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Phytochrome B and PHYTOCHROME INTERACTING FACTOR8 modulate seasonal growth in trees

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Summary

- The seasonally synchronized annual growth cycle that is regulated mainly by photoperiod and temperature cues is a crucial adaptive strategy for perennial plants in boreal and temperate ecosystems.
- Phytochrome B (phyB), as a light and thermal sensor, has been extensively studied in *Arabidopsis*. However, the specific mechanisms for how the phytochrome photoreceptors control the phenology in tree species remain poorly understood.
- We characterized the functions of *PHYB* genes and their downstream PHYTOCHROME INTERACTING FACTOR (PIF) targets in the regulation of shade avoidance and seasonal growth in hybrid aspen trees. We show that while phyB1 and phyB2, as phyB in other plants, act as suppressors of shoot elongation during vegetative growth, they act as promoters of tree seasonal growth. Furthermore, while the *Populus* homologs of both *PIF4* and *PIF8* are involved in the shade avoidance syndrome (SAS), only PIF8 plays a major role as a suppressor of seasonal growth.
- Our data suggest that the *PHYB-PIF8* regulon controls seasonal growth through the regulation of *FT* and *CENL1* expression while a genome-wide transcriptome analysis suggests how, in *Populus* trees, phyB coordinately regulates SAS responses and seasonal growth cessation.

Introduction

In boreal and temperate ecosystems, perennial woody trees have evolved an adaptive mechanism that allows them to survive harsh winter conditions by alternating between active growth and dormancy according to seasonal climate changes. Photoperiod and temperature are the primary environmental cues regulating the seasonal synchronization of the critical developmental transitions in the annual growth cycle (Singh *et al.*, 2017). In the model plant hybrid aspen, the timing of growth cessation in the autumn is governed primarily by photoperiod, while temperature controls the bud break in spring. Studies have shown that seasonal growth cessation and flowering time in perennial plants share conserved genetic pathways controlled by *FLOWERING LOCUS T* (*FT*) genes (Ding & Nilsson, 2016). *FT* genes promote flowering and suppress short-day (SD)-induced growth cessation and bud set (Bohlenius *et al.*, 2006). The *CONSTANS/FLOWERING LOCUS T* (*CO/FT*) module plays a central role in the photoperiodic control of Arabidopsis' flowering time (Valverde *et al.*, 2004). Interestingly, in *Populus*, GIGANTEA (GI) plays a critical role in SD-induced growth cessation via *FT2* in a *CO*-independent manner (Ding *et al.*, 2018). Downstream of *FT2* and *FD-LIKE1* (*FDL1*), LIKE-APETALA1 (LAP1) directly triggers the expression of *AINTEGUMENTA-LIKE 1* (*AIL1*) to promote the cell-cycle progression, which antagonizes the SD-induced growth cessation (Kaufmann *et al.*, 2010; Karlberg *et al.*, 2011; Azeez *et al.*, 2014). Another target gene of *LAP1*, *BRANCHED1* (*BRC1*), triggers growth cessation by antagonizing FT2 function via protein–protein interaction. After growth cessation, the continuation of SDs induces bud dormancy establishment before winter (Maurya *et al.*, 2020). Recent studies have shown that abscisic acid (ABA) promotes dormancy establishment by inducing expression of *SHORT VEGETATIVE PHASE-LIKE* (*SVL*). SVL then upregulates the expression of *CALLOSE SYNTHASE 1* (*CALS1*) to restrict access to growth-promotive signals via plasmodesmata closure (Tylewicz *et al.*, 2018).

Once dormancy is established, buds no longer respond to growth-promotive signals unless exposed to an extended period of cold, following which growth can be reactivated by warm temperature as visibly manifested by bud break. Also, SVL regulates bud break by antagonistically acting on the ABA and gibberellic acid (GA) pathways, which are the two crucial plant hormones involved in seasonal growth (Singh *et al.*, 2018, 2019). A functional divergence has been suggested for the two paralogous *FT* genes in *Populus* (Bohlenius *et al.*, 2006; Hsu *et al.*, 2006, 2011). While *FT2* plays a critical role in growth cessation (Bohlenius *et al.*, 2006), *FT1* is strongly induced by cold in the winter (Hsu *et al.*, 2011; Rinne *et al.*, 2011, 2018; Singh *et al.*, 2018) and has

been hypothesized to control both reproductive onset (Hsu *et al.*, 2011) and to be involved in the release of dormancy and to stimulate bud break (Rinne *et al.*, 2011, 2018; Singh *et al.*, 2018). Other genes that have been found to be involved in regulating the trees' seasonal bud break include *CENTRORADIALIS-LIKE 1* (*CENL1*)/*TERMINAL FLOWER 1* (*TFL1*) and *EARLY BUD BREAK 1* (*EBB1*) (Mohamed *et al.*, 2010; Yordanov *et al.*, 2014).

So far, the specific mechanisms for how the phytochrome photoreceptors control the regulation of seasonal growth and dormancy in perennial trees remain poorly understood. Alterations in *PHYTOCROME A* (*PHYA*) expression levels affect the timing of growth cessation and bud set in hybrid aspens (Olsen *et al.*, 1997; Kozarewa *et al.*, 2010), while *PHYB2* has been reported to be associated with quantitative trait loci (QTLs) controlling growth cessation and bud set in *Populus* (Frewen *et al.*, 2000), It is also known that red (R) or far red (FR) light night breaks can inhibit SD-induced growth cessation (Howe *et al.*, 1996). These findings suggest that phytochromes have important roles in photoperiod-regulated seasonal growth in trees. However, the molecular function of *PHYB* genes in the photoperiodic response remains to be characterized in trees. In *Arabidopsis thaliana* (Arabidopsis), phyA promotes flowering by stabilizing the CO protein, while phyB destabilizes CO to inhibit floral initiation (Lin, 2000; Yanovsky & Kay, 2002; Cseke *et al.*, 2003; Valverde *et al.*, 2004). Interestingly, as either FR or R light can suppress SD-induced growth cessation in *Populus* (Howe *et al.*, 1996), and CO appears to play a minor role in the day-length response (Hsu *et al.*, 2012), this indicates an evolutionary divergence in the photoperiodic pathways between Arabidopsis and *Populus*. What, then, are the downstream factors in the phytochrome-controlled growth cessation? In Arabidopsis, the perception of light signals by phytochromes induces transcriptional network responses via phytochrome-interacting factors (PIFs) (Leivar & Quail, 2011; Legris *et al.*, 2016; Pham *et al.*, 2018). PIFs are inhibitors of phytochrome-induced responses, and phytochromes act by inhibiting PIF activity (Lorrain *et al.*, 2008; Leivar *et al.*, 2008, 2012). In Arabidopsis, PIF4, PIF5 and PIF7 promote hypocotyl and petiole elongation in response to shade (low R : FR ratio) which is part of the shade avoidance syndrome (SAS) (Lorrain *et al.*, 2008; Li *et al.*, 2012; Mizuno *et al.*, 2015). PIF4, PIF5 and PIF7 are also activators of shade-induced flowering through positive regulation of the expression of *FT* and its close homolog *TWIN SISTER OF FT* (*TSF*) (Kumar *et al.*, 2012; Galvao *et al.*, 2019; Zhang *et al.*, 2019). Besides, phyB also functions as a thermosensor in Arabidopsis (Jung *et al.*, 2016; Legris *et al.*, 2016). And both PIF4 and PIF7 play roles in thermomorphogenesis (Quint *et al.*, 2016; Fiorucci *et al.*, 2020). Hence it is important to investigate the role of PIFs in SD-induced growth cessation and warm temperature-induced bud break in trees.

Here we characterized the functions of *PHYB* genes and their downstream targets PIF4 and PIF8 in regulating seasonal growth in hybrid aspen. We show that phyB1 and phyB2 act as promoters of seasonal growth and that PIF8 has an important role in the same pathway as a suppressor, while PIF4 only has a minor role. We found that the *PHYB-PIF8* regulon controls SD-induced growth through the regulation of *FT2* expression, while it affects thermal-induced bud break, possibly through regulation of the expression of *FT1* and *CENL1* genes. Furthermore, genome-wide transcriptome analysis suggests that in *Populus* trees, phyB coordinately regulates the expression of genes involved in the SAS responses and seasonal growth cessation.

Materials and Methods

Plant material and growth conditions

Hybrid aspen *Populus tremula* × *tremuloides*, clone T89, were used as wild-type (WT) control and plant transformation (Nilsson *et al.*, 1992). Plants were grown in controlled-growth chambers at 21°C under long days (18 h : 6 h, light : dark; LD^{18h}) or SDs (14 h : 10 h, light : dark; SD^{14h}). Illumination was from 'Powerstar' lamps (HQI-T 400W/D BT E40; Osram, Munich, Germany) giving an R/FR ratio of 2.9. After 12 wk of SDs, plants were exposed to low temperatures (4°C, SD^{8h}) for 8 wk to release dormancy and subsequently to the warm LD conditions (21°C/LD^{18h}).

Generation of vector constructs and transformation

For *PHYB*RNAi vector construction, a fragment similar to both *PHYB1* and *PHYB2* was amplified from hybrid aspen with the primers dsphyBF/dsphyBR and cloned into the pDONR201 vector (Invitrogen), and then transferred to the final destination vector pK7GWIWG2(I) or pH7GWIWG2(I) (Karimi *et al.*, 2002). For the construction of oe*PHYB1* and oe*PHYB2* overexpression vectors, full-length cDNAs of *PHYB1* and *PHYB2* were amplified with two pairs of primers oxphyB1F/R and oxphyB2F/R respectively and cloned into the entry vector pDONR201. The fragments were then transferred to the destination vector pK2GW7 (Karimi *et al.*, 2002). *PHYB1*KO and *PHYB2*KO trees were generated by CRISPR/Cas9-mediated gene editing using vectors from the golden gate-based GreenGate cloning system (Lampropoulos *et al.*, 2013). The final constructs contain p*35S::AthCas9:trbcs* assembled from GreenGate modules A, B and C, the sgRNAs under the control of the *A. thaliana U6* promoter (GreenGate modules D and E), and a *pNOSk::KanRl:tNOS* cassette (GreenGate module F). All the modules were finally cloned into the destination vector pGreen-IIS backbone.

To generate overexpression construct of oe*PIF4a* and oe*PIF8a*, full-length cDNAs of *PIF4a* and *PIF8a* were amplified from hybrid aspen T89 leaves using the primer pairs, oxPIF4aF/oxPIF4aR and oxPIF8aF/oxPIF8aR. The PCR products were cloned into entry vector pDONR201. The fragment was then transferred to destination vector pK2GW7. To construct *PIF4*RNAi and *PIF8*RNAi plants, fragments were amplified using primer pairs dsPIF4F/dsPIF4R and dsPIF8F/dsPIF8R by using the full-length *PIF4a* and *PIF8a* cDNAs as a template, respectively. Similarly, PCR products were cloned into pDONR201 and then transferred to the destination vector pK7GWIWG2 (I) (Karimi *et al.*, 2002). All these constructs were individually transformed into the Agrobacterium strain GV3101

(pMP90RK). Primers used for construct generation are listed in Supporting information Table S1. All cloning reactions were performed according to the manufacturer's instructions. Hybrid aspen was transformed as previously described (Nilsson *et al.*, 1992).

Induction of growth cessation, bud dormancy and bud flush scoring

Plants were grown in a growth chamber for *c.* 4 wk in LD conditions (LD^{18h}, at 21°C) and then shifted to SD conditions (SD^{14h}, at 21°C). Scoring of growth cessation and bud set started after transfer to SD conditions. For scoring of bud break, after 12 wk of SD treatment, plants were transferred to a cold room with short days (SD^{8h}, 4°C) for 8 wk and returned to warm LD conditions (LD^{18h}, 21°C). Bud set and bud break were scored as previously described (Rohde *et al.*, 2011). Two or three independent and representative transgenic lines and eight plants of each line were analyzed.

RNA-seq sampling and bioinformatics

*PHYB*RNAi and WT plants were grown in controlled-growth chambers for *c.* 4 wk (LD^{18h}, 21°C) and then transferred to short days (SD^{14h}, 21°C) in growth chambers. Leaf and shoot apices were collected from LD^{18h} and 2 wk after SD^{14h} treatments from three independent plants of each genotype at 4 h after light (ZT4). The 10^{th} and 11^{th} fully expended leaves counted from the top were taken. A total of eight different types of samples were taken. Samples were named sequentially by genotype (T = WT, R = *PHYB*RNAi), day length (L, LD; S, SD) and tissue (L, leaf; S, shoot), giving the following abbreviations: TLL, WT-LD-leaf; RLL, *PHYB*RNAi-LD-leaf; TLS, WT-LD-shoot; RLS, *PHYB*RNAi-LD-shoot; TSL, WT-SD-leaf; RSL, *PHYB*RNAi-SD-leaf; TSS, WT-SD-shoot; RSS, *PHYB*RNAi-SD-shoot. For transcriptome analysis of oe*PIF8a*, apical dormant buds of oe*PIF8a* and WT plants treated for 2 months in a cold room (SD^{8h}, 4°C) were collected. Total RNA was extracted using the CTAB-LiCl method (Xu *et al.*, 2009) and was then quantified using a Nanodrop 1000 (Thermo Scientific, Waltham, MA, USA). The quality was assessed with the Agilent 2100 Bioanalyzer using Pico chips (both Agilent Technologies, Chandler, AZ, USA). Samples were sequenced at the National Genomic Infrastructure (NGI) from SciLife Laboratory (Uppsala, Sweden) on an Illumina HiSeq2500 in a paired-end 125 bp read length. The RNA-seq data were analyzed using a previously developed pipeline for quality control, read mapping and expression quantification (Rahmatallah *et al.*, 2016). RNA-seq data were filtered by presence criteria and subjected to ANOVA. Genes with a false discovery rate < 0.05 and fold-change > 2 were selected for further analysis.

RNA quantification

For diurnal expression pattern analysis of genes in this study, leaves from transgenic and WT plants were grown in LD^{18h} conditions and transferred to SD^{14h} conditions for 2 wk before the start of sampling. For dormancy samples, shoot apical buds were collected at different stages of dormancy. Total RNA were extracted with the CTAB-LiCl method (Xu *et al.*, 2009). cDNA synthesis was performed using the iScript cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's instructions. Quantitative real-time PCR analyses were carried out with a Roche LightCycler 480 II instrument, and the measurements were obtained using the relative quantification method (Livak & Schmittgen, 2001). Results were normalized to the expression of *UBQ* RNA. A complete list of primers used in real-time PCR analysis is presented in Table S1.

Protein extraction and immunoblot

Protein extraction and immunoblots were carried out as previously described (Zhang *et al.*, 2017). In brief, protein was extracted with extraction buffer (0.1% w/v sodium dodecyl sulfate, 0.1% v/v Triton X-100, 1 mM phenylmethylsulfonyl fluoride (Sigma), 14 mM 2-mercaptoethanol, and 2× complete protease cocktail (Roche)). Immunodetection of PIF8a was performed using the 16B12 anti-HA-POD monoclonal antibody (Roche), and a SuperSignal West Femto chemiluminescence kit (Pierce, Rockford, IL, USA) was used for the detection of the band signals. The intensities of Western blot band signals were collected from a LAS-3000 Imaging System (Fujifilm, Tokyo, Japan). Rubisco bands displayed in the gel were used as a control.

Results

phyBs affect hybrid aspen vegetative growth

As previously shown for *Populus* species (Howe *et al.*, 1998; Olsen, 2010; Karve *et al.*, 2012), the *P. tremula* genome contains three phytochrome-like genes: *PHYA*, *PHYB1* and *PHYB2* (Fig. S1). To address the functions of phyB in *Populus*, we generated transgenic hybrid aspen plants where expression of *PHYB1* and *PHYB2* were either downregulated (*PHYB*RNAi) or increased (oe*PHYB1* and oe*PHYB2*) (Fig. S2a–c). *PHYB*RNAi lines displayed a constant SAS response under LD^{18h} growth conditions, including longer internodes, smaller leaf lamina size, and hyponastic growth compared with WT plants (Figs 1a, S3). By contrast, both the oe*PHYB1* and oe*PHYB2* lines showed shorter internodes and reduced hyponastic growth (Figs S3, S4a). The overexpressing plants also displayed an increased sylleptic branching compared with the WT plants (Fig. S4b). These results suggest that hybrid aspen *PHYBs* are negative regulators of shoot elongation during vegetative growth and provoke a SAS response when downregulated, consistent with their role in other plants (Franklin & Quail, 2010).

phyBs suppress SD-induced growth cessation and bud set

PHYB2 was previously reported to coincide with a QTL associated with variation in bud set timing in *P. trichocarpa* × *deltoides* (Frewen *et al.*, 2000). Therefore, we carried out a detailed study under controlled-growth conditions to investigate phyB function

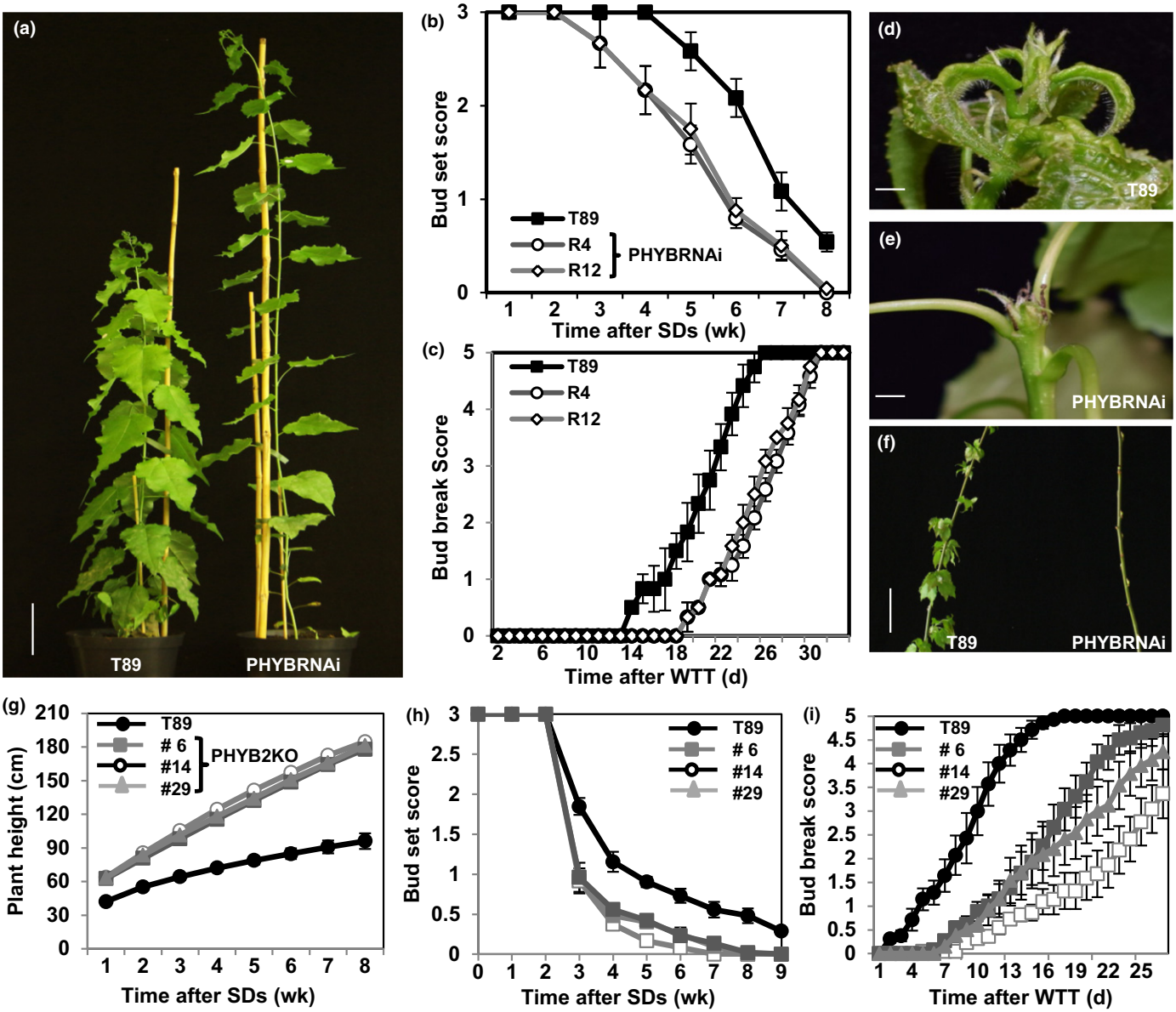


Fig. 1 Altered expression of *PHYBs* affects seasonal growth in hybrid aspen. (a) Overview of *PHYB*RNAi and wild-type (WT) plants grown in long-day conditions (18 h : 6 h, light : dark; LD^{18h}) for 2 months. Bar, 10 cm. (b) Bud set score of *PHYB*RNAi and WT plants after transfer from LD^{18h} to short-day conditions (14 h : 10 h, light : dark; SD^{14h}). (c) Bud break score (Rohde *et al*., 2011) of *PHYB*RNAi and WT plants after transfer from cold, SD conditions (8 h 4°C : 16 h 4°C, light : dark; SD^{8h}, 4°C) to warm, LD conditions (LD^{18h}, 21°C). Data shown are mean values from six plants of each line. Error bars represent ±SE. (d, e) Shoot apices of WT and *PHYB*RNAi plants after 25 d of SD treatment (SD^{14h}): while the WT is still in active growth (d) *PHYB*RNAi plants have formed a terminal bud (e). Bar, 0.2 mm. (f) Bud flush of WT and *PHYB*RNAi plants after 24 d of LD conditions (LD^{18h}), 21°C treatment. The WT has flushed its buds (f, left) while *PHYB*RNAi displays no signs of bud break (f, right). Bar, 10 cm. (g) Growth rates of *PHYB2*KO and WT plants under LD^{18}. (h, i) Bud set and bud break score of *PHYB2*KO plants. *PHYB2*KO trees display an earlier growth cessation and a delayed bud break compared with the WT. Data shown are mean values from nine plants of each line. Error bars represent ±SE. WTT, warm temperature treatment (18 h : 6 h, light : dark; LD^{18h}; 21°C).

during SD-induced growth cessation and bud set. WT and *PHYB*RNAi plants were first grown under LD^{18h} for 1 month and then transferred to 14 h short days (SD^{14h}). WT plants ceased growth 4 wk after the shift to SD (Fig. 1b). However, the *PHYB*RNAi plants displayed a hypersensitivity in their response to the day-length shift and started growth cessation about 2 wk earlier (Fig. 1b,d,e). Consistently, the oe*PHYB1* and oe*PHYB2* plants showed hyposensitivity with 1 wk later growth cessation compared with WT plants (Figs S4c, 4e–g; see later). This shows that phyBs act as suppressors of SD-induced growth cessation in hybrid aspen.

phyBs promote thermo-controlled bud break

In *Populus*, warm temperature triggers bud break in spring irrespective of photoperiod (Dillen *et al*., 2010). We investigated if the alteration in *PHYB* expression levels affects the thermoregulated bud break in hybrid aspen. After 12 wk of SD^{14h} treatment,

PHYB transgenic and WT plants were shifted to 4°C in SD^{8h} for 10 wk to release the dormancy. The trees were then returned to LD^{18h} at 21°C and scored for the timing of bud break. WT plants' buds flushed after 12 d in the warm temperature, and the shoots fully expanded after 24 d (Fig. 1c). By contrast, *PHYB*RNAi plants broke buds almost one week later than WT plants, and the buds fully expanded after 1 month (Fig. 1c,f). Consistently, the *oePHYB1* and *oePHYB2* transgenic trees initiated bud break 2–4 d earlier compared with WT plants (Fig. S4d,h). Overall, these results suggest that phyB promotes thermoregulated bud break in hybrid aspen. However, to what extent phyB functions as an actual thermosensor in *Populus*, similar to what has been suggested in Arabidopsis (Jung *et al.*, 2016; Legris *et al.*, 2016), needs to be investigated further.

phyB2 is the dominant hybrid aspen phyB

To further understand the *Populus PHYB* paralogs' individual roles, we generated single knockout mutants of *PHYB1* and *PHYB2*, respectively, using CRISPR/Cas9 technology (Fig. S2d). Similar to the *PHYB*RNAi plants, *PHYB2* knockout mutants (*PHYB2*KO) displayed a strikingly increased height growth compared with the WT (Fig. 1g), while *PHYB1* knockout mutants (*PHYB1*KO) displayed only a slight increase in height (Fig. S5a). We further monitored the seasonal growth cessation and bud break of these transgenic plants. Similarly, we only observed earlier SD-induced growth cessation and delayed bud break in the *PHYB2*KO plants (Fig. 1h,i), while the *PHYB1*KO plants behaved as the WT (Fig. S5b,c). To further investigate the role of *PHYB1*, we then generated *PHYB1B2* double knockout transgenic plants in the *PHYB2*KO background, using the same guide RNAs for the construction of single knockout plants (Fig. S2d). In all, 27 out of 34 independently regenerated transgenic shoots failed to form roots and died in tissue culture. However, we noticed that a few surviving *PHYB1B2*KO lines already terminated growth and formed a bud in tissue culture (Fig. S5d). Such a strong growth cessation phenotype in *PHYB1B2*KO plants is similar to that seen in *GI* knockdown (*GI*RNAi) plants described previously (Ding *et al.*, 2018), and is a much more marked response than in *PHYB2*KO plants. Overall, these results suggest that while *PHYB2* encodes the dominant phyB in controlling seasonal growth and elongation growth in *Populus* trees, phyB1 can at least partially compensate for the lack of phyB2 activity.

PIF8 is a major regulator of seasonal growth

As PIFs are key regulators of phytochrome-controlled photomorphogenic development in Arabidopsis (Ni *et al.*, 1998; Leivar & Monte, 2014), we wanted to investigate their role in the *PHYB*-dependent regulation of seasonal growth in *Populus* trees. Phylogenetic analysis of Arabidopsis and *Populus* PIFs identified 11 PIF-encoding genes in the *Populus* genome (Fig. S6a). Six of the proteins contain an active phytochrome binding (APB) domain, in other species shown to mediate interaction with light-activated phyB (Pham *et al.*, 2018), including PtPIF1, PtPIF3a, PtPIF3b, PtPIF4a, PtPIF8a and PtPIF8b (Fig. S6b). The phylogenetic analysis of *Populus* PIF proteins suggested that *Populus* lacks an AtPIF7 homolog (Fig. S6a). Instead, we identified two homologs of AtPIF8, called PtPIF8a and PtPIF8b. In Arabidopsis, PIF7 and PIF8 proteins showed different molecular behaviors upon light exposure. A recent study showed that AtPIF8 protein was stabilized by far-red light (FRc) but destabilized by red light (Rc) (Oh *et al.*, 2020). Unlike any other PIFs in Arabidopsis, AtPIF7 is a light-stable protein, although the function of PIF7 is similar to other PIFs (Leivar *et al.*, 2008). Like Arabidopsis PIF7, we found that PtPIF8a protein was light-stable in response to either Rc or FRc (Fig. S7). These results suggest an evolutionary divergence of the regulation of the PIF8 proteins.

We then generated RNAi and overexpressing transgenic plants of *PtPIF4* and *PtPIF8*, respectively (Fig. S8). *PIF4a* overexpression plants (oe*PIF4a*) grew normal in tissue culture but had a poor survival when transferred to soil (Fig. S9a). By contrast, plants with a downregulation of *PIF4* displayed a slight decrease in plant height, both in a WT and *PHYB*RNAi background (Fig. S9b). Likewise, while the *PIF8*RNAi plants displayed a decreased internode length and epinastic growth similar to oe*PHYB* plants (Figs 2a, S3), the oe*PIF8a* plants showed a strong SAS response similar to the *PHYB*RNAi lines (Figs 2a, S3). We then characterized their roles in seasonal growth. Downregulation of *PIF4* expression had no effect either on SD-induced growth cessation or thermal-controlled bud break (Fig. S9c–f). By contrast, downregulation of *PIF8* expression clearly delayed growth cessation and induced earlier bud break (Figs 2b–d, 2f–g), while oe*PIF8a* plants stopped growth earlier and flushed their buds later than WT plants (Figs 2b,c, 2e–g). These results suggest that while both *PIF4* and *PIF8* contribute to the regulation of shoot elongation and the SAS response, only *PIF8* has a major role in regulating seasonal growth.

The *PHYB-PIF8* module regulates the expression of *FT* and *CENL1* genes

Populus FT/CENL1 family genes have been found to play key roles in regulating seasonal growth. *FT2* is a key regulator of SD-induced growth cessation and bud set (Bohlenius *et al.*, 2006), while *FT1* and *CENL1* have been suggested to be involved in the regulation of dormancy release and bud break in hybrid aspen (Ruonala *et al.*, 2008; Mohamed *et al.*, 2010; Rinne *et al.*, 2011; Singh *et al.*, 2018). To test whether *PHYB-PIF8* affects growth cessation and bud set by regulating *FT2*, we analyzed the expression of *FT2* in *PHYB*RNAi, *PIF8*RNAi and oe*PIF8a* plants under LD^{18h} conditions and 2 wk after shifting to SD^{14h}. *FT2* displayed a diurnal expression pattern and was downregulated in both *PHYB*RNAi and oe*PIF8a* plants compared with WT plants (Fig. 3a–d). Two weeks of SD treatment induced a drastic downregulation of *FT2* expression in the WT, while *PIF8*RNAi plants still maintained high expression levels (Fig. 3d). Both *FT1* and *CENL1* have strong seasonal expression patterns. In the WT, they are specifically induced by cold treatment and are quickly repressed after a shift from cold (5°C) to warm temperature (Fig. 3e–h) (Rinne *et al.*, 2011; Ding *et al.*, 2018). However, both *FT1* and *CENL1* in *PHYB*RNAi and oe*PIF8a* plants

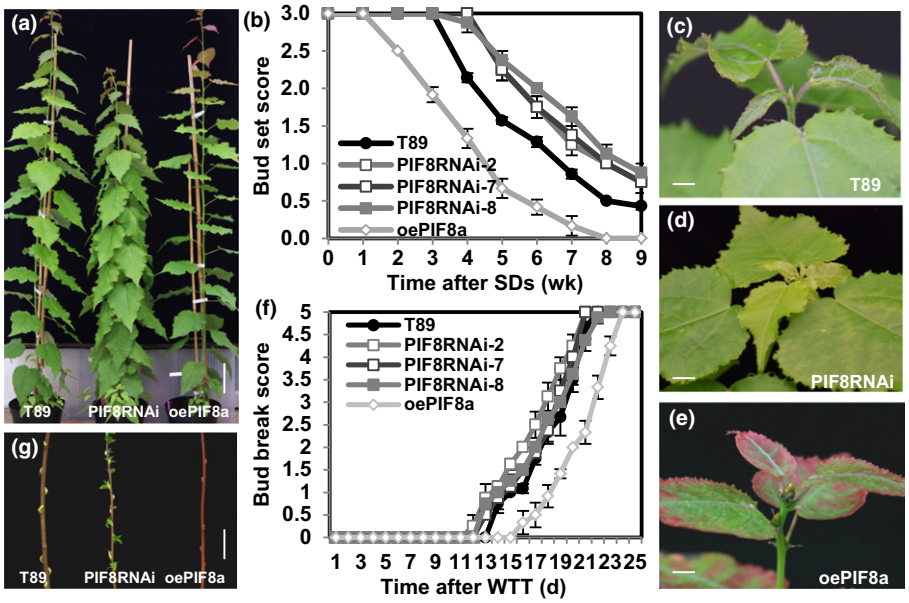


Fig. 2 Altered *PIF8* expression affects seasonal growth in hybrid aspen. (a) Overview of wild-type (WT) (left), *PIF8*RNAi (centre) and oe*PIF8a* (right) trees grown in long-day conditions (18 h : 6 h, light : dark; LD^{18h}) for 2 months. Bar, 10 cm. (b) Bud set score of WT, *PIF8*RNAi and oe*PIF8a* plants after transfer from LD^{18h} to short-day conditions (14 h : 10 h, light : dark; SD^{14h}). Data shown are mean values from six plants of each line. Error bars represent ±SE. (c–e) Shoot apices of WT, *PIF8*RNAi and oe*PIF8a* plants after 6 wk of SD^{14h} treatment: oe*PIF8a* has formed a terminal bud (e), WT has just started to set bud (c), while *PIF8*RNAi has not yet stopped growing (d). Bar, 0.4 mm. (f) Bud break score of WT, *PIF8*RNAi and oe*PIF8a* plants after transfer from cold, SD conditions (8 h 4°C : 16 h 4°C, light : dark; SD^{8h}, 4°C) to warm, LD (LD^{18h}, 21°C) conditions. The buds of *PIF8*RNAi plants break slightly earlier than the WT, while oe*PIF8a* plants break 1 wk later than the WT. Data shown are mean values from six plants of each line. Error bars represent ±SE. (g) WT (left), *PIF8*RNAi (centre) and oe*PIF8a* (right) trees after 2 wk of LD^{18h}, 21°C treatment. Bar, 10 cm.

maintained higher expression levels after 2 wk of warm temperature compared with the WT (Fig. 3e–h). On the contrary, they showed significantly lower expression in *PIF8*RNAi plants (Fig. 3f,h). Overall, these results suggest that the *PHYB-PIF8* module is involved in controlling seasonal growth, during both the autumnal growth cessation and spring bud break, possibly through the regulation of *FT2* and *FT1*/*CENL1* expression, respectively.

Transcriptome analysis reveals the coordination of SAS- and SD-induced growth cessation by *Populus* phyBs

Our genetic analysis results showed that phyBs have dual roles in regulating plant growth in *Populus* trees. As in other plants, they inhibit shade avoidance by repressing internode elongation, but they can also play a completely opposite role by suppressing the SD-induced growth cessation and bud set of shoots in the fall. We performed a transcriptome analysis to determine how phyBs coordinate the SAS response with SD-induced growth cessation. Leaves and shoot apices of WT and *PHYB*RNAi plants grown under LD^{18h} or SD^{14h} were taken for RNA-seq analysis. Principal component analysis (PCA) showed that the first principal component (explained 53.9% of the variance) separates tissue-specific samples (Fig. S10). In comparison, the second and third principal components (explaining 8.7% and 19.7% of the variance, respectively) separate the samples associated with the SD-induced growth cessation status in leaves and apices (Fig. S10). Differentially expressed (DE) genes among the treatments or genotypes ($P < 0.05$, fold-change > 2) were used for further analysis (Fig. 4a; Table S2). During active growth, we only identified 152 DE genes in the LD shoot apex samples of *PHYB*RNAi lines (Fig. 4a). By contrast, there were 1068 DE genes in leaves (Fig. 4a), suggesting that the SAS response in *PHYB*RNAi plants mainly affects the leaf transcriptome (Figs 1a, S3). Under SD conditions, the DE gene number increased significantly in both leaf and shoot apical samples, suggesting that downregulation of *PHYB* expression has largely altered the plant's perception of the SD signal (Fig. 4a). Most of the DE genes were specifically grouped by tissue, which suggested that *PHYB* regulates the photoperiodic response in a spatial manner (Fig. 4b; Table S3). Gene ontology (GO) analysis showed that DE genes in group A were mainly involved in biological processes that are typical for the response to shade such as photosystem genes, response to light and regulation of hormone concentrations (Fig. 4c,d; Table S3). By contrast, group B genes were mainly involved in cell wall organization, cell cycle and cell division corresponding to the morphological changes that occur during growth cessation and early bud development (Fig. 4c,d; Table S3). Finally, group C contains many genes that have been associated with both SAS and the regulation of growth cessation, such as genes involved in flavonoid signals and cell wall organization (Fig. 4c, d; Table S3). Overall, these results suggest that *Populus* phyBs regulate SAS- and SD-induced growth cessation in both common and distinct pathways, and largely in a tissue and day-length-dependent manner.

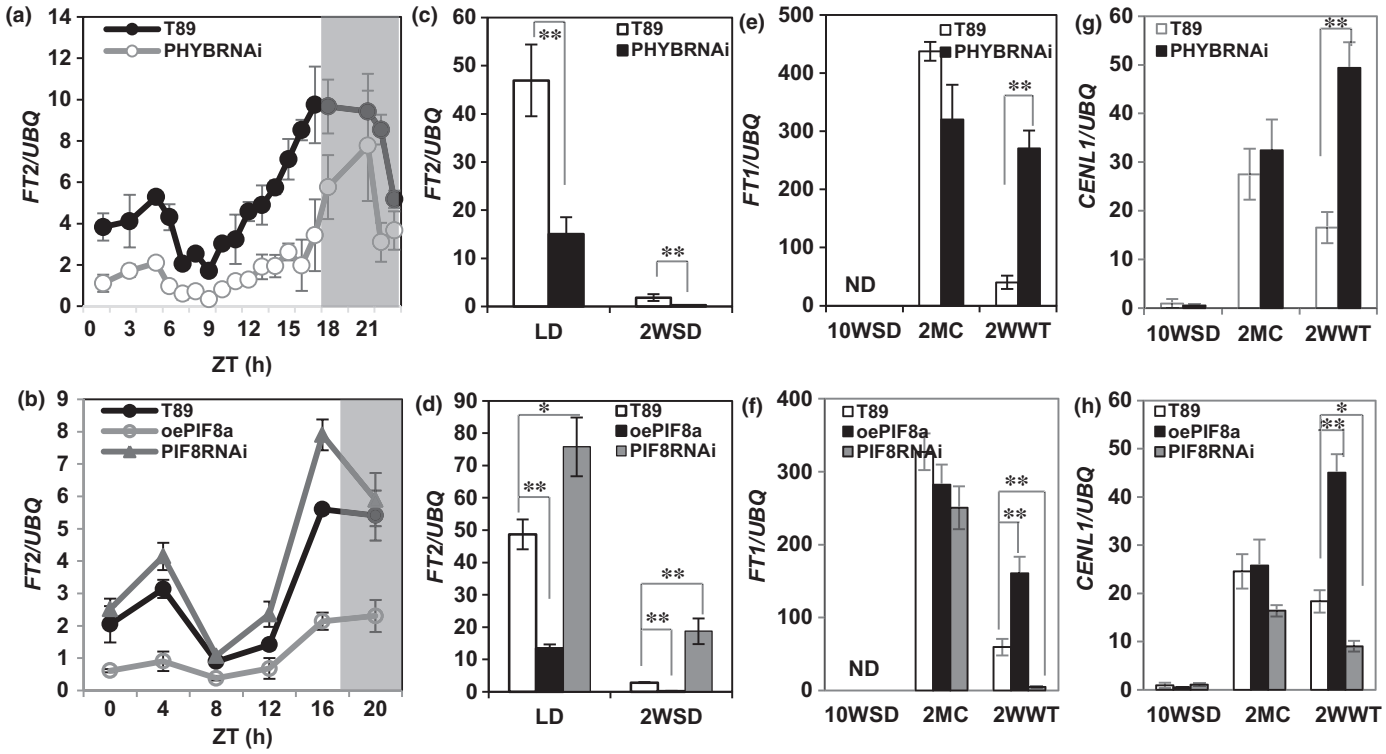
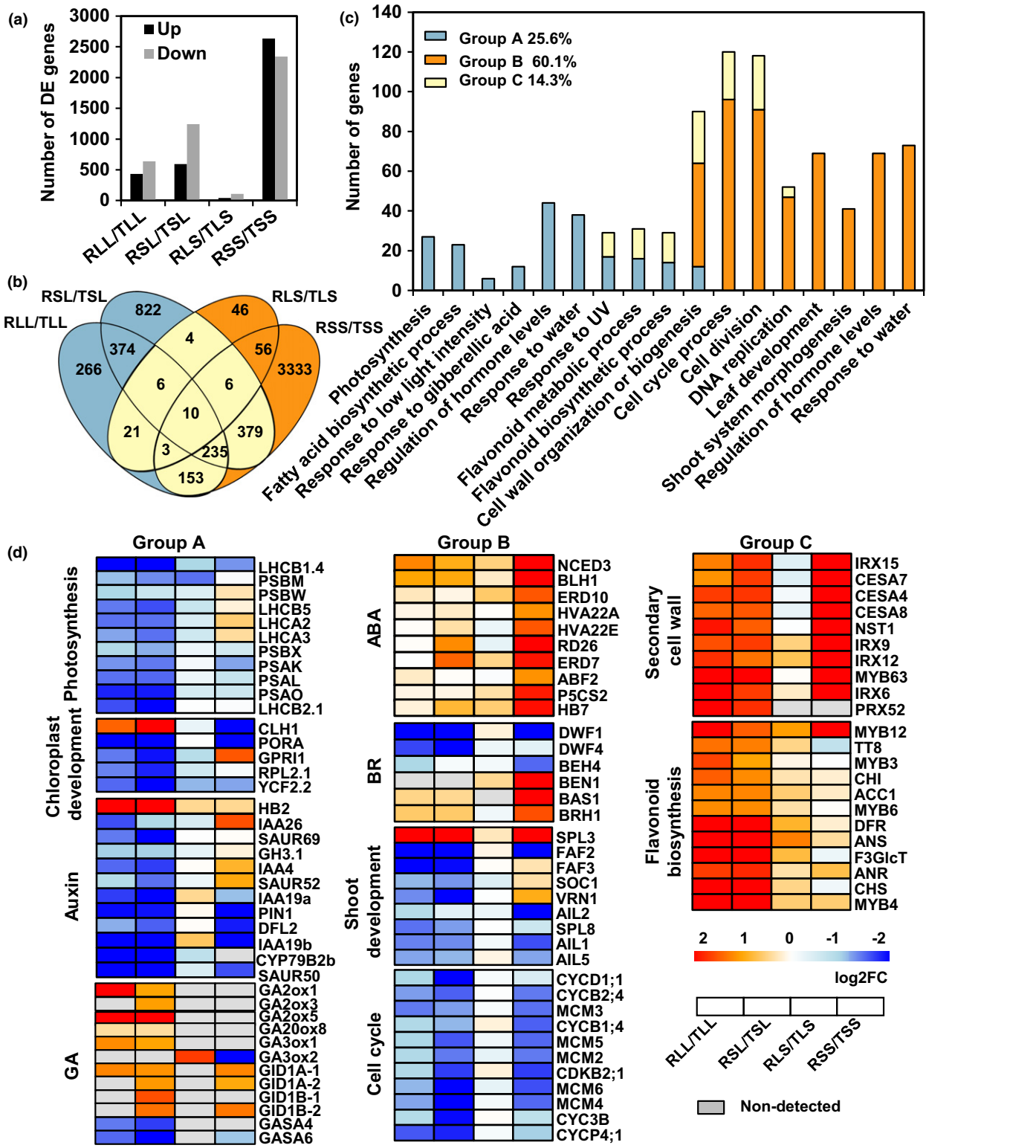


Fig. 3 The *PHYB-PIF8* module regulates the expression of *FT* and *CENL1* genes in hybrid aspen. (a, b) Diurnal expression analysis of *FT2* in leaves of wild-type (WT) (a, b), *PHYB*RNAi (a) and *PIF8*RNAi and oe*PIF8a* (b) plants grown under long-day conditions (18 h : 6 h, light : dark; LD^{18h}). Gray boxes, night; white boxes, day. (c, d) Comparative expression analysis of *FT2* in leaves of WT (c, d), *PHYB*RNAi lines (c) and *PIF8*RNAi and oe*PIF8a* lines (d) grown under LD^{18h} conditions and 2 wk after transfer to short-day conditions (14 h : 10 h, light : dark; SD^{14h}). (e, f) Comparative expression analysis of *FT1* in apical buds of WT (e, f), *PHYB*RNAi (e) and *PIF8*RNAi and oe*PIF8a* (f) plants after 10 wk in SD^{14h} (10WSD), 2 months of cold treatment (8 h 4°C : 16 h 4°C, light : dark; SD^{8h}, 4°C; 2MC) and 2 wk of LD^{18h} warm temperature treatment (2WWT). (g, h) Comparative expression analysis of *CENL1* in apical buds of WT (g, h), *PHYB*RNAi (g), *PIF8*RNAi and oe*PIF8a* (h) plants after 10WSD, 2MC and 2WWT (21°C). Data are mean values from three biological replicates. Error bars represent ±SE. ZT, zeitgeber time. Asterisks denote significant differences between transgenic and WT events as determined by *t*-test (*, $P < 0.05$; **, $P < 0.01$).

PHYB-PIF8 regulon-dependent genome-wide transcriptome changes in seasonal growth

To investigate the *PIF8*-mediated bud break control, we performed transcriptomic analysis using apical buds taken from both WT and oe*PIF8a* after 2 months of cold treatment. A total of 844 DE genes were identified (Table S4). To investigate potential target genes downstream of the *PHYB-PIF8* regulon, we compared the transcriptome between the oe*PIF8a* dormant buds with *PHYB*RNAi apices after SD treatment (RSS/TSS). We found that these two samples share a total of 215 common DE genes, including 134 upregulated and 81 downregulated genes (representing 25.5% of all WT/*oePIF8a* DE genes) (Fig. 5a; Table S5). Interestingly, we found a significant enrichment of a G-box motif, a potential PIF binding site, in the promoter regions of the 215 common DE genes ($P = 0.0065$; Table S6). GO analysis indicated that upregulated genes were mainly associated with defense response or abiotic stress-related phytohormone responses such as ABA, jasmonic acid (JA) and salicylic acid (SA) responses, while the downregulated genes were associated with active growth processes such as cell proliferation, cell cycle, meristem activity and organ development (Fig. 5b). Interestingly, most of these genes have been reported to have opposite expression trends between SD-induced growth cessation and thermoregulated bud break (Ruttink *et al.*, 2007; Yordanov *et al.*, 2014). These results suggest that the *PHYB-PIF8* module regulates the growth cessation in 'autumn' and bud break in 'spring' through a series of common regulons. We further confirmed the changes of *BRC1* and *AIL1* expression in WT, *PHYB*RNAi, *PIF8*RNAi and oe*PIF8a* apices during growth cessation and bud break by quantitative PCR (Fig. 5c–h). Both genes have been suggested to play important roles in the regulation of trees' seasonal growth (Karlberg *et al.*, 2011; Rameau *et al.*, 2014; Muhr *et al.*, 2016, 2018; Singh *et al.*, 2018; Wang *et al.*, 2019; Maurya *et al.*, 2020; Vayssieres *et al.*, 2020). Consistent with the transcript profiling, *BRC1* was upregulated in both *PHYB*RNAi and oe*PIF8a* dormant buds, while its expression was downregulated in *PIF8*RNAi plants (Fig. 5c–e). By contrast, *AIL1* was downregulated in both *PHYB*RNAi and oe*PIF8a* dormant buds while being upregulated in *PIF8*RNAi plants (Fig. 5f–h). In all, these results suggest that, besides the *FT/CENL1* gene family, the *PHYB-PIF8* regulon mediates SD-induced growth cessation in 'autumn' and thermoregulated bud break in 'spring' through common phytohormone response signals and meristem regulation pathways, including *BRC1*, *AIL1* and other meristem activity genes.



Discussion

The function of the phyB-PIF regulon in trees

The seasonally synchronized annual growth cycle regulated mainly by photoperiod and temperature cues is a crucial adaptive strategy for perennial plants in boreal and temperate ecosystems. The molecular mechanisms underlying this phenomenon are starting to be deciphered. Genetic variation in *PHYB*, encoding a photoreceptor, and in Arabidopsis, also reported to function as a thermal sensor, has been reported to be associated with variation in SD-induced growth cessation in *Populus* (Frewen *et al.*, 2000).

Fig. 4 Genome-wide transcriptome analysis reveals the coordination of shade avoidance syndrome (SAS) and short-day (SD)-induced growth cessation by *Populus* phyBs. (a–c) Transcriptome dynamic analysis of *PHYB*RNAi and wild-type (WT) plants responding to SD-induced growth cessation. RNA-seq data from leaf and shoot apices of *PHYB*RNAi and WT plants taken from long-day conditions (18 h : 6 h, light : dark; LD^{18h}) and 2 wk after short-day (14 h : 10 h, light : dark; SD^{14h}) treatment. Samples were named sequentially by genotype (T, WT; R, *PHYB*RNAi), day length (L, LD; S, SD) and tissue (L, leaf; S, shoot), resulting in the following abbreviations: TLL, WT-LD-leaf; RLL, *PHYB*RNAi-LD-leaf; TLS, WT-LD-shoot; RLS, *PHYB*RNAi-LD-shoot; TSL, WT-SD-leaf; RSL, *PHYB*RNAi-SD-leaf; TSS, WT-SD-shoot; RSS, *PHYB*RNAi-SD-shoot. (a) Number of differentially expressed (DE) genes between WT and *PHYB*RNAi leaf and shoot apices in different day-length conditions. (b) Venn diagram showing that most DE genes are organ-specific. Groups A–C represent leaf-specific, shoot-specific and the overlap between leaf and shoot DE genes, respectively. (c) Gene ontology analysis of three groups of DE genes in terms of biological processes; group A (blue), group B (orange) and group C (yellow). (d) Heat map analysis of selected DE genes in groups A–C between *PHYB*RNAi and WT, representing different biological processes. Color represents the $\log_2$fold changes ($\log_2$FC). The gene FC-value was calculated from the ratio of *PHYB*RNAi to WT (R/T). The comparison groups order was RLL/TLL, RSL/TSL, RLS/TLS, RSS/TSS. The grey box indicates undetected in RNA-seq data.

It is also known that *PHYB* overexpression in *Populus* suppresses FR-induced elongation of shoots and petioles (Karve *et al.*, 2012). However, we still largely lack genetic and molecular evidence of phyB function in trees, including loss-of-function analysis, to understand its role in regulating seasonal growth and the SAS response. Here we characterized the function of the two *Populus PHYB* paralogs and downstream *PIF* genes in regulating seasonal growth in hybrid aspen. Application of both gain- and loss-of-function approaches confirmed that the *PHYB-PIF* regulon acts as a mediator of photoperiod and temperature signals. Its function covers several stages of seasonal growth, including the SAS response, SD-induced growth cessation and thermoregulated dormancy release and bud break. We propose a model for how *PHYB* and its downstream targets form a genetic network regulating seasonal growth in hybrid aspen (Fig. 6). According to this model, the *PHYB-PIF* regulon in *Populus* trees has a conserved signaling pathway in regulating the SAS response: PHYB negatively regulates SAS through PIF genes. PIF4 and PIF8 act redundantly downstream of PHYB. By contrast, in the regulation of seasonal growth, PIF8 plays a primary role. Under SDs, *PHYB* act as a negative regulator of growth cessation and the *PHYB-PIF8* module inhibits growth by repressing the expression of

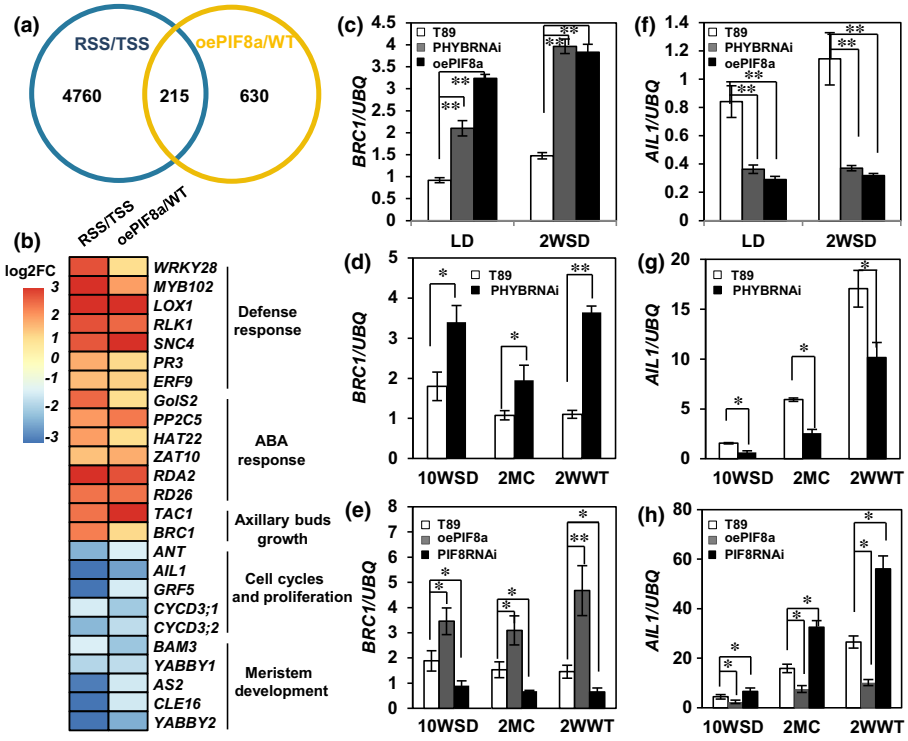


Fig. 5 Genome-wide analysis of *PHYB-PIF8* regulon target genes in hybrid aspen. (a) Venn diagram of the common differentially expressed (DE) genes in *PHYB*RNAi short-day (SD) shoots (RSS, *PHYB*RNAi-SD-shoot; TSS, WT-SD-shoot) and in 2-month cold-treated apical buds of oe*PIF8a* (oe*PIF8a*/WT). (b) Heat map of selected common DE genes from different biological processes associated with seasonal growth regulation. Color represents the $\log_2$fold-changes. (c, f) Comparative expression analysis of *BRC1* and *AIL1* in WT, oe*PIF8a* and *PHYB*RNAi shoot apices in long-day (LD) conditions (18 h : 6 h, light : dark; LD^{18h}) and 2 wk after short-day treatment (2WSD) (14 h : 10 h, light : dark; SD^{14h}). (d, e) Comparative expression analysis of *BRC1* in WT (d, e), *PHYB*RNAi (d), *PIF8*RNAi and oe*PIF8a* (e) apical buds after 10 wk in SD^{14h} conditions (10WSD), after 2 months of cold treatment (SD^{8h}, 4°C; 2MC) and after 2 wk of reactivation in warm temperature (LD^{18h}, 21°C; 2WWT). (g, h) Comparative expression analysis of *AIL1* in apical buds of WT (g, h), *PHYB*RNAi (g), *PIF8*RNAi and oe*PIF8a* (h) in 10WSD, 2MC and 2WLD treatments. Data are mean values from three biological replicates. Error bars ± SE. Asterisks denote significant differences between transgenic and WT events as determined by *t*-test (*, $P < 0.05$; **, $P < 0.01$).

FT2. During dormancy release and bud break, the *PHYB-PIF8* module affects bud break timing, possibly through regulation of *CENL1* expression. We do not include *FT1* in the model, as it is still unclear what role *FT1* plays in the release of dormancy or bud break regulation. The expression of both *FT1* and *CENL1* is reduced in response to warm temperature, and our data show that this temperature response is likely to be mediated through *PHYB-PIF8*. The *PHYB-PIF8* module also shares common targets in mediating SD-induced growth cessation in 'autumn' and thermoregulated bud break in 'spring', including phytohormone response and meristem regulation pathways such as *BRC1*, *AIL1* and other meristem activity signals.

Evolutionarily conservation and diversification of the phyB-PIF module in *Populus*

Phytochromes are present in all land plants and most green algal lineages, except in the chlorophytes (Li *et al.*, 2015). The number of phytochromes is species-dependent. In Arabidopsis, there are five phytochrome members designated as phyA to phyE, while rice contains three different phytochromes, designated as phyA to phyC. The two counterparts phyD and phyE were once thought to be dicot-specific phytochromes (Pham *et al.*, 2018). However, there are only three phytochrome genes in *Populus*, including *PHYA* and two paralogs of *PHYB* (Howe *et al.*, 1998; Olsen, 2010; Karve *et al.*, 2012) (Fig. S1). In Arabidopsis, phyA and phyB mostly display antagonistic action in multiple light signaling pathways, but they have synergistic functions (Su *et al.*, 2015). For example, phyA and phyB act antagonistically in flowering time regulation: phyA induces flowering time while phyB represses it (Cerdan & Chory, 2003). As there is a remarkable conservation of the genetic pathways regulating the photoperiodic response in *Populus*, SD-induced growth cessation and bud set, and the photoperiodic pathway controlling flowering time in Arabidopsis (Ding & Nilsson, 2016), it was reasonable to speculate that phyA and phyB would act antagonistically in the regulation of tree phenology. However, all previous studies and our genetic results confirm that phyA and phyB function synergistically in the regulation of tree phenology. Both of them act as positive regulators of tree growth (Howe *et al.*, 1996; Olsen *et al.*, 1997). Moreover, in Arabidopsis, phyA and phyB antagonistically regulate flowering time by stabilizing or destabilizing the CO protein, which plays a central role in the induction of flowering in LDs (Yanovsky & Kay, 2002; Cseke *et al.*, 2003; Valverde *et al.*, 2004). By contrast, in *Populus*, CO seems to have only a minor function in regulating SD-induced growth cessation (Bohlenius *et al.*, 2006; Hsu *et al.*, 2012). Overall, these results suggest that despite their conserved functions, *Populus* phyBs have evolved new signaling pathways in the regulation of trees' seasonal growth.

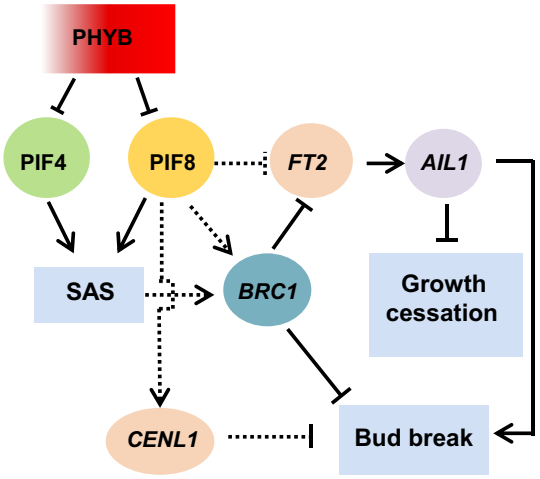


Fig. 6 Model of how the *PHYB-PIF8* regulon affects seasonal growth in hybrid aspen. During the growing season, phyB negatively regulates shade avoidance syndrome (SAS) through both PIF4 and PIF8, while only PIF8 plays a major role downstream of phyB in regulating seasonal growth. phyB acts as a negative regulator, and PIF8 as a positive regulator, of short-day (SD)-induced growth cessation in the autumn, at least partly through the regulation of the expression of *FT2*, the key gene involved in SD-induced growth cessation in trees. On the other hand, *PHYB* (positively) and PIF (negatively) induce dormancy release and bud break in spring, possibly through the regulation of expression of *CENL1*, a key gene involved in thermoregulated bud break. The *PHYB-PIF8* regulon regulates seasonal growth cessation and bud break through common integrators such as *BRC1* and *AIL1*. *BRC1* could also have roles in both SAS- and SD-induced growth cessation, as *BRC1* has been reported to act as a key regulator of shade-inhibited axillary bud outgrowth. Solid lines represent direct genetic interactions or verified effects on growth processes, and dotted lines represent connections that need to be characterized further.

Like phytochromes, the primary signaling partners for phytochromes, PIFs, have been discovered in various plant lineages from bryophytes to angiosperms (Lee & Choi, 2017). Arabidopsis has eight PIFs (PIF1–PIF8), while there are 11 PIFs in *Populus* (Fig. S6). The phyB-PIF signaling module has been investigated extensively and is involved in a diversity of biological processes (Pham *et al.*, 2018). PIF4 has been found to act as a central signaling hub that integrates environmental cues, including light and temperature, to regulate growth and development (Choi & Oh, 2016). In the beginning, we therefore focused on the *Populus PIF4* ortholog and characterized its function in seasonal growth. However, *PtPIF4* seems to have a minor role in SD-induced growth cessation and thermal-controlled bud break, as *PtPIF4*RNAi plants are similar to WT plants during these processes (Fig. S9). Instead, we found that the *PtPIF8* genes, orthologs of Arabidopsis *PIF7/PIF8*, play a major role in regulating seasonal growth in hybrid aspen (Fig. 2). The light response results (Fig. S7), together with the genetic results (Fig. 6), suggest that *Populus* PIF8 might be more similar in function to Arabidopsis PIF7 than to PIF8.

In Arabidopsis, PIF4 and PIF7 are the primary regulators downstream of phyB during shade-induced flowering. In the shade, they induce flowering by activating the expression of *FT* and its close homolog *TSF* (Kumar *et al.*, 2012; Galvao *et al.*, 2019; Zhang *et al.*, 2019). In hybrid aspen, *PIF4* and *PIF8* have conserved and redundant roles in the SAS response. However, only PIF8 has a role in photoperiod-induced growth cessation, but, in contrast to the situation in Arabidopsis, through repression of the expression of *FT2*. Interestingly, a similar phenomenon was found in another perennial plant, alfalfa (*Medicago sativa*). Shade treatment of alfalfa triggers a classical SAS

response, with increased internode and petiole lengths, but with delayed flowering instead of early flowering normally seen in annual plants (Lorenzo *et al.*, 2019). Therefore, one can speculate that, as an adaptation to the perennial growth strategy, the phyB-PIF module in perennial plants has evolved new mechanisms to uncouple the SAS response from the photoperiodic regulation of flowering time or growth cessation (Lorenzo *et al.*, 2019).

phyB regulates the tree SAS response and seasonal growth through overlapping and distinct pathways

The genetic analysis showed that phyB has dual roles in regulating shade avoidance and seasonal growth in trees. Our genome-wide transcriptome analysis suggests that these two pathways could be tissue- and day-length-dependent (Fig. 4). Under LD conditions, the main differences between the transcriptomes of WT and *PHYB*RNAi plants are in the leaf. Typical SAS-related pathways, such as cell wall, photosystem, and phytohormone (GA, auxin etc.) pathways, were overrepresented. However, the shoot apex transcriptome responded very differently in WT and *PHYB*RNAi trees when they were subjected to SDs as a result of the *PHYB*RNAi trees arresting growth and setting bud prematurely compared with the WT. In addition to the distinct pathways regulating shade avoidance and SD-induced growth cessation, we also found that the pathways have common targets. One major crosstalk between SAS and growth cessation is hormonal pathways. Many hormones have roles in both SAS- and SD-induced growth cessation. For example, the ABA biosynthetic gene *NINE-CIS-EPOXYCAROTENOID DIOXYGENASE 3* (*NCED3*) and a series of the ABA response genes were upregulated in *PHYB*RNAi plants (Fig. 4d). This result is consistent with previous reports that shade induces increased ABA concentrations (Reddy *et al.*, 2013). ABA has important roles in the development of tree buds and dormancy (Rohde *et al.*, 2002; Ruttink *et al.*, 2007; Tylewicz *et al.*, 2018). GA's roles in axillary bud (AXB) outgrowth and SAS response are dynamic and mostly depend on organ and developmental stage (Kohnen *et al.*, 2016; Katyayini *et al.*, 2020). It was reported that the quiescence of AXBs in hybrid aspen is mainly correlated with high levels of expression of the GA catabolic gene *GIBBERELLIN 2-OXIDASE 1* (*GA2ox1*) (Katyayini *et al.*, 2020). Among DE genes, we found that the expression levels of three GA2ox genes (*GA2ox1*, *GA2ox3*, *GA2ox5*) were upregulated in *PHYB*RNAi plants, and four *GA* receptor genes (*GID1A-1*, *GID1A-2*, *GID1B-1* and *GID1B-2*). These results indicate that GA availability is low because *GID1* expression levels are known to increase when GA concentrations decrease as a result of a homeostatic adjustment (Hedden & Thomas, 2012). Consistently, the GA-induced genes *GAST1 PROTEIN HOMOLOG4* (*GASA4*) and *GAST1 PROTEIN HOMOLOG6* (*GASA6*) were downregulated in *PHYB*RNAi plants (Fig. 4d). We notice that the expression levels of three GA biosynthesis genes (*GA20ox8*, *GA3ox1* and *GA3ox2*) were altered in *PHYB*RNAi as well (Fig. 4d). *GA20ox8* and *GA3ox1*, which are predominantly expressed in leaves, were upregulated in *PHYB*RNAi plants. Interestingly, another GA biosynthesis gene, *GA3ox2*, which is mainly expressed in the shoot apex, has an opposite expression trend in *PHYB*RNAi plants compared with WT plants under LD and SD conditions. In LD shoots, the expression of *GA3ox2* is higher in *PHYB*RNAi plants compared with WT plants, while in SD shoots the expression of *GA3ox2* becomes lower in *PHYB*RNAi plants. Therefore, the expression pattern of *GA3ox2* may partially explain how phyB oppositely modulates shoot apex growth under LD and SD conditions. Also, the brassinosteroid (BR) biosynthesis genes *DWARF 1* (*DWF1*), *DWARF 4* (*DWF4*), and the *BR*-signaling gene *BES1/BZR1 HOMOLOG 4* (*BEH4*) were downregulated, while the BR catabolism genes *PHYB ACTIVATION TAGGED SUPPRESSOR 1* (*BAS1*), *BRASSINOSTEROID-RESPONSIVE RING-H2* (*BRH1*) and *BRI1-5 ENHANCED 1* (*BEN1*) were upregulated in *PHYB*RNAi plants (Fig. 4d). In trees, both GA and ABA have been well characterized as key hormones in regulating seasonal growth (Rinne *et al.*, 2011; Zawaski & Busov, 2014; Tylewicz *et al.*, 2018). The roles of BRs in trees' seasonal growth are not well understood, but they are involved in regulating seed dormancy and germination in other plants (Kim *et al.*, 2019). Besides, expression of many transcription factors involved in shoot meristem activities were changed in *PHYB*RNAi plants, including *BRC1*, a key integrator of different hormonal and light signaling pathways that are involved in the control of bud outgrowth in many plants as well as seasonal growth in trees (Rameau *et al.*, 2014; Muhr *et al.*, 2016, 2018; Singh *et al.*, 2018; Wang *et al.*, 2019; Maurya *et al.*, 2020; Vayssieres *et al.*, 2020). At the same time, *Populus* homologs of the Arabidopsis genes *ANT*, *AIL1* and *AIL2*, known to be targets of BRC1, were downregulated in *PHYB*RNAi plants (Fig. 4d). Thus, this suggests that phyBs coordinate SAS- and SD-induced growth cessation through an intricate regulation of different hormonal signaling pathways, the effector BRC1 and downstream genes active in the shoot apex.

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Author contributions

ON and JD designed the research. JD, BZ, YL and DA, performed the experiments. JD, BZ and YL collected and analyzed the data. JD and ON wrote the manuscript. All authors read and edited the manuscript before publication.

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Data availability

The transcriptome data have been deposited in NCBI SRA with accession number PRJNA706169.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Phylogenetic analysis of phytochrome genes.

Fig. S2 Expression of *PHYB* in transgenic lines and *PHYB* knockout strategy.

Fig. S3 Phenotypic comparison of WT, *PHYB* and *PIF8* transgenic lines.

Fig. S4 Ectopic expression of *PHYBs* affects seasonal growth in hybrid aspen.

Fig. S5 Seasonal growth analysis of *PHYB1*KO plants and overview of *PHYB1B2*KO plants.

Fig. S6 *PHYTOCHROME INTERACTING FACTOR* (*PIF*) genes in *P. tremula*.

Fig. S7 Rc and FRc responsiveness of PIF8a protein.

Fig. S8 Expression of *PIF4* and *PIF8* in transgenic lines.

Fig. S9 PIF4 regulates plant growth downstream of PHYB.

Fig. S10 Principal component analysis (PCA) of RNA-seq data from leaf and shoot apices of *PHYB*RNAi and WT plants in LD and SD conditions.

Tables S1 Primers used in this study.

Tables S2 List of DE genes between WT and *PHYB*RNAi plants described in Fig. 4(a).

Tables S3 Gene ontology (GO) analysis of DE genes between WT and *PHYB*RNAi plants described in Fig. 4(c,d).

Tables S4 List of DE genes between WT and oe*PIF8* apical buds.

Tables S5 List of shared DE genes between RSS/TSS and oe*PIF8*/WT.

Tables S6 Putative PIF8 targets.

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