

Photoperiodic growth control in perennial trees

Abdul Azeez^{1,*} and Aniruddha P Sane²

¹Plant Molecular Biology Laboratory; Jain R&D Jain Irrigation Systems Ltd; Maharashtra, India; ²CSIR-National Botanical Research Institute; Lucknow, India

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Plants have to cope with changing seasons and adverse environmental conditions. Being sessile, plants have developed elaborate mechanisms for their survival that allow them to sense and adapt to the environment and reproduce successfully. A major adaptive trait for the survival of trees of temperate and boreal forests is the induction of growth cessation in anticipation of winters. In the last few years enormous progress has been made to elucidate the molecular mechanisms underlying SDs induced growth cessation in model perennial tree hybrid aspen (*Populus tremula* × *P. tremuloides*). In this review we discuss the molecular mechanism underlying photoperiodic control of growth cessation and adaptive responses.

Introduction

In temperate regions, the advent of winter is associated with a gradual fall in temperature and day length. Most of the perennial trees anticipate the advent of winter by sensing the reduction in day length while some trees such as apple and pear utilize a reduction in temperature as a signal for the advent of winter. Yet others use a combination of both day length and temperature signal to prepare themselves for winter.¹⁻³ Trees of temperate climates perceive SDs signal and respond by ceasing growth well in advance of winter and protect their apical meristem and leaf primordia within an apical bud.⁴ In the last decade several studies have been carried out to unveil the molecular mechanism of photoperiodic controlled growth cessation and adaptation using the perennial tree hybrid aspen (*Populus tremula* × *P. tremuloides*) as a model plant.⁵⁻⁸ In this review, we discuss photoperiodic control of growth cessation and adaptive response in such trees and describe the unexpected similarities between photoperiodic growth cessation program in trees and flowering time control in model plant *Arabidopsis thaliana*.

Environmental consequences on seasonal plant growth

Perennial trees with life spans of a few to several hundred years are among the longest living organisms on the earth. One of the keys to their survival is the evolution of highly complicated and sensitive mechanisms to perceive changing environment and modulate their growth accordingly. In boreal and temperate

regions where temperatures drop below -30°C during winter, perennial trees stop their growth well ahead of winter to ensure their survival. During autumn, when the winter is approaching, both day length and temperature fall gradually. However, there can be considerable variations in the temperatures in a given season in each year and hence the fall in temperature is not as robust a cue for approaching winter. In contrast, the daily fall in day length prior to winter is constant year after year and is therefore a major robust cue for plants in boreal and temperate regions to understand that winter is approaching in contrast to the fall in temperature as a signal. When day length falls below a critical value, plants undergo growth cessation and stop making new leaves and form an apical bud to protect the Shoot Apical Meristem (SAM). The critical day length value that determines the timing of growth varies with geographical area and comes earlier at higher latitudes⁹ which face winters earlier. Since it is critical for plants to get the maximum period for their development and reproduction and also enough time for undergoing adaptations to survive the winter, the timing of growth cessation is a trait of major adaptive significance.

In addition to growth cessation, the perennating organs like SAM and leaf primordia, enclosed within the apical bud, initiate physiological processes such as acquisition of cold hardiness (cumulatively referred to as adaptive response) in order to cope with extremely low temperatures (LTs) of temperate and boreal region that are crucial for their survival.⁴ Without cold hardiness, plants may be subjected to serious injuries due to inter-cellular ice formation which may damage the cellular membrane during winter. LT may also inactivate enzymes thereby affecting essential physiological processes like respiration, photosynthesis etc.¹⁰⁻¹⁴ Indeed, failure to undergo growth cessation and acquire cold hardiness severely compromises survival as shown in hybrid aspen plants that fail to sense or respond to SDs.¹⁵ Additionally, following growth cessation, SAM and leaf primordia acquire dormancy which is also induced by SDs. Dormancy is considered to be part of the mechanism contributing to survival by preventing precocious activation of growth before spring. In contrast to growth cessation, photoperiodic control of dormancy remains poorly understood and therefore this review focuses primarily on photoperiodic control of growth cessation and its co-ordination with acquisition of cold hardiness.

Molecular mechanism underlying SDs induced growth cessation and adaptation

Plants modulate their growth in response to changes in day length by receiving light signals through photoreceptors like

*Correspondence to: Abdul Azeez; Email: azeezabdul78@gmail.com
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phytochromes that detect red (R)/far-red (FR) light and cryptochromes that detect blue light signal. Molmann et al.¹⁶ investigated the impact of light qualities on growth cessation and bud set of Norway spruce (*Picea abies*). They have shown that blue light treatment delays growth cessation and bud set but cannot prevent it completely. Phytochromes have been shown to mediate short day induced growth cessation and bud set in *Populus*.¹⁷ PHYTOCHROME A (PHY A) seems to be important in the perception of short days and the subsequent response. Transgenic poplar (hybrid aspen) ectopically expressing oat *PHY A* had a perturbed SDs response and thus failed to cease growth and bud set.^{15,18} On the other hand, down regulation of endogenous *PHY A* in hybrid aspen led to an early response to SDs and an earlier bud set than control plants.¹⁸

However, the major breakthrough in how photoperiod controls seasonal growth in trees came through analysis of tree orthologs of Arabidopsis flowering time regulator *FT*. The expression of *FT* is modulated by photoperiod with long days inducing the transition from vegetative phase to reproductive phase through

expression of *FT* in Arabidopsis and other plants.¹⁹⁻²¹ *FT* is regulated by CONSTANS (CO) protein in a photoperiod dependent manner. *CO* expression displays a diurnal expression pattern with a peak toward the end of the day. In SDs (long nights) *CO* expression peaks at night. Since *CO* protein is highly unstable in dark, *CO* can only induce *FT* expression, and thereby flowering, when days are long, the *CO* protein is stable and accumulates at levels high enough to activate *FT*.²²

The CO/*FT* module also plays a central role regulating SDs mediated growth cessation in perennial trees.⁵ After SDs signal perception in trees, *FT2* down-regulation is required to induce growth cessation.²³ Transgenic poplar over-expressing either *FT1* or *FT2* show a perturbed SD response that abolishes growth cessation and bud set. In contrast, downregulation of *FT2* results in an earlier SD response and plants show early growth cessation and bud set in comparison to control plants.^{7,24} In addition to down-regulation of *FT*, additional components also contribute to regulation of the timing of growth cessation since hybrid poplar clones that display similar downregulation of *FT* expression nevertheless differ in their timing of growth cessation in response to SDs.²⁵

Until recently, the downstream targets of CO/*FT* module in SD mediated growth cessation were not known. Moreover, since *FT* by itself lacks DNA binding ability, it was not clear how *FT* would control the downstream targets. A large scale screen designed to identify new factors involved in photoperiodic growth cessation identified the tree ortholog of the MADS-box protein floral meristem identity gene *APETALA1* (*API*), noted as *LAPI* (*Like-API*), as the main downstream target of CO/*FT* module.⁷ Like *FT1* and *FT2* over-expressors, *LAPI* over-expressors demonstrated a perturbed SDs response and failed to cease growth and bud set, whereas RNAi-mediated down-regulation of *LAPI* led to an earlier response to SDs and earlier growth arrest and bud set than control plants.⁷ Moreover, *LAPI* was shown to be regulated by *FT* and its downregulation could suppress the phenotype of *FT* over-expressors clearly indicating *LAPI* as a key regulator in SDs mediated growth cessation in hybrid aspen.⁷ Furthermore, *LAPI* was found to control the expression of a key cell

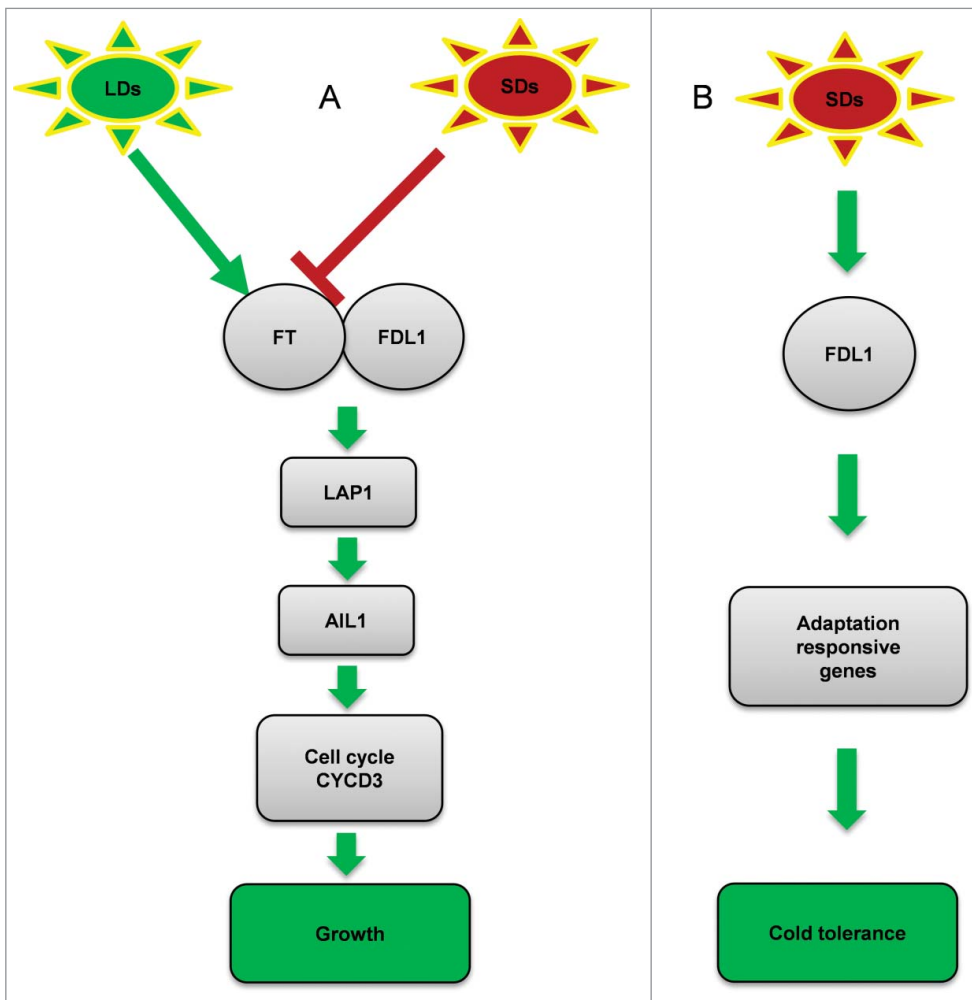


Figure 1 Model for photoperiodic growth control in perennial trees (adopted from Azeez et al.⁷ and Tylewicz et al.⁸)

cycle regulator *AINTEGUMENTA-LIKE1* (*AIL1*), thereby explaining how *FT* down-regulation by SDs could induce growth cessation. These observations lead us to suggest that day length mediates 2 different developmental pathways namely flowering in annuals such as *Arabidopsis* and growth cessation in trees. These two pathways are conserved till AP1 and diverge downstream of AP1/LAP1 rather than FT.

Another recent finding is that the flowering time regulator FD, a partner of FT, is also involved in photoperiodic control of seasonal growth. Since FT lacks DNA binding ability it was an open question as to how FT could control *LAP1* expression. Although FT lacks DNA binding capacity, it makes a complex with the bZIP transcription factor FLOWERING LOCUS D (FD) and the protein 14-3-3 to make a Florigen Activating Complex (FAC) that binds the regulatory region of the downstream component to induce transition to flowering in the SAM.²⁶ Recently, 2 closely related FD homologs, *FDL1* (*FD-LIKE1*) and *FDL2* (*FD-LIKE2*) with distinct roles have been identified in hybrid aspen.⁸ FDL1 makes a complex with FT2 and mediates photoperiodic control of growth through control of *LAP1* which is the downstream target of this complex.

Intriguingly, FDL1 is also involved in SDs induced transcriptional changes in adaptive responses and bud maturation of these 2 pathways.⁸ Functional analysis of *FDL1* showed that in over-expressors, expression of the adaptive response marker genes such as *OSM* (*OSMOTIN*) and *LEA* (*LATE EMBRYOGENESIS ABUNDANT*), was much higher than in control plants after SDs treatment, whereas in FDL1-RNAi plants, the expression of these marker genes was lower than in control plants after SDs.⁸ SDs induced bud maturation is accompanied by accumulation of the phenylpropanoids in the bud scales that gives a dark color to the bud and functions as a good marker. The expression of phenylpropanoid marker genes such as *CHS* (*CHALCONE SYNTHASE*), *C4H* (*CINNAMATE 4-HYDROXYLASE*) was up-regulated after SDs in control plants but not as prominently in *FDL1* over-expressors after SDs. The buds in *FDL1* over-expressors were also greener compared to controls. FDL1-RNAi plants showed the opposite phenotype,⁸ suggesting the role of FDL1 in SDs mediated transcriptional control of adaptive responses and bud maturation pathways.

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Conclusions

Timing of growth cessation before the advent of winter in perennial trees of temperate and boreal forests is a major adaptive trait. Photoperiodic signals are perceived in leaves and transduced via CO/FT module to control growth. Repression of *FT2* expression is required to induce SDs mediated growth cessation and bud set. Expression of the MADS-box transcription factor *LAP1* is positively regulated by FAC, while reduction in *FT* expression causes reduction in *LAP1* expression which results in the down-regulation of *AIL1*. Since core cell-cycle genes are positively regulated by *AIL1*, its down-regulation results in reduction in expression of core cell-cycle genes which induces growth cessation and bud set. Based on the molecular information available for photoperiodic growth control in perennial trees we propose a model which is adopted from Azeez et al.⁷ and modified according to Tylewicz et al.⁸ (Fig. 1A). According to this model, short days lead to growth cessation through suppression of FTs but SDs mediated transcriptional control of adaptive responses to cold and bud maturation pathways requires FDL1 (Fig. 1B).

Although the major components of SDs mediated growth control are now known, the site of signal perception is not yet understood. FT in *Arabidopsis* is expressed in leaves and then transported to the shoot apex where it induces flowering. But there is still no information on FT movement in trees to regulate growth. Another major lacuna is regarding hormonal control of photoperiodic growth. For example, gibberellins (GA) and auxin²⁷ play a role in photoperiodic control of growth but the interplay between CO/FT and GA (or auxin) signaling is still not entirely clear. These and other questions not covered here such as the role of clock will hopefully be answered in the coming years.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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