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A long photoperiod relaxes energy management in *Arabidopsis* leaf six

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ABSTRACT

Plants adapt to the prevailing photoperiod by adjusting growth and flowering to the availability of energy. To understand the molecular changes involved in adaptation to a long-day condition we comprehensively profiled leaf six at the end of the day and the end of the night at four developmental stages on *Arabidopsis thaliana* plants grown in a 16 h photoperiod, and compared the profiles to those from leaf 6 of plants grown in a 8 h photoperiod. When *Arabidopsis* is grown in a long-day photoperiod individual leaf growth is accelerated but whole plant leaf area is decreased because total number of rosette leaves is restricted by the rapid transition to flowering. Carbohydrate measurements in long- and short-day photoperiods revealed that a long photoperiod decreases the extent of diurnal turnover of carbon reserves at all leaf stages. At the transcript level we found that the long-day condition has significantly reduced diurnal transcript level changes than in short-day condition, and that some transcripts shift their diurnal expression pattern. Functional categorisation of the transcripts with significantly different levels in short and long day conditions revealed photoperiod-dependent differences in RNA processing and light and hormone signalling, increased abundance of transcripts for biotic stress response and flavonoid metabolism in long photoperiods, and for photosynthesis and sugar transport in short photoperiods. Furthermore, we found transcript level changes consistent with an early release of flowering repression in the long-day condition. Differences in protein levels between long and short photoperiods mainly reflect an adjustment to the faster growth in long photoperiods. In summary, the observed differences in the molecular profiles of leaf six grown in long- and short-day photoperiods reveal changes in the regulation of metabolism that allow plants to adjust their metabolism to the available light. The data also suggest that energy management is in the two photoperiods fundamentally different as a consequence of photoperiod-dependent energy constraints.

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1. Introduction

Plants as light-dependent, autotrophic organisms have adapted to the regular light–dark cycles resulting from the rotation of the earth. The length of the light period, or photoperiod, depends on the latitude and time of the year. Plants must adjust to changes in day-length to optimize growth in varying photoperiod lengths. Although this requires tight control of physiological and molecular processes, the underlying regulatory mechanisms are still poorly understood. It is now well established that the circadian clock synchronizes metabolism with the changing photoperiods [1–4]. Photoperiod length affects net daily photosynthesis and starch metabolism [5,6] and adjusts seasonal growth [7–9]. However, the molecular integration of photoperiod, clock and metabolic control during leaf development remains a challenging problem.

Arabidopsis is a facultative long-day plant whose flowering is controlled by the photoperiod pathway [7,8,10,11] in concert with molecular, hormonal and environmental signals [10]. Interactions between the circadian clock and photoperiod length during vegetative growth affect leaf number and size, as well as their morphological and cellular properties [12–16]. Plants in which the vegetative to floral growth transition is accelerated by increasing day-length or repression of regulatory genes have fewer leaves, increased single leaf areas, and a higher epidermal cell number in individual leaves compared to late flowering plants [12,15,16]. While these adaptations to photoperiod are well documented at the phenotypic level, little is known about how concerted regulation of photoperiod-dependent gene expression and protein levels is achieved during diurnal cycles and at different stages of leaf development.

We therefore asked how phenotypic changes are related to molecular profiles in a single leaf of *Arabidopsis* plants growing in a long-day (LD; 16 h light, 8 h dark) or short-day (SD; 8 h light, 16 h dark) condition. These two photoperiods cause consistent phenotypic changes in the number and morphology of successive leaves on the rosette [12,16]. Because size and shape of successive leaves vary during *Arabidopsis* development [17] we decided to focus the analysis on leaf number 6, which is the first adult leaf of the *Arabidopsis* (Col-4) rosette in short-day conditions. Leaf 6 was used previously to generate molecular data for *Arabidopsis* grown in SD [18]. To gain insights into the molecular pattern underlying the phenotypic changes between photoperiods, we therefore analyzed transcript and protein levels of leaf number 6 grown in LD at four developmental stages, both at the end of the day (EOD) and end of the night (EON). We then compared the data with the corresponding previously established molecular data for leaf 6 of *Arabidopsis* grown in SD either under optimal watering (SOW) or a 40% water deficit (SWD) [18]. Integration and comparative analyses of the quantitative proteomics and transcriptomics data revealed that fewer genes have significant diurnal transcript level fluctuations in LD than SD. Transcripts and proteins with significantly different levels in SD and LD validate the hypothesis that a short photoperiod requires a tight energy management, which is relaxed in a long photoperiod.

2. Material and methods

2.1. Plant material, leaf 6 and rosette growth measurements

Arabidopsis thaliana accession Col-4 (N933) plants were grown in a growth chamber equipped with the PHENOPSIS automaton [19] as described previously [18] with the exception that day length in the growth chamber was fixed at 16 h. In brief, seeds were sown in pots filled with a mixture (1:1, v/v) of a loamy soil and organic compost at a soil water content of 0.3 g water/g dry soil and just before sowing 10 ml of a modified one-tenth-strength Hoagland solution were added to the pot surface. After 2 days in the dark, day length in the growth chamber was adjusted to 16 h at ~220 μmol/m^2/s incident light intensity at the canopy. Plants were grown at an air temperature of 21.1 °C during the light period and 20.5 °C during the dark period with constant 70% humidity. During the germination phase water was sprayed on the soil to maintain sufficient humidity at the surface. Beginning at plant germination, each post was weighed twice a day to calculate the soil water content, which was adjusted to 0.4 g water/g dry soil by the addition of appropriate volumes of nutrient solution. The experiment was repeated independently three times and each leaf 6 sample was prepared by bulking material from numerous plants. The frozen plant material was sent to the MPI in Golm, where it was ground and aliquotted using a cryogenic grinder (German Patent No. 8146.0025U1).

Growth-related traits of leaf 6 at single leaf and cellular scales were measured as described [20]. Five rosettes were harvested and dissected every 2–3 days during each experiment. Leaf 6 area [mm^2] was measured after imaging with a binocular magnifying