PLANT SCIENCE

Photoperiodic control of seasonal growth is mediated by ABA acting on cell-cell communication

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In temperate and boreal ecosystems, seasonal cycles of growth and dormancy allow perennial plants to adapt to winter conditions. We show, in hybrid aspen trees, that photoperiodic regulation of dormancy is mechanistically distinct from autumnal growth cessation. Dormancy sets in when symplastic intercellular communication through plasmodesmata is blocked by a process dependent on the phytohormone abscisic acid. The communication blockage prevents growth-promoting signals from accessing the meristem. Thus, precocious growth is disallowed during dormancy. The dormant period, which supports robust survival of the aspen tree in winter, is due to loss of access to growth-promoting signals.

Dormancy protects meristematic cells of perennial plants in temperate and boreal ecosystems by preventing growth during winter. Release from dormancy enables reinitiation of growth when favorable conditions return in spring (*1*). Shorter photoperiods as winter approaches (*2*) induce growth cessation, formation of a bud that encloses the arrested leaf primordia and shoot apical meristem (SAM) (Fig. 1A), and bud dormancy (*3*, *4*). Longer photoperiods alone cannot promote growth in dormant buds; prolonged exposure to low temperatures is required to release dormancy (*5*, *6*). We show that blockage of symplastic communication mediated by the action of abscisic acid (ABA) is part of the photoperiodically controlled dormancy mechanism in hybrid aspen.

Short photoperiods induce expression of ABA receptors and increase ABA levels in hybrid aspen buds (*4*, *7*). ABA regulates dormancy (*8*). Therefore, we probed ABA's role in photoperiodic control of bud dormancy. First, we generated hybrid aspen plants with reduced ABA responses by expressing the dominant-negative *abi1-1* allele of *ABI1*, a key ABA-signaling gene (*9*). Those hybrid aspens that expressed *abi1-1* had reduced ABA responses, manifested by weak induction of the ABA-inducible gene *KIN2*, compared with that of wild-type (WT) controls (fig. S1). We then assessed bud dormancy by exposing WT and *abi1-1* plants to 11 weeks of short photoperiod followed by transfer to long photoperiod without the low-temperature treatment required for dormancy release. Both WT and *abi1-1* plants ceased growth and set buds after 4 weeks of short photoperiod (Fig. 1, A to C), but after 11 weeks of short photoperiod followed by long photoperiod, WT buds remained dormant, whereas *abi1-1* buds reactivated growth within 11 to 15 days (Fig. 1, D to F). Thus, attenuation of ABA responses compromised photoperiodic control of bud dormancy and not growth cessation.

We investigated transcriptomic responses to short photoperiod in WT and *abi1-1* apices in order to understand ABA-mediated control of dormancy. After 6 and 10 weeks of short photoperiod, respectively, we detected 9290 and 3053 differentially expressed genes in WT and 10,514 and 2149 differentially expressed genes in *abi1-1* (line 1) apices (table S1). A large number of transcripts for plasmodesmata-associated proteins responded to short photoperiod. Plasmodesmata closure (by callosic dormancy sphincters) correlates with dormancy and their opening with dormancy release in diverse plants, including hybrid aspen and charophycean algae such as Chara (*6*, *10*, *11*). Of 187 poplar homologs of *Arabidopsis* genes encoding proteins enriched in plasmodesmata (*12*), 62 and 47 were induced after 6 and 10 weeks in WT apices, respectively, and of these, 53.2 and 76.6% were differentially expressed in *abi1-1* relative to WT apices at these time points (table S2). Expression of *GERMIN-LIKE 10*; *REMORIN-LIKE 1* and *2*, which are implicated in plasmodesmata function (*13*); and *CALLOSE SYNTHASE 1*, which is required for callose deposition (*6*), was progressively up-regulated, whereas that of *GH17-39*, a glucanase implicated in sphincter removal (*6*), was down-regulated in WT apices after 6 and 10 weeks of short photoperiod. These genes showed an altered response to short photoperiod in *abi1-1* plants (fig. S2). Thus, ABA mediates short-photoperiod response of the plasmodesmata-related transcriptome.

Transcriptomic analysis prompted us to investigate ABA's role in plasmodesmata closure (Fig. 1, G to O). Under long photoperiod, WT and *abi1-1* lines 1 and 3 had similar frequencies of "closed" plasmodesmata with dormancy sphincters (12.5 versus 17.4 and 13.5%, respectively). After 5 weeks of short photoperiod, corresponding frequencies were 78% in WT and 5.5 and 17.4% in *abi1-1* apices, respectively, and after 10 weeks, frequencies increased to 83.6% in WT plants but fell to 2.2 and 0.5% in *abi1-1* lines 1 and 3, respectively. Thus, ABA mediates plasmodesmata closure in response to short photoperiod. Plasmodesmata closure is not required for growth cessation (because growth cessation occurs in *abi1-1* plants) and indicates association of plasmodesmata closure with bud dormancy, both being mediated by the same factor, ABA.

To investigate ABA-mediated plasmodesmata closure's role in short photoperiod–induced dormancy, we overexpressed PDLP1 (PLASMODESMATA-LOCATED PROTEIN 1), which impairs trafficking via plasmodesmata (*14*) and phenocopying plasmodesmata blockage by dormancy sphincters, in *abi1-1* plants (fig. S3). Both *abi1-1*/*PDLP1* double transformants and parental *abi1-1* plants ceased growth and formed buds under short photoperiod (Fig. 2, A to C), but subsequent exposure to long photoperiod only reactivated growth in the latter (Fig. 2, D to F). Thus, PDLP1 expression suppressed *abi1-1* plants' bud dormancy phenotype, although *KIN2* expression responses to ABA remained attenuated in *abi1-1*/*PDLP1* (fig. S4). Thus, expression of PDLP1 was sufficient to restore bud dormancy in *abi1-1*/*PDLP1* plants without the restoration of general ABA responses.

PICKLE (*PKL*) is an antagonist of polycomb repression complex 2, which is implicated in seed dormancy (*15*, *16*). *PKL* expression was down-regulated in WT plants but up-regulated in *abi1-1* plants under short photoperiod (fig. S5). Hence, we investigated whether *PKL* could be involved in plasmodesmata closure and dormancy regulation mediated by ABA. Thus, we examined plasmodesmata in *abi1-1* plants with suppressed *PKL* activity (*abi1-1*/*PKL*RNAi) (RNAi, RNA interference) (fig. S6). Under long photoperiod, frequencies of plasmodesmata with dormancy sphincters were comparable in *abi1-1* (13.1%) and *abi1-1*/*PKL*RNAi lines 9 (19.4%) and 11 (18.4%) (Fig. 3, A to C). After 5 weeks of short photoperiod, the frequencies increased in the *abi1-1*/*PKL*RNAi lines (to 34.4 and 28.5%, respectively), but not *abi1-1* plants (16.4%) (Fig. 3, D to F). After 10 weeks of short photoperiod, the frequencies further increased in *abi1-1*/*PKL*RNAi lines 9 and 11 to 84.6 and 74.5%, respectively, but fell in *abi1-1* plants (5.2%) (Fig. 3, G to I). *PKL* down-regulation in *abi1-1*/*PKL*RNAi also suppressed expression defects of plasmodesmata

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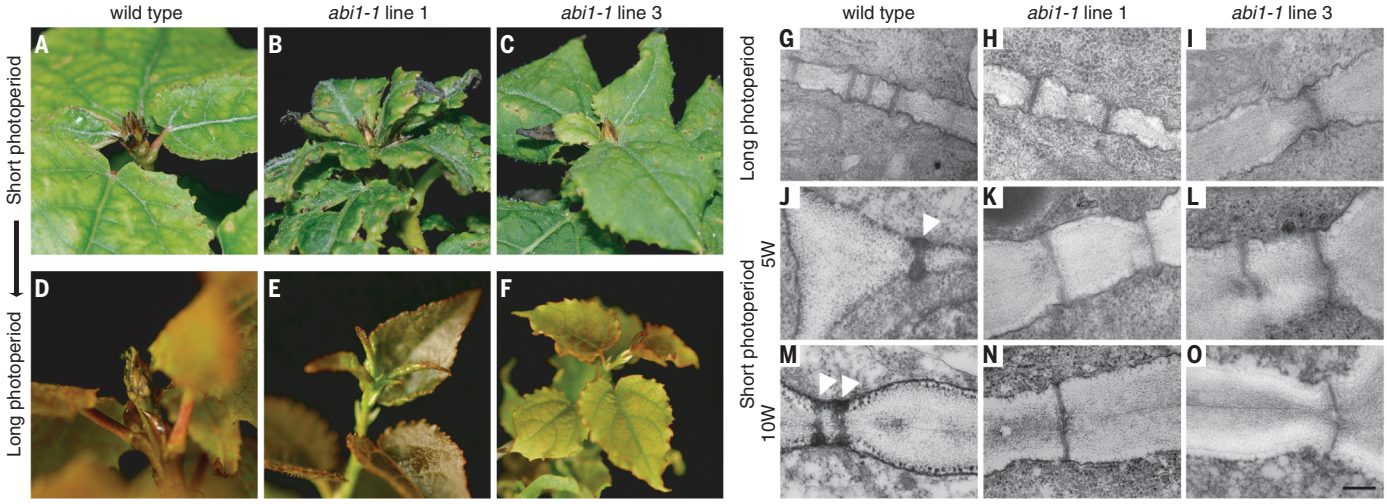

Fig. 1. Hybrid aspen plants with attenuated ABA responses fail to establish dormancy. (**A** to **C**) Buds of (A) wild type, (B) *abi1-1* line 1, and (C) *abi1-1* line 3 after 11 weeks of short photoperiod. (**D** to **F**) Unlike in (D) WT, buds burst in (E) *abi1-1* line 1 and (F) *abi1-1* line 3. (**G** to **I**) Transmission electron microscopy (TEM) micrographs of apices of actively growing (G) WT plants, (H) *abi1-1* line 1, and (I) *abi1-1* line 3, showing plasmodesmata lacking electron-dense dormancy sphincters. (**J** to **O**) Sphincters are observed after 5 and 10 weeks of short photoperiod in apices of [(J) and (M)] wild-type plants (indicated with arrowheads), but not [(K) and (N)] *abi1-1* line 1 or [(L) and (O)] *abi1-1* line 3. Scale bar, 200 nm.

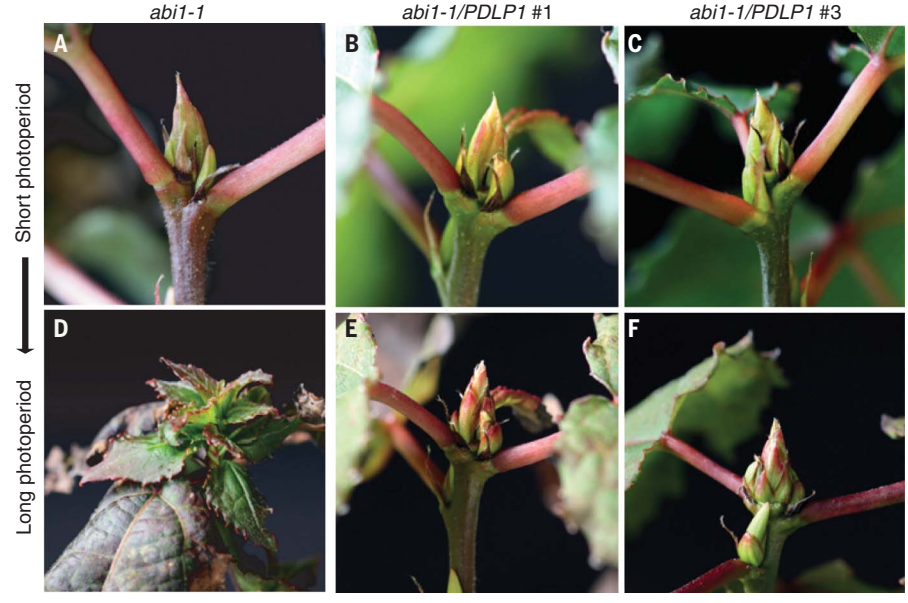

Fig. 2. *PDLP1* expression restores bud dormancy in *abi1-1* plants. (**A** to **C**) Buds of (A) *abi1-1*, (B) *abi1-1/PDLP1* line 1, and (C) *abi1-1/PDLP1* line 3 after 11 weeks of short photoperiod. (**D** to **F**) Transfer to long photoperiod results in bud burst in (D) *abi1-1* plants but not in (E) *abi1-1/PDLP1* line 1 or (F) *abi1-1/PDLP1* line 3.

markers evident in *abi1-1* plants (fig. S7). Although both *abi1-1* and *abi1-1*/*PKL*RNAi plants ceased growth and set buds (Fig. 3, J to L), *abi1-1*/*PKL*RNAi buds remained dormant and did not reactivate growth (unlike nondormant *abi1-1* buds) after long photoperiod exposure after 11 weeks of short photoperiod (Fig. 3, M to O). Thus, *PKL* down-regulation restores plasmodesmata closure and bud dormancy defects in *abi1-1* plants, suggesting that ABA mediates plasmodesmata closure and bud dormancy by suppressing *PKL*.

Plasmodesmata closure could mediate dormancy by limiting access of SAM to growth-promotive signals. We investigated this hypothesis by analyzing responses of WT and *abi1-1* buds to *FLOWERING LOCUS T1* (*FT1*), a seasonal growth regulator induced during dormancy release and before bud growth resumes (*6*, *17*). We grafted scions of WT and *abi1-1* plants exposed to 10 weeks of short photoperiod (in order to induce plasmodesmata closure and dormancy) onto rootstocks of *FT1*-expressing plants (*18*). Although buds of WT scions did not reactivate growth, new leaves emerged from buds of *abi1-1* scions under a continued short photoperiod (Fig. 4). Thus, plasmodesmata closure, as in WT plants, was associated with buds' failure to respond to FT1 or FT1-derived growth-promotive signals, corroborating the involvement of plasmodesmata in photoperiodic control of ABA-mediated bud dormancy.

Thus, short photoperiods suppress FT2, which causes growth cessation and amplifies the ABA response by enhancing levels of ABA and ABA receptors (*4*, *7*). ABA suppresses PKL and induces callose synthase to block plasmodesmata and maintains these blockages by repressing antagonistic glucanases (fig. S8). Hence, attenuating ABA responses not only results in a failure to induce plasmodesmata closure at dormancy onset but also in fewer subsequently closed plasmodesmata. Plasmodesmata closure through *PKL* down-regulation or *PDLP1* expression, which both target cell-cell communication, suppresses dormancy defects in *abi1-1* plants. *PDLP1* expression restores dormancy without suppressing ABA response defects in *abi1-1* plants. Thus, plasmodesmata closure is essential to dormancy and occurs downstream of ABA-mediated control of dormancy in response to shorter photoperiods.

With plasmodesmata closed, growth arrest is maintained even in the presence of growth-promoting signals. Reopening of closed plasmodesmata in dormant buds occurs slowly and only after prolonged exposure to low temperature. Hence, dormancy prevents precocious activation of growth. On the other hand, in the absence of dormancy and plasmodesmatal closure, growth

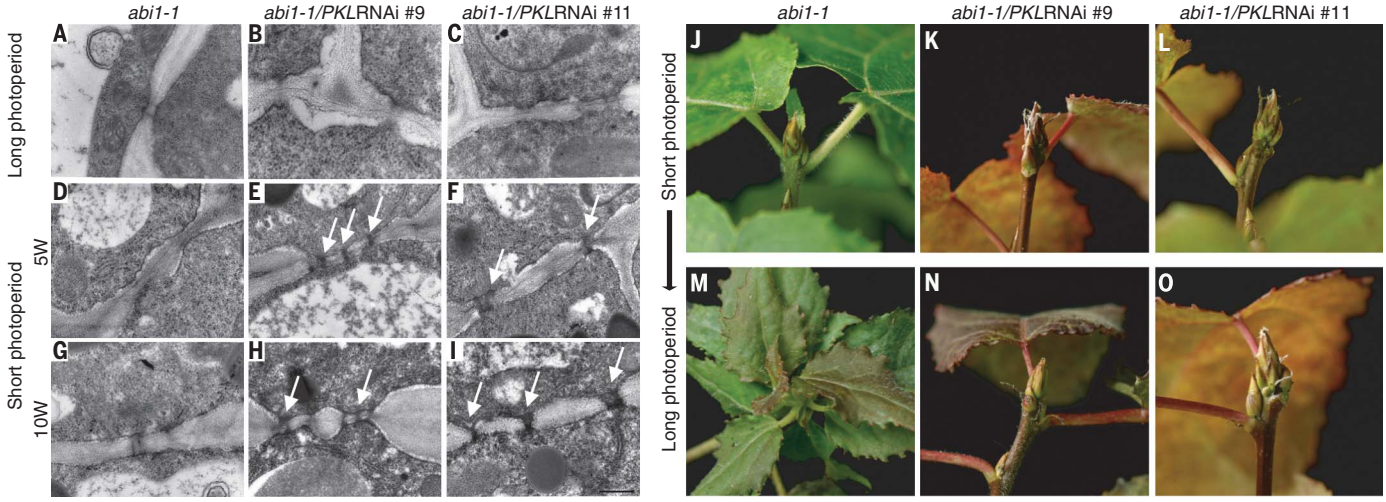

Fig. 3. *PKL* down-regulation restores dormancy sphincters and bud dormancy in *abi1-1* plants. (**A** to **C**) TEM micrographs of apices of actively growing (A) *abi1-1* plants, (B) *abi1-1/PKL*RNAi line 9, and (C) *abi1-1/PKL*RNAi line 11, showing plasmodesmata lacking electron-dense dormancy sphincters. (**D** to **I**) After 5 and 10 weeks of short photoperiod, sphincters were not observed in [(D) and (G)] *abi1-1* apices but were present in *abi1-1/PKL*RNAi apices of [(E) and (H)] lines 9 and [(F) and (I)] 11 (arrows). Scale bar, 500 nm. (**J** to **L**) Buds of (J) *abi1-1* plants, (K) *abi1-1/PKL*RNAi line 9, and (L) *abi1-1/PKL*RNAi line 11 after 11 weeks of short photoperiod. (**M** to **O**) After a shift to long photoperiod, buds burst in (M) *abi1-1* plants but not in (N) *abi1-1/PKL*RNAi line 9 or (O) *abi1-1/PKL*RNAi line 11.

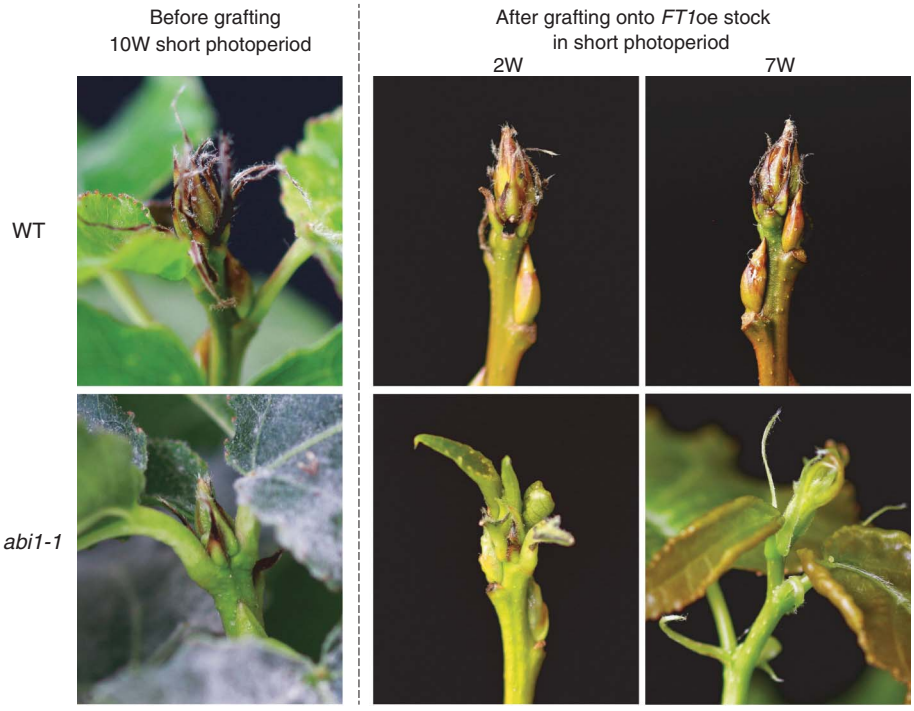

Fig. 4. *FT1*-expressing stocks can reactivate growth in *abi1-1* scions under short photoperiod. WT and *abi1-1* buds after 10 weeks of short photoperiod before grafting, and a further 2 and 7 weeks of short photoperiod after grafting of WT and *abi1-1* scions on *FT1*-expressing stocks. Buds remained dormant in WT scions but burst in *abi1-1* scions.

cessation induced by short photoperiod can be quickly reversed. Thus, dormancy, unlike growth cessation, adds robustness to the mechanism that is crucial for perennial survival and longevity in the face of changing seasons.

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ACKNOWLEDGMENTS

Funding: Grants from Vetenskapsrådet (VR-2016-04430) and the Knut and Alice Wallenberg Foundation (2014-0032) to R.P.B. are gratefully acknowledged. **Author contributions:** S.T., A.P., S.M., P.M., A.A., R.K.S., and J.I. performed experiments. N.M. and T.R.H. performed the transcriptomics analysis. R.P.B., D.M.E., J.L.B., and Y.H. designed experiments and provided intellectual input. All authors contributed to writing the manuscript. **Competing interests:** The authors declare no competing interests. **Data and materials availability:** Raw RNA sequencing reads are available at the European Nucleotide Archive (www.ebi.ac.uk/ena) under accession no. PRJEB23073. All other data needed to evaluate the conclusions in the paper are present in the paper or the supplementary materials.

SUPPLEMENTARY MATERIALS

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Materials and Methods
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30 May 2017; resubmitted 18 December 2017
Accepted 26 February 2018
Published online 8 March 2018
10.1126/science.aan8576