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Vernalization shapes shoot architecture and ensures the maintenance of dormant buds in the perennial Arabis alpina

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Summary

- Perennials have a complex shoot architecture with axillary meristems organized in zones of differential bud activity and fate. This includes zones of buds maintained dormant for multiple seasons and used as reservoirs for potential growth in case of damage. The shoot of Arabis alpina, a perennial relative of Arabidopsis thaliana, consists of a zone of dormant buds placed between subapical vegetative and basal flowering branches. This shoot architecture is shaped after exposure to prolonged cold, required for flowering.
- To understand how vernalization ensures the maintenance of dormant buds we performed physiological and transcriptome studies, followed the spatiotemporal changes of auxin, and generated transgenic plants.
- Our results demonstrate that the complex shoot architecture in A. alpina is shaped by its flowering behavior, specifically the initiation of inflorescences during cold treatment and rapid flowering after subsequent exposure to growth-promoting conditions. Dormant buds are already formed before cold treatment. However, dormancy in these buds is enhanced during, and stably maintained after, vernalization by a BRC1-dependent mechanism. Post-vernalization, stable maintenance of dormant buds is correlated with increased auxin response, transport, and endogenous indole-3-acetic acid (IAA) levels in the stem.
- Here we provide a functional link between flowering and the maintenance of dormant buds in perennials.

Key Words: Arabis alpina, bud dormancy, dormant bud bank, flowering, perennial, polycarpic, shoot architecture, vernalization

Introduction

Perennial plants live for many years and reproduce several times during their lifetime, whereas annuals die after the first reproduction. Growth after flowering in perennials is maintained by vegetative meristems, which in the following year will develop either into vegetative branches or stay dormant for multiple seasons. Perennials persist through harsh environmental conditions during the winter. Thus, axillary and apical buds in a perennial plant transition through the various stages of dormancy before they resume active organogenesis the following spring (Rohde & Bhalerao, 2007).

A bud can become dormant and enter a state of low metabolic activity in response to several cues, which is a phenomenon observed in annuals and more often in perennial species. During development the outgrowth of axillary buds close to the shoot apical meristem is repressed by apical dominance. This is a classic example where the development of an organ is influenced by another organ and has also been described as correlative inhibition, latency or paradormancy in annual and perennial species (Cline, 1991; Considine & Considine, 2016). This form of dormancy is not definitive and buds can resume growth when the inhibiting organ, which is often the main shoot apex, is removed (Snow, 1925). Buds in trees and herbaceous perennials also enter two other forms of dormancy, endo- and eco- dormancy (Rohde & Bhalerao, 2007). Endodormancy is regulated by endogenous signals within the bud whereas ecodormancy is imposed by unfavorable environmental conditions (Lang et al., 1987). Thus ecodormant buds are able to activate growth in response to decapitation (removal of the main shoot apex) only under growth promotive conditions whereas endodormant buds are not (Lang et al., 1987). During the life cycle of a perennial plant, apical or axillary buds experience winter in the endodormant state and later become ecodormant so that they will actively grow only during favorable environmental conditions. It is, however, very common in perennials to maintain dormant axillary buds across multiple seasons. These dormant buds serve as a backup bud bank and, in case of damage, are used as reservoirs for potential growth facilitating a bet-hedging mechanism (Nilsson et al., 1996; Vasconcelos et al., 2009). Interestingly, dormant buds and actively growing (vegetative or reproductive) axillary branches are organized in zones in a species-specific pattern (Costes et al., 2014; Lazaro et al., 2018).

The outgrowth of an axillary bud after decapitation involves two phases, firstly the rapid release from dormancy and secondly its sustained growth (Wang et al., 2018). Auxin, strigolactones, cytokinin and sugar fine-tune this process by regulating the expression of the TCP transcription factor BRANCHED 1 (BRC1) (Aguilar-Martínez et al., 2007; Rameau et al., 2015).

Decapitation causes an elevation of sucrose levels followed by a depletion of the endogenous indole-3-acetic acid (IAA) in the polar auxin transport stream, which influences the auxin flux out of the axillary bud contributing to its sustainable growth (Prusinkiewicz et al., 2009; Crawford et al., 2010; Shinohara et al., 2013; Mason et al., 2014; Barbier et al., 2015). The transition to flowering triggers the activation of the upper most axillary buds in a basipetal sequence having a similar effect to decapitation (Prusinkiewicz et al., 2009; Dierck et al., 2016). In an annual plant, the relation between flowering and bud activation is easy to trace as its life cycle ends within one growing season. However, many perennials spread the flowering process over several years and, although they initiate flowering during the summer or autumn, these plants usually flower the following spring (Vasconcelos et al., 2009; Hsu et al., 2011; Kurokura et al., 2013).

Here we used the perennial Arabis alpina, a close relative of Arabidopsis thaliana, to investigate the link between flowering and the maintenance of a dormant bud bank observed in many perennial species. A. alpina plants flower in response to prolonged exposure to cold, a process known as vernalization (Wang et al., 2009). Interestingly, floral buds in A. alpina are initiated during cold exposure and the duration of cold treatment is important to ensure floral commitment before plants experience growth-promoting conditions again (Wang et al., 2009; Lazaro et al., 2018). The shoot architecture of A. alpina consists of branches that undergo flowering, others that remain vegetative and nodes with dormant axillary buds (Wang et al., 2009). Similar to other perennials, this architecture is organized in zones with consequent nodes of dormant axillary buds (V2) being placed between subapical axillary vegetative branches (V3) and basal axillary flowering branches (V1) (Fig. 1; Costes et al., 2014; Lazaro et al., 2018).

As this architecture appears only in plants that flower in response to prolonged cold, we hypothesized that vernalization shapes shoot architecture and the maintainance of dormant axillary buds. We explored this hypothesis by characterizing the zonation pattern of A. alpina in conditions that influence flowering. We showed that the zone of V2 dormant buds is present only in flowering plants exposed to a sufficient length of cold treatment which ensures flowering. We also compared the transcriptome of V2 buds to the one of V3 buds during and post-vernalization. Our analysis indicated that dormancy in V2 buds is enhanced during vernalization and is maintained when plants are returned to growth-promoting conditions. We showed that during cold exposure, IAA transport in the stem is low and that the growth of V2 buds is inhibited by ecodormancy. V2 axillary buds stay dormant after plants return to growth-promoting conditions, being dominated by the outgrowing inflorescence and V3 vegetative

axillary branches. The A.alpina BRC1 also contributes to the maintenance of dormancy in V2 buds, during and after vernalization.

Materials and Methods

Plant material, growth conditions and phenotyping

All our experiments were performed with the Arabis alpina accession Pajares or the pep1-1 mutant described by Wang et al. (2009). Seeds were stratified for 4 days at 4°C and transferred to soil in a long day (LD, 16h light:8h dark) greenhouse at 18-20°C. After 8 weeks of growth, cold treatments were carried out in a short day (SD, 8h light: 16h dark) growth chamber at 4°C for different durations (depending on the experiment) before plants were moved back to a LD greenhouse. For 1-naphthaleneacetic acid (NAA) and 1-N-naphthylphthalamic acid (NPA) treatments, plants were sprayed immediately after 12 weeks of cold treatment and one week after the return to a LD greenhouse with 100µM NAA (Sigma-Aldrich), 100µM NPA (Chem Service) or DMSO supplemented with 0.2% (v/v) Tween-20. For the simple decapitation method (Fig. 5), the apex was removed directly above the highest extended internode (corresponding to nodes 11–14 within the V2 zone). Excision of inflorescence and/or V3 buds at the end of vernalization was performed under a stereo microscope by removing all flowering buds and/or the eight nodes below the lowest flowering bud (corresponding to V3 nodes). The shoot architecture at different time points was scored by recording bud fate (flowering, vegetative, dormant) and branch length at each node, and the number and type of branches per zone. At least 10-12 plants were used for each experiment.

Plasmid constructs

The DR5:GUS fragment was excised from a plasmid provided by Tom Guilfoyle and introduced into the GATEWAY-compatible pEarleyGate 301 plasmid containing the BASTA resistance gene. For the 35S:AaBRC1 dsRNAi constructs, three cDNA fragments of AaBRC1 (Fragment 1-3; Table S1) were amplified and introduced into the GATEWAY-compatible pJawohl-8-RNAi plasmid. The names of the A. alpina 35S:AaBRC1 dsRNAi lines correspond to the fragments introduced. For each construct, homozygous transgenic A. alpina lines carrying single copy transgenes were generated using the floral dip method (Clough & Bent, 1998).

GUS staining

V3 buds for the GUS staining assays were harvested from 8 nodes below the lowest flowering bud, whereas V2 buds were harvested from the nodes below to the V3 nodes. V2 stem samples for GUS staining assays were harvested from the upper three extended internodes in the main stem within the V2 zone. Samples were placed directly into 90% ice-cold acetone, incubated for 1h on ice, washed in 50mM phosphate buffer (pH 7.0) and submerged in 2.5mL GUS staining solution under moderate vacuum for 20min (Scarpella et al., 2004). After a 37°C incubation in the dark for maximum 16h, chlorophyll was removed by transferring the samples through an ethanol series. GUS activity was observed in whole stem tissues, transverse stem sections or longitudinal leaf axil sections 50–60µm prepared on a Leica VT1000S vibratome in samples immobilized on 6% (w/v) agarose. Representative photographs from two different biological experiments were taken using the stereo microscope Nikon SMZ18 and Nikon Digital Sight camera (DS-Fi2) for whole stem segments, and the Zeiss Axio Imager microscope with the Zeiss Axio Cam 105 color camera for cuttings.

RNA extraction, cDNA synthesis and quantitative real-time PCR

For RNA-Seq transcript profiling and quantitative RT-PCR analysis, V2 and V3 buds were harvested under a stereo microscope from nodes indicated in each experiment. For quantification of the GUS expression of the DR5:GUS lines, the upper three extended internodes within the V2 zone in the main stem were harvested and the axillary buds were removed. Each experiment consisted of three independent biological replicates. Total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen) and subjected to DNase treatment using the Ambion DNA-free kit (Invitrogen). Total RNA (2 µg) was used for the synthesis of cDNA by reverse transcription with SuperScript II Reverse Transcriptase (Invitrogen) and oligo dT (18) primers.

For qRT-PCR analysis, three technical replicates were prepared using 26 ng of cDNA for each reaction. Relative gene expression values were calculated using ΔCt and the mean of the two reference gene expression values (AaUBI10 and AaPP2A) according to Pfaffl et al. 2001. The ΔCt values were scaled to the average of the control. Primers used are listed in Table S1.

RNA sequencing transcript profiling

Poly(A) RNA enrichment, library preparation and sequencing were carried out at the MPIPZ Genome Center, Cologne, Germany using 1 µg total RNA. Poly(A) RNA was isolated using the NEBNext Poly(A) mRNA Magnetic Isolation Module (New England Biolabs) and

quantified using capillary electrophoresis (TapeStation, Agilent Technologies) and a fluorometer (Qubit, Thermo Fisher Scientific). Libraries were prepared using the NEBNext Ultra Directional II RNA Library Prep Kit for Illumina (New England Biolabs) and sequenced using a HiSeq3000 (Illumina) with 1 x 150bp single read length.

Sequence reads were mapped and aligned to the reference genome using HISAT2 followed by assembly and quantification of expression levels using STRINGTIE. Gene counts for all samples were obtained using Python (http://ccb.jhu.edu/software/stringtie/dl/prepDE.py). The quality of the samples was assessed using dispersion plots. Differentially expressed genes with more than 2-fold change and a corrected p-value below 0.05 were obtained using DESeq2 and selected for further analysis. Transcriptome data from this study is deposited in the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) under the accession number GSE126944. Gene Ontology (GO) enrichment was performed using DAVID (Huang et al., 2009) for biological processes 5, with Benjamini correction (Pvalue < 0.05) and plotted using the BACA package in R (Fortino et al., 2015; http://r-project.org/) only for the Arabidopsis annotated orthologs. Data were analyzed using hierarchical clustering. FPKM values were extracted from the STRINGTIE-created GTF files and log2 transformed. For the clustering we used Cluster 3.0, in which we included only the genes that met both of the following criteria with: i) a log2 FPKM value equal or higher than 2 in at least one sample and ii) a difference between the maximum and minimum expression value of 1.5 or higher. The log2 FPKM values of selected genes were median-centered by transcript. Hierarchical clustering was performed using uncentered correlation and average linkage. Data were visualized using Java TreeView (http://doi.org/10.5281/zenodo.1303402). Venn diagrams were constructed using Venny v2.1 (http://bioinfogp.cnb.csic.es/tools/venny/).

Quantification of IAA

For the free IAA quantification of V2 samples, the upper three extended internodes within the V2 zone in the main stem were harvested and the axillary buds were removed. For the free IAA quantification of inflorescence (I) and V3 branches, 2cm stem cuttings from the base of each branch were harvested. Plant material (15mg fresh weight) was purified as previously described in Andersen et al. (2008), and 500pg $^{13}C_6$-IAA internal standard was added to each sample before homogenization and extraction. Free IAA was quantified in the purified samples using combined gas chromatography - tandem mass spectrometry.

^{3}H-IAA transport assay

For the auxin transport capacity assay, we harvested the top elongated stem segment within the V2 zone (21mm) from plants after 8 weeks in LDs (8wLD), after 12 weeks cold treatment (+0) and 5 days after the return to LD greenhouse conditions (+5d). Stem segments were placed on wet paper and transferred to 30µl 0.05% MES (pH 5.5–5.7) containing 100nM ^{3}H-IAA with the apical part being submerged into the solution for 10min (Hartmann Analytic). Samples were then transferred to fresh 0.05% MES containing 1µM IAA for 90min (Lewis & Muday, 2009). Samples harvested at the end of cold treatment were incubated with 1µM IAA at 4°C. Subsequently, samples were cut into 3mm segments, with 0-3mm being the apical part of the sample, and immersed in Rotiszint eco plus (Roth) for 16h before the quantification by scintillation for 2min using a LS6500 Multi-Purpose Scintillation Counter (Beckman Coulter). CPM values were scaled to the average for the 8wLD samples at 6-9mm or for 8wLD total samples.

Statistical analysis

Data was analyzed using ANOVA followed by a Tukey post hoc test for pairwise multiple comparisons using the R platform (http://r-project.org/). Pairwise comparisons were analyzed using Student's t test.

Results

Vernalization in A. alpina correlates with the formation of a complex shoot architecture and the maintenance of dormant buds

The A. alpina accession Pajares flowers in response to prolonged cold treatment (Wang et al., 2009). We scored bud activity and fate in Pajares (wild type) plants grown in long days for 8 weeks (8wLD), exposed to 12 weeks cold treatment (+0) and subsequently returned back to a LD greenhouse for up to 9 weeks (Fig. 2a,b,c,d,e,f). Plants before cold treatment developed axillary vegetative branches only in the lower nodes (1-8), whereas upper nodes (9-21) contained dormant buds (Fig 2e; 8wLD; U. Ponraj & K. Theres, unpublished). After cold treatment, plants flowered and already at 3 weeks post-vernalization had established a complex architecture consisting of basal flowering and vegetative axillary branches (Fig. 2a,b; yellow and green boxes in nodes 1–12 in Fig. 2e), subapical vegetative axillary branches (Fig. 2b; green boxes in nodes 22–29 in Fig. 2e) and apical inflorescence branches (Fig. 2a; yellow boxes in nodes 30–40 in Fig. 2e). Basal flowering branches differ from the inflorescence branches by only partially senescing at the end of flowering. Nodes 13-21 were not occupied by an axillary

branch or contained a bud that did not grow more than 0.5 cm even after 9 weeks post-vernalization (Fig. 2c,d; grey and brown boxes in Fig. 2e). This result suggests that buds in nodes 13-21 are dormant before and after cold treatment. Similar to other woody perennials, the A. alpina stem was organized in zones of differential bud activity and fate according to their position on the main stem (Figs 1, 2e; Costes et al., 2014; Lazaro et al., 2018). Nodes 13-21 represent the V2 dormant bud zone and were the last nodes initiated before cold treatment. This zone is located between the subapical V3 zone of axillary vegetative branches (nodes 22-29) and the V1 zone of basal flowering branches (nodes 1-12) (Figs 1, 2e; Lazaro et al., 2018).

To check whether this architecture is determined by vernalization response we scored the shoot architecture in the perpetual flowering 1 (pep1) mutant which carries lesions in the ortholog of the MADS box transcription factor FLOWERING LOCUS C (FLC) and does not require cold treatment to flower (Michaels & Amasino, 1999; Sheldon et al., 2000; Wang et al., 2009). All axillary buds in pep1-1 developed into flowering branches and similar to wild type plants were activated acropetally (Fig. 2f,g; Wang et al., 2009). pep1-1 also lacked the zones of dormant axillary buds and vegetative axillary branches. In wild type plants, buds were gradually activated in an acropetal manner giving rise to an axillary branch even in nodes that stay stably dormant after exposure to cold treatment (Fig. 2g). This result suggests that vernalization in A. alpina is required to ensure the stable maintenance of dormant buds.

To further assess the relationship between vernalization and shoot architecture, we exposed wild type plants to an insufficient duration of cold treatment that does not ensure flowering and scored bud activity and fate (Fig. 3). Similar to previous studies, plants grown continuously in LDs or cold-treated only for 3 weeks did not flower, whereas plants exposed to 8 weeks cold treatment showed extreme floral reversion phenotypes (e.g. inflorescences produced leaves instead of flowers) (Fig. S1; Wang et al., 2009; Lazaro et al., 2018). We compared the length of axillary branches at each leaf axil at 3 weeks post-cold treatment or after 11 weeks in LDs for the non-cold-treated plants. Branch length in LD-grown plants and in plants exposed to 3 weeks of cold was reduced acropetally (treatment 0 and 3 in Fig. 3c). Interestingly, nodes 13–19 (corresponding to the V2 zone) were completely inhibited only in plants exposed to 12 weeks of cold and which flowered (treatment 12 in Fig. 3b,c). In plants exposed to 8 weeks of cold treatment, axillary branches developed in nodes 13–19 (V2 zone) but their length was shorter than branches in nodes 1-12 (V1 zone) or in nodes 20-29 (V3 zone) (treatment 8 in Fig. 3b,c). Interestingly, V3 vegetative branches appeared only in flowering plants and were always located just above the nodes with the inhibited buds. This result suggests that the presence of

V3 branches might be correlated with the repression of bud growth in the V2 zone but only a period of 12 weeks of cold treatment secures the stable inhibition of V2 buds.
Overall, these results suggest that vernalization is linked to the complex architecture in A. alpina and genotypes that flower without cold treatment lack the zone of dormant buds.

Transcriptome analysis suggests that V3 buds are not dormant during vernalization, whereas dormancy in V2 buds is ensured during and post-vernalization

To investigate the molecular mechanisms that lead to the activation of V3 buds and the inhibition of growth in V2 buds, we performed a transcriptome profiling. To identify an early developmental stage post-vernalization in which the V2 and V3 buds are differentiated, we developed transgenic A. alpina plants carrying the DR5 promoter fused to the reporter gene β-glucuronidase (GUS). In A. thaliana and other species bud growth has been demonstrated using the auxin inducible synthetic promoter DR5 (Prusinkiewicz et al., 2009; Barbier et al., 2015). In A. alpina DR5:GUS transgenic plants, we detected the GUS signal only in V3 buds at 5 days post-vernalization (Fig. 4a,b,c,d). This result suggests that, although at 5 days post-vernalization there is no obvious development of V3 branches, the growth of V3 buds is activated. We then harvested axillary buds from nodes 16-19 to represent V2 buds and axillary buds from nodes 23-26 to represent V3 buds. Buds were harvested from plants immediately after 12 weeks of cold treatment (+0) and 5 days post-vernalization (+5d). As expected, the transcriptome of V2 and V3 buds at 5 days post-vernalization was the most dissimilar (1984 genes; +5dV2 vs +5dV3; Fig. 4e; Table S2). Interestingly, the transcriptome of V2 and V3 buds differed also at the end of vernalization (1128 genes for +0V2 vs +0V3; Fig. 4e; Table S2) suggesting that the V2 and V3 buds were differentiated already during cold treatment. Almost all genes that were differentially expressed between V2 and V3 buds at the end of cold treatment were also differentially expressed in the other comparisons (Fig. 4e). Likewise, Gene Ontology (GO) enrichment analysis indicated that all GOs enriched for the differentially expressed genes between V2 and V3 buds at the end of cold treatment were also identified in the other comparisons (Fig. S2; Table S3). GO terms common to all comparisons were mainly associated with `water deprivation´ and `hormone responses´ such as abscisic acid, ethylene and jasmonic acid.

To identify genes that share similar expression patterns, we performed hierarchical clustering analysis. Low expressed and invariant genes were filtered out and from 25817 genes whose

transcripts were found accumulated at least in one of the conditions tested, 4983 could be classified using hierarchical clustering (Fig. 4f). Our biological replicates were clustered together (Fig. 4f). Interestingly, samples harvested at the end of cold treatment (+0) were separated from samples harvested from plants 5 days after the return to greenhouse conditions (+5d) suggesting a general transcriptional reprogramming in both V2 and V3 buds when plants were moved from cold to warm conditions (Fig. 4f). We identified 34 co-expressed clusters, which were assigned into two higher level clusters I and II (Fig. 4f; Table S4,). The separation of these higher level clusters was shaped by the expression of genes in the V3 buds during vernalization. Genes in Cluster I showed low and genes in Cluster II showed high transcript accumulation in V3 buds at the end of vernalization (Fig. 4f). Genes in Cluster I3 and I6 showed higher transcript accumulation in V3 buds post-vernalization accounting for putative candidate genes involved in the activation of growth in V3 buds (Fig. 4g,h). These included homologs of genes involved in cell expansion EXPANSIN B1 (EXPB1), IAA biosynthesis YUCCA2 and the auxin signaling factors INDOLE-3-ACETIC ACID 7 and 14 (IAA7, IAA14) (Fig. 4g,h; Zhao et al., 2001; Sharova, 2007; Paponov et al., 2008). Interestingly, most enriched GO terms in Cluster I3 were associated with developmental processes (e.g. "trichome differentiation", "plant epidermis morphogenesis" and "cell fate commitment") (Table S5). Genes in Clusters II7 and II8 showed higher transcript accumulation in V3 buds compared to V2 buds already during cold treatment (Fig. 4j). Both clusters were enriched for GO terms related to cell division (e.g. "DNA metabolic processes", "DNA replication" and "nuclear division") and included cell cycle regulators such as PROLIFERATING CELLULAR NUCLEAR ANTIGEN 1 (PCNA1) shown to be upregulated during bud activation in other species (Fig. 4j; Table S4 and S5; Roman et al., 2016; Holalu & Finlayson, 2017). The enrichment of genes involved in cell cycle and transcriptional machinery detected to be upregulated in V3 buds during vernalization suggests that V3 buds are not dormant during cold treatment.

Clusters I14, I15, I20 and I21 showed higher transcript accumulation in V2 buds compared to V3 buds during and after cold treatment (Fig. 4i). Interestingly, all these clusters were enriched for GOs related to abscisic acid and water deprivation (Fig. 4i; Table S5). These clusters contained genes related to ABA signaling such as ABA INSENSITIVE RING PROTEIN 2 (AIRP2), ABSCISIC ACID RESPONSIVE ELEMENT-BINDING FACTOR 1 and 2 (ABF1, ABF2), ABA INSENSITIVE 1 and 5 (ABI1, ABI5), KINASE 2 (KIN2), ABI FIVE BINDING PROTEIN 1 and 3 (AFP1, AFP3), BEL1-LIKE HOMEODOMAIN 1 (BLH1) and PROTEIN PHOSPHATASE 2CA (PP2CA) or ABA biosynthesis (e.g. NINE-CIS-EPOXYCAROTENOID DIOXYGENASE 3, NCED3) shown to be associated with bud dormancy in several species (Fig.

4i; Destefano-Beltrán et al., 2006; Zheng et al., 2015; Chao et al., 2017; Singh et al., 2018). We also detected the dehydrin coding genes EARLY RESPONSIVE TO DEHYDRATION 10 (ERD10), ERD14 and RESPONSIVE TO ABA 18 (RAB18), which are induced by ABA and suggested to prevent water dehydration during tree winter dormancy (Fig. 4i; Arora et al., 2003). Transcript accumulation of homologs of genes associated with the repression of the cytokinin level and response such as CYTOKININ OXIDASE/DEHYDROGENASE 1 (CKX1) and KISS ME DEADLY 1–4 (KMD1–4) were also detected in these clusters (Fig. 4i; Werner et al., 2001; Kim et al., 2013). We also identified the homologs of genes that have been previously shown in A. thaliana to respond to conditions that trigger dormancy and therefore are considered as dormancy markers (Tarancón et al., 2017). This includes the A. alpina homologs of HOMEOBOX 21 and 53 (HB21, HB53), PLANT PEPTIDE CONTAINING SULFATED TYROSINE 1 (PSY1), NAC DOMAIN CONTAINING PROTEIN 29 (NAC029), SENESCENCE-ASSOCIATED GENE 21 (SAG21), HISTONE H1-3 (HIS1-3) and EIN3-BINDING F BOX PROTEIN 2 (EBF2) (genes highlighted in bold in Fig. 4i; Table S6). Interestingly, genes in Cluster I21 showed high transcript upregulation in V2 buds specifically post-vernalization (Fig. 4i). In this cluster, we detected the homologs of the strigolactone signaling genes DWARF 14 (D14) and SUPPRESSOR OF MAX2 1 (SMAX1). D14 in A. thaliana and its homologs in other species regulate bud dormancy (Arite et al., 2009; Waters et al., 2012). Overall, these results suggest that dormancy in V2 buds is regulated during and post-vernalization which and involved the activation of ABA and strigolactone signaling genes.

V2 buds become ecodormant during cold exposure and are under the control of correlative inhibition after plants return to growth-promoting conditions

To assess whether cold imposes an endodormant or ecodormant state on V2 buds we decapitated plants immediately after being exposed to different durations of cold treatment by removing the primary stem above the nodes 11-14 depending on the treatment (Fig. 5a). Plants cold-treated for less than 12 weeks failed to maintain dormant buds even in non-decapitated controls (Fig. 5c). Irrespective of the cold treatment, the length of axillary branches that developed after plants returned to greenhouse conditions was longer in decapitated plants compared to non-decapitated controls (Fig. 5b). Endodormant buds do not respond to decapitation (Lang et al., 1987), and therefore we conclude that V2 buds are not endodormant during cold treatment. To assess the effect of cold on bud outgrowth we decapitated plants and exposed them to 12 weeks of cold treatment (Fig. 5d,e,f). Buds in decapitated and intact plants

did not grow during cold treatment suggesting that cold inhibits growth and imposes an ecodormant state in V2 buds (Fig. 5e,f).

Since V2 buds remain dormant also after plants return to growth-promoting conditions, we tested whether the outgrowth of V2 buds post-vernalization is determined by other parts of the plant (Fig. 6a). For this we performed a series of decapitation and excision experiments by excising buds or branches in vernalized plants before returning to the greenhouse (Fig. 6a). Excision of the inflorescence buds (I) or V1 branches separately reduced the number of dormant buds in the V2 zone (nodes 11–16; Fig. 6a,b). The biggest effect on bud outgrowth in the V2 zone was obtained when we excised both inflorescence and V3 buds (I+V3) together (Fig. 6a,b). These results suggest that post-vernalization the outgrowth of buds within the V2 zone is influenced by other parts of the plant.

To validate that the genes we identified in our transcriptome profiling regulate dormancy in V2 buds we tested their expression patterns after decapitation (Fig. 6c). For this, we vernalized plants and removed the inflorescence and V3 buds (I+V3) before transferring them back to greenhouse conditions. Transcript accumulation of selected genes was tested in the axillary buds within the V2 zone at 5 days after the excision of the inflorescence and V3 buds (I+V3). Decapitation, resulted in a reduced transcript level of all genes tested. This result suggests that post-vernalization the expression of these genes is regulated by an apical signal (Fig. 6c).

In many species, bud inhibition by apical dominance is regulated by polar auxin transport (Wang et al., 2018). To assess whether changes in auxin dynamics are important for the maintenance of dormancy in V2 buds post-vernalization we performed several tests (e.g. we measured endogenous IAA levels, auxin response and auxin transport). We first tested auxin response in the topmost elongated internodes within the V2 region of the main stem using the DR5:GUS transgenic *A. alpina* plants. We decapitated plants immediately after 12 weeks of cold treatment and afterwards transferred them to greenhouse conditions. Stems from decapitated plants at 5 days post-vernalization (Figs 7c,f, S3) and intact plants at the end of cold treatment (Fig. 7a,d, S3) did not show a strong GUS signal. In contrast, we detected a GUS signal in non-decapitated plants 5 days post-vernalization (Fig. 7b,e, S3). This result suggests that auxin response is enhanced in the V2 region of the main stem post-vernalization. We also measured the levels of endogenous IAA during the *A. alpina* life cycle. Similar to DR5:GUS results, IAA levels in the main stem (in the internodes within the V2 region) were low during vernalization and were transiently increased post-vernalization (Fig. 7g). Interestingly, IAA levels at 2 weeks post-vernalization were very high which correlated with high IAA levels in the stems of the actively growing inflorescence stems (Fig. 7h) and V3 branches (Fig. 7i).

Decapitation by removal of the inflorescence and V3 buds (I+V3) at the end of cold treatment did not result in an increase of the endogenous IAA levels in the V2 stem measured 5 days post-vernalization (Fig. 7j). This result suggests that the outgrowth of the inflorescence and V3 branches post-vernalization induces a higher IAA level in the V2 main stem. To test whether the transient increase of the endogenous IAA level in the stem represents an increase in auxin transport we measured IAA transport capacity using acropetal ^{3}H-IAA treatment in excised stem segments of the main stem corresponding to the V2 region. We compared the same V2 stem region from plants grown for 8 weeks in a LD greenhouse (8wLD), after 12 weeks of cold treatment (+0) and subsequently after 5 days in greenhouse conditions (+5d). ^{3}H-IAA levels in the V2 zone were higher before and post-vernalization, compared to at the end of vernalization (Fig. 7m,n). This result suggests that prolonged exposure to cold leads to a decrease in auxin transport in the V2 zone which is re-established again after the return to growth-promoting conditions. We finally applied the synthetic auxin NAA and the auxin transport inhibitor NPA to plants cold-treated for 12 weeks before being transferred back to the greenhouse. NAA treatment increased, whereas NPA treatment reduced the number of buds in the V2 zone compared to mock-treated plants (Fig. 7o,p). In addition, NPA strongly impaired the development of the inflorescence and V1 branches (Fig. 7p). These results confirmed the importance of auxin levels and auxin transport for the inhibition of buds in the V2 zone post-vernalization.

Altogether we conclude that the outgrowth of the inflorescence and of the V3 vegetative branches post-vernalization correlates with an enhancement of endogenous IAA levels, auxin response and transport in the V2 zone which may stably repress the outgrowth of V2 buds. During cold treatment, although auxin transport is low, the development of V2 axillary buds is inhibited by ecodormancy.

AaBRC1 ensures the maintenance of dormancy in V2 buds post-vernalization

As BRC1 (and to a lesser extent BRC2) are the major regulators of bud dormancy in *A. thaliana*, we also followed the expression patterns of their homologs (AaBRC1 and AaBRC2) in *A. alpina* (Fig. S4; Aguilar-Martínez et al., 2007; González-Grandío et al., 2013). AaBRC1 transcript levels were higher in V2 compared to V3 buds 5 days after vernalization (Fig. S4b). Similar to the expression patterns of the genes detected in our transcriptome, AaBRC1 transcript levels in V2 buds were reduced in response to decapitation (Figs 6c, S4b). AaBRC2 expression levels did not differ between V2 and V3 buds and in response to decapitation (Fig. S4b). We

subsequently tested the expression of candidate genes from our transcriptome and AaBRC1 on V2 buds across the A. alpina life cycle. Buds from the V2 zone (nodes 17-20) were harvested from plants at different developmental stages; after being grown for 8 weeks in a LD greenhouse (8wLD), cold-treated for 12 weeks (+0) and subsequently transferred back to a LD greenhouse for 5 days (+5d). AaBRC1 transcript levels in V2 buds increased during cold treatment and remained at a high level after plants were returned to greenhouse conditions (Fig. 8). This expression pattern was shared for most genes tested, including the A. alpina homolog of HB53 (AaHB53) which is a direct target of BRC1 in A. thaliana (González-Grandío et al., 2017). Interestingly, transcript accumulation of AaHIS1-3, which in A. thaliana has a BRC1-dependent expression pattern, increased only post-vernalization (Fig. 8). These results suggest that dormancy in V2 buds is enhanced during vernalization and is maintained post-vernalization after plants experience growth-promoting conditions.

We also created transgenic lines with reduced expression of AaBRC1. Plants of 35S:AaBRC1 dsRNAi lines 1 and 2 showed significant downregulation of AaBRC1 expression whereas line 3 did not (Fig. S5). We characterized these lines at different developmental stages with plants grown for 8 weeks in long days (8wLD), exposed to 12 weeks of cold treatment (+0) and subsequently returned to a LD greenhouse for 5 weeks (+5w) (Fig. 9). Before vernalization we observed no difference in branch number and branch length (Figs 9a,b,c, S6a). Transgenic lines also flowered with a similar total number of leaves compared to control plants (Fig. S6 d,e,f,g,h). Lines 1 and 2 showed a minor difference in branch number at the end of vernalization (Fig. 9a,c). The branching phenotype in Lines 1 and 2 became stronger post-vernalization and showed an increased number of branches in nodes 12-16 (corresponding to nodes in the V2 zone) (Figs 9a,d,e, S6c). In these two lines, transcript levels of AaHB53 were reduced in V2 buds at the end of and post-vernalization but not before vernalization (Fig. S5). Interestingly, transcript accumulation of AaHIS1-3 and AaAIRP2 in the 35S:AaBRC1 dsRNAi lines was similar to control plants in all developmental stages (Fig. S5). These results suggest that AaBRC1 regulates the activity of V2 buds during and post-vernalization. However, an AaBRC1-independent pathway regulating bud dormancy might also exist.

Discussion

Perennial plants flower multiple times during their life cycle by restricting senescence only to reproductive branches and maintaining growth through vegetative axillary branches and dormant buds. Bud dormancy in general serves as a strategy, which is followed by annual and

perennial species, to enable plants to re-grow after harsh environmental conditions or after damage due to biotic and abiotic cues. In herbaceous plants, buds in the axils of younger leaves that are close to the shoot apical meristem are often temporarily dormant (Kebrom, 2017). This phenomenon is explained by apical dominance, in which after the removal of the shoot apical meristem, buds can resume growth (Cline, 1991). Apical dominance in trees and shrubs is more complex. In trees the highest buds have the tendency to grow out, whereas in shrubs usually the lowest buds develop into a branch (Cline, 1991). Thus often dormant axillary buds are not necessarily located in the axils of leaves close to the shoot apical meristem and leaves with dormant axillary buds in their axils are often older compared to the ones with flowering or vegetative branches (Costes & Guédon, 2002; Costes et al., 2014; Lazaro et al., 2018). Nevertheless, the fate of these buds is still influenced by other parts of the plant (Costes et al., 2014).

We demonstrated that a major factor that determines the complex shoot architecture and the positioning of dormant buds along the shoot is flowering. Specifically, the separation in time of the different steps of flowering (floral induction, flower bud initiation and anthesis), which is seen in many perennial species, is crucial for shaping a complex shoot architecture. Perennials often initiate floral buds prior to winter, either during the summer or early autumn, and flower the following spring (Vasconcelos et al., 2009; Hsu et al., 2011; Koskela et al., 2012; Kurokura et al., 2013). Wild type A. alpina plants initiate flower buds during cold treatment and flower rapidly after subsequent exposure to growth-promoting conditions (Wang et al., 2009). V2 axillary buds that stay stably dormant are formed before the onset of vernalization (U. Ponraj & K. Theres, unpublished). In this study we show that irrespective of what happens to the shoot apical meristem, V2 buds become ecodormant during cold exposure. Floral bud initiation during cold treatment determines the fate of V2 buds at later developmental stages, after plants have been exposed again to growth-promoting conditions.

In annuals and certainty in some perennial species, the induction to flowering changes the pattern of axillary bud initiation (from acropetal to basipetal) and new buds are initiated in leaf axils adjacent to the shoot apical meristem (Prusinkiewicz et al., 2009; Dierck et al., 2016). In perennials, these buds do not commit to reproductive development and the following year give rise to vegetative structures (Carmona et al. 2002; Foster et al., 2003). As A. alpina plants initiate flowering during vernalization, new buds (V3) are initiated during cold exposure and are designated to ensure the maintenance of vegetative growth after flowering giving rise to vegetative axillary branches (Wang et al., 2009; U. Ponraj & K. Theres, unpublished). Maintenance of vegetative growth in V3 branches is ensured by the floral repressor PEP1, as

PEP1 mRNA levels are upregulated in the V3 branches after vernalization irrespective of the duration of cold treatment (Wang et al., 2009; Lazaro et al., 2018). We showed that flowering in the main shoot apex is always linked to the presence of the V3 vegetative axillary branches. In addition, the transcripts of genes previously shown to correlate with the release of bud dormancy are enhanced in V3 buds already during cold exposure (Devitt & Stafstrom, 1995; Campbell et al., 1996; Horvath et al., 2002; Freeman et al., 2003). These results suggest that V3 buds are not dormant during and after cold-exposure. Similarly, in tulip bulbs axillary buds located close to the flowering shoot apex never arrest growth (Moreno-Pachon et al., 2018). The link between flowering and paradormancy has been demonstrated in rice, in which the flowering time regulator Heading date 3a (Hd3a), the ortholog of FLOWERING LOCUS T (FT) in A. thaliana, acts as the systemic signal for flowering and axillary bud activation (Tsuji et al., 2015). In A. alpina, the flowering time regulator PEP1 might orchestrate the crosstalk between flowering and bud activity, as the pep1-1 mutant has a clear branching phenotype. In rice, it has also been shown that another flowering time regulator, the transcription factor SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 14 (OsSPL14), which is the ortholog of SPL15 regulates shoot branching and panicle branching (Miura et al., 2010). However, this role of SPL15 is not conserved in A. alpina, as the activity of V2 buds is not affected in Aaspl15-knockout lines (Hyun et al., 2019).

Flowering might indirectly determine the fate of V2 buds by simply giving an advantage to the inflorescence and V3 buds formed during cold treatment to outgrow rapidly when plants experience growth-promoting conditions. Consequently, the development of these buds might be at the expense of the V2 buds. V3 branches and the inflorescence may act as strong nutrient sinks during and after vernalization causing sugar diversion from the V2 buds and thereby contributing to growth inhibition. This is in line with the fact that BRC1 is inhibited by sugars in both perennials and annuals and that carbon-starvation induced dormancy is observed in annual and perennial plants (Mason et al., 2014; Barbier et al., 2015; Tarancón et al., 2017). From the transcript levels of dormancy marker genes and our physiological studies we can conclude that dormancy in V2 buds is enhanced during vernalization and is maintained post-vernalization. After the return to warm temperatures V2 buds enter a latent state mainly being dominated by the inflorescence and V3 branches. The auxin canalization model postulates that polar auxin transport from an apical auxin source inhibits sufficient polar auxin stream from lateral buds (Prusinkiewicz et al., 2009). In our system, the fact that V3 vegetative branches are positioned in the axils of the leaves just above the V2 zone might not be random and they might act as an auxin source to determine the activity of V2 buds. This is in line with our results as

we observed enhancement of basipetal auxin transport, increased auxin response and endogenous indole-3-acetic acid (IAA) levels in the stem after vernalization.

Our study links the pattern of flowering observed in temperate perennials with the maintenance of dormant buds across multiple seasons and provides the framework of how perennials ensure long-term growth.

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Author contributions

AV, PM and MCA planned and designed the research, AV, AR, KL, UN performed experiments, AV, PM, AR, MCA analysed data, AV and MCA wrote the manuscript. PM and AR contributed equally to this work.

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Supporting information

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

Fig. S1 Duration of cold treatment influences floral commitment in the shoot apical meristem.
Fig. S2 GO enrichment analysis in differentially regulated genes in V2 and V3 buds at the end of vernalization (+0) and five days after vernalization (+5d).
Fig. S3 GUS transcript accumulation in transgenic DR5:GUS A. alpina lines.
Fig. S4 AaBRC1 is the homolog of the A. thaliana BRC1 and its expression is downregulated in respond to decapitation.
Fig. S5 Transcript accumulation of dormancy-associated genes is reduced after vernalization in 35S:AaBRC1 dsRNAi lines.
Fig. S6 35S:AaBRC1 dsRNAi lines do not show major differences in branch length and total leaf number at flowering.
Table S1. Primers Used in this Article.
Table S2. List of genes whose transcript levels have been identified to be differentially expressed between V2 and V3 buds at the end of vernalization (+0) and 5 days post-vernalization (+5d).
Table S3. GO enrichment categories identified in genes whose transcript levels have been identified to be differentially expressed between V2 and V3 buds at the end of vernalization (+0) and 5 days post-vernalization (+5d).
Table S4. List of coexpressed clusters obtained after hierarchical clustering of the transcript accumulated in V2 and V3 buds at the end of vernalization (+0) and 5 days post-vernalization (+5d).
Table S5. GO enrichment categories identified in the different coexpressed clusters.

Table S6. Homologs of A. thaliana genes identified as a "bud dormancy" Genes in Tarancón et al 2017 differentially regulated in our Transcriptome Analysis.

Figure Legends

Fig. 1 Diagram of plant architecture of a flowering Arabis alpina wild type plant. Axillary meristems are organized in zones of differential bud activity and fate: V1, flowering axillary branches that partially senesce; V2, dormant buds; V3, vegetative axillary branches; I, inflorescence. Yellow circle denotes flowering in the inflorescence or axillary branches, grey circles indicate dormant buds, green triangle the presence of a vegetative branch.

Fig. 2 Vernalization determines shoot architecture in Arabis alpina. (a) to (e) Analysis of branch formation in wild type Pajares plants (wt) cold-treated for 12 weeks. (a) A flowering branch. (b) A vegetative branch. (c) A leaf axil without a branch (d) A leaf axil with a branch smaller than 0.5 cm. Bar size indicates 1 cm. (e) Analysis of branch formation in a set of wt plants grown in long days (LDs) for 8 weeks (8wLD), cold-treated for 12 weeks (+0) and transferred back to LDs for 1, 2, 3 and 9 weeks (+1wLD, +2wLD, +3wLD, +9wLD). Zones are mentioned on the right as described in Fig. 1. (f) and (g) Analysis of branch formation in pep1-1 mutant growing continuously in LDs. (f) Analysis of branch formation in a set of pep1-1 plants scored for up to 13 weeks (5, 9, 10 and 13wLD) in a LD greenhouse. (g) Branch length in each node in pep1-1 and wt plants. In (e) and (f) each column represents a single plant and each square within a column represents an individual leaf axil numbered from the oldest to the youngest node. Yellow or green colors indicate the presence of a flowering (as in a) or vegetative axillary branch (as in b) in the particular leaf axil. Grey or brown colors indicate the absence of an axillary branch (as in c) or the presence of a branch smaller than 0.5 cm (as in d) in the particular leaf axil. n=12. In (g) The boxes indicate the interquartile range, the vertical line in the middle is the median, the horizontal lines (whiskers) correspond to the highest or lowest value within 1.5 times the interquartile range, the dots are the outliers.

Fig. 3 The duration of cold treatment in Arabis alpina determines shoot architecture and the maintenance of a zone of dormant buds. (a) Diagram illustrating the experimental design. Plants were grown for 11 weeks in long days (LDs) (0), or for 8 weeks in LDs and subsequently cold-

treated for 3 (3w), 8 (8w) or 12 (12w) weeks. Plants were scored 3 weeks after they were returned to a LD greenhouse. Solid line box represents LD greenhouse conditions and dotted line box vernalization at 4^{o}C under short days (SDs). (b) Analysis of branch formation in plants exposed to different durations of cold treatment or to continuous LDs for 11 weeks. As in Fig. 2, each column represents a single plant and each square within a column represents an individual leaf axil numbered from the oldest to the youngest node. Yellow or green colors indicate the presence of a flowering or vegetative axillary branch in the particular leaf axil. Grey or brown colors indicate the absence of an axillary branch or the presence of a branch smaller than 0.5 cm in the particular leaf axil. (c) Branch length of axillary branches in each node in plants exposed to different durations of cold treatment or to continuous LDs for 11 weeks. The boxes in (c) indicate the interquartile range, the vertical line in the middle is the median, the horizontal lines (whiskers) correspond to the highest or lowest value within 1.5 times the interquartile range, the dots are the outliers. Nodes corresponding to V2 and V3 zones are indicated. n=12.

Fig. 4 The transcriptomes of V2 and V3 buds are already differentiated during vernalization. (a) to (d) GUS activity in longitudinal sections of axillary buds harvested from the nodes in V2 and V3 zones in *DR5:GUS* Arabis *alpina* plants. (a) in V2 buds at the end of 12 weeks of vernalization (+0). (b) in V2 buds 5 days post-vernalization (+5d). (c) in V3 buds at the end of 12 weeks of vernalization (+0). (d) in V3 buds 5 days post-vernalization (+5d). Arrows indicate GUS signal and scale bars 250µm. (e) Venn diagram showing the overlap of significantly regulated genes in V2 and V3 buds at the end of vernalization (+0) and 5 days post-vernalization (+5d). (f) Heat map representing the hierarchical clustering of 4983 coexpressed transcripts between samples. Coexpressed clusters were assigned into two higher level clusters (I and II). (g) to (j) Expression profiles of selected clusters using FPKM values. Error bars represent the SE of all FPKM values of genes within the cluster obtained in a particular sample. (g) and (h) Clusters that show higher transcript accumulation only in V3 buds post-vernalization. (i) Clusters that show higher transcript accumulation in V2 buds during and post-vernalization. (j) Clusters that show higher transcript accumulation in V3 buds during and post-vernalization. The selected GO terms shown in (g) and (h) are predominant in each cluster. The selected GO terms shown in (i) and (j) are representative and shared between clusters in each group. Selected GO terms had p-values after Benjamini correction lower than 0.05. Numbers in brackets indicate the number of genes present in each cluster. Above each cluster selected representative genes which belong to common GOs and which are related to development or dormancy are

indicated. Genes highlighted in bold were suggested in Tarancon *et al*. (2017) to be "*bud dormancy*" genes as their expression patterns are correlated with bud dormancy.

Fig. 5 V2 buds are ecodormant during vernalization. (a) to (c) Decapitation experiment to assess whether V2 buds are endodormant during cold treatment. (a) Diagram illustrating the experimental design for results obtained from decapitation experiments presented in (b) and (c). Arabis alpina plants were grown for 8 weeks in long days (8wLD; solid line box) and subsequently cold-treated for 0, 3, 8 and 12 weeks (dotted line box). Plants were decapitated prior to being returned to warm temperatures (solid line box). Control plants are the same as in Fig. 3. (b) Branch length of new branches of control (white) or decapitated plants (grey) was scored 3 weeks after decapitation. (c) Number of leaf axils without a branch in control (white) or decapitated plants (grey) scored 3 weeks after decapitation. Nodes 1-11 scored for treatment 0; nodes 1-12 scored for treatment 3, nodes 1-13 scored for treatment 8 and nodes 1-14 scored for treatment 12. (d) to (f) Decapitation experiment to assess whether V2 buds are ecodormant during vernalization. (d) Diagram illustrating the experimental design for results obtained from decapitation experiments presented in (e) and (f). Plants were grown for 8 weeks in long days (8wLD) and decapitated prior to being subjected to 12 weeks of cold treatment. (e) Analysis of branch formation in plants at the end of the 12 weeks of cold treatment in control and decapitated plants. As in Fig. 2, each column represents a single plant and each square within a column represents an individual leaf axil numbered from the oldest to the youngest node. Green color indicates the presence of a vegetative axillary branch in the particular leaf axil. Grey or brown colors indicate the absence of an axillary branch or the presence of a branch smaller than 0.5 cm in the particular leaf axil. (f) Number of leaf axils without a branch (represented with brown and grey boxes in e) at the end of 12 weeks of cold treatment in control (white) and decapitated plants (grey). Only nodes 1-11 were scored in control plants. The boxes in (c) and (f) indicate the interquartile range, the horizontal line in the middle is the median, the vertical lines (whiskers) correspond to the highest or lowest value within 1.5 times the interquartile range, the dots are the outliers. Asterisks indicate significant differences using student's t test $P<0.05$ between control and decapitated plants. n=12. ns, not significant.

Fig. 6 Dormancy of V2 buds post-vernalization is regulated by correlative inhibition. (a) Analysis of branch formation after excision of axillary branches or buds belonging to different zones. As in Fig. 5a (treatment 12) Arabis alpina plants were decapitated immediately at the end of 12 weeks of cold treatment and subsequently moved to a long days (LDs) greenhouse

for 3 weeks. In all treatments V3 and inflorescence (I) buds were dissected under the binocular. Control indicates intact plants, I indicates plants in which the inflorescence buds have been dissected, V1 indicates plants in which V1 branches have been removed, I+V3 indicates plants in which the inflorescence and V3 buds have been dissected. As in Fig. 2, each column represents a single plant and each square within a column represents an individual leaf axil numbered from the oldest to the youngest node. Yellow or green colors indicate the presence of a flowering or vegetative axillary branch in the particular leaf axil. Grey or brown colors indicate the absence of an axillary branch or the presence of a branch smaller than 0.5 cm in the particular leaf axil. (n= 10 or 12). (b) Number of leaf axils without a branch (represented with brown and grey boxes in a) after the excision of buds or branches in different zones. Letters show significant differences between conditions ($P<0.05$, n=3) using ANOVA followed by pairwise multiple comparison using Tukey. (c) Relative transcript accumulation of AaHB53, AaDRM2, AaNAC029, AaAIRP2, AaABI1, AaHIS1-3 and AaEFB2 in V2 buds in response to decapitation of inflorescence and V3 buds together (I+V3) compared to the control at five days after return to greenhouse conditions. Expression levels of all genes were normalized with AaPP2A and AaUBI10. The boxes in (b) indicate the interquartile range, the horizontal line in the middle is the median, the vertical lines (whiskers) correspond to the highest or lowest value within 1.5 times the interquartile range, the dots are the outliers. Asterisk indicates significant differences between control and decapitated plants using student's t test ($P<0.05$, n=3). Errors indicate SD.

Fig. 7 Auxin response, endogenous indole-3-acetic acid (IAA) level and IAA transport capacity in the V2 main stem is decreased during vernalization and is increased post-vernalization. Modification of this pattern changes the size of the V2 zone. (a) to (f) GUS activity within the V2 region on the main stem. (a) V2 main stem region at the end of vernalization. (b) V2 main stem region at 5 days post-vernalization. (c) V2 main stem region at 5 days post-vernalization in plants in which the inflorescence and V3 buds were dissected immediately at the end of the 12 weeks of cold treatment (see also Fig. 5a). (d) Transversal section of V2 main stem region at the end of vernalization. (e) Transversal section of V2 main stem region at 5 days post-vernalization. (f) Transversal section of V2 main stem at 5 days post-vernalization in plants in which the inflorescence and V3 buds were dissected immediately at the end of the 12 weeks of cold treatment (see also Fig. 5a). (g) to (j) IAA level in pg/mg of Fresh Weight (FW). (g) in V2 main stem region before vernalization (8wLD), at the end of vernalization (+0), 3 and 5 days (+3d and +5d), and 1, 2, 3 and 9 weeks post-vernalization (+1w, +2w, +3w, and +9w). (h) at

the base of the inflorescence stem, (i) at the base of the V3 axillary vegetative branches. (j) in V2 main stem region in plant in which the inflorescence and V3 buds have been dissected immediately at the end of the 12 weeks of cold treatment. Samples were harvested from plants transferred to a long day (LD) greenhouse 5 days after decapitation. Letters indicate significant differences between conditions ($P<0.05$, n=3) using ANOVA followed by pairwise multiple comparison using Tukey. Asterisk in (j) indicates significant differences between control and decapitated plants using student's t test ($P<0.05$, n=3). Errors indicate SD. (m) to (n) IAA transport capacity in V2 main stem region in 8 week old plants grown in LD (8wLD), at the end of vernalization (+0) and 5 days post-vernalization (+5d). (m) ^{3}H-IAA measured in mm of stem from the ^{3}H-IAA acropetal source relative to the ^{3}H-IAA measured in the 6-9 mm of stems from 8wLD plants. (n) Total ^{3}H-IAA in stem (6-21 mm) at the end of vernalization (0) and 5 days after vernalization (5d) relative to the ^{3}H-IAA in stems from 8wLD plants. Letters indicate significant differences between conditions ($P<0.05$, n=3) using ANOVA followed by pairwise multiple comparison using Tukey. Errors indicate SE. (o) Number of leaf axils without a branch (represented with brown and grey boxes in p) in Arabis alpina plants vernalized for 12 weeks and subsequently sprayed with mock, 100μM NAA or 100μM NPA immediately at the end of 12 weeks of cold treatment and one week after the return to greenhouse conditions. Plants were scored 5 weeks after they were returned to the greenhouse. Letters indicate significant differences between conditions ($P<0.05$, n=12) using ANOVA followed by pairwise multiple comparison using Tukey. (p) Analysis of branch formation after mock, NAA or NPA treatment. Branch scoring is presented as in Fig. 2. Each column represents a single plant and each square within a column represents an individual leaf axil numbered from the oldest to the youngest node. Yellow or green colors indicate the presence of a flowering or vegetative axillary branch in the particular leaf axil. Grey or brown colors indicate the absence of an axillary branch or the presence of a branch smaller than 0.5 cm in the particular leaf axil. The boxes in (o) indicate the interquartile range, the horizontal line in the middle is the median, the vertical lines (whiskers) correspond to the highest or lowest value within 1.5 times the interquartile range, the dots are the outliers.

Fig. 8 Transcript accumulation of dormancy related genes in Arabis *alpina* V2 buds is increased during and maintained post-vernalization. Relative transcript accumulation of *AaBRC1, AaBRC2, AaHB53*, *AaDRM2, AaNAC029, AaAIRP2*, *AaABI1, AaHIS1-3*, *AaKMD1* and *AaSAG21* in 8-week-old plants grown in long days (LDs) (8wLD; light grey), at the end of vernalization (+0; grey) and five days after vernalization (+5d, dark grey). Transcript levels of

all genes are normalized with *AaPP2A* and *AaUBI10*. (*n*=3). Letters indicate significant differences between conditions (*P*<0.05, *n*=3) using ANOVA followed by pairwise multiple comparison using Tukey. Errors indicate SD.

Fig. 9 AaBRC1 ensures the maintenance of dormancy in V2 buds post-vernalization. (a) Analysis of branch formation in wild type (wt) Arabis alpina plants and in 35S:AaBRC1 dsRNAi lines 1 to 3 in 8-week-old plants grown in long days (LDs) (8wLD), at the end of vernalization (+0) and five weeks post-vernalization (+5wLD). As in Fig. 2, each column represents a single plant and each square within a column represents an individual leaf axil numbered from the oldest to the youngest node. Yellow or green colors indicate the presence of a flowering or vegetative axillary branch in the particular leaf axil. Grey or brown colors indicate the absence of an axillary branch or the presence of a branch smaller than 0.5 cm in the particular leaf axil. n=12. (b) Branch number per plant in 8-week-old plants grown in LDs (8wLD). (c) Branch number per plant in plants at the end of vernalization (+0). (d) Branch number per plant in plants at 5 weeks after vernalization (+5wLD). (e) Number of leaf axils without a branch at 5 weeks after vernalization (+5w; represented with brown and grey boxes in a). The boxes in (b-e) indicate the interquartile range, the horizontal line in the middle is the median, the vertical lines (whiskers) correspond to the highest or lowest value within 1.5 times the interquartile range, the dots are the outliers. Letters indicate significant differences between conditions (P<0.05) using ANOVA followed by pairwise multiple comparison using Tukey.

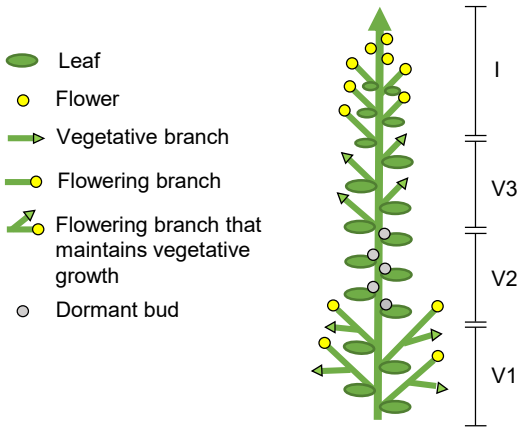
Leaf
Flower
Vegetative branch
Flowering branch
Flowering branch that maintains vegetative growth
Dormant bud
I
V3
V2
V1

Fig. 1

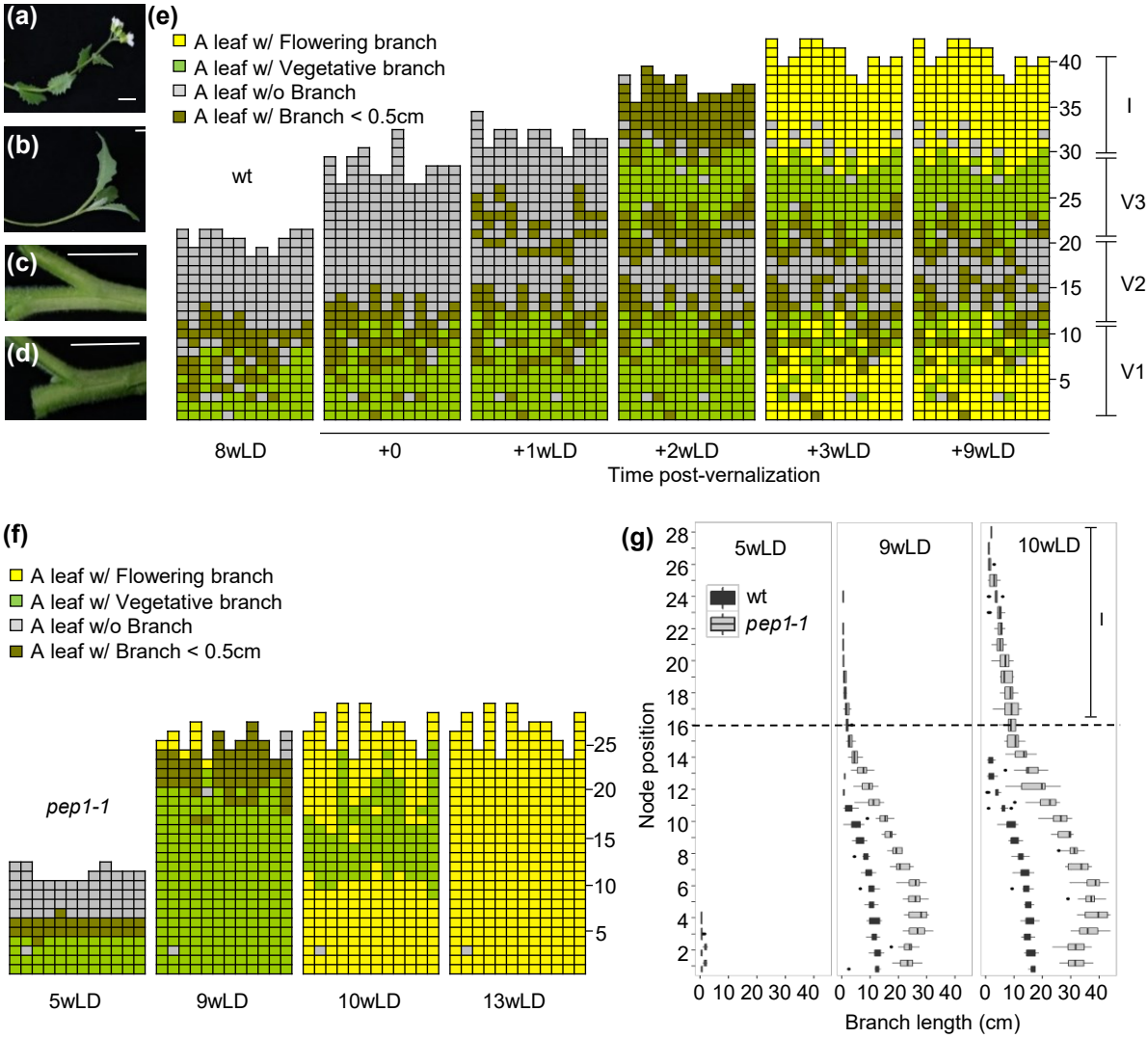
(a)
(b)
(c)
(d)
(e)
A leaf w/ Flowering branch
A leaf w/ Vegetative branch
A leaf w/o Branch
A leaf w/ Branch < 0.5cm
wt
40
35
30
25
20
15
10
5
I
V3
V2
V1
8wLD
+0
+1wLD
+2wLD
+3wLD
+9wLD
Time post-vernalization
(f)
A leaf w/ Flowering branch
A leaf w/ Vegetative branch
A leaf w/o Branch
A leaf w/ Branch < 0.5cm
pep1-1
25
20
15
10
5
5wLD
9wLD
10wLD
13wLD
(g)
5wLD
9wLD
10wLD
wt
pep1-1
I
Node position
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0 10 20 30 40
Branch length (cm)

Fig. 2

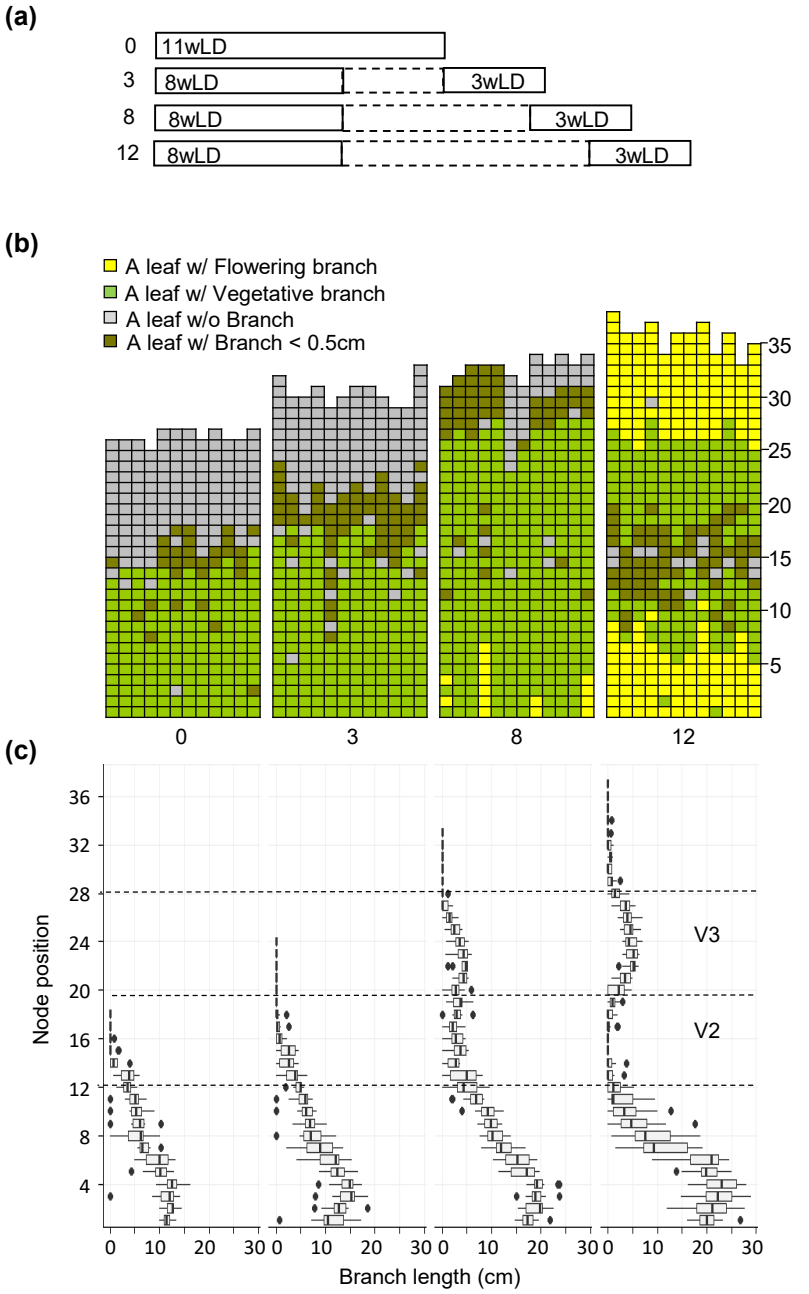
(a)
0
11wLD
3
8wLD
3wLD
8
8wLD
3wLD
12
8wLD
3wLD
(b)
A leaf w/ Flowering branch
A leaf w/ Vegetative branch
A leaf w/o Branch
A leaf w/ Branch < 0.5cm
35
30
25
20
15
10
5
0
3
8
12
(c)
Node position
36
32
28
24
20
16
12
8
4
V3
V2
0
10
20
30
Branch length (cm)

Fig. 3

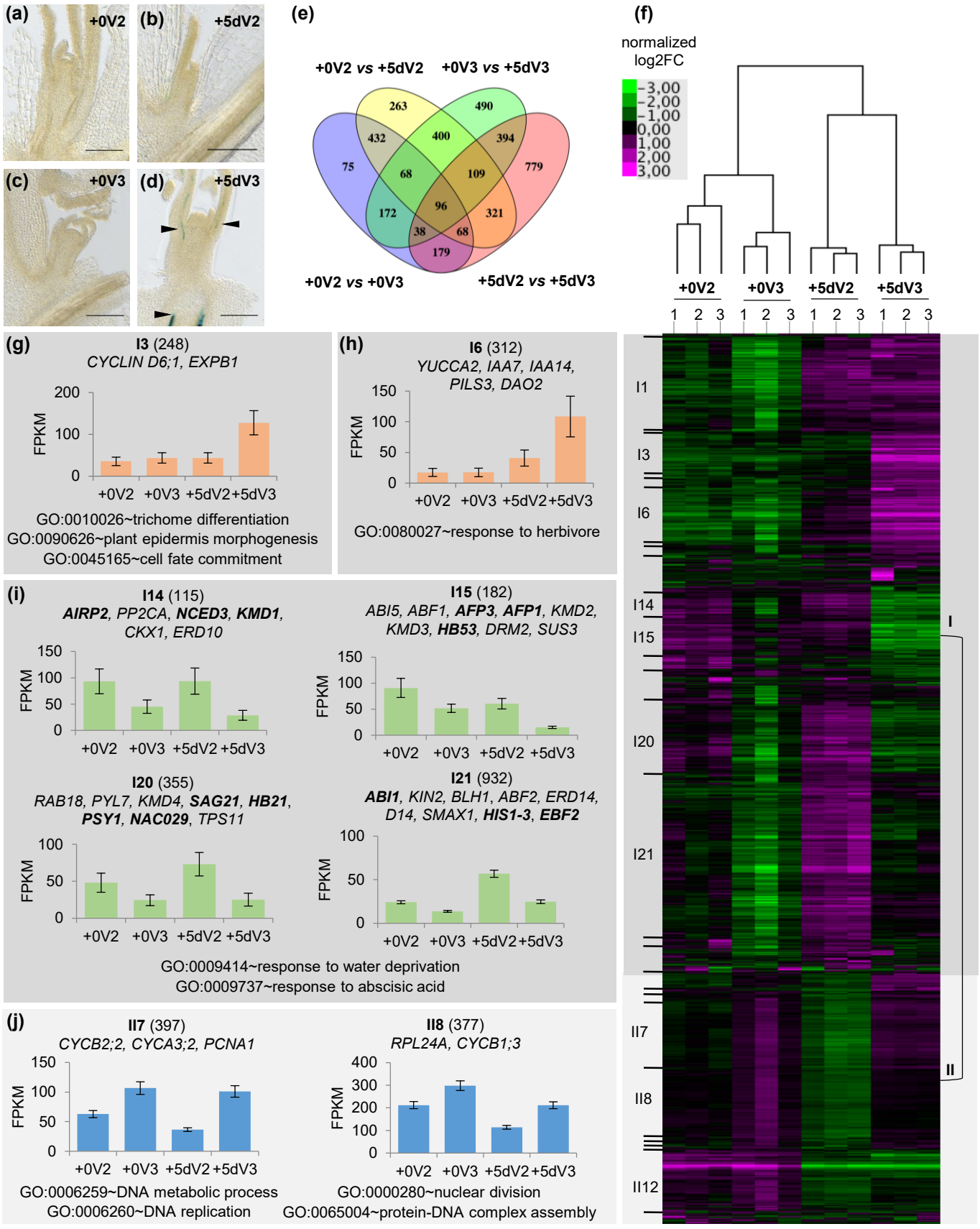
(a)
+0V2
(b)
+5dV2
(c)
+0V3
(d)
+5dV3
(e)
+0V2 vs +5dV2
+0V3 vs +5dV3
263
490
432
400
394
75
68
109
779
96
172
321
38
68
179
+0V2 vs +0V3
+5dV2 vs +5dV3
(f)
normalized log2FC
-3,00
-2,00
-1,00
0,00
1,00
2,00
3,00
+0V2
+0V3
+5dV2
+5dV3
1 2 3 1 2 3 1 2 3 1 2 3
I1
I3
I6
I14
I15
I20
I21
II7
II8
II12
I
II
(g)
I3 (248)
CYCLIN D6;1, EXPB1
FPKM
200
100
0
+0V2 +0V3 +5dV2 +5dV3
GO:0010026~trichome differentiation
GO:0090626~plant epidermis morphogenesis
GO:0045165~cell fate commitment
(h)
I6 (312)
YUCCA2, IAA7, IAA14, PILS3, DAO2
FPKM
150
100
50
0
+0V2 +0V3 +5dV2 +5dV3
GO:0080027~response to herbivore
(i)
I14 (115)
AIRP2, PP2CA, NCED3, KMD1, CKX1, ERD10
FPKM
150
100
50
0
+0V2 +0V3 +5dV2 +5dV3
I15 (182)
ABI5, ABF1, AFP3, AFP1, KMD2, KMD3, HB53, DRM2, SUS3
FPKM
150
100
50
0
+0V2 +0V3 +5dV2 +5dV3
I20 (355)
RAB18, PYL7, KMD4, SAG21, HB21, PSY1, NAC029, TPS11
FPKM
100
50
0
+0V2 +0V3 +5dV2 +5dV3
I21 (932)
ABI1, KIN2, BLH1, ABF2, ERD14, D14, SMAX1, HIS1-3, EBF2
FPKM
100
50
0
+0V2 +0V3 +5dV2 +5dV3
GO:0009414~response to water deprivation
GO:0009737~response to abscisic acid
(j)
II7 (397)
CYCB2;2, CYCA3;2, PCNA1
FPKM
150
100
50
0
+0V2 +0V3 +5dV2 +5dV3
GO:0006259~DNA metabolic process
GO:0006260~DNA replication
II8 (377)
RPL24A, CYCB1;3
FPKM
400
300
200
100
0
+0V2 +0V3 +5dV2 +5dV3
GO:0000280~nuclear division
GO:0065004~protein-DNA complex assembly

Fig. 4

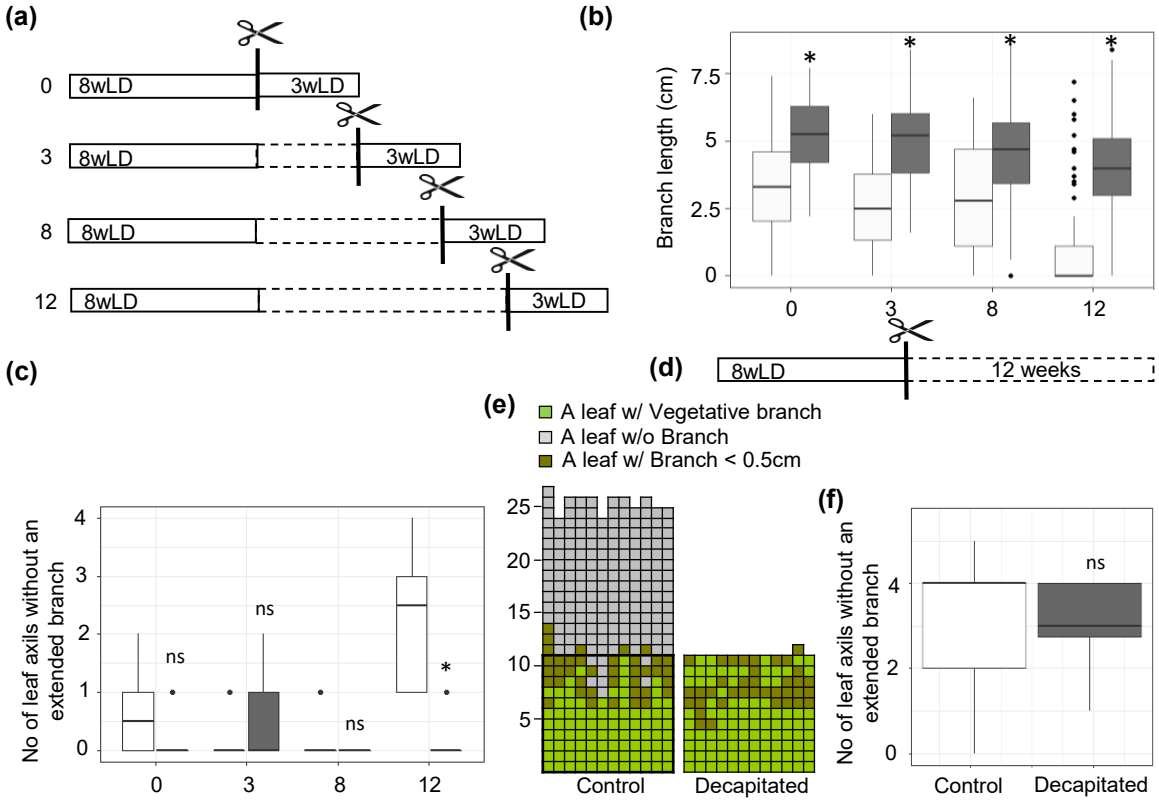
(a)
0
8wLD
3wLD
3
8wLD
3wLD
8
8wLD
3wLD
12
8wLD
3wLD
(b)
Branch length (cm)
7.5
5
2.5
0
0
3
8
12
(c)
No of leaf axils without an extended branch
4
3
2
1
0
ns
ns
ns
*
0
3
8
12
(d)
8wLD
12 weeks
(e)
A leaf w/ Vegetative branch
A leaf w/o Branch
A leaf w/ Branch < 0.5cm
25
20
15
10
5
Control
Decapitated
(f)
No of leaf axils without an extended branch
4
2
0
ns
Control
Decapitated

Fig. 5

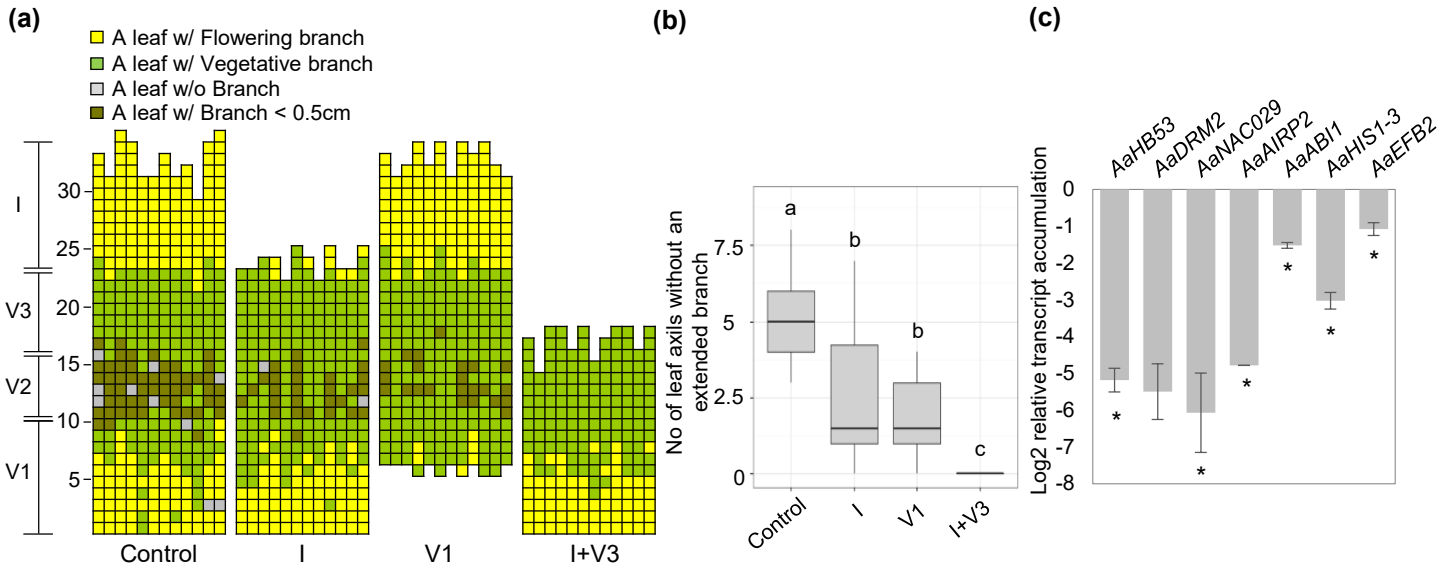
(a)
A leaf w/ Flowering branch
A leaf w/ Vegetative branch
A leaf w/o Branch
A leaf w/ Branch < 0.5cm
I
V3
V2
V1
30
25
20
15
10
5
Control
I
V1
I+V3
(b)
No of leaf axils without an extended branch
7.5
5
2.5
0
a
b
b
c
Control
I
V1
I+V3
(c)
Log2 relative transcript accumulation
0
-1
-2
-3
-4
-5
-6
-7
-8
AaHB53
AaDRM2
AaNAC029
AaAIRP2
AaABI1
AaHIS1-3
AaEFB2
*

Fig. 6

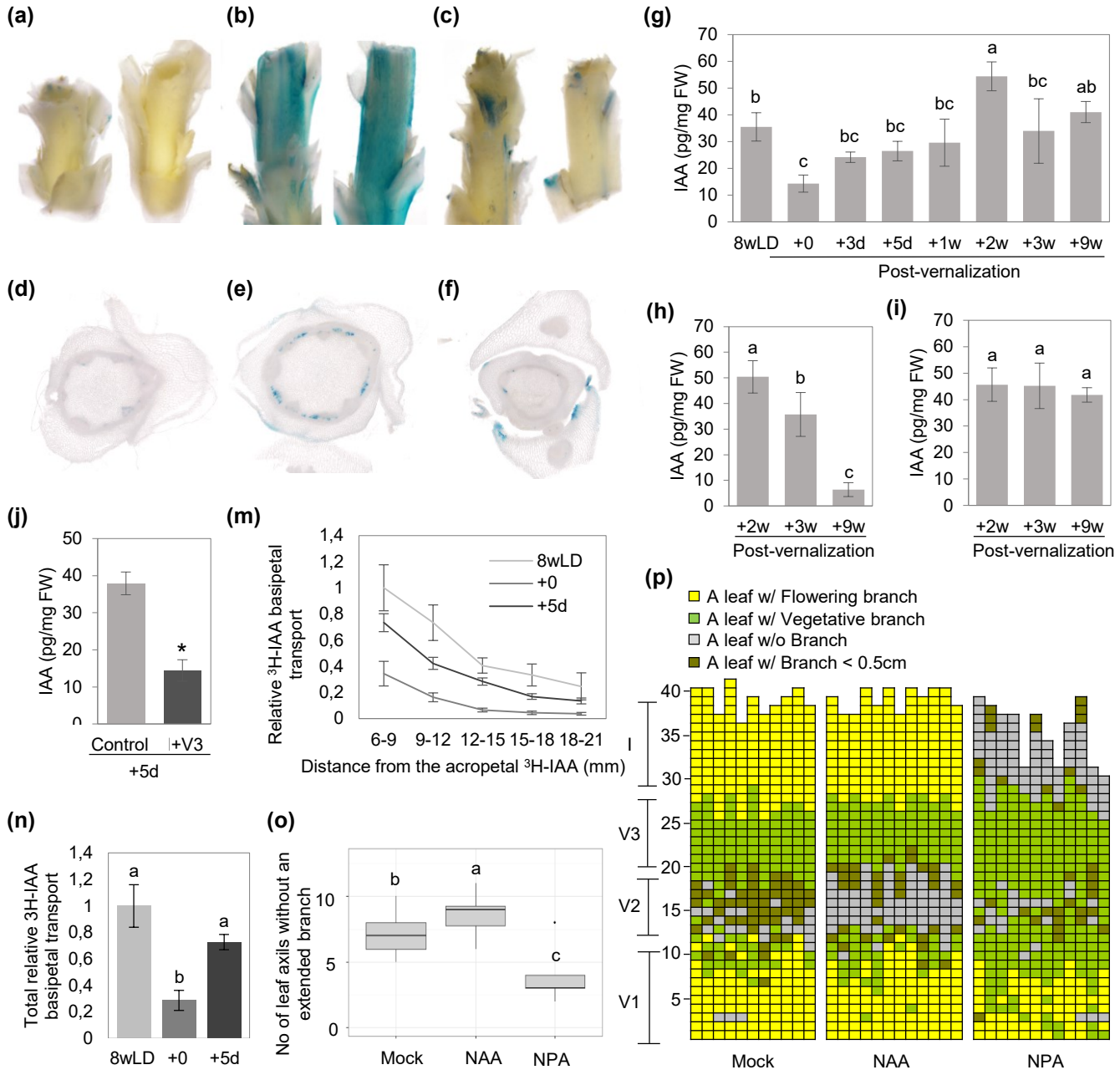
(a)
(b)
(c)
(d)
(e)
(f)
(g)
IAA (pg/mg FW)
8wLD +0 +3d +5d +1w +2w +3w +9w
Post-vernalization
(h)
+2w +3w +9w
Post-vernalization
(i)
+2w +3w +9w
Post-vernalization
(j)
Control +V3
+5d
(m)
Relative ³H-IAA basipetal transport
8wLD
+0
+5d
6-9 9-12 12-15 15-18 18-21
Distance from the acropetal ³H-IAA (mm)
(n)
Total relative 3H-IAA basipetal transport
8wLD +0 +5d
(o)
No of leaf axils without an extended branch
Mock NAA NPA
(p)
A leaf w/ Flowering branch
A leaf w/ Vegetative branch
A leaf w/o Branch
A leaf w/ Branch < 0.5cm
I
V3
V2
V1
Mock NAA NPA

Fig. 7

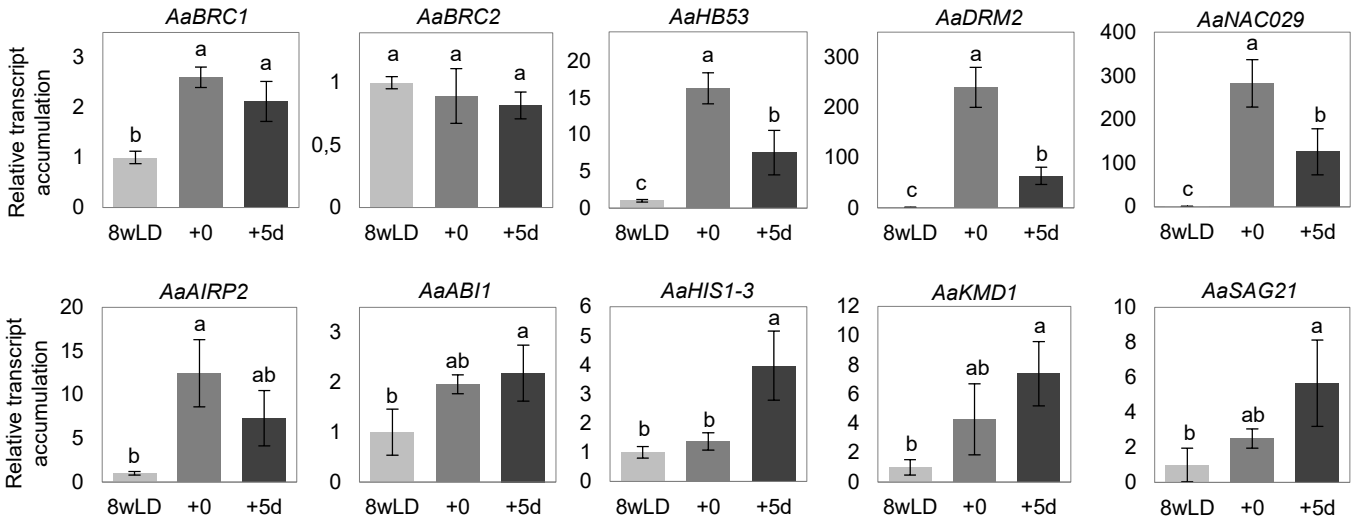
AaBRC1
AaBRC2
AaHB53
AaDRM2
AaNAC029
AaAIRP2
AaABI1
AaHIS1-3
AaKMD1
AaSAG21
Relative transcript accumulation
8wLD
+0
+5d

Fig. 8.

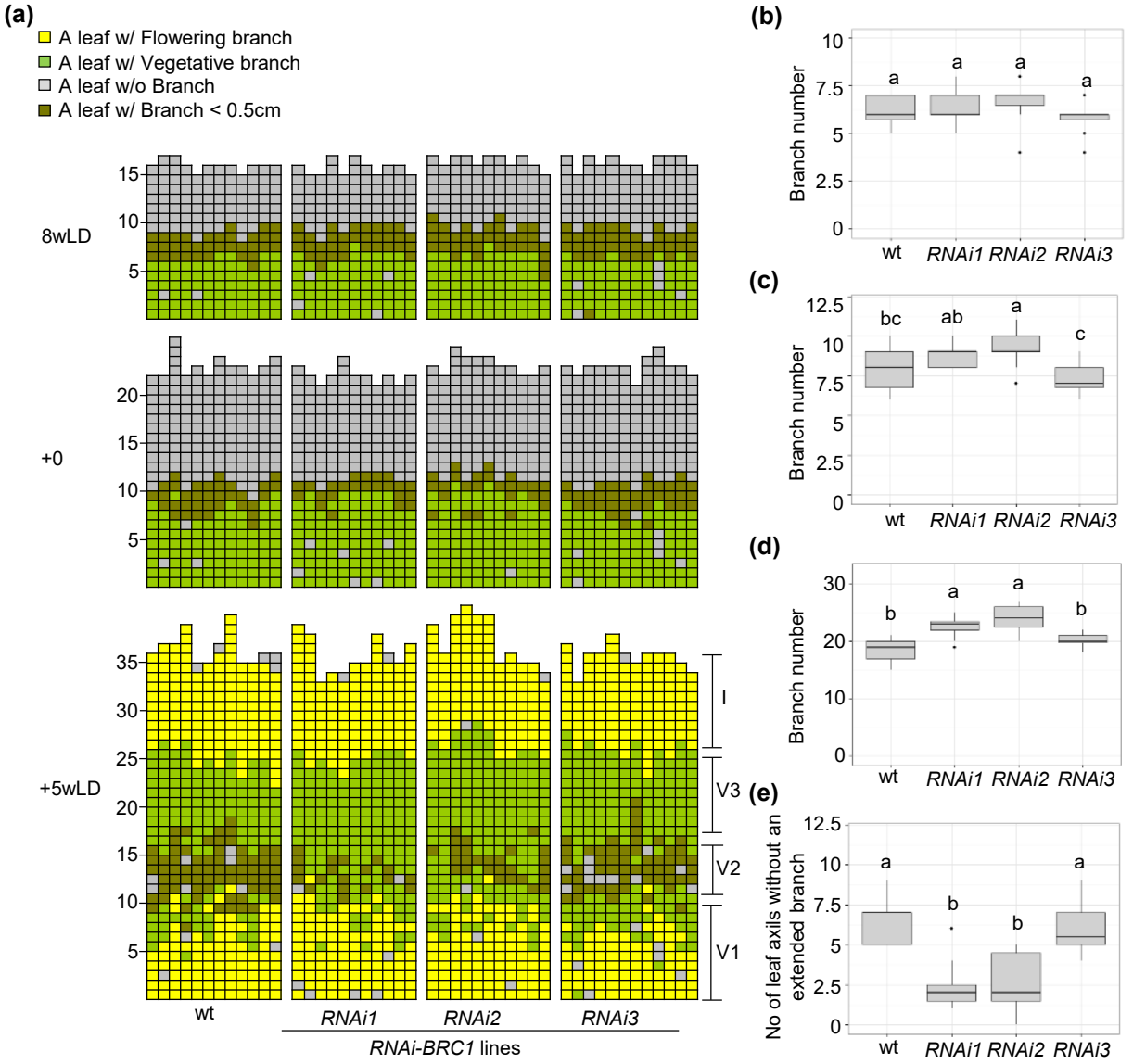
(a)
A leaf w/ Flowering branch
A leaf w/ Vegetative branch
A leaf w/o Branch
A leaf w/ Branch < 0.5cm
8wLD
+0
+5wLD
I
V3
V2
V1
wt
RNAi1
RNAi2
RNAi3
RNAi-BRC1 lines
(b)
Branch number
(c)
Branch number
(d)
Branch number
(e)
No of leaf axils without an extended branch
wt
RNAi1
RNAi2
RNAi3

Fig. 9