiently to 5 LDs (Torti et al., 2012) or 7-week-old plants grown in hydroponic conditions were shifted in LD for 1 day (Bouché et al., 2016). To investigate the physiological and molecular changes in rosette leaves, we induced flowering with a single SD-LD switch on 4-week-old plants, which allowed harvesting enough leaf material in a short time scale. We confirmed that the number of total leaves did not differ significantly between plants exposed to SD-LD conditions and plants transiently exposed to LDs (Torti et al., 2012). In our conditions, we further recorded the expression of key flowering genes, which enabled us to define the floral transition window between day 0 and day 5, with the expression of the API::GUS reporter observed at the end of this window, pointing at completion of the developmental switch.

**SD-LD switch allows uncoupling the bolting and floral transition**

Using our transfer system, we characterised the reproductive phase change, which is classically quantified by the leaf number and the bolting time. We observed that the older the plants were when exposed to LD, the quicker they initiate bolting after the transfer in LD, suggesting that the bolting process is mainly dependent on the age of the plants. The floral transition was dependent on the time of the LD exposure: the larger the number of light hours perceived in LD, the smaller the total number of leaves. Thus, the bolting transition is dependent on age-related processes and the floral transition on the LD stimulus itself. Both transitions are involved in the reproductive phase switch but it seems that a decoupling of the two could occur, suggesting that they could be regulated by different factors or by the same signal but in a different temporal window. The developmental switch requires energy due to the biogenesis processes that take place and the capability to produce new metabolites might be directly related to the mass or age of the plant. It was demonstrated that the LD stimulus
increases leaf sucrose level and expression of genes regulating sucrose synthesis, participating to the florigenic signal (King et al., 2008; Ortiz-Marchena et al., 2015; Corbesier et al., 1998). However, sucrose is not the only metabolite involved in the floral transition since other sources of carbon, phosphorous, nitrogen or sulphur have been shown to impact this process (Bernier and Périlleux, 2005; Bernier et al., 1993; Schulze et al., 1994). Whether the increased production of metabolites is important for bolting or for the florigenic stimulus per se is still an open question.

The floral transition window defined by the expression pattern of key flowering genes

After the LD shift we observed a rapid increase in CO expression, starting after 1 day, for the plants grown 3 weeks in SD, with a peak at 3 dat. This was followed by FT expression starting at 2 dat and also peaking at 3 dat. Our data are consistent with previous observations of FT upregulation in LD occurring after CO expression (Kardailsky et al., 1999; Kobayashi et al., 1999; Suárez-López et al., 2001). In response to LD, FT is upregulated but if the plants return to SD, FT transcription is rapidly repressed (Corbesier et al., 2007). However, a short upregulation appears sufficient to induce the flowering commitment of the apical shoots (Torti et al., 2012). At the rosette level, we found an FT peak after 3 days, followed by a reduction of the mRNA level, thus it seems that it is not necessary to keep a high FT expression for flowering. FT expression decreases in young leaves in 17-day-old plants (Wigge et al., 2005), however, the decrease we observed could also result from the production over time of new small leaves, since we recorded the expression in the whole rosette. During the vegetative phase FT is stably repressed by different transcription factors, among which is the MADS-box protein SVP. The observed transient downregulation of SVP may also participate to the FT induction (Lee et al., 2007; Jang et al., 2009; Li et al., 2008).

SOC1 encodes a transcription factor with a major role in floral induction, floral patterning but also floral meristem determinacy. Its expression is activated by FT in the SAM, and coincides with the change of the meristem identity (Samach et al., 2000; Borner et al., 2000; Lee and Lee, 2010). Following SOC1
expression profile until 9 dat we observed small fluctuations in its expression with a repression after 1 dat and then a weak re-activation. The increase of \textit{SOC1} mRNA level at 3 dat may reflect its expression in meristem; the less pronounced peak may be due to the fact that only a small number of specialized cells in the whole rosette are involved in \textit{SOC1} expression. Furthermore, \textit{SOC1} expression depends strongly on a continuous photoperiodic stimulus (Torti et al., 2012), and its expression is maintained over time in LD. However, our results showed that \textit{SOC1} expression was not always maintained and we did not observe an increase of its mRNA level. Recently, it has been demonstrated that \textit{SOC1}, as \textit{FT}, is expressed in stomata guard protoplasts with a role in the enhancement of stomata aperture (Kimura et al., 2015). The shift to LD may induce changes in the photosynthetic activity of the leaves and thus a stomata adaptation is required. The weak transient fluctuations of \textit{SOC1} within the first 3 days after the LD transfer may also reflect these photosynthetic changes.

\textbf{Relationship between SD-LD switch, leaf growth and endoreduplication}

The relationship between the developmental programs and endoreduplication is still elusive. A number of studies reported links between endoreduplication and several cell variables, such as cell size or cell number, but the different data do not converge toward a clear consensus about the presence and nature of the relationship. Using an extensive analysis on 200 genotypes from a mutant collection and a RIL population, Massonnet et al., (2011) described a positive relationship between endoreduplication factor (varying from 0.33 to 2.17) and the area of the rosette or the area of leaf 6, but no correlation was observed between endoreduplication and the rosette leaf number. Consistently with this study, we found a positive correlation between leaf growth induced by LD exposure and endoreduplication. The LD switch seems to induce an acceleration of leaf growth after 9 days on the 3\textsuperscript{rd} and 4\textsuperscript{th} leaf that correlates with an increase in the endoreduplication levels, reaching 4 endocycles (32C). This pair of leaves shows a less pronounced growth from 0 to 5 days, yet, the average DNA content in response to LD exposure increases. The rise in ploidy may participate to a complementary strategy to increase leaf metabolism or to an endogenous signalling for floral transition. Endoreduplication in plants occurs in tissues that
develop quickly and have high metabolic activity (Inzé and De Veylder, 2006; Kondorosi et al., 2000). The increase in cell volume without division could be seen as a more efficient way for the leaves to maximize the surface area in order to absorb more light during the photosynthesis and accumulate more metabolites. We can speculate that the observed increase in endoreduplication during the floral transition may help the plant to provide the energy required during the developmental switch.

Recent studies have demonstrated that the cell-cycle-related genes and the spindle-assembly checkpoint (SAC) genes are other important regulators of floral transition (Klepikova et al., 2015; Bao et al., 2014). Bao et al., (2014) showed that the *A. thaliana* SACs genes can interact with the flowering gene *SUPPRESSOR OF FRIGIDA 4 (SUF4)*, to regulate both the flowering time through the modulation of the transcriptional state of *FLC* and the endocycle number by changing the timing of the cell cycle phases. *CYCD3;1*, a marker gene of endoreduplication, is deregulated in the meristem during the floral transition (Klepikova et al., 2015) and its mutation induces abnormal flowering time and altered ploidy levels in the leaves (Dewitte et al., 2007; Schnittger et al., 2002). The two processes can be co-regulated by the same genes but further analyses of flowering time and cell cycle mutants are required to provide more insight about the relationship between these two processes and their direction of causality, if there is one.

**Future perspectives**

Our data highlight physiological processes occurring simultaneously with the florigenic signalling (Figure 6). Further transcriptome analysis of the corresponding gene sets involved in endoreduplication, cell division control and leaf differentiation as well as photosynthetic responses will precise the gene networks that control the molecular events accompanying the inductive LD switch on mature leaves. Comparisons with transcriptome data in shoots and roots in the same developmental window will complete the knowledge of floral transition at the whole-plant level.
Figure 6: Chronological progression of the main events occurring during the SD-LD switch.
Plants were grown during 4 weeks in SD and then transferred in LD. The peak of CO and FT expression and the start of the expression of the floral meristem marker AP1 are indicated. Plants are bolting 13 days after the transfer in LD.
Experimental procedure

Plant materials and Growth Conditions

All *Arabidopsis thaliana* lines in this study were in the Col-0 background. Plants were grown in soil, in growth chamber under white fluorescent light, under short-day (8 hours light/16 hours dark, SD) or long-day (16 hours light/8 hours dark, LD) conditions. Temperature in SD was 21°C during the light period and 18°C during the dark, humidity (65%) remained constant. In LD, temperature (21°C) and humidity (70%) remained constant. Plants were cultured for 3, 4 or 5 weeks in individual pot, in SD then transferred in LD. Plants were analysed at different time points before and after the transfer, and material was collected before dusk, at Zeitgeber time 15 (ZT15) considering ZT 0 the switched on of the light.

Expression Analysis

Total RNAs were prepared from rosette material, treated and reverse transcribed, as previously described (Le Roux et al., 2014). Quantitative real-time PCR was performed on a BioRad CFX96 apparatus using the SYBR green Master Mix (BioRad) following manufacturer’s instructions. For each primer pair, a dilution series was used as standard, to calculate the slope and intercept. *UBIQUITIN10 (UBQ10)* was used as reference gene. Primers are in Supplemental table 3.

For GUS histochemical staining, plants were collected in the staining solution (1 mM X-Gluc (5-bromo-4-chloro-3-indolyl-ß-D-glucuronide), 0.1 M sodium phosphate buffer, pH 7.0, 2 mM potassium ferrocyanide, 2 mM potassium ferricyanide, and 0.5% Triton X-100), infiltrated under vacuum 3 times, for 5 minutes each, and incubated at 37°C overnight. After staining, samples were washed in 70% ethanol and observed under a light microscope.
Leaf Growth Analysis

Individual leaves were harvested at different time points, flattened on white paper and then digitally scanned. Leaf areas (blade and petiole) were calculated from the binary images using ImageJ software (http://rsb.info.nih.gov/ij/). Leaves from 10 to 15 plants were analysed.

Ploidy Analysis

Leaves 1 to 4 were harvested at different time points, chopped with a razor blade in 800 µl of buffer (45 mM MgCl2, 30 mM sodium citrate, 20 mM MOPS, pH 7.0, and 1% Triton X-100) (Galbraith et al., 1991), filtered over a 30 µm mesh, and 150 µl of a propidium iodide solution (100 µg/ml) was added. The nuclear DNA content was analysed using a CyFlow cytometer (Partec, Germany) and the FloMax software (Partec, Germany). Endoreduplication factor was calculated by the formula: EF= (% of 2C nuclei x 0) + (% of 4C nuclei x 1) + (% of 8C nuclei x 2) + (% of 16C nuclei x 3) + (% of 32C nuclei x 4).

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Supporting Information

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<th>Number of weeks in SD</th>
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<td>Rosette leaves</td>
<td>31.1 ± 1.6</td>
<td>35.2 ± 0.7</td>
<td>42.3 ± 1.5</td>
</tr>
<tr>
<td>Cauline leaves</td>
<td>7.1 ± 0.4</td>
<td>7.4 ± 0.8</td>
<td>8.9 ± 0.2</td>
</tr>
<tr>
<td>Total leaves</td>
<td>38.1 ± 2.1</td>
<td>42.7 ± 1.6</td>
<td>51.2 ± 1.7</td>
</tr>
<tr>
<td>% Cauline leaves</td>
<td>18.3 ± 1.6</td>
<td>17.4 ± 1.3</td>
<td>17.3 ± 1.8</td>
</tr>
<tr>
<td>Number of days to bolt from sowing</td>
<td>36 ± 1</td>
<td>42 ± 1</td>
<td>49 ± 1</td>
</tr>
<tr>
<td>Number of days to bolt from transfer</td>
<td>15 ± 1</td>
<td>14 ± 1</td>
<td>14 ± 1</td>
</tr>
</tbody>
</table>

**Supplemental table 1: Flowering time of AP1::GUS plants.**

Rosette, cauline and total leaf numbers were recorded on bolted plants when first flower appeared. The bolting time was recorded when the stem was 0.5 cm high. Three biological replicates were performed with 12 plants, each.
Supplemental table 2: Endoreduplication factors of the leaves in response to SD or SD-LD conditions.
Plants were grown 4 weeks in SD, and then either kept in SD (dwt) or transferred in LD (dat) for 15 days. Endoreduplication factor was calculated in the first two pairs of leaves.

<table>
<thead>
<tr>
<th>Time</th>
<th>1&lt;sup&gt;st&lt;/sup&gt;-2&lt;sup&gt;nd&lt;/sup&gt; leaves</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt;-4&lt;sup&gt;th&lt;/sup&gt; leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 dbt</td>
<td>1.59 ± 0.07</td>
<td>1.26 ± 0.01</td>
</tr>
<tr>
<td>0</td>
<td>1.46 ± 0.03</td>
<td>1.34 ± 0.06</td>
</tr>
<tr>
<td>2 dwt</td>
<td>1.67 ± 0.04</td>
<td>1.58 ± 0.01</td>
</tr>
<tr>
<td>3 dwt</td>
<td>1.69 ± 0.05</td>
<td>1.64 ± 0.01</td>
</tr>
<tr>
<td>5 dwt</td>
<td>1.61 ± 0.18</td>
<td>1.5 ± 0.08</td>
</tr>
<tr>
<td>9 dwt</td>
<td>2.03 ± 0.30</td>
<td>1.92 ± 0.01</td>
</tr>
<tr>
<td>15 dwt</td>
<td>2.08 ± 0.02</td>
<td>1.95 ± 0.01</td>
</tr>
<tr>
<td>2 dat</td>
<td>1.87 ± 0.01</td>
<td>1.79 ± 0.05</td>
</tr>
<tr>
<td>3 dat</td>
<td>1.89 ± 0.02</td>
<td>1.77 ± 0.01</td>
</tr>
<tr>
<td>5 dat</td>
<td>1.86 ± 0.04</td>
<td>1.95 ± 0.03</td>
</tr>
<tr>
<td>9 dat</td>
<td>2.10 ± 0.04</td>
<td>2.14 ± 0.05</td>
</tr>
<tr>
<td>15 dat</td>
<td>2.04 ± 0.09</td>
<td>2.35 ± 0.03</td>
</tr>
</tbody>
</table>

Supplemental table 3: Primer list for RT-QPCR experiments.
References


Molecular and morphological characterisation of the floral transition in Arabidopsis thaliana induced by photoperiodic switch


