Report

Current Biology

A Tree Ortholog of *SHORT VEGETATIVE PHASE* Floral Repressor Mediates Photoperiodic Control of Bud Dormancy

Highlights

- *SHORT VEGETATIVE PHASE* ortholog *SVL* mediates photoperiodic control of dormancy
- *SVL* acts downstream of ABA in dormancy regulation
- *SVL* promotes dormancy by suppressing the growth-promotive gibberellic acid pathway
- *SVL* activates *CALLOSE SYNTHASE* expression, a key mediator of plasmodesmatal closure

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In Brief

Singh et al. have identified *SHORT VEGETATIVE PHASE* ortholog *SVL* as a key component in ABA-mediated photoperiodic control of bud dormancy. They show that *SVL* simultaneously suppresses the growth-promotive GA pathway and induces the expression of *CALLOSE SYNTHASE 1* (*CALS1*), which mediates plasmodesmatal closure to promote dormancy.

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A Tree Ortholog of *SHORT VEGETATIVE PHASE* Floral Repressor Mediates Photoperiodic Control of Bud Dormancy

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SUMMARY

Perennials in boreal and temperate ecosystems display seasonally synchronized growth. In many tree species, prior to the advent of winter, exposure to photoperiods shorter than a critical threshold for growth (short days; SDs) induces growth cessation, culminating in the formation of an apical bud that encloses the shoot apical meristem and arrested leaf primordia [1–4]. Following growth cessation, subsequent exposure to SDs induces transition to dormancy in the shoot apex [5]. Establishment of dormancy is crucial for winter survival and is characterized by the inability of the shoot meristem to respond to growth-promotive signals [6]. Recently, SDs were shown to induce bud dormancy by activating the abscisic acid (ABA) pathway. ABA upregulates expression of *CALLOSE SYNTHASE 1* (*CALS1*) and suppresses glucanases that break down callose to induce the blockage of intracellular conduits (plasmodesmata; PDs) with callosic plugs called "dormancy sphincters" that by restricting access to growth-promotive signals promote dormancy [7]. However, components downstream of ABA in dormancy regulation remain largely unknown, and thus there are significant gaps in our understanding of photoperiodic control of bud dormancy. Here we demonstrate that *SVL*, orthologous to *Arabidopsis* floral repressor *SHORT VEGETATIVE PHASE* (*SVP*), is a mediator of photoperiodic control of dormancy downstream of the ABA pathway in hybrid aspen. *SVL* downregulation impairs dormancy, whereas *SVL* overexpression suppresses dormancy defects resulting from ABA insensitivity. Downstream, *SVL* induces *callose synthase* expression and negatively regulates the gibberellic acid (GA) pathway to promote dormancy, thus revealing the regulatory module mediating photoperiodic control of dormancy by ABA.

RESULTS AND DISCUSSION

To identify downstream components of abscisic acid (ABA) pathways involved in dormancy regulation, we compared gene expression after exposure to short days (SDs) in apices of wild-type (WT) hybrid aspen plants and *abi1-1* plants [7, 8]. The hybrid aspen *abi1-1* plants have reduced response to ABA, as they express the cDNA for *Arabidopsis* dominant-negative abi1-1 allele and are defective in dormancy regulation in response to SDs [7, 8]. Gene expression analysis indicated that *SVL* (*SVP-LIKE*), a hybrid aspen ortholog of *SHORT VEGETATIVE PHASE* (*SVP*), a floral repressor in *Arabidopsis thaliana* and related to *DORMANCY ASSOCIATED MADS* (*DAM*) genes from various tree species [9, 10], was induced by SDs in apices, correlating with the transition to bud dormancy (Figure 1A). Importantly, SDs failed to induce *SVL* expression in *abi1-1* apices, in contrast with the wild-type (Figure 1B). These findings indicate that ABA mediates SD induction of *SVL* expression during bud dormancy.

ABA promotes dormancy by suppressing expression of *PICKLE* (*PKL*), a chromodomain protein [7], and defects in dormancy regulation resulting from ABA insensitivity can be suppressed by downregulating *PKL* expression in *abi1-1* plants. Hence, we investigated whether ABA mediates *SVL* induction by suppressing *PKL* after SDs. *SVL* expression was weaker in *abi1-1* apices after 10 weeks of SDs than in wild-type plants, whereas downregulation of *PKL* in *abi1-1* plants (*PKL*RNAi/*abi1-1*) resulted in restoration of *SVL* expression to almost wild-type levels after SDs (Figure 1B). Thus, SD-mediated induction of *SVL* in the apex is dependent on ABA and requires suppression of *PKL*.

The correlation of *SVL* expression with bud dormancy and attenuation of *SVL* expression in *abi1-1* plants prompted us to investigate *SVL*'s role in photoperiodic control of bud dormancy. We addressed this by investigating bud dormancy in *SVL*RNAi hybrid aspen plants [10] with reduced *SVL* expression. Both wild-type and *SVL*RNAi plants were exposed to 10 weeks of SDs and responded by termination of growth and bud formation. However, following subsequent exposure to long days (LDs) without chilling, buds of wild-type plants maintained growth arrest, indicating the establishment of bud dormancy (0% growth reactivation; n = 10) (Figure 1C), whereas buds of *SVL*RNAi plants resumed growth (100% reactivation; n = 10), indicating

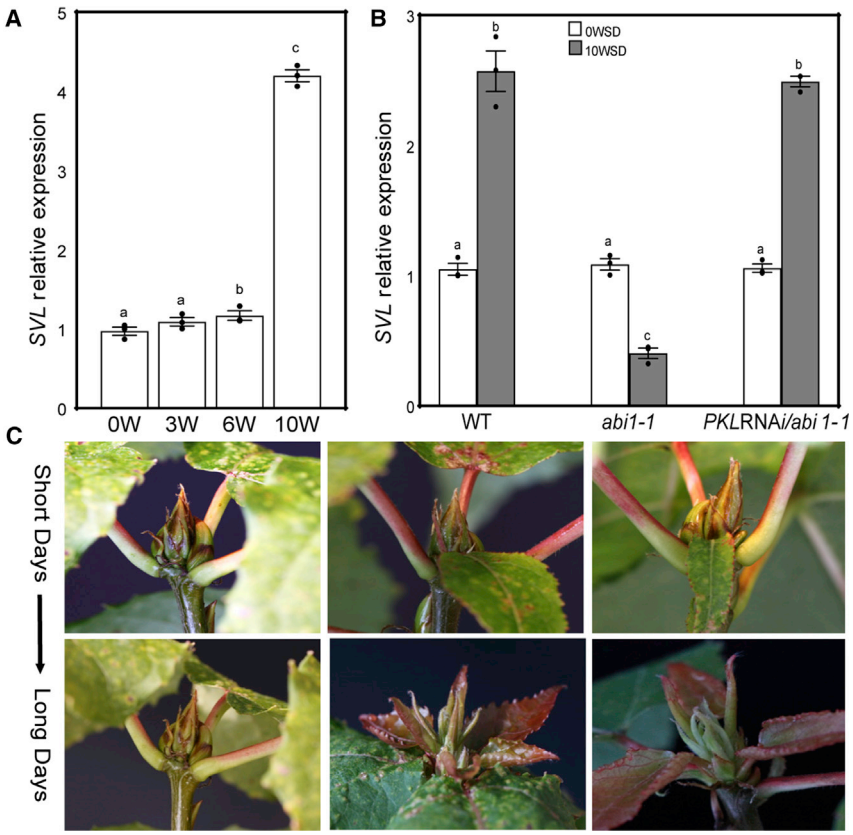

Figure 1. *SVL* Mediates in Photoperiodic Control of Bud Dormancy

(A) Expression of *SVL* in wild-type (WT) apices after short days.

(B) *SVL* expression is altered in ABA-insensitive (*abi1-1*) lines that also have dormancy establishment defects [7]. *SVL* suppression in *abi1-1* lines is reversed by *PKL* downregulation in *abi1-1* (*PKL*RNAi/*abi1-1*) plants. Relative transcript levels of *SVL* in wild-type, *abi1-1*, and *PKL*RNAi/*abi1-1* lines before (0WSD) and after (10WSD) 10 weeks of SDs.

Expression of the cited genes is shown relative to the reference gene *UBQ*, with 0W wild-type set to 1. Individual values (dots) and means (bars) with horizontal lines as SEM of three independent biological replicates are shown. Different letters (a–c) over the bars indicate significant differences at $p < 0.05$. Statistical analysis is done using one-way ANOVA implying Tukey's multiple comparison test.

(C) Apical buds of wild-type plants and *SVL*RNAi lines 2 and 10 after 10 weeks of SDs (upper panels). Unlike in wild-type plants, buds burst in *SVL*RNAi lines 2 and 10 after exposure to LDs without chilling (lower panels).

a defect in dormancy regulation. Thus, these results indicate that *SVL* mediates photoperiodic control of bud dormancy.

ABA mediates photoperiodic control of *SVL* expression, and *SVL*RNAi plants phenocopy *abi1-1* plants [7]. Therefore, we tested the hypothesis that defects in dormancy regulation in *abi1-1* plants are due to reduction in *SVL* expression by overexpressing *SVL* in *abi1-1* plants (Figure S1). Whereas *abi1-1* buds restarted growth (100% reactivation; n = 10), buds of the resulting *SVL*-overexpressing (*SVL*oe)/*abi1-1* plants maintained growth arrest (0% growth reactivation; n = 10) after exposure to LDs following 10 weeks of SDs. Thus, enhancing *SVL* expression in *abi1-1* plants can suppress defects in dormancy regulation evident in the parental *abi1-1* plants (Figure 2A).

We next identified the downstream targets of *SVL* to understand how it mediates in dormancy. Transition to dormancy by SDs involves the induction plasmodesmatal closure by callosic dormancy sphincters and downregulation of the gibberellic acid (GA) pathway [7, 10]. Because *SVL* acts downstream of ABA, we hypothesized that *SVL* could mediate in dormancy by transcriptional regulation of genes involved in plasmodesmatal closure and GA metabolism that are implicated in dormancy and whose regulation is altered in dormancy-defective *abi1-1* plants after SDs [7]. Therefore, we investigated the expression of *CALLOSE SYNTHASE 1* (*CALS1*), involved in plasmodesmatal closure via callose deposition, and those in the GA pathway, *GA20 oxidase*, a key enzyme in GA biosynthesis, and *GA2 oxidase*, that promotes GA catabolism, in *SVL* transgenics. Gene expression analysis indicated that *CALS1* and *GA2 oxidase 8* were downregulated in *SVL*RNAi (Figure 2B), whereas their expression was upregulated in *SVL*oe plants compared to wild-type (Figure 2C). Contrastingly, the expression of GA biosynthesis-related genes *GA20 oxidase 1* and *2* was upregulated in *SVL*RNAi apices (Figure S2A) and downregulated in *SVL*oe plants compared to wild-type after 10 weeks of SDs (Figure S2B). Thus, the defects in transcriptional regulation of *CALS1* and the GA pathway after SDs in *SVL*RNAi phenocopy those in *abi1-1* plants. We then investigated whether *SVL* can bind to the promoter of the *CALS1* and GA pathway genes. Chromatin immunoprecipitation (ChIP)-qRT-PCR experiments using DNA from shoot apices of transgenic hybrid aspen plants expressing Myc-tagged *SVL* (*Myc-SVL*) and wild-type control showed that *SVL* can directly bind at the CArG motif present in the *CALS1* and *GA2 oxidase 8* promoter (Figure 2D), whereas no significant binding was observed with the *GA20 oxidase 1* and *2* promoters (data not shown). These results indicate that *SVL* mediates in transcriptional regulation of key components involved in photoperiodic dormancy regulation by ABA.

We then took a genetic approach to examine the role of GA in ABA- and *SVL*-mediated control of bud dormancy. We overexpressed a *GA2 oxidase* (whose levels are reduced in *abi1-1* and *SVL*RNAi plants) in *SVL*RNAi and *abi1-1* plants and investigated bud dormancy in the resulting *GA2oxoe/SVL*RNAi and *GA2oxoe/abi1-1* plants (Figure S3). The buds in parental *SVL*RNAi and *abi1-1* plants showed dormancy defects, restarting growth after exposure to LDs following 10 weeks of SDs without chilling (100% growth reactivation; n = 10) (Figure 3). In contrast, *GA2oxoe/SVL*RNAi and *GA2oxoe/abi1-1* buds were dormant, because they maintained growth arrest during this treatment (0% reactivation of growth; n = 10) (Figure 3), suggesting the GA pathway as a downstream target of SDs in dormancy regulation.

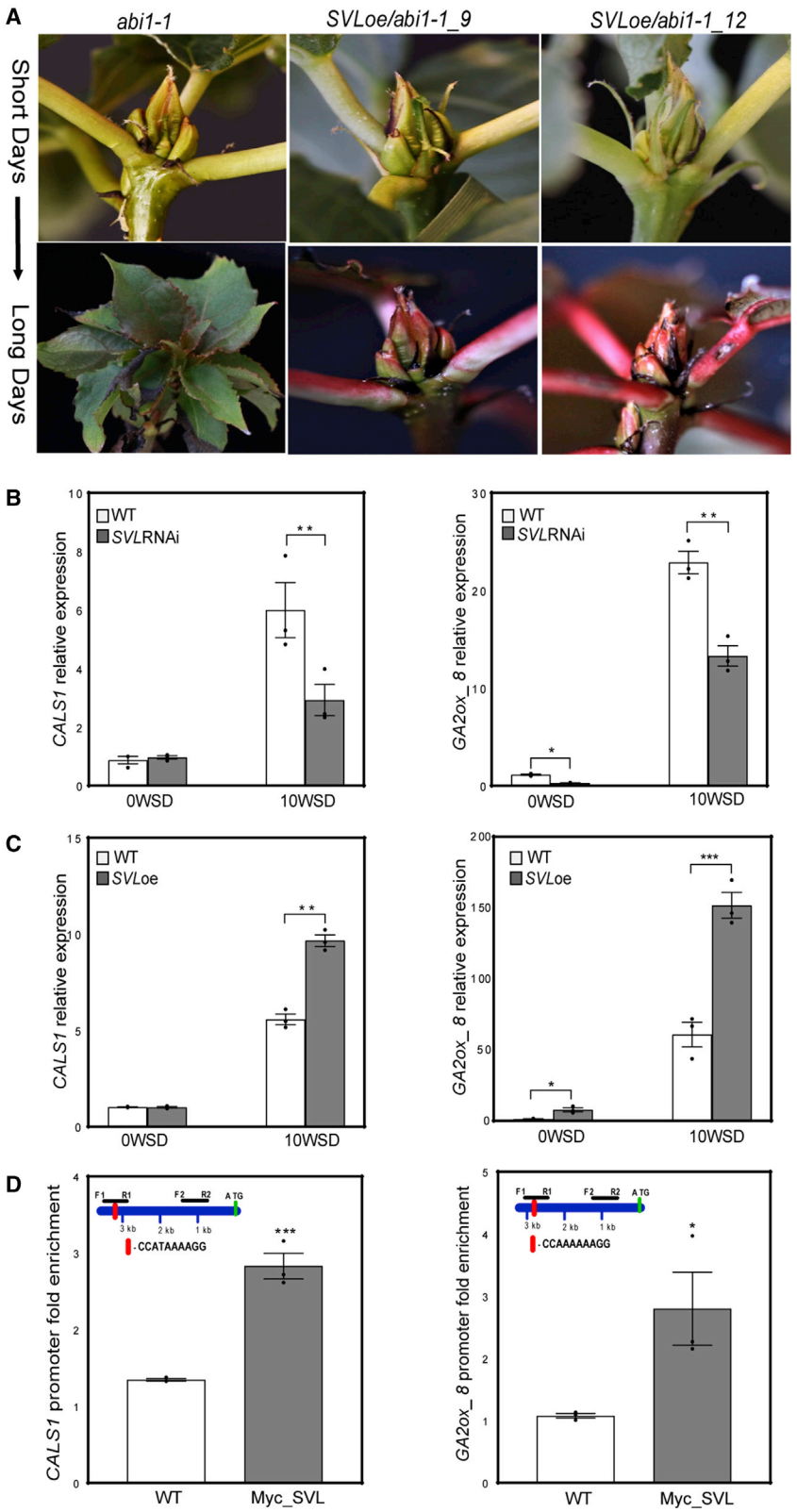

Figure 2. *SVL* Overexpression Restores Dormancy Defects in ABA-Insensitive (*abi1-1*) Plants, and *CALS1* and *GA2 oxidase 8* Are Downstream Targets of *SVL* in Dormancy Regulation

(A) Apical buds of parental *abi1-1* and *SVL*-over-expressing *abi1-1* (*SVL*oe/*abi1-1*) lines 9 and 12 after 10 weeks of SDs (upper panels). Unlike *abi1-1* plants, *SVL*oe/*abi1-1* lines 9 and 12 do not restart growth after exposure to LDs without chilling (lower panels).

(B) Expression of *CALS1* and *GA2 oxidase 8* in apices of wild-type and *SVL*RNAi plants before (0WSD) and after (10WSD) 10 weeks of SDs.

(C) Expression of *CALS1* and *GA2 oxidase 8* in apices of wild-type and *SVL*oe plants before (0WSD) and after (10WSD) 10 weeks of SDs.

Expression of the cited genes is shown relative to the reference gene UBQ, with 0W wild-type set to 1. Individual values (dots) and means ± SEM (bars ± horizontal lines) of three independent biological replicates are shown. Asterisks indicate significant (*$p < 0.05$), highly significant (**$p < 0.01$), and extremely significant differences (***$p < 0.001$) calculated using the t test.

(D) Enrichment of the DNA fragments containing the CArG motif quantified by ChIP-qRT-PCR for *CALS1* and *GA2 oxidase 8*. Presented values were first normalized by their respective input values, and then fold enrichments in wild-type and *SVL*-Myc plants relative to negative controls were calculated. Insets: diagrammatic representation of the *CALS1* and *GA2 oxidase 8* promoters showing the CArG motif and their positions within a 3.5-kb region. F1-R1 indicates positions of DNA fragments used to assess DNA-protein interactions in ChIP assays, and F2-R2 indicates positions of DNA fragments with no CArG motif used as negative controls in the assays. Asterisks indicate significant (*$p < 0.05$) and highly significant differences (***$p < 0.001$) calculated using the t test.

See also Figures S1 and S2.

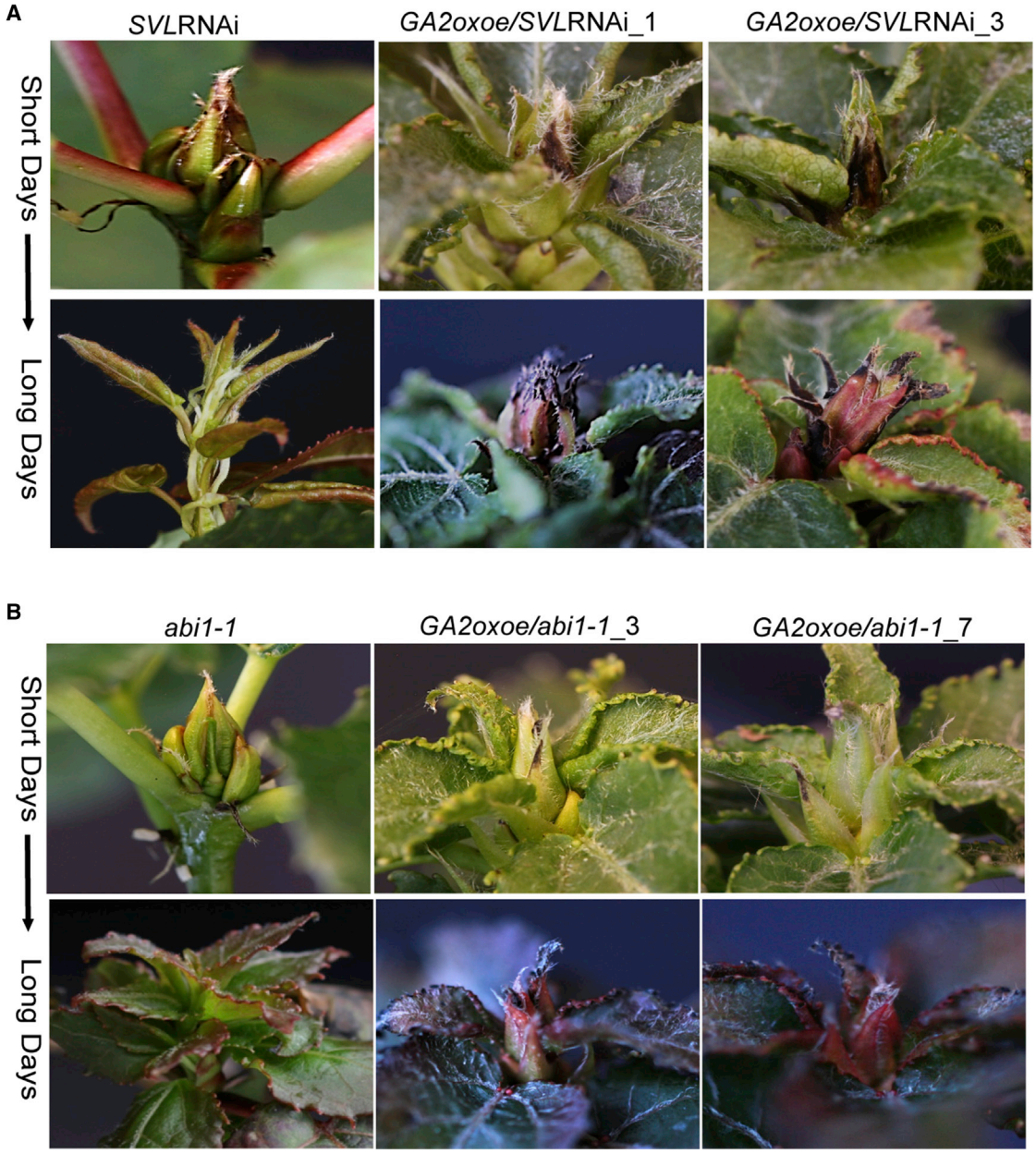

Figure 3. Reduction of GA Levels by Overexpression of *GA2 oxidase* Leads to Dormancy Restoration in *SVL*RNAi and *abi1-1* Plants

(A) Apical buds of *SVL*RNAi and *GA2oxoe/SVL*RNAi lines 1 and 3 (upper panels) after 10 weeks of SDs. Unlike *SVL*RNAi, apical buds of *GA2oxoe/SVL*RNAi lines 1 and 3 (lower panels) did not start growth after exposure to LDs without chilling.

(B) Apical buds of *abi1-1* and *GA2oxoe/abi1-1* lines 3 and 7 (upper panels) after 10 weeks of SDs. Unlike *abi1-1*, apical buds of *GA2oxoe/abi1-1* lines 3 and 7 (lower panels) did not start growth after exposure to LDs without chilling.

See also Figure S3.

In summary, bud dormancy in hybrid aspen, which is known to be regulated by ABA, is mediated by activation of *SVL* after SDs. ABA induces *SVL* expression after SDs and *SVL* promotes dormancy downstream of ABA by transcriptionally regulating the expression of genes, e.g., *CALS1*, that mediate in plasmodesmatal closure and those of GA metabolism. By suppressing expression of GA biosynthesis genes and inducing expression of a *GA2 oxidase* gene, *SVL* induction can ensure that the levels of active GA, a potent growth promoter [11, 12], cannot be enhanced after SDs, thereby lowering the potential for reactivation of growth in the buds and promoting dormancy (Figure 4).

Establishment of bud dormancy is a key developmental adaptation that enables perennials to survive harsh winter conditions. ABA mediates photoperiodic control of bud dormancy [7], and thus is an evolutionarily conserved dormancy regulator in seeds and apical buds [13]. Although extensive analysis of mutants of

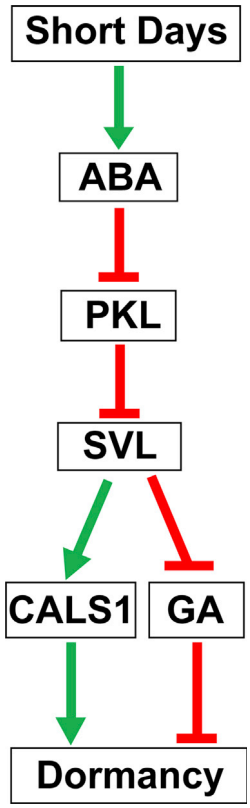

Figure 4. Model for Dormancy Regulation in Hybrid Aspen Buds
SDs induce growth cessation and increase levels of ABA. Upon exposure to SDs, ABA suppresses *PKL* to induce *SVL* expression. *SVL* induces *CALS1* expression and represses GA metabolism to ensure that GA levels cannot be upregulated nor reach shoot apical meristem (SAM) after SDs, thereby suppressing a key requirement for reinitiation of growth, and thus promoting dormancy.

the model plant *Arabidopsis* has uncovered the genetic network underlying ABA-mediated control of seed dormancy [14, 15], corresponding networks in buds remain poorly characterized. Thus, our understanding of ABA's action in photoperiodic control of bud dormancy remained incomplete. Here we identified a tree ortholog of *Arabidopsis* floral repressor *SVP* as a target of ABA in photoperiodic control of bud dormancy. Induction of *SVL* expression in response to SDs is ABA dependent and associated with bud dormancy. Moreover, genetic evidence clearly shows that *SVL* plays a key role in transcriptional control of bud dormancy by photoperiodic signals downstream of ABA.

SVL is a member of a subgroup of MADS-box transcription factors of which *SVP*, in *Arabidopsis*, was best characterized as a repressor in temperature-mediated control of floral transition [10, 16–18]. In trees, much less was known about the function of *SVP*-like genes. However, recently *SVL*, and *DAM* genes in peach and other tree species (that are also closely related to SVP) [10, 19–25], were shown to function in temperature-mediated control of bud break. Bud break occurs only after dormancy is released by exposure to low temperature [26] and, interestingly, low temperature represses the expression of *SVL* and certain *DAM* gene [10, 27]. Moreover, genetic data showed that *SVL* and *DAM* genes act as negative regulators of bud break [10, 20, 21, 27]. Our data demonstrating *SVL* as a promoter of dormancy thus serve to clarify the role of low temperature in dormancy release and in inducing transition to bud break by suppressing *SVL* expression. Thus, the antagonistic regulation of *SVL* by photoperiodic and temperature signals can control the switch to dormancy and its subsequent release to facilitate bud break.

Our results suggest an interesting regulatory parallel between bud dormancy and floral transition, because the *SVL* ortholog of *SVP* suppresses floral transition in *Arabidopsis* also by suppressing GA pathways [28]. Thus, negative regulation of GA by SVP and its orthologs has been conserved in trees and *Arabidopsis*. However, in trees, *SVL* is positively regulated by ABA, whereas *SVP* regulation by ABA in *Arabidopsis* has not been reported so far. Thus, the action of *SVL* downstream of ABA appears to have enabled negative regulation of GA to be employed for dormancy regulation in buds in trees and flowering in *Arabidopsis* in response to environmental cues such as photoperiod. This is a striking example of evolutionary strategy based on using a conserved signaling module in the regulation of distinct developmental transitions.

We previously showed that dormancy regulation involves blockage of cell-cell communication via plasmodesmata [7]. Here we reveal that SDs acting through ABA activate *SVL*, a transcriptional factor negatively regulating growth-promotive GA pathways. Thus, SDs by inducing plasmodesmatal closure restrict the movement of growth-promotive signals in the apex, and this mechanism is complemented by *SVL*-mediated suppression of the growth-promotive GA pathway, lowering the potential for reactivation of growth and providing robustness to dormancy regulation. In summary, our results provide mechanistic insight into photoperiodic control of bud dormancy and reveal a new function for a tree ortholog of the *Arabidopsis* floral repressor *SVL* in bud dormancy regulation.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures and one table and can be found with this article online at https://doi.org/10.1016/j.cub.2018.11.006.

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AUTHOR CONTRIBUTIONS

R.K.S. and R.P.B. designed the experiments. R.K.S., P.M., and J.P.M. performed the experiments. R.K.S. and R.P.B. contributed to the writing of the manuscript. All the authors reviewed and approved the final version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

1. Ruttink, T., Arend, M., Morreel, K., Storme, V., Rombauts, S., Fromm, J., Bhalerao, R.P., Boerjan, W., and Rohde, A. (2007). A molecular timetable for apical bud formation and dormancy induction in poplar. Plant Cell *19*, 2370–2390.
2. Cooke, J.E.K., Eriksson, M.E., and Junttila, O. (2012). The dynamic nature of bud dormancy in trees: environmental control and molecular mechanisms. Plant Cell Environ. *35*, 1707–1728.
3. Ding, J., and Nilsson, O. (2016). Molecular regulation of phenology in trees—because the seasons they are a-changin'. Curr. Opin. Plant Biol. *29*, 73–79.
4. Singh, R.K., Svystun, T., AlDahmash, B., Jönsson, A.M., and Bhalerao, R.P. (2017). Photoperiod- and temperature-mediated control of phenology in trees—a molecular perspective. New Phytol. *213*, 511–524.
5. Heide, O.M. (1974). Growth and dormancy in Norway spruce ecotypes: II. After-effects of photoperiod and temperature on growth and development in subsequent years. Physiol. Plant. *31*, 131–139.
6. Rohde, A., and Bhalerao, R.P. (2007). Plant dormancy in the perennial context. Trends Plant Sci. *12*, 217–223.
7. Tylewicz, S., Petterle, A., Marttila, S., Miskolczi, P., Azeez, A., Singh, R.K., Immanen, J., Mähler, N., Hvidsten, T.R., Eklund, D.M., et al. (2018). Photoperiodic control of seasonal growth is mediated by ABA acting on cell-cell communication. Science *360*, 212–215.
8. Petterle, A. (2011). ABA and chromatin remodelling regulate the activity-dormancy cycle in hybrid aspen. PhD thesis (Swedish University of Agricultural Sciences).
9. Horvath, D.P., Chao, W.S., Suttle, J.C., Thimmapuram, J., and Anderson, J.V. (2008). Transcriptome analysis identifies novel responses and potential regulatory genes involved in seasonal dormancy transitions of leafy spurge (*Euphorbia esula* L.). BMC Genomics *9*, 536.
10. Singh, R.K., Maurya, J.P., Azeez, A., Miskolczi, P., Tylewicz, S., Stojkovič, K., Delhomme, N., Busov, V., and Bhalerao, R.P. (2018). A genetic network mediating the control of bud break in hybrid aspen. Nat. Commun. *9*, 4173.
11. Eriksson, M.E., Hoffman, D., Kaduk, M., Mauriat, M., and Moritz, T. (2015). Transgenic hybrid aspen trees with increased gibberellin (GA) concentrations suggest that GA acts in parallel with FLOWERING LOCUS T2 to control shoot elongation. New Phytol. *205*, 1288–1295.
12. Zawaski, C., and Busov, V.B. (2014). Roles of gibberellin catabolism and signaling in growth and physiological response to drought and short-day photoperiods in *Populus* trees. PLoS ONE *9*, e86217.
13. Maurya, J.P., and Bhalerao, R.P. (2017). Photoperiod- and temperature-mediated control of growth cessation and dormancy in trees: a molecular perspective. Ann. Bot. *120*, 351–360.
14. Née, G., Xiang, Y., and Soppe, W.J. (2017). The release of dormancy, a wake-up call for seeds to germinate. Curr. Opin. Plant Biol. *35*, 8–14.
15. Shu, K., Liu, X.D., Xie, Q., and He, Z.H. (2016). Two faces of one seed: hormonal regulation of dormancy and germination. Mol. Plant *9*, 34–45.
16. Hartmann, U., Höhmann, S., Nettesheim, K., Wisman, E., Saedler, H., and Huijser, P. (2000). Molecular cloning of SVP: a negative regulator of the floral transition in *Arabidopsis*. Plant J. *21*, 351–360.
17. Lee, J.H., Ryu, H.S., Chung, K.S., Posé, D., Kim, S., Schmid, M., and Ahn, J.H. (2013). Regulation of temperature-responsive flowering by MADS-box transcription factor repressors. Science *342*, 628–632.
18. Lee, J.H., Yoo, S.J., Park, S.H., Hwang, I., Lee, J.S., and Ahn, J.H. (2007). Role of SVP in the control of flowering time by ambient temperature in *Arabidopsis*. Genes Dev. *21*, 397–402.
19. Hao, X., Chao, W., Yang, Y., and Horvath, D. (2015). Coordinated expression of FLOWERING LOCUS T and DORMANCY ASSOCIATED MADS-BOX-like genes in leafy spurge. PLoS ONE *10*, e0126030.
20. Wu, R., Tomes, S., Karunairetnam, S., Tustin, S.D., Hellens, R.P., Allan, A.C., Macknight, R.C., and Varkonyi-Gasic, E. (2017). *SVP*-like MADS box genes control dormancy and budbreak in apple. Front. Plant Sci. *8*, 477.
21. Wu, R., Wang, T., Warren, B.A.W., Allan, A.C., Macknight, R.C., and Varkonyi-Gasic, E. (2017). Kiwifruit SVP2 gene prevents premature budbreak during dormancy. J. Exp. Bot. *68*, 1071–1082.
22. Wu, R.M., Walton, E.F., Richardson, A.C., Wood, M., Hellens, R.P., and Varkonyi-Gasic, E. (2012). Conservation and divergence of four kiwifruit SVP-like MADS-box genes suggest distinct roles in kiwifruit bud dormancy and flowering. J. Exp. Bot. *63*, 797–807.
23. Leida, C., Conesa, A., Llácer, G., Badenes, M.L., and Ríos, G. (2012). Histone modifications and expression of DAM6 gene in peach are modulated during bud dormancy release in a cultivar-dependent manner. New Phytol. *193*, 67–80.
24. Li, Z., Reighard, G.L., Abbott, A.G., and Bielenberg, D.G. (2009). Dormancy-associated MADS genes from the EVG locus of peach [*Prunus persica* (L.) Batsch] have distinct seasonal and photoperiodic expression patterns. J. Exp. Bot. *60*, 3521–3530.
25. Yamane, H., Ooka, T., Jotatsu, H., Hosaka, Y., Sasaki, R., and Tao, R. (2011). Expressional regulation of PpDAM5 and PpDAM6, peach (*Prunus persica*) dormancy-associated MADS-box genes, by low temperature and dormancy-breaking reagent treatment. J. Exp. Bot. *62*, 3481–3488.
26. Saure, M. (1985). Dormancy release in deciduous fruit trees. Hortic. Rev. (Am. Soc. Hortic. Sci.) *7*, 239–300.
27. Jiménez, S., Reighard, G.L., and Bielenberg, D.G. (2010). Gene expression of DAM5 and DAM6 is suppressed by chilling temperatures and inversely correlated with bud break rate. Plant Mol. Biol. *73*, 157–167.
28. Andrés, F., Porri, A., Torti, S., Mateos, J., Romera-Branchat, M., García-Martínez, J.L., Fornara, F., Gregis, V., Kater, M.M., and Coupland, G. (2014). SHORT VEGETATIVE PHASE reduces gibberellin biosynthesis at the *Arabidopsis* shoot apex to regulate the floral transition. Proc. Natl. Acad. Sci. USA *111*, E2760–E2769.
29. Azeez, A., Miskolczi, P., Tylewicz, S., and Bhalerao, R.P. (2014). A tree ortholog of APETALA1 mediates photoperiodic control of seasonal growth. Curr. Biol. *24*, 717–724.
30. Karlberg, A., Bako, L., and Bhalerao, R.P. (2011). Short day-mediated cessation of growth requires the downregulation of AINTEGUMENTALIKE1 transcription factor in hybrid aspen. PLoS Genet. *7*, e1002361.
31. Saleh, A., Alvarez-Venegas, R., and Avramova, Z. (2008). An efficient chromatin immunoprecipitation (ChIP) protocol for studying histone modifications in *Arabidopsis* plants. Nat. Protoc. *3*, 1018–1025.
32. Gendrel, A.V., Lippman, Z., Martienssen, R., and Colot, V. (2005). Profiling histone modification patterns in plants using genomic tiling microarrays. Nat. Methods *2*, 213–218.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
anti-MYC monoclonal antibody	Abcam	ab32, Mouse monoclonal to c-Myc - ChIP Grade, GR255064; RRID: AB_303599
Bacterial and Virus Strains		
Agrobacterium tumifaciens GV3101pmp90RK	[19]	N/A
Chemicals, Peptides, and Recombinant Proteins		
Murashige and Skoog (MS) medium	Duchefa	M0222
Critical Commercial Assays		
pENTR/D-TOPO Cloning kit	Life Technologies	Cat#: K2400-20
Gateway LR Clonase II Enzyme mix	Life Technologies	Cat#: 11791100
Spectrum Total Plant RNA isolation kit	Sigma	Cat#: STRN250
RNeasy Plant mini kit	QIAGEN	Cat#: 74904
iScript cDNA Synthesis Kit	Bio-Rad	Cat#: 170-8891
Maxima Sybr green qPCR mix	Life Technologies	Cat#: K0253
Experimental Models		
Hybrid aspen (*Populus tremula x tremuloides*) clone T89 (wildtype/WT)		N/A
Oligonucleotides		
See Table S1		N/A
Recombinant DNA		
pENTR/D-TOPO	Invitrogen	Cat#: K240020
pH2GW7	[20]	N/A
pK2GW7	[20]	N/A
pK7GWIWG2 (I)	[20]	N/A
Software and Algorithms		
GraphPad Prism version 7	GraphPad software	https://www.graphpad.com/

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Rishikesh P Bhalerao (rishi.bhalerao@slu.se).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Hybrid aspen (*Populus tremula x tremuloides*) clone T89 was used as experimental model.

METHOD DETAILS

Plant material and growth conditions

Hybrid aspen (*Populus tremula x tremuloides*) clone T89 (wild-type) and the transgenic plants were cultivated in half-strength MS medium (Duchefa) under sterile conditions for 5 weeks then transferred to soil and grown for another 4 weeks in the greenhouse (16 hr light, 22°C and 66% relative humidity). For dormancy tests, plants were grown in growth chambers, initially under LD conditions (16 hr, 20°C light/8 hr, 15°C dark cycles) for one week for acclimatization and subsequently under SD conditions (8 hr, 20°C light/16 hr, 15°C dark cycles) for 10 weeks. Following SD treatment, plants were exposed to LD conditions again, and bud burst was monitored for at least 8 weeks to assess establishment of bud dormancy [7]. 10 plants from wild-type and each transgenic lines were tested for dormancy. Percentage of plants restarting growth was calculated using number of plants started/ total no. of plants multiplied by 100. Samples of apices were taken for expression analysis before exposing plants to SDs (at 0 weeks, 0W) and after plants had ceased growth and developed dormancy, i.e., after 10 weeks of SDs (10WSD).

Generation of plasmid constructs and plant transformation

The transgenic hybrid aspen plants overexpressing (*SVL*oe) and with reduced *SVL* expression (*SVL*RNAi) have been described before [10]. Briefly, full length cDNA for *SVL* was amplified using primers described in Table S1 and cloned into vector pK2GW7 for transformation of hybrid aspen clone T89 to generate *SVL* overexpressers. A 156 bp fragment of *SVL* cDNA was amplified using primers described in Table S1 and cloned into pK7GWIWG2 (I) vector and used for downregulation of *SVL* (*SVL*RNAi). For overexpression of *GA2 oxidase*, full length cDNAs were amplified using primers listed in Table S1 and cloned in plant expression gateway vector pH2GW7. The generated plant transformation vectors were subsequently transformed into Agrobacterium strain GV3101pmp90RK and used for transformation of *SVL*RNAi and *abi1-1* plants, as previously described [10, 29] to generate *SVL*oe/*abi1-1*, *GA2oxoe*/*SVL*RNAi and *GA2oxoe*/*abi1-1* lines, respectively. A MYC tag was introduced in forward primer used to amplify full length *SVL* cDNA and cloned into pK2GW7 vector to obtain Myc-SVL fusion construct. Methods and details for *abi1-1* and *PKL*RNAi/*abi1-1* is mentioned previously [7].

RNA isolation and quantitative real-time PCR (qRT-PCR) analysis

Total RNA was extracted using a Sigma Spectrum Total Plant RNA isolation kit from samples of tissues taken at the same time of day. Portions (10 μg) of total RNA were treated with RNase-Free DNase (QIAGEN) and cleaned using an RNeasy Mini Kit (QIAGEN). One μg of the RNA from each sample was used to generate cDNA using an iScript cDNA synthesis kit (BioRad). Selected (UBQ-like) reference genes were validated using GeNorm Software. qRT-PCR analyses were carried out with a Roche LightCycler 480 II instrument and relative expression values were calculated using the Δ-ct-method, as previously described [30]. A complete list of primers used in qRT-PCR analysis is presented in Table S1.

Chromatin Immunoprecipitation (ChIP) Assays

The ChIP assays were carried out as previously described by [31] and [32] with some modifications [10]. Briefly, apex from actively growing hybrid aspen wild-type and Myc-*SVL* plants were harvested and cross-linked using 1% formaldehyde. 1gm of sample were ground into fine powder in precooled nuclei isolation buffer and then filtered through two layers of Miracloth. The homogenized, filtered mixtures were then centrifuged and the pellets obtained were re-suspended in nuclei lysis buffer. Chromatin was sheared to about 0.3-0.5 kb fragments by sonication (Bioruptor UCD-300, Diagenode). After sonication, the samples were centrifuged again to remove cell debris and each supernatant was transferred to a new tube (after retaining 10% of each sonicated sample used as Input DNA control in the Q-PCR analyses. After centrifugation, the supernatant was precleared with 40 μL protein A-magnetic beads for 60 min at 4°C with gentle agitation and shaking. Fifteen microgram of anti-MYC monoclonal antibody (Abcam, Cambridge, UK, Cat no. ab32; GR255064) was added to each supernatant and the resulting mixtures were further incubated overnight at 4°C. Protein A-magnetic beads (Dynabeads, Invitrogen) were then added again and incubation was continued for 2 hr. The magnetic beads were washed two times each with low salt buffer, high salt buffer, LiCl buffer and TE buffer. The immunocomplexes were collected from beads with 250 μL of elution buffer and incubated at 65°C for 20 min with agitation. 0.3 M NaCl was added to each tube (and Input DNA control) and cross-linking was reversed by incubation at 65°C overnight. Residual protein was degraded by incubation with 20 μg of Proteinase K in 10 mM EDTA and 40 mM Tris-HCl, pH 8.0, at 45°C for 1 hr. After proteinase treatment precipitated DNA was purified using a ChIP DNA clean and concentrator kit according to the manufacturer's protocol (Zymo Research Corp.). Both immunoprecipitated DNA and input DNA were analyzed by real-time PCR (Light Cycler; Roche Applied Science) using primers for *GA2 oxidase* and *CALS1* listed in Table S1. Promoter fragment amplified using (F1-R1) pair of primers contains CArG motif and was used to assess DNA-protein interactions in ChIP assays, and fragment amplified from primer pair (F2-R2) with no CArG motif was used as negative controls in the assays.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis was performed in Graphpad Prism 7. Statistical significance was tested using the standard t test. The sample sizes and p values are reported in the figure legends.