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Evolution of the DELLA gene family in plants and functional characterization of PmDELLA-like in the regulation of plant architecture in Prunus mume

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Abstract

DELLA proteins are central negative regulators of gibberellin (GA) signaling in plants; however, the evolutionary dynamics and functional roles of the DELLA gene family remain poorly understood. Here, we performed a comprehensive phylogenetic analysis of 807 DELLA proteins across 324 species and functionally characterized *PmDELLA-like*, a DELLA-like member in *Prunus mume*. Phylogenetic analysis revealed that DELLA proteins diverged into two monophyletic clades, DELLA I and DELLA-like, in seed plants, with lineage-specific expansion of DELLA I being a dominant trend in angiosperms. Ancestral sequence reconstruction uncovered a three-stage evolutionary pattern of the DELLA motif: a highly divergent pre-DELLA state in bryophytes, establishment of the conserved "DELLA" sequence in the common ancestor of vascular plants, and subsequent functional divergence into "DKELA/DELLA" (DELLA I) and "DGLLA" (DELLA-like) in seed plants. In *P. mume*, *PmDELLA-like* is highly expressed in shoot apical meristems and leaf primordia, and responds to GA treatment. Overexpression of *PmDELLA-like* in poplar resulted in a dwarf and multi-branched phenotype with shortened internodes, reduced endogenous GA content, and altered expression of genes involved in GA metabolism, circadian rhythm, and cell elongation. These findings suggest that PmDELLA-like modulates plant architecture by integrating GA signaling with light-responsive and cell elongation pathways, thereby playing a key role in coordinating growth and branching in woody plants. This study provides new insights into the evolution of the DELLA gene family and the regulatory mechanisms of plant architecture formation in woody plants, offering a theoretical basis for molecular breeding of ornamental tree species.

Background

Plant architecture is a core agronomic and horticultural trait that governs morphological architecture, biomass allocation strategy, and environmental adaptability of plants(Zhou and Yin, 2014;Liu et al., 2025;Wang et al., 2025). In fruit and ornamental tree production, plant architecture directly influences canopy structure, planting density, light interception, and ultimately fruit yield and ornamental value (Ku et al., 2015;Farooque et al., 2017). Understanding the genetic mechanisms underlying height regulation has therefore long been a central pursuit in plant biology and breeding (Niu et al., 2023).

Among the endogenous hormones controlling plant growth, gibberellins (GAs) are recognized as the primary promoters of internode elongation(Wang et al., 2017;Wang and Wang, 2022). GAs can drive stem growth by promoting cell division and elongation, regulating cell wall plasticity and vascular arrangement. Abnormalities in the GA signaling pathway often leads to plant dwarfing or excessive elongation, making it a target for studying height regulation and crop genetic improvement (Chen et al., 2014;Zhang et al., 2025). The GA signaling pathway has been systematically elucidated as a "synthesis–perception–response" module (Gallego-Giraldo et al., 2008;Hartweck, 2008). In this module, DELLA proteins function as central repressors that integrate GA signals with multiple developmental and environmental cues (Golldack et al., 2013). Upon GA binding to its receptor GID1, DELLA proteins are recognized by the SCF^(SLY1/GID2) E3 ubiquitin ligase complex and subsequently degraded via the 26S proteasome (Bethke and Jones, 1998;Peng et al., 1999). This degradation releases growth-promoting

transcription factors—such as PIFs—that activate downstream genes driving cell division and elongation (Li et al., 2024). Therefore, DELLA proteins play a pivotal role, serving not only as the central switch for GA response but also as a key node known to integrate multiple hormones (such as auxin, ethylene, and brassinolide) and environmental signals to coordinate growth and development (Briones-Moreno et al., 2023).

Phylogenetic analyses have revealed that DELLA proteins originated from the ancestral GRAS family in early land plants over 400 million years ago (Yasumura et al., 2007;Jiao et al., 2020;Huang et al., 2024). The canonical GID1–DELLA signaling module was established in vascular plants, so its emergence predates the establishment of the complete GA metabolic pathway and GID1 receptor in plants (Bomke and Tudzynski, 2009;Shani et al., 2024). Despite the well-established role of DELLA proteins as central repressors in the GA signaling pathway, how the key molecular interfaces required for GA perception and signal transduction evolved across plant lineages remains poorly understood(Ji et al., 2026). The N-terminal DELLA domain, which contains two conserved motifs—the DELLA motif essential for GID1 recognition and the TVHYNP motif that reinforces GID1–DELLA interaction—constitutes the molecular basis of GA-dependent DELLA degradation (Hirano et al., 2010;Zhao et al., 2017;Yu and Wang, 2020). This raises a fundamental question: what was the functional state of the DELLA domain before the GA–GID1–DELLA signaling module was established? Comparative sequence analysis of DELLA proteins across diverse plant lineages, combined with ancestral sequence reconstruction, offers an opportunity to trace the evolutionary history of these critical functional motifs and to determine whether their sequence changes coincided with the stepwise assembly of the GA signaling pathway.

Notably, prior to angiosperm radiation, the DELLA lineage split into two subclades (Hernández-García et al., 2019). Before angiosperm differentiation, DELLA genes diverged into two subclades, among which the DELLA1 subclade retains intact DELLA and TVHYNP motifs, is recognized and degraded by GID1, and performs the canonical GA signaling repressor function. In contrast, the DELLA2 (DELLA-like) subclade has variations in the N-terminal DELLA and TVHYNP motifs, cannot bind to GID1, cannot be degraded by GA induction, and can constitutively repress GA responses. DELLA-like proteins have received considerably less attention; in particular, their functional divergence in angiosperms remains poorly understood.

Prunus mume is a renowned ornamental and fruit tree with a cultivation history of over 3,000 years in China. It is cherished for its early spring blossoms, diverse flower colors, and elegant branching habits (Fan et al., 2024;Meng et al., 2026). Despite its horticultural importance, dwarf and highly branched *P. mume* cultivars remain scarce, limiting its applications in potted culture and bonsai. Breeding compact, freely branching Mei varieties is therefore a priority goal. However, the regulatory mechanisms underlying plant height and branching in *P. mume* remain largely unknown. Our preliminary genomic analyses identified a *DELLA-like* gene, designated *PmDELLA-like*, in the *P. mume* genome and we investigated its roles in regulating plant architecture.

Methods

Plant materials

'*Feilve*'Mei, *Populus alba × P. glandulosa* (84K poplar), and *Nicotiana benthamiana* were cultivated in the greenhouse of Qingdao Agricultural University. The growth conditions were set to a 16-hour light/8-hour dark photoperiod with a temperature of (25 ± 2) ℃. Three-year-old potted Mei plants were used in this study. One-year-old branches were selected for hormone spraying treatments. Nodes from these one-year-old branches were excised for tissue culture to induce lateral bud germination.

Shoot tips (approximately 3 cm segments centered on a leaf bud) from '*Feilve*' Mei were surface-sterilized (rinsed with running water for 2 hours, followed by immersion in 75% ethanol for 30 seconds and 2% sodium hypochlorite solution for 10 minutes until the cut ends turned white). The sterilized explants were then inoculated onto Mei seedling-strengthening medium (MS + 0.8 mg/L 6-BA + 0.3 mg/L NAA).

Shoot tips from the branches were collected for RNA extraction, while newly emerged leaves from the branches were used for DNA extraction. For RNA extraction, shoot tips were collected from 84K poplar. The fifth internode from the top was harvested for paraffin sectioning. Leaves of *Nicotiana benthamiana* were used for Agrobacterium-mediated transient transformation to observe subcellular localization.

DELLA proteins identification and phylogenetic analysis

Genome assemblies of 324 embryophyte species were retrieved from the public database NCBI (https://www.ncbi.nlm.nih.gov), GDR (https://www.rosaceae.org/), and PGDD (https://chibba.agtec.uga.edu/). Candidate protein sequences were screened using the PF12041(DELLA domain) and PF03514 (GRAS domain) from the Pfam (http://pfam.xfam.org/) and HMMER (v3.0) with an E-value cut-off of 1e − 5, followed by refinement via BLAST + alignment against *Arabidopsis* DELLA reference sequences. Species lacking DELLA proteins were removed, and those with BUSCO completeness scores below 70% were also excluded. Finally, DELLA protein sequences from 324 species were retained for downstream analyses (Supplementary information data 1).

The protein sequences of 324 species were aligned using MAFFT (v7.526). The multisequence alignment results were further filtered with TrimAl (v1.5) to eliminate regions with low alignment quality and high gapratios, yielding high-quality alignment results. The final alignment comprised 807 sequences and 409 amino acid sites. Phylogenetic analyses were performed using IQ-TREE v2.1.4 (Minh et al. 2020) under the LG + F+G model with 1000 ultrafast bootstrap replicates. Based on the phylogenetic tree and amino acid alignment of the DELLA gene family, we reconstructed the amino acid sequences at key nodes of the gene tree using the LG + F+G model and the -asr option. The N-terminal conserved sequences of the ancestral sequences were extracted for visualization. And protein conserved motifs were predicted using the online website MEME (https://meme-suite.org/meme/).

Hormone treatments

During the leaf expansion stage from leaf buds of '*Feilve*' Mei, shoots were sprayed with either a 200 mg/L gibberellin (GA_3) solution or a paclobutrazol (PBZ) solution. Shoots sprayed with distilled water served as the control. Each treatment group consisted of three biological replicates. Spraying was conducted once every seven days for four consecutive weeks. After the treatment period, shoot length, number of lateral branches, number of internodes, and internode length were measured.

DNA extraction, RNA extraction, and gene cloning

Stem tip tissues (approximately 1 cm in length) were collected from GA_3-treated and water-treated *P. mume* 'Feilve' plants, as well as from PmDELLA-like-overexpressing poplar and wild-type 84K poplar plants, and newly emerged leaves on *P. mume* branches. Samples were immediately frozen in liquid nitrogen and stored at -80°C. DNA was extracted from *P. mume* 'Feilve' leaves using the DNA Extraction Kit (DC104-01; Vazyme; Nanjing; China) for promoter cloning. Total RNA was extracted from *P. mume* using the RNA Extraction Kit (RC411-01; Vazyme; Nanjing; China). First-strand cDNA was synthesized from total RNA of *P. mume* 'Feilve' using the Hiscript 1st strand cDNA Synthesis Kit (R211-02; Vazyme; Nanjing; China). Referring to the *P. mume* genome, the full-length coding sequence (CDS) of *PmDELLA-like* (*Pm027468*) was cloned. Primer sequences used in this study were listed in Table S1.

Vector construction and genetic transformation

Unfortunately, a stable genetic transformation system for *P. mume* is not available. Therefore, to validate gene function, we performed heterologous transformation using a woody model plant. The CDS sequences of *PmDELLA-like* was inserted into the overexpression vector pCAMBIA1300-GFP, to construct the pCAMBIA1300-*PmDELLA-like*-GFP overexpression vector. This vector was introduced into 84K poplar via Agrobacterium tumefaciens-mediated leaf disc transformation. Transgenic lines were selected on kanamycin-containing medium and confirmed by PCR and qRT-PCR to obtain stably overexpressing transgenic lines.

To observe the subcellular localization of *PmDELLA-like*, its coding sequence was inserted into the pCAMBIA1300-GFP vector. The constructed vector and control were transformed into Agrobacterium tumefaciens strain GV3101 and transiently expressed in leaves of *N. benthamiana*. Fluorescent signals were detected using a laser scanning confocal microscope (TCsp5 ; Agilent; America).

Phenotypic measurement and microstructural observation

Positively identified overexpression plants were transferred from culture bottles to a soil substrate and grown in the greenhouse for 12 weeks. Plant height, stem diameter, branch number, internode number, and internode length were measured for both transgenic and wild-type plants. To observe microstructure, paraffin sectioning was performed. Stem segments from overexpression and wild-type

plants were fixed in FAA, followed by dehydration, clearing, wax infiltration, embedding, and sectioning at a thickness of 8 μm. Sections were stained with a 10% toluidine blue solution. Cell morphology and arrangement were observed under an optical microscope, and cell length and width were measured using ImageJ software.

RNA in situ hybridization

Leaf buds of *P. mume* at the leaf expansion stage were collected and fixed with 4% paraformaldehyde to preserve the RNA in situ hybridization. Subsequently, the tissues are embedded in paraffin and sectioned to facilitate probe penetration. The experiment employed a digoxigenin-labeled antisense RNA probe synthesized by in vitro transcription targeting *PmDELLA-like*. The sections were incubated with the probe overnight in a hybridization buffer containing 50% formamide at an appropriate temperature, followed by washing steps to remove nonspecific binding. Finally, color development is performed using the alkaline phosphatase-conjugated anti-digoxigenin antibody system with NBT/BCIP substrate for signal detection.

Transcriptome sequencing

Total RNA was isolated from the apical 1-cm stem tips of both wild-type *Populus alba × P. glandulosa* (WT) and transgenic lines overexpressing *PmDELLA-like*, using the RNAprep Pure Plant Plus Kit (DP441, TIANGEN Biochemical Technology Co., Ltd., Beijing, China). Three biological replicates were performed for each genotype. Sequencing was carried out on the Illumina HiSeq™ 2000 platform. Raw data were subjected to strict quality filtering with fastp v0.20.0 (Chen et al., 2018). High-quality clean reads were then aligned to the reference genome using HISAT2 (Kim et al., 2015), and gene annotation was performed against the *P. alba* reference genome (GCF_005239225.1_ASM523922v1). The featureCounts tool was used to quantify raw read counts per gene, after which expression levels were normalized as FPKM (Fragments Per Kilobase of transcript per Million mapped fragments). Differential expression analysis was conducted on the raw read counts using DESeq2 v1.30.0, which normalizes counts and estimates statistical significance. Genes with $|\log_2(\text{fold change})| \geq 1$ and FDR < 0.05 were considered differentially expressed. Additionally, all assembled unigenes were subjected to BLAST searches against the KEGG Ortholog (KO) and Gene Ontology (GO) databases using BLAST2GO, with a cutoff E-value of 10^{-6} (Kanehisa et al., 2025; Götz et al., 2008). The raw transcriptome sequencing data reported in this study have been deposited in the Genome Sequence Archive (GSA) at the National Genomics Data Center (NGDC), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences, under accession number CRA040489.

Statistical analysis

All experiments were performed with at least three biological replicates. Data are presented as mean ± standard deviation. Statistical analysis was performed using GraphPad Prism 9 software, employing Student's t-test or one-way ANOVA.

Results

Divergence of DELLA proteins into DELLA I and DELLA-like clades in seed plants and expansion of DELLA I genes as a major trend in angiosperm evolution

A total of 807 DELLA subfamily members from 324 terrestrial plant genomes were identified, including bryophytes (45 species), ferns (7 species), lycophytes (2 species), gymnosperms (10 species), basal angiosperms (19 species), monocots (41 species), and eudicots (200 species); the number of DELLA proteins in each specie is shown in Table S2. Phylogenetic analysis resolved three clades: pre-DELLA, DELLA I, and DELLA II (DELLA-like) (Fig. 1), and DELLA protein sequences used to constructed phylogenetic tree were showed in supplementary information data 1. The non-monophyletic pre-DELLA clade contained 77 genes from 59 bryophyte, lycophyte, and fern species. Most bryophytes had a single pre-DELLA gene, except two *Sphagnum* species with two. The two lycophytes had expanded repertoires (*Huperzia asiatica*: 7; *Diphasiastrum complanatum*: 6. Ferns varied from one (*Marsilea*, *Brainea*) to four (*Azolla filiculoides*, *Alsophila spinulosa*) pre-DELLA genes (Table S2). These patterns suggest a large-scale expansion of pre-DELLA genes in lycophytes.

In gymnosperms and angiosperms, DELLA proteins formed two monophyletic clades: DELLA I and DELLA-like. Gymnosperm genes were basal in both clades and copy numbers were largely conserved: all gymnosperms except *Ginkgo biloba* had one DELLA α gene, and all except *Gnetum montanum*, *Pinus albicaulis*, and *Welwitschia mirabilis* had one DELLA-like gene. DELLA α was absent in 42.1% of basal angiosperm species (Figure S1A). Thus, DELLA copy numbers remained relatively conserved in early seed plants, with independent losses in some lineages.

Monocot and eudicot DELLA-like genes formed two distinct monophyletic clades. Monocot DELLA I was sister to the eudicot DELLA I clade, which further split into DELLA 1 and DELLA 2 clades containing basal eudicot, superrosid, and superasterid members. In seed plants, DELLA I copy numbers varied considerably, showing an initial increase followed by a decline in angiosperms; mean numbers per species ranged from 0.84 to 2.54, peaking in superrosids (2.54) and decreasing to 1.47 in superasterids (Figure S1B). DELLA-like gene numbers ranged from 0 to 4, with mean values of 0.7–1.1 per species except in basal eudicots (Figure S1C). DELLA-like genes accounted for 56.8% of DELLA genes in basal angiosperms, whereas DELLA I genes accounted for > 67% in monocots, basal eudicots, superrosids, and superasterids, reaching 74.4% in superrosids; basal eudicots completely lost DELLA-like genes (Figure S1A). Thus, DELLA I underwent pronounced lineage-specific expansion, whereas DELLA-like numbers remained relatively stable. Notably, Rosaceae had a mean of 4.27 DELLA genes per species, significantly higher than other superrosids; within Rosaceae, Maleae had a mean of 6, significantly higher than Rosoideae (3.5) and Amygdaleae (2.0), with a 3:1 DELLA I to DELLA-like ratio in two Maleae species, indicating synchronous, proportional duplication. This likely reflects the known paleopolyploidization in *Malus* (Velasco et al., 2010).

Ancestral node reconstruction of the N-terminal DELLA conserved motif in DELLA proteins

The DELLA motif is essential for GID1 recognition, while TVHYNP enhances the GID1–DELLA interaction. Sequence alignment revealed marked variation in the DELLA motif across groups (Fig. 2A). The pre-DELLA proteins varied considerably; 40% of fern sequences had the canonical DELLA sequence, identical to angiosperm DELLA I. DELLA I motifs were highly conserved in angiosperms (82.75% DELLA), whereas gymnosperm sequences differed markedly: DKELA (44.44%), DMELA (33.33%), and DRELA (22.22%). Among seed plants, DGLLA predominated in DELLA-like proteins (58%).

Ancestral state reconstruction based on the 807-protein phylogeny (Fig. 2B) showed that the ancestral DELLA-domain motif in land plants was DGQSA, resembling extant ferns, lycophytes, and bryophytes. The canonical “DELLA” sequence emerged in the common ancestor of vascular plants and persisted until the seed plant ancestor, then diverged into “DGLLA” (ancestral DELLA-like) and “DKELA” (ancestral DELLA). DKELA appeared only at the ancestral node of gymnosperm DELLA proteins, whereas all key ancestral nodes of angiosperm DELLA proteins harbored the "DELLA" motif. Overall, the N-terminal conserved sequence evolved from an early non-conserved stage (bryophytes), through a brief conserved transition (vascular plant ancestor), to conserved and non-conserved forms in seed plants.

Identification and phylogenetic analysis of the DELLA subfamily in Rosaceae

A total of 38 DELLA proteins were identified from nine Rosaceae species (representing seven tribes and nine genera). A phylogenetic tree with *Arabidopsis* sequences resolved two clades: DELLA I (31 proteins) and DELLA-like (14 proteins) (Fig. 3A), DELLA protein sequences used to constructed phylogenetic tree were listed in Supplementary information data 2. Arabidopsis lacked DELLA-like members, whereas each Rosaceae species had one to four. DELLA I clustered with Arabidopsis GAI and RGA1; DELLA-like clustered with RGA-like1–3. Motifs 1, 4, 5, 7, 8, and 9 were present in all members, with motifs 5 and 9 in the DELLA domain and motifs 7, 8, 1, and 4 in the GRAS domain (Fig. 3B). All DELLA-like members lacked motifs 11 and 13, and motif 14 was N-terminal in DELLA-like but central in DELLA I, indicating significant differences in conserved motif architecture. Except for Pm027468 (PmDELLA-like), Gtro4g122227.t2, and Rmu_co8349301.1, all other DELLA proteins contained motifs 2, 3, 6, 10, and 12, suggesting possible functional divergence in these three members.

(A) and conserved motifs (B) of DELLA proteins in Rosaceae.

The expression of PmDELLA-like was upregulated after gibberellin treatment in the shoot apical meristem of P. mume

The cloning and sequence analysis of *PmDELLA-like* play a significant role in regulating the plant height of *P. mume*. The gene coding sequence of *PmDELLA-like* was cloned using cDNA reverse-transcribed

from the RNA of apical buds of the *P. mume* 'Fei Lve'. Fluorescence quantitative analysis indicated that *PmDELLA-like* is highly expressed in the stems and leaf buds of *P. mume* (Fig. 4A), and its expression level gradually increased during leaf bud development (Fig. 4B). Additionally, through exogenous GA_3 spray treatment on the shoot tips of *P. mume* branches, it was observed that the expression level of *PmDELLA-like* was upregulated at 30 minutes, 1 hour, 2 hours, and 6 hours after treatment (Fig. 4C). The in situ hybridization results indicated that *PmDELLA-like* is highly expressed in the shoot apical meristem, leaf primordia, and vascular bundles (Fig. 4E). And the *PmDELLA-like* protein is localized in the nucleus (Fig. 4F). These results indicated that *PmDELLA-like* is primarily expressed in the stem, as well as in leaf primordia, the shoot apical meristem, and vascular bundles within leaf buds, and that its expression responded to GA_3 treatment.

(A) Expression levels of *PmDELLA-like* in various tissues of *P. mume*. (B) Expression level of *PmDELLA-like* at different development stages of leaf buds. (C) Expression level of *PmDELLA-like* in shoot sips of branches at different time points after exogenous spraying with GA_3. (D) In situ hybridization of the *PmDELLA-like* sense strand probe in leaf bud; (E), In situ hybridization of the *PmDELLA-like* antisense strand probe in leaf bud; LP, leaf primordia, SAM, shoot apical meristem, Pi, pith, VB denotes vascular bundle, Xy, xylem, Ph, phloem, and Co, cortex. (F) Subcellular localization of PmDELLA-like protein.

Overexpression of PmDELLA-like in poplar leads to reduced plant height and increased branch number

To further validate its biological function, we constructed an overexpression vector of *PmDELLA-like* and introduced it into 84K poplar (Fig. 5A). Compared to wild-type (WT) plants, the overexpression plants exhibited reduced plant height (Fig. 5B), increased branch number (Fig. 5C), decreased stem diameter (Fig. 5D), a higher number of internodes (Fig. 5E), shorter internode length (Fig. 5F). To further observe the cytological structure, we prepared paraffin sections (Fig. 5G-J) and performed statistical analysis using Image J software. The results showed that compared with the WT, the cortex and xylem of *PmDELLA-like*-overexpressing plants were significantly narrower, while the phloem was thicker (Fig. 5K). Statistical analysis of epidermal cells and cortex cells revealed that the epidermal cells of *PmDELLA-like*-overexpressed poplar were shorter and narrower, and the cortex cells were narrower than those of the WT plants (Fig. 5L). These results indicate that *PmDELLA-like* can influence plant architecture formation by inhibiting cell growth and division as well as differentiation.

(A) The 3 months of *PmDELLA-like*-OE and WT poplar. (B) Plant height. (C) Branch number. (D) Stem diameter. (E) Internode number. (F) Internode length. (G) Transverse sections of stems from WT poplar. (H) Transverse sections of stems from *PmDELLA-like*-OE poplar. (I) Longitudinal sections of stems from WT poplar. (J) Longitudinal sections of stems from *PmDELLA-like*-OE poplar. (K) Comparison of cortex, phloem, xylem width between *PmDELLA-like*-OE and WT poplar. (L) Comparison of epidermal and cortex cells between *PmDELLA-like*-OE and WT poplar. (M) Gibberellin contents in the stems tips of WT and *PmDELLA-like*-OE poplars. Pi, pith; Xy, xylem; Ph, phloem; Co, cortex; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, ns indicates no significant difference.

Transcriptomic Profiling and Regulatory Target Identification of PmDELLA-like

Stem tips (1 cm) from the apex of branches of *PmDELLA-like*-overexpressing poplar plants were sampled for transcriptome sequencing, with stem tips from wild-type plant branches serving as the control. A total of 1,183 upregulated genes and 2,041 downregulated genes were detected (Fig. 6A). KEGG enrichment analysis revealed that the differentially expressed genes (DEGs) were primarily enriched in pathways such as plant circadian rhythm, metabolic pathways, diterpenoid biosynthesis, and plant MAPK signaling (Fig. 6B). And GO enrichment analysis showed the DEGs involved to circadian rhythm (GO:0007623) and light response, including response to red light (GO:0010114), response to blue light (GO:0009637), cellular response to blue light (GO:0071483), response to light intensity (GO:0009642), response to red or far red light (GO:0009639), cellular response to light stimulus (GO:0071482), cellular response to radiation (GO:0071478), response to high light intensity (GO:0009644) were changed (Fig. 6C). These results indicated that PmDELLA-like may be involved in the regulatory network connecting light signaling and the circadian rhythm. Thus the heatmaps of DEGs related to gibberellin biosynthesis and circadian rhythm pathways were displayed in Fig. 6D and Fig. 6E, respectively. Beside to LOC118060709 (GA20OX), three GA biosynthesis genes were down-regulated, including two KAO (LOC118063426 and LOC118049585) and one GA20OX (LOC118043535) encoding genes in stem tips of PmDELLA-like-overexpressing poplar. However, for the gibberellin degradation genes GA2OXs, one gene (LOC118030385) was up-regulated, while two genes (LOC118062430 and LOC118035057) were down-regulated (Fig. 6D). A total of 33 genes were up-regulated and 46 down-regulated in circadian rhythm pathway (Fig. 6E).

To assess whether DELLA overexpression affects downstream cell elongation processes, we analyzed the expression profiles of genes known to be involved in cell wall remodeling and cell expansion (e.g., EXPA, XTH, TUB). In *PmDELLA-like*-overexpressed poplar, 61 genes related to cell elongation were also found to be altered, such as genes encoding expansin (*LOC118053381, LOC118042962, LOC118061995, LOC118035613*), xyloglucan endotransglucosylase/hydrolase (*LOC118033641, LOC118054391, LOC118060905, LOC118029594*), pectinesterase/pectinesterase inhibitor (*LOC118041091, LOC118061174, LOC118060156, LOC118054161, LOC118041326, LOC118056334, LOC118040163, LOC118048524, LOC118028572*), phytochrome-interacting factor (*LOC118041138, LOC118028327, LOC118050400, LOC118048518, LOC118061713, LOC118041333, LOC118056546, LOC118052071, LOC118035461, LOC118035770*) et al (Figure S2).

A total of 274 differenttially expressived transcription factors (DETFs) in *PmDELLA-like*-overexpressing poplar were identified, among which 73 were upregulated and 201 were downregulated. These transcription factors belong to diverse gene families including AP2/ERF-AP2 (30), MYB (30), bHLH (24), C2C2 (19), NAC (18), WRKY (16), bZIP (16), GRAS (10) and HD-ZIP (7) families (Fig. 7).

(A) Volcano plot of DEGs. Red indicates upregulation, and blue indicates downregulation. (B) KEGG pathway enrichment analysis of DEGs. (C) GO pathway enrichment analysis of DEGs. (D) Heatmap of DEGs involved in gibberellin biosynthesis and metabolism pathway. *KAO*, ent-kaurenoic acid oxidase;

GA2OXs, Gibberellin 2-oxidases; GA20OXs, Gibberellin 20-oxidases. (E) Heatmap of DEGs involved in circadian rhythm. Black font indicates substances involved in the gibberellin biosynthesis and metabolism pathway. Green background indicates bioactive gibberellins. Log_2FC denotes log_2(Fold Change).

Discussion

A three-stage evolutionary trajectory of the DELLA motif reflects the stepwise assembly of GA signaling

Ancestral sequence reconstruction across 807 DELLA proteins revealed a three-stage evolutionary trajectory of the DELLA motif, which closely mirrors the stepwise assembly model of the plant GA signaling pathway (Hernández-García et al., 2021). In the common ancestor of land plants, the ancestral DELLA domain was reconstructed as DGQSA, a highly divergent sequence lacking a recognizable GID1-interaction interface. This finding aligns with the absence of functional GID1 orthologs in bryophytes (Hirano et al., 2007), and with previous reports that moss DELLA proteins (e.g., PpDELLAa) exhibit intrinsic growth-repressive activity independent of GA perception when heterologously expressed in *Arabidopsis* (Yasumura et al., 2007). The high sequence diversity of pre-DELLA motifs across extant bryophytes further supports the hypothesis that prior to the emergence of GID1, the DELLA domain was not under purifying selection to maintain a conserved recognition interface, and instead functioned as a hormone-independent transcriptional regulatory hub (Briones-Moreno et al., 2023).

Strikingly, the canonical "DELLA" motif emerged precisely at the ancestral node of vascular plants, coinciding with the evolutionary origin of GID1 and the establishment of the complete GA biosynthetic pathway (Gazara et al., 2018;Hernández-García et al., 2021). In extant ferns, 40% of DELLA proteins already carry the canonical DELLA sequence, and this motif was stably retained through the common ancestor of seed plants. The temporal congruence between DELLA motif fixation and GID1 emergence provides sequence-level molecular evidence supporting the hypothesis that the GA–GID1–DELLA signaling module was established in a single evolutionary event in early vascular plants. The structural basis for this conservation is straightforward: high-affinity GID1 binding requires precise residue complementarity within the DELLA motif (notably the conserved aspartate and leucine residues) (Murase et al., 2008;Hirano et al., 2010), which imposed strong purifying selection that drove rapid sequence standardization immediately following GID1 acquisition.

Importantly, our analysis uncovered an unanticipated intermediate state in the refinement of this signaling module. The "DKELA" motif was reconstructed exclusively at the gymnosperm ancestral node of DELLA , whereas the canonical "DELLA" motif was fixed at all key ancestral nodes of angiosperm DELLA proteins. Extant gymnosperms predominantly retain non-canonical DELLA variants (DKELA, DMELA, or DRELA), suggesting that optimization of the GID1–DELLA interaction interface continued beyond the origin of vascular plants, and underwent further functional refinement during angiosperm evolution. This progressive refinement of the recognition interface has not been captured by previous

evolutionary models (Hernández-García et al., 2021), and may partially explain the generally less pronounced GA responsiveness observed in gymnosperms relative to angiosperms.

In seed plants, DELLA Ⅰ and DELLA-like diverged into distinct lineages with contrasting evolutionary fates: DELLA Ⅰ retained the canonical DELLA motif and GA-dependent degradability, whereas DELLA-like accumulated N-terminal mutations (predominantly yielding the "DGLLA" variant) that abolished GID1 binding, converting it into a constitutive growth repressor (Peng et al., 1997;Chai et al., 2022). Subsequent lineage-specific expansion of DELLA Ⅰ in angiosperms paralleled the copy number expansion of the GID1 receptor family from one to two or three copies (Gazara et al., 2018). The joint retention of DELLA I and DELLA-like genes across most seed plant lineages is likely driven by their complementary regulatory functions—GA-responsive dynamic repression versus constitutive basal repression—which confer selective advantages for fine-tuning growth regulation across complex developmental and environmental contexts.

PmDELLA-like responds to GA signals and modulates GA homeostasis despite its inability to bind the GA receptor GID1

In Arabidopsis, canonical DELLA proteins such as GAI repress GA responses and modulate endogenous GA contents, thereby coordinating plant growth and development (Peng et al., 1997;Fu et al., 2001). Previous work in *P. mume* has identified three DELLA family members, and heterologous overexpression of PmDELLA and PmRGL2 in Arabidopsis both conferred dwarf phenotypes (Lu, 2015;Gao et al., 2025). The Arabidopsis ortholog of PmDELLA-like, RGL3, also exhibits transcriptional responsiveness to GA_3 treatment. In this study, exogenous GA_3 application rapidly upregulated *PmDELLA-like* transcription in the *P. mume* shoot apical meristem, indicating that although DELLA-like proteins escape GA-dependent proteolysis, their encoding genes remain transcriptionally responsive to GA signals. This pattern is consistent with findings in sugarcane, where expression of the DELLA-like gene *ScGAIL* was significantly upregulated after 1 h of 100 μM GA_3 treatment (Chai et al., 2022).

We propose that GA-induced upregulation of DELLA-like transcription represents a compensatory feedback mechanism within the GA signaling pathway. When GA_3 activates signaling and triggers degradation of canonical DELLA I proteins, cells may upregulate DELLA-like transcription as a molecular "brake" to prevent excessive GA responses and maintain growth homeostasis. In support of this model, we observed significantly reduced endogenous GA contents in shoot tips of *PmDELLA-like*-overexpressing poplar, alongside widespread transcriptional changes in GA biosynthesis and inactivation genes. These results demonstrate that PmDELLA-like participates in the regulation of GA metabolic homeostasis. This finding is congruent with observations in *ScGAIL*-overexpressing Arabidopsis, where GA receptor genes (*GID1a/b/c*) and biosynthesis genes (*KO*, *GA3ox1*, *GA20ox1*) were all dysregulated, leading to disrupted GA metabolic profiles and altered relative abundances of different GA species (Chai et al., 2022).

We speculate that DELLA-like-mediated regulation of GA biosynthesis and metabolism arises as a homeostatic feedback response: when GA signaling is constitutively repressed by DELLA-like, plants

attempt to restore signaling balance by modulating the abundance of bioactive GA. In addition, canonical DELLA proteins can function not only through protein–protein interactions to repress growth, but also as transcriptional regulators to modulate the expression of downstream target genes (Park et al., 2013;Huang et al., 2024). For example, Arabidopsis DELLA proteins directly repress *AtGA3OX1* expression to regulate plant height (Boccaccini et al., 2014), and modulate *AtEXP8*, *AtPRE1* and *AtPRE5* to inhibit cell elongation (de Lucas et al., 2008). Our transcriptomic data showing altered expression of GA metabolic genes align with this regulatory paradigm, suggesting that PmDELLA-like may directly or indirectly regulate the transcription of GA pathway genes, despite its divergence from canonical DELLA I proteins at the N-terminal motif.

PmDELLA-like represses plant height and modulates branching by inhibiting cell elongation and integrating light/circadian regulatory networks

Heterologous overexpression of *PmDELLA-like* in poplar produced a dwarf, multi-branched phenotype with shortened internodes, which is consistent with the dwarfing effects reported for *AtGAI* overexpression in rice (Fu et al., 2001), *RoDELLA* overexpression in *Pelargonium × domesticum* (Hamama et al., 2012), and *LhSLRL* overexpression in Arabidopsis (Liu et al., 2020). At the cytological level, epidermal and cortical cells were significantly reduced in size in *PmDELLA-like*-overexpressing poplar, and transcriptomic analysis revealed downregulation of multiple cell wall remodeling genes (including *EXPA*, *XTH* and pectin esterase genes) and cell elongation-related genes. Combined, these results indicate that PmDELLA-like represses cell expansion to drive dwarfing, a functional property shared with canonical DELLA I proteins, which bind and inhibit growth-promoting transcription factors such as PIF4 to block activation of downstream cell growth genes (de Lucas et al., 2008).

Photoperiod and the circadian clock are core regulators of shoot elongation and branching in *P. mume*. The circadian clock and GA signaling engage in reciprocal regulation(Ma et al., 2025): GA signals feed back to modulate circadian period, while core clock components such as TOC1 and PRR-family proteins regulate the expression of GA metabolism-related genes (Blazquez et al., 2002;Arana et al., 2011). In *PmDELLA-like*-overexpressing poplar, differentially expressed genes were significantly enriched in circadian rhythm and light response pathways, suggesting that PmDELLA-like coordinates plant height and branching development in woody plants by interfacing with photoperiod and circadian clock networks. This regulatory role is in line with the established function of DELLA I proteins in photomorphogenesis, where DELLAs integrate light signals through direct interactions with PIFs and photoreceptors to coordinate growth and development (Achard et al., 2007). Recent work further demonstrates that the blue light receptor CRY1 stabilizes DELLA proteins by physically interacting with GID1 to repress GA signaling during photomorphogenesis (Xu et al., 2021), providing a mechanistic framework linking DELLA function to light-responsive growth regulation.

Collectively, our findings suggest that PmDELLA-like may act as a tunable GA-responsive regulatory node that integrates hormonal, light and circadian signals to shape woody plant architecture. Future work should focus on identifying the direct downstream targets and protein interaction partners of PmDELLA-

like in *P. mume*, and elucidating how this evolutionarily divergent DELLA variant rewires the growth regulatory network to accommodate the developmental complexity of perennial woody species. Such studies will advance our understanding of how ancient growth-regulatory modules have been co-opted to shape the specialized architecture of woody horticultural crops.

Conclusions

This study systematically depicts the evolutionary trajectory of the DELLA gene family across land plants, revealing a three-stage evolution pattern of the core DELLA motif that mirrors the stepwise assembly of the gibberellin signaling pathway. Functional characterization of *PmDELLA-like* in *Prunus mume* demonstrates that this DELLA-like member regulates plant architecture by modulating gibberellin homeostasis, cell elongation, and light/circadian regulatory networks. These findings deepen our understanding of the functional divergence of DELLA family members in woody plants, and provide candidate genes and theoretical support for molecular breeding of compact plant architecture in ornamental tree species.

Abbreviations

6-BA
6-benzyladenine
ANOVA
analysis of variance
AP2/ERF
APETALA2/ethylene-responsive factor
bHLH
basic helix-loop-helix
BUSCO
Benchmarking Universal Single-Copy Orthologs
bZIP
basic leucine zipper
C2C2
C2C2-type zinc finger transcription factor
cDNA
complementary DNA
CDS
coding sequence
Co
cortex
CRY1
cryptochrome 1
DEGs

differentially expressed genes
DETFs
differentially expressed transcription factors
DNA
deoxyribonucleic acid
EXPA
expansin
FAA
formalin-acetic acid-alcohol
GA
gibberellin
GA_3
gibberellin A_3
GA2OXs
Gibberellin 2-oxidases
GA20OXs
Gibberellin 20-oxidases
GID1
GIBBERELLIN INSENSITIVE DWARF1
GO
Gene Ontology
GRAS
GAI/RGA/SCR transcription factor family
HD-ZIP
homeodomain-leucine zipper
HMMER
Hidden Markov Model-based sequence alignment tool
IQ-TREE
phylogenetic tree inference software
KAO
ent-kaurenoic acid oxidase
KEGG
Kyoto Encyclopedia of Genes and Genomes
LP
leaf primordia
Log2FC
log2 (Fold Change)
MAFFT
Multiple Alignment using Fast Fourier Transform
MEME

Multiple Em for Motif Elicitation
MS
Murashige and Skoog medium
MYB
myeloblastosis transcription factor
NAA
1-naphthaleneacetic acid
NAC
NAM/ATAF/CUC transcription factor
NBT/BCIP
nitro blue tetrazolium / 5-bromo-4-chloro-3-indolyl phosphate
OE
overexpression
PBZ
paclobutrazol
PCR
polymerase chain reaction
Pfam
Protein family
Ph
phloem
Pi
pith
PIFs
phytochrome-interacting factors
PRR
pseudo-response regulator
qRT-PCR
quantitative real-time polymerase chain reaction
RGL
RGA-like
RNA
ribonucleic acid
SAM
shoot apical meristem
SCF < sup>SLY1/GID2</sup>
SCF E3 ubiquitin ligase complex containing SLY1/GID2
ScGAIL
Saccharum officinarum GA-insensitive DELLA-like protein
TOC1

TIMING OF CAB EXPRESSION 1
TrimAl
alignment trimming tool
TUB
tubulin
VB
vascular bundle
WT
wild-type
WRKY
WRKY transcription factor
XTH
xyloglucan endotransglucosylase/hydrolase
Xy
xylem

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the Genome Sequence Archive (GSA) at the National Genomics Data Center (NGDC) repository, [CRA040489]

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

The authors confirm contribution to the paper as follows: study conception and design: Li L, Guo S; data collection: Zhang C, Guo S, Jiang P; analysis and interpretation of results: Lu Y, Li W; draft manuscript

preparation: Guo S; funding acquisition and resources: Li L, Zhang Q. All authors reviewed the results and approved the final version of the manuscript.

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Figures

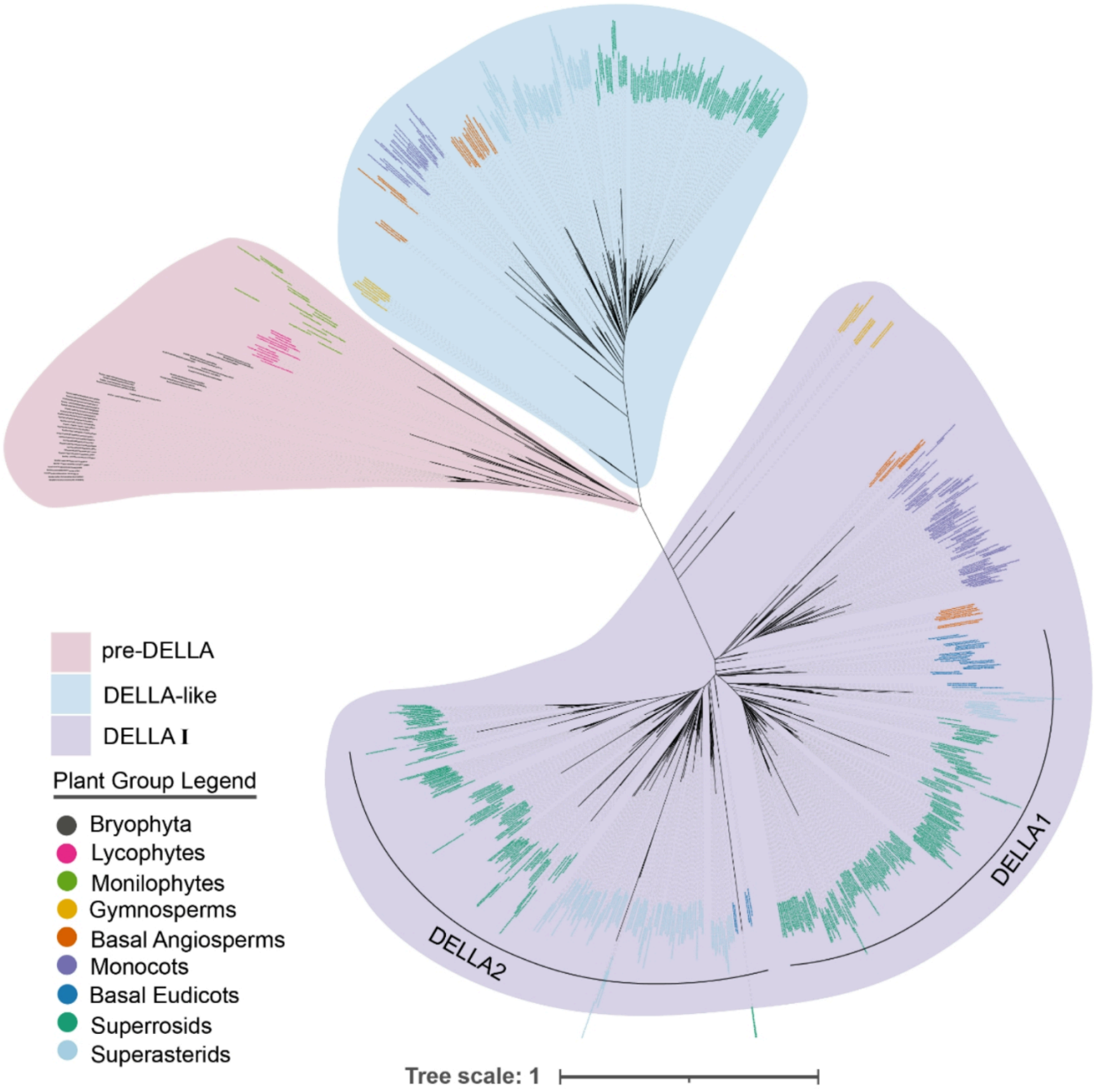


Figure 1

Phylogenetic tree based on 807 DELLA proteins from 324 terrestrial plants

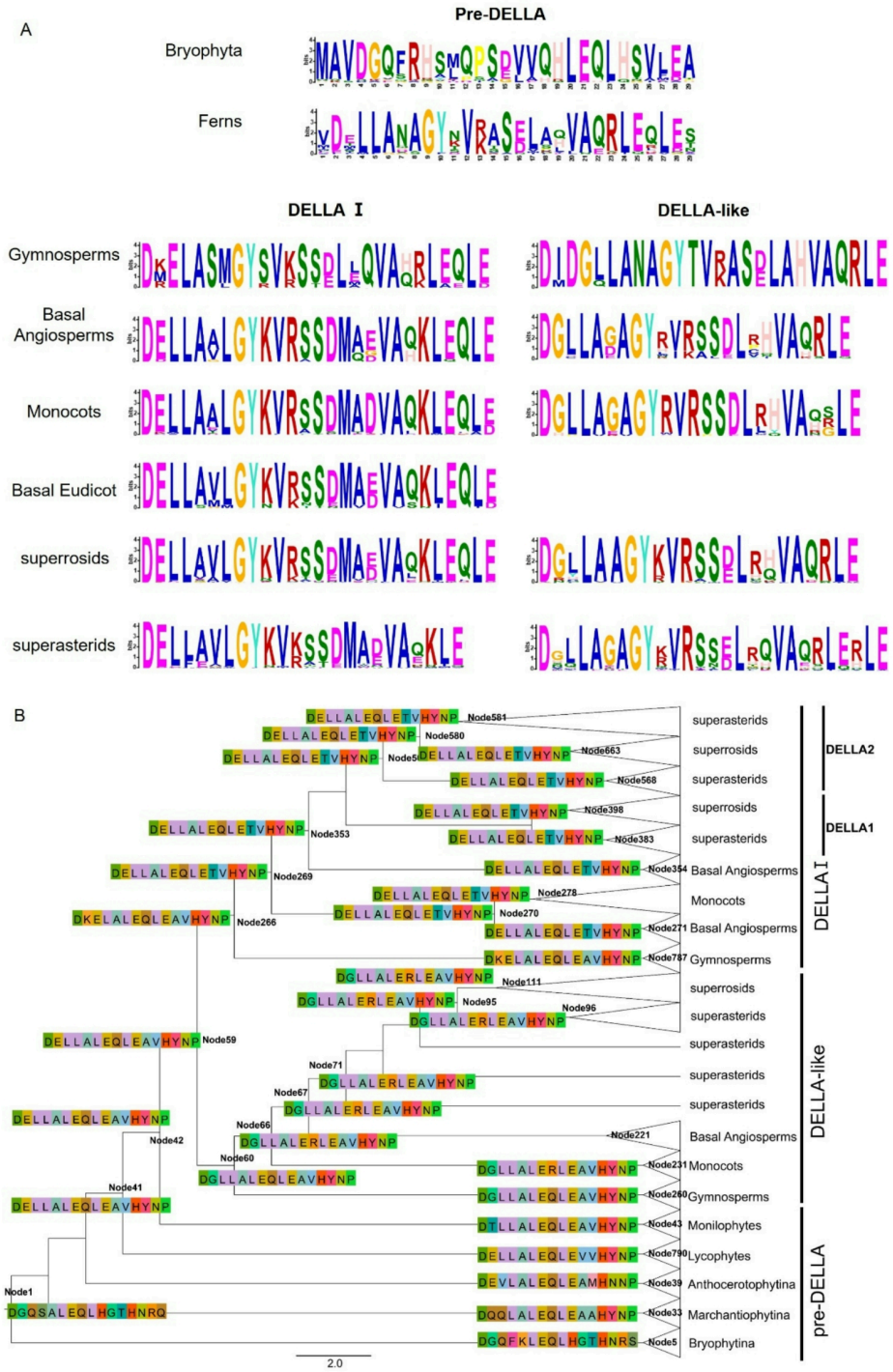


Figure 2

Sequence model of the conserved DELLA motif in the N-terminus of DELLA proteins (A) and ancestral state reconstruction (B).

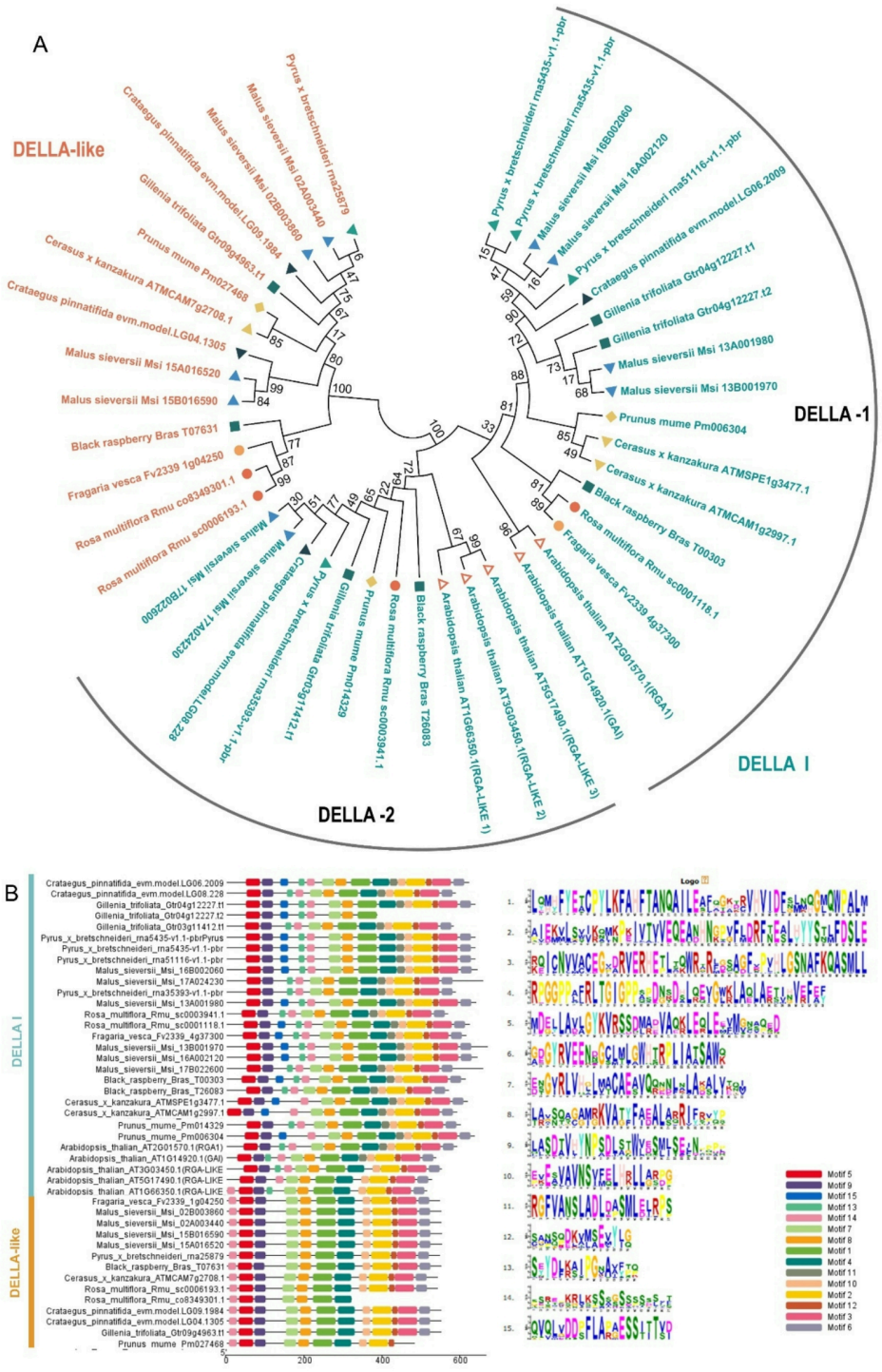


Figure 3

Phylogenetic analysis

(A) and conserved motifs (B) of DELLA proteins in Rosaceae.

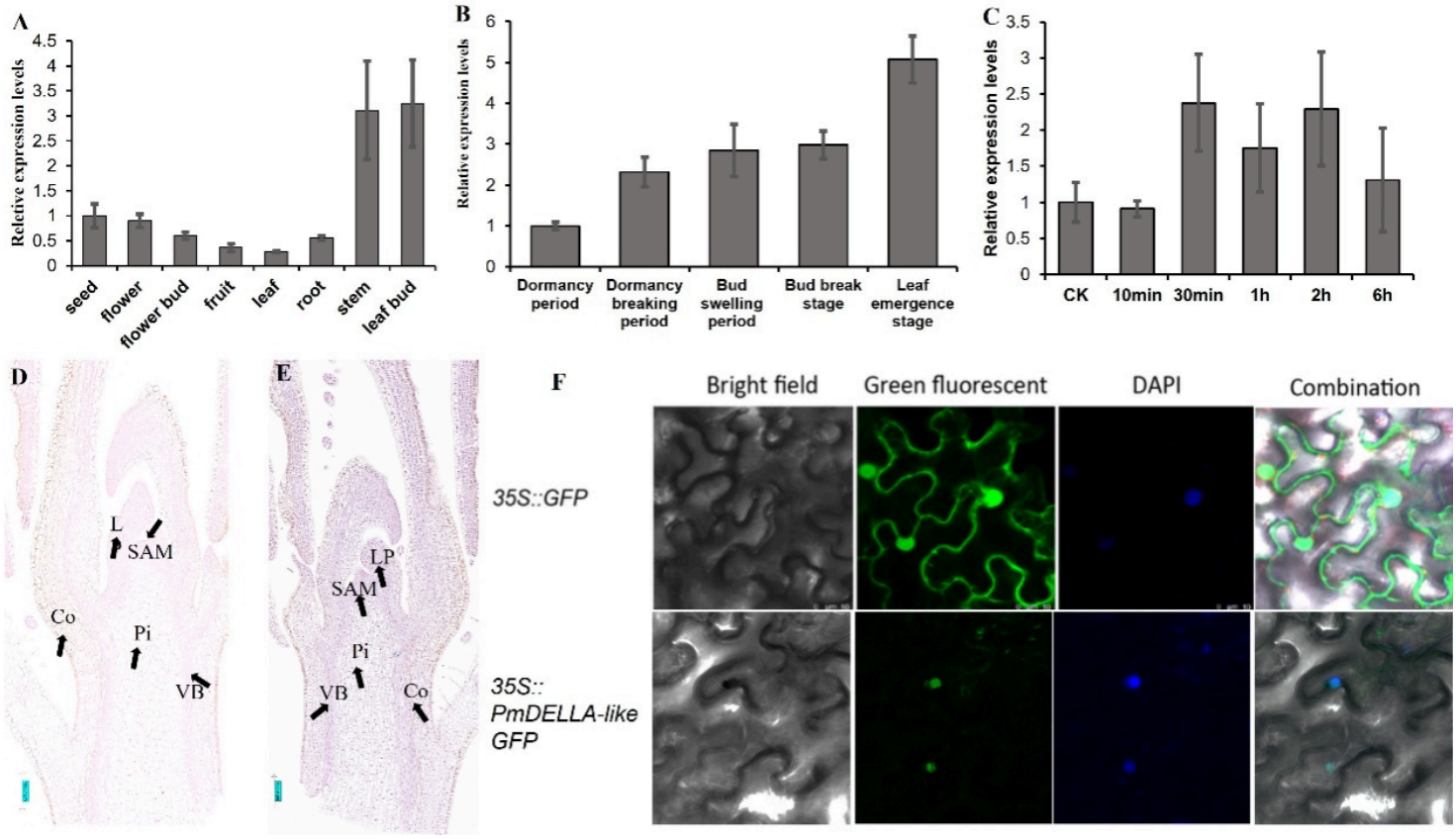


Figure 4

(A) Expression levels of *PmDELLA-like* in various tissues of *P. mume*. (B) Expression level of *PmDELLA-like* at different development stages of leaf buds. (C) Expression level of *PmDELLA-like* in shoot sips of branches at different time points after exogenous spraying with GA_3. (D) In situ hybridization of the *PmDELLA-like* sense strand probe in leaf bud; (E), In situ hybridization of the *PmDELLA-like* antisense strand probe in leaf bud; LP, leaf primordia, SAM, shoot apical meristem, Pi, pith, VB denotes vascular bundle, Xy, xylem, Ph, phloem, and Co, cortex. (F) Subcellular localization of PmDELLA-like protein.

Analysis of *PmDELLA-like* expression patterns in *P. mume*.

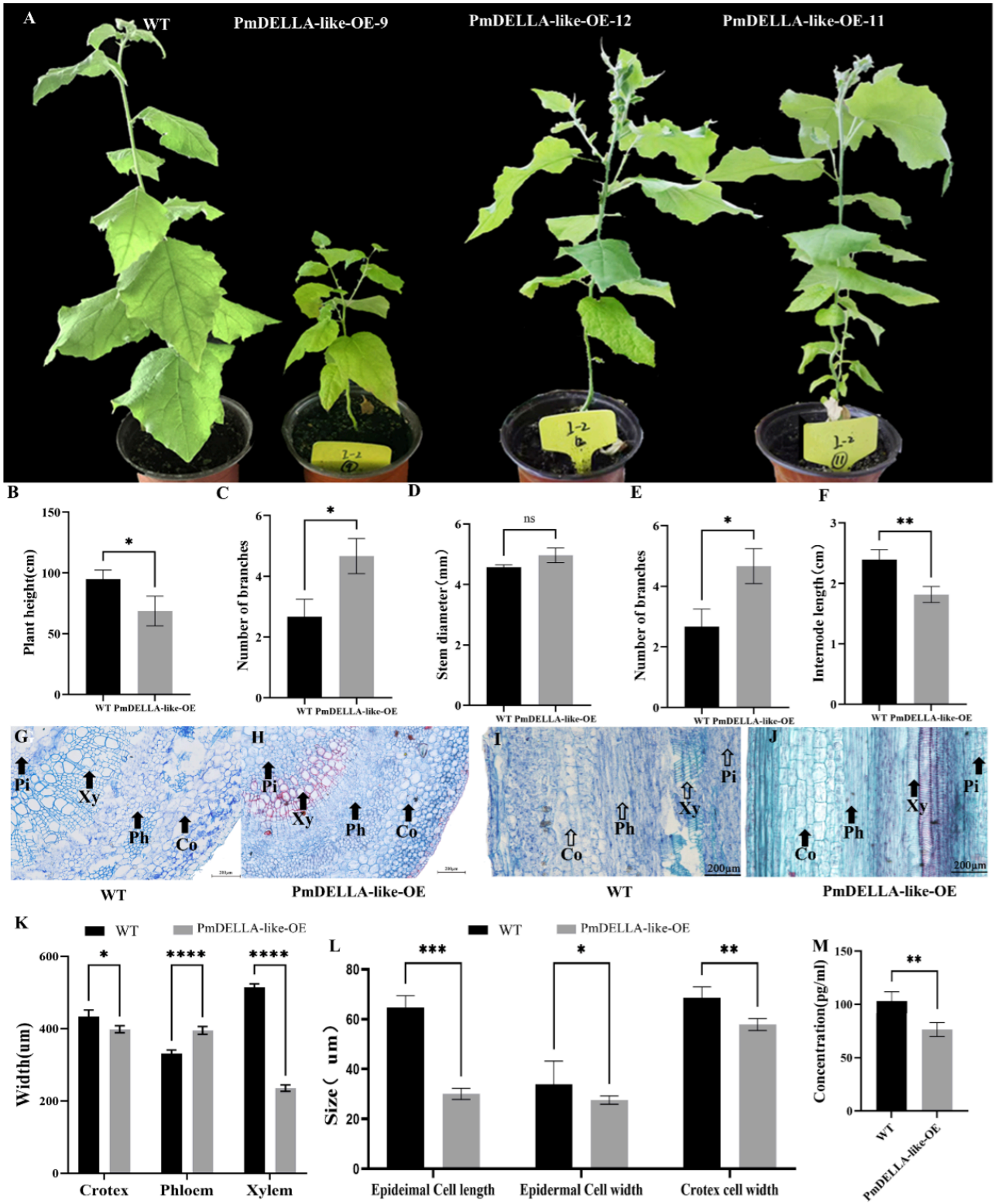


Figure 5

Phenotype of *PmDELLA-like*-overexpressed (*PmDELLA-like*-OE) and wild (WT) poplar

(A) The 3 months of *PmDELLA-like*-OE and WT poplar. (B) Plant height. (C) Branch number. (D) Stem diameter. (E) Internode number. (F) Internode length. (G) Transverse sections of stems from WT poplar. (H) Transverse sections of stems from *PmDELLA-like*-OE poplar. (I) Longitudinal sections of stems from

WT poplar. (J) Longitudinal sections of stems from *PmDELLA-like*-OE poplar. (K) Comparison of cortex, phloem, xylem width between *PmDELLA-like*-OE and WT poplar. (L) Comparison of epidermal and cortex cells between *PmDELLA-like*-OE and WT poplar. (M) Gibberellin contents in the stems tips of WT and *PmDELLA-like*-OE poplars. Pi, pith; Xy, xylem; Ph, phloem; Co, cortex; *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, ns indicates no significant difference.

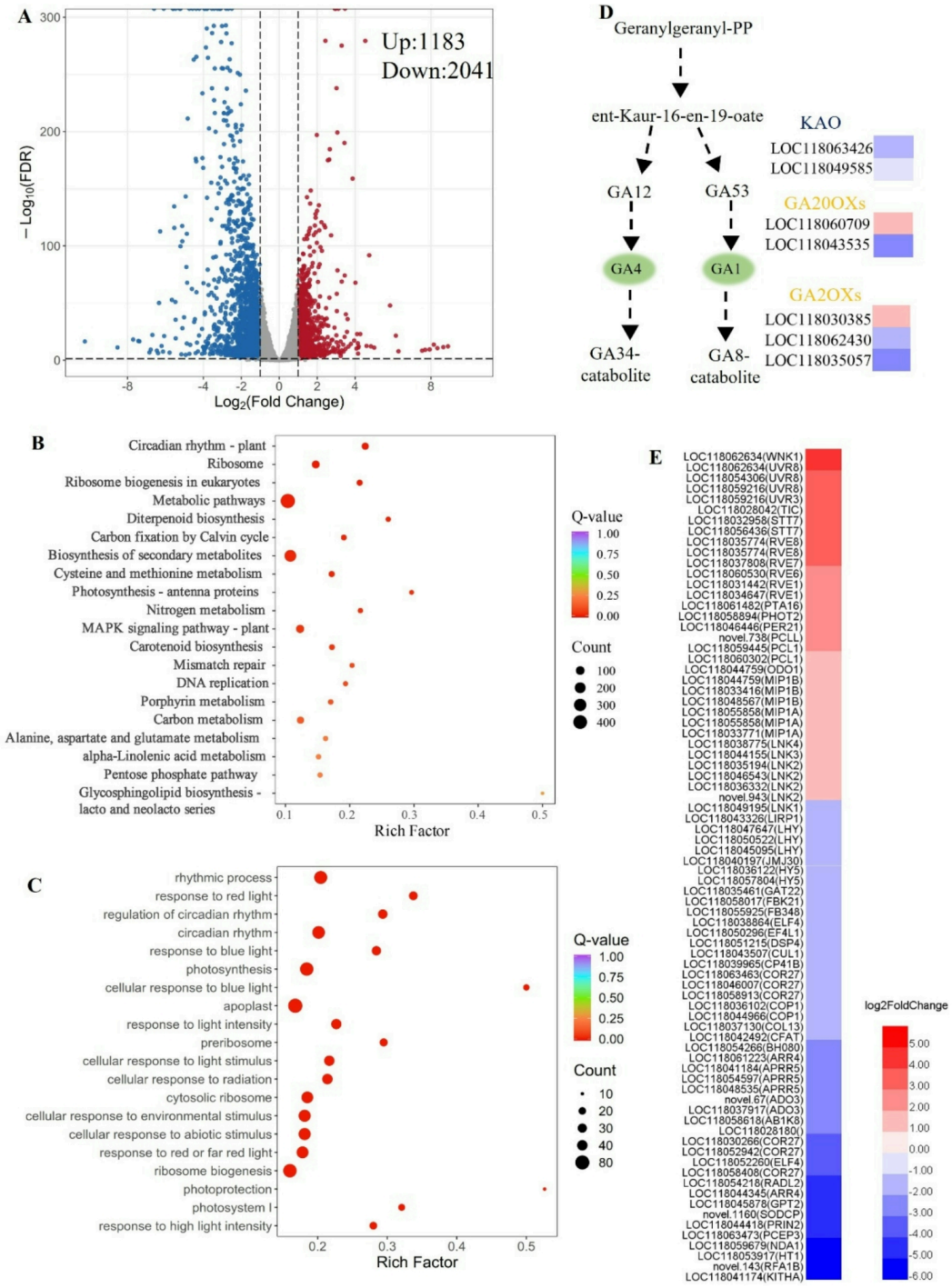


Figure 6

Figure 6. Transcriptomic analysis of shoot apical meristems from *PmDELLA-like*-overexpressing poplar and analysis of differentially expressed genes (DEGs).

(A) Volcano plot of DEGs. Red indicates upregulation, and blue indicates downregulation. (B) KEGG pathway enrichment analysis of DEGs. (C) GO pathway enrichment analysis of DEGs. (D) Heatmap of DEGs involved in gibberellin biosynthesis and metabolism pathway. *KAO*, ent-kaurenoic acid oxidase; GA2OXs, Gibberellin 2-oxidases; GA20OXs, Gibberellin 20-oxidases. (E) Heatmap of DEGs involved in circadian rhythm. Black font indicates substances involved in the gibberellin biosynthesis and metabolism pathway. Green background indicates bioactive gibberellins. Log_2FC denotes log_2(Fold Change).

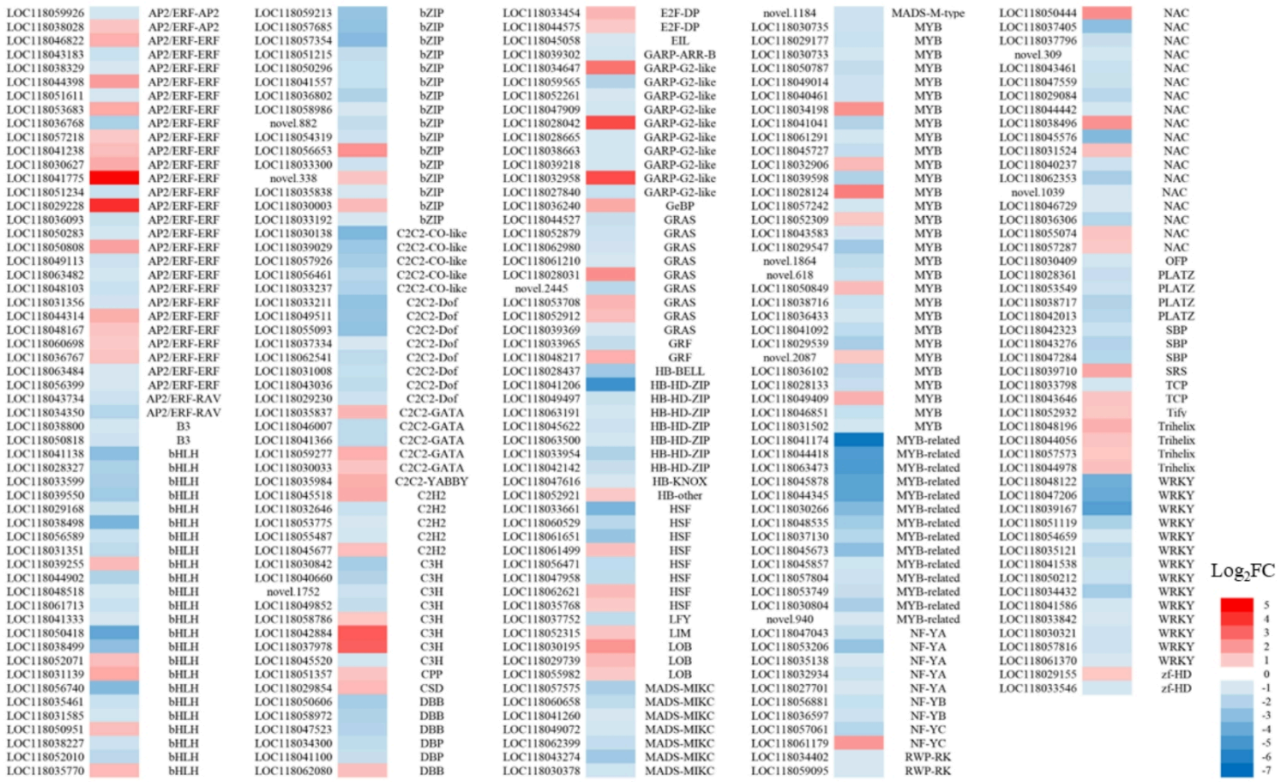


Figure 7

Heatmap showing expression of transcription factors associated with plant height regulation. Log_2FC denotes log_2(Fold Change).

Supplementary Files

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- supplementarymaterials1.pdf