Genome-wide Identification and Characterization of the DELLA Subfamily in *Prunus mume*

Jiuxing Lu, Weiru Yang, and Qixiang Zhang¹

Beijing Key Laboratory of Ornamental Plants Germplasm Innovation & Molecular Breeding, National Engineering Research Center for Floriculture, Beijing Laboratory of Urban and Rural Ecological Environment and College of Landscape Architecture, Beijing Forestry University, Beijing, 100083, China

ADDITIONAL INDEX WORDS. mei, bioinformatics, functional analysis, breeding, gibberellins

ABSTRACT. Control of development is an important issue in the production of ornamental plants. Gibberellins (GAs) play a key role in regulating plant growth and development. DELLA is nuclear negative regulators of GA signaling. We identified two DELLA homologous genes, *PmDELLA1* and *PmDELLA2*, in the genome of mei (*Prunus mume*) genome. We analyzed the structure, expression patterns and molecular functions of both genes. Tissue expression analysis showed that both genes were transcriptionally active. *PmDELLA1* showed higher expression in seeds than *PmDELLA2*. This indicated that *PmDELLA2* plays different roles from *PmDELLA1* in seed germination. The expressions of both genes at various flowering stages were relatively low. We speculated that *PmDELLAs* might be positive regulators of flowering by releasing the repression of GA during floral blooming. Transgenic arabidopsis (*Arabidopsis thaliana*) lines overexpressing the two genes showed dwarf and delayed flowering. We confirmed that the two *PmDELLAs* were partially conserved with genes encoding DELLA proteins in arabidopsis. Our bioinformatics and functional analyses provide information that may be valuable to improve the economic, agronomic and ecological properties of mei and other Rosaceae fruit trees.

Gibberellins are phytohormones that promote important aspects of growth, such as seed germination, leaf expansion, stem elongation, flowering, and fruit development (Davies, 1995). In woody plants, many GA-mediated processes are agriculturally important. GA levels are routinely manipulated by chemical means to produce desirable effects in a number of species (Looney, 1997; Rademacher, 2000). For example, exogenous GA is used to control flowering time and increase fruit size, and inhibitors of GA biosynthesis are applied to restrict vegetative growth. Mei, domesticated in China for more than 3000 years, is one of the most important ornamental flower and fruit trees among the Rosaceae. In East Asia, the flower and young fruit of mei can tolerate low temperatures (–4 to –2 °C) in early spring (Chen, 1996). Mei blooms in late winter or early spring (typically late January or February) before the new leaves grow. Its special fragrance makes it popular with Chinese people (Chen, 1996). However, it is too big in size for us to put common mei indoors. We need to minimize the size of mei to meet customer demands. In addition, regulation of flowering time is also key problems for us. So, it is necessary to investigate the functions of GA-associated transcriptional regulators, such as DELLA, in mei.

DELLA is a key component of the GA signaling pathway, acting as negative elements. They constitute a subgroup of the GRAS gene family (whose name was derived from the first three functionally characterized genes, *GAI*, *RGA*, and *SCR*), which encode plant-specific transcription factors. DELLA was named from a short stretch of amino acids (D-E-L-L-A) in their N-terminal region, which is highly conserved among plant species (Locascio et al., 2013) (Fig. 1A). They also have

additional conserved motifs, such as the TVHYNP domain; a poly(serine/threonine) stretch; two leucine heptad repeats, which may mediate protein–protein interactions; a putative nuclear localization signal; and a putative SH2 phosphotyrosine-binding domain (Locascio et al., 2013) (Fig. 1A). Mutants lacking DELLA motif show a phenotype similar to that of continuous gibberellin application (Cao et al., 2005; Cheng et al., 2004; Dill and Sun, 2001; Tyler et al., 2004). For example, the stable mutants *gai-1* and *rga-17* show a dwarf phenotype in arabidopsis equivalent to that caused by gibberellin deficiency (Dill and Sun, 2001; Peng et al., 1997). Similar functions of DELLA were found in rice (*Oryza sativa*) (*SLR1*, *DLT*), wheat (*Triticum aestivum*) (*RhtB1b*, *RhtD1b*), maize (*Zea mays*) (*D8*), barley (*Hordeum vulgare*) (*SLN1*), and grape (*Vitis vinifera*) (*VvGAI*) (Boss and Thomas, 2002; Chandler et al., 2002; Ogawa et al., 2000; Peng et al., 1999). For example, the semidominant dwarf wheat mutants that were the basis of the ‘Green Revolution’ wheat are caused by a mutation in the DELLA region of the *Rht-B1* and *Rht-D1* genes (Ogawa et al., 2000; Peng et al., 1999). The highly productive, semidwarfed ‘Pinot Meunoir’ grape contains a single amino acid change in the DELLA domain of *VvGAI* (Boss and Thomas, 2002; Chandler et al., 2002). In many species, mutations of the DELLA motif are highly conserved (Dill and Sun, 2001; Itoh et al., 2002). There are five genes (*GAI*, *RGA*, *RGL1*, *RGL2*, and *RGL3*) encoding DELLA protein in arabidopsis (Dill and Sun, 2001; Lee et al., 2002; Silverstone et al., 1998). Phenotypic analysis of the five proteins revealed their unique roles in the plant, although with a certain degree of overlapping functions. For example, *GAI* and *RGA* function to control cell expansion in hypocotyls, shoots and roots, while *RGL2* is the major DELLA regulating germination (Lee et al., 2002; Tyler et al., 2004; Wen and Chang, 2002). Recent research speculated that the functional diversification of the different DELLA genes in arabidopsis probably reflect changes in their regulatory sequences (Gallego-Bartolomé et al., 2010).

Received for publication 15 Oct. 2014. Accepted for publication 4 Mar. 2015. The research was supported by Ministry of Science and Technology (grants 2011AA100207 and 2013AA102607) and Special Fund for Beijing Common Construction Project.

¹Corresponding author. E-mail: zqxjbfu@126.com.

Fig. 1. (A) Key domains structure map of DELLA proteins. (B) Gene structure maps of *PmDELLA1* and *PmDELLA2*. (C) Multiple alignments results of the deduced amino sequences of DELLA in *Prunus mume* (*PmDELLA1* and *PmDELLA2*), *Malus × domestica* (*MdRGL1a*, *MdRGL2a*), and *Arabidopsis thaliana* (*RGL2*) are shown. Gray blocks: key domains of the GRAS protein family. Black blocks indicate DELLA subfamily-specific domains. Overhead lines indicate conserved motifs. Dashes indicate gaps to maximize alignment.

In this study, two genes likely encoding DELLA proteins (*PmDELLA1* and *PmDELLA2*) were identified from the mei genome database. They were cloned and their gene expression patterns and the effects of their transformation were observed. We aimed 1) to identify and characterize *PmDELLAs* in mei; 2) to analyze deduced amino acid sequences of *PmDELLAs* relationships among plants through comparative genomics; 3) to analyze their expression patterns in mei; 4) to perform functional analysis of *PmDELLAs* in transgenic arabidopsis; and 5) to provide useful information for further breeding of mei, such as that related to flowering time and plant size.

Materials and Methods

SEQUENCE RETRIEVAL. The genome files were downloaded from the mei genome project (Zhang et al., 2012b). Using the five DELLA protein sequences from arabidopsis as queries, the mei genomic database was blasted using local blast software. Default parameters were used in the analyses, and false hits were removed by manual inspection. All obtained protein sequences were subjected to domain analysis using protein sequence analysis and classification software [InterProScan (Jones et al., 2014)] to detect the presence of the DELLA domain, using default parameters. Protein sequences lacking the DELLA domain were rejected. The physicochemical properties of the identified protein sequences were predicted by the expert protein analysis system server (Artimo et al., 2012). Information on previously reported DELLA proteins of arabidopsis was retrieved from the arabidopsis information resource (Rhee et al., 2003). The genome data of apple (*Malus ×domestica*), strawberry (*Fragaria vesca*), cucumber (*Cucumis sativus*), barrel medic (*Medicago truncatula*), black cottonwood (*Populus trichocarpa*), chinese cabbage (*Brassica rapa*), orange (*Citrus sinensis*), cocoa (*Theobroma cacao*), grape, tomato (*Solanum lycopersicum*), rice, maize, spikemoss (*Selaginella moellendorffii*), bryophyte (*Physcomitrella patens*), green alga (*Chlamydomonas reinhardtii*), red alga (*Cyanidioschyzon merolae*), and yeast (*Saccharomyces cerevisiae*) were collected from plant genome database [Phytozome (Goodstein et al., 2012)]. Genome data on banana [*Musa acuinata* (D'Hont et al., 2012)] were also collected. We used an online blast tool to identify DELLA protein sequences, using default parameters and manual removal of false hits. The protein family database [Pfam (Finn et al., 2013)] was used to identify the DELLA proteins sequences.

MULTIPLE ALIGNMENT ANALYSES. Multiple sequence alignment of the full-length DELLA protein sequences of different species was performed using the ClustalX 2.1 program with its default settings (Larkin et al., 2007). Phylogenetic trees were constructed using the neighbor-joining method in the MEGA5.1 software (Tamura et al., 2011). Forty-one protein sequences were downloaded from the national center for biotechnology information website (NCBI) (Supplemental Table 1). We tested the reliability of the obtained tree using bootstrapping with 1000 replicates.

MRNA SEQUENCING AND ANALYSIS. We conducted gene expression analysis using RNA sequencing (RNA-seq) technology (Illumina, San Diego, CA). Five organs were obtained from 'Zangmei' mei in Tibet, China. Roots were harvested from 2-year-old plants of 'Zangmei' mei. Stems were obtained from a young stem tip. We collected new-sprouting young leaves in the spring. Fruit were obtained after 5 weeks

of blooming. Organs were collected and immediately stored in liquid nitrogen.

Total RNA was isolated from the plant organs using an Easy-spin™ kit (Aidlab, Beijing, China), following the instructions of the manufacturer. The sequencing and assembly were performed by the Beijing Genomics Institute (Beijing, China).

GENE CLONING. Young leaves of 'Yu die' mei from Jiufeng international plum blossom garden (Beijing, China), were collected and placed immediately in liquid nitrogen for subsequent cloning of the full-length cDNAs of *PmDELLA1* and *PmDELLA2*. Gene-specific primers (Supplemental Table 2) were designed based on identified gene sequences from genome data. The full-length cDNAs were amplified using the following program: one cycle at 94 °C for 5 min; 30 cycles at 98 °C for 5 s, at 60 °C for 15 s, at 72 °C for 1 min 30 s; and hold at 72 °C for 10 min. The resulting PCR product was separated on a 1% agarose gel and extracted using a BIOMIGAgel Purification Kit (Biomiga, San Diego, CA). Purified fragments were then cloned into the pGEM T-easy vector (Takara Biotechnology, Otsu, Japan) and transformed into *Escherichia coli* Top 10 cells (Tiangen, Beijing, China). Positive colonies were confirmed by PCR analysis and sequencing of the inserted DNA (Taihe, Beijing, China).

QUANTITATIVE PCR. The expressions of *PmDELLAs* in different organs and at different developmental stages were examined using quantitative PCR. We extracted total RNA from roots, stems, leaves, seeds, flowers at three developmental stages (Flower A, B, and C; corresponding to bud, full-bloom stage, and end-bloom stages, respectively), and fruit at three developmental stages (Fruit A, B, and C; corresponding to 10, 45, and 90 d after blooming, respectively) using the TRIzol reagent (Invitrogen, Carlsbad, CA) following the instruction of the manufacturer. First-strand cDNA was synthesized from 2 µg total RNA using the TIANScript First Strand cDNA Synthesis Kit (Tiangen), according to the instruction of the manufacturer. Quantitative PCR was performed using the PikoReal real-time PCR system (Thermo Fisher Scientific, Waltham, MA). Reactions were performed in a 20-µL volume containing 1 µL of cDNA, 300 nM of each primer (Supplemental Table 2) and 10 µL of SYBR Premix Ex Taq II (Takara Biotechnology). The reactions were performed under the following conditions: 30 s at 95 °C; followed by 40 cycles of 5 s at 95 °C and 30 s at 60 °C.

The relative quantification values were calculated using a combination of two reference genes, the protein phosphatase 2A gene (PP2A), and ubiquitin-conjugating enzyme E2 (UBC). According previous study of the selection of reference genes in mei, PP2A and UBC were stably expressed in different organs and genotypes in mei (Wang et al., 2014b). The modified mathematical model was used to calculate the relative quantification (Wang et al., 2014a). The amplification efficiency of primer pairs of target genes and reference genes were derived from a standard curve generated from a serial dilution of the mixed cDNA from all tested samples as the template performed for each candidate reference gene in triplicate. Normalization factor for the two reference genes were calculated by geNorm V. 3.5 software (Vandesompele et al., 2002). There were three replicates of each organ and development stage.

PLANT TRANSFORMATION. The full-length *PmDELLA1* and *PmDELLA2* cDNAs were cloned separately into the pCAMBIA1304 plant transformation vector, containing the CaMV35S promoter, octopine synthase terminator, and

Table 1. Identity of *PmDELLA1* and *PmDELLA2* from a local blast similarity search against *Prunus mume* genome and predicted physicochemical properties of deduced amino acid sequences of *PmDELLA1* and *PmDELLA2*.

<i>P. mume</i> gene ^z										Query gene		Results	
Gene identity	Name	Length (aa)	MW (kDa)	PI	GRAVY	Chr	Position (Mbp)	DELLA motif start	DELLA motif end	Name	GenBank no.	Identity (%)	E-value
Pm006304	PmDELLA1	633	70.0	5.33	-0.316	A2	16.0	52	124	GAI	AT1G14920	74	0.0
										RGA	AT2G01570	63	0.0
										RGL1	AT1G66350	69	0.0
										RGL2	AT3G03450	66	0.0
										RGL3	AT5G17490	66	0.0
Pm014329	PmDELLA2	597	64.7	5.09	-0.251	A4	11.7	47	115	GAI	AT1G14920	64	0.0
										RGA	AT2G01570	63	0.0
										RGL1	AT1G66350	64	0.0
										RGL2	AT3G03450	61	0.0
										RGL3	AT5G17490	61	0.0

^zaa = amino acid; MW = molecular weight; PI = isoelectric point; GRAVY = grand average of hydropathy; Chr = chromosome number.

Fig. 2. Summary of the numbers of DELLA proteins and genome sizes of 20 representative species. The tree was constructed according to five kingdom classification system. The genome information of the 20 species, which represent the major evolutionary lineages of *Prunus* species, was acquired from plant genome database. Online blast tool of protein family database were used to identify DELLA proteins. Triangles indicate woody plants.

a hygromycin resistance gene as the selection marker. *Agrobacterium tumefaciens* strain EHA105 was used for floral dip transformation of arabidopsis [Columbia ecotype (Clough and Bent, 1998)]. Resultant seeds were plated on Murashige and Skoog (MS) culture medium containing 30 µg·mL⁻¹ hygromycin to select for transformants. Total RNA was extracted from the leaves of mature plants and transgene expression levels were analyzed by quantitative PCR, using specific primers (Supplemental Table 1). Phenotypic analyses were performed on transgenic lines of T₃ and T₄ (third and fourth generations self-pollinated) with wild-type arabidopsis as the control (CK). For the analysis of leaf size and plant height, transformed T₃ seedlings and CK were grown for 25 d in long-day conditions (16 h light/8 h dark). For recording bloomed ratio, T₃ seedlings and CK were grown for 60 d in short-day condition (8 h light/16 h dark). Plant height was measured along the primary inflorescence axis and flowering was scored when petals were visible. For each treatment (long-day condition and short-day condition), we conducted in three repetitions. Data are collected from 20 plants per line for each repetition.

Seeds (T₄) from transgenic lines and CK were vernalized at 4 °C for 4 d, sterilized, rinsed, and then plated onto 0.5 × MS

medium or media containing 10⁻⁴ M GA₃ (Biomiga). Seedlings were grown in a growth room maintained at 20 °C with a 16-h light/8-h dark cycle using fluorescent light for 7 d. Seedlings were placed in a drop of water on a glass slide and the hypocotyl lengths (from the cotyledon shoulders to the collet of the root hairs) were measured from calibrated digital images (S8AP0 stereomicroscope with DFC500 camera; Leica, Wetzlar, Germany). The seedlings were measured for 25–30 plants per line for each repetition. This experiment was performed three times with similar results.

Results

IDENTIFICATION AND CHARACTERIZATION OF PmDELLAS IN MEI. Two genes encoding DELLA proteins from the entire mei genome were identified (Table 1). We named the two genes *PmDELLA1* and *PmDELLA2*, and deduced amino sequences *PmDELLA1* and *PmDELLA2*. The predicted *PmDELLA1* and *PmDELLA2* ORFs were 1899 and 1791 bp, respectively. Alignment with arabidopsis DELLA protein sequences showed that *PmDELLA1* and *PmDELLA2* were 61% to 74% homologous to the five arabidopsis DELLA proteins. Alignment

Fig. 3. Neighbor-joining distance tree showing similarity of DELLA protein sequences from *Prunus mume* (PmDELLA1 and PmDELLA2), *Arabidopsis thaliana* [AtGAI (CAA75492), AtRGA (CAA72177), AtRGL1 (NP_176809), AtRGL2 (NP_186995), AtRGL3 (NP_197251)], *Brassica rapa* [BrRGA2 (Q5BN23), BrRGA1 (Q5BN22)], *Cucurbita maxima* [CmGAI (Q6EI06), CmGAI-B (Q6EI05)], *Gossypium hirsutum* [GhGAI (Q84TQ7), GhRGA (AAY28970)], *Lactuca sativa* [LsDELLA1 (BAG71200), LsDELLA2 (BAG71201)], *Lycopersicon esculentum* [LeGAI (Q7Y1B6)], *Malus ×domestica* [MdRGL1 (AAY56752), MdRGL2 (AAY56750)], *Malus hupehensis* [MhDELLA (ABS50250), MhGAI1 (ABL61270)], *Oryza sativa* [OsSLR1 (NP_001051032)], *Phaseolus vulgaris* [PvDELLA1 (BAF62636), PvDELLA2 (BAF62637)], *Physcomitrella patens* [PpDELLA1 (XM_001774262), PpDELLA2 (XM_001754038)], *Pisum sativum* [PsCRY (ABI34432), PsDELLA (ABI30654)], *Populus trichocarpa* [PtDELLA1 (XM_002312414), PtDELLA2 (XM_002314763), PtDELLA3 (XM_001774262), PtDELLA4 (XM_001754038)], *Ricinus communis* [RcGAI (XP_002534030), RcGAI-B (XP_002527794)], *Saccharum officinarum* [SoGAI (AAZ08571)], *Selaginella kraussiana* [SkDELLA (ABU63412)], *Selaginella moellendorffii* [SmDELLA (ABX10758)], *Sorghum bicolor* [SbDELLA (XP_002466594)], *Sphagnum palustre* [SpDELLA (ABU63411)], *Triticum aestivum* [TaRhtD1a (Q9ST59)], *Vitis vinifera* [VvDELLA1 (XM_002534030), VvGAI1 (XM_002284612)], and *Zea mays* [ZmD8 (Q9ST48), ZmD9 (ABI84225)]. Numbers at nodes indicate bootstrap values (1000 replicates). Red dots indicate PmDELLA1 and PmDELLA2.

of PmDELLA1 with PmDELLA2 showed that they shared 64% identity. The grand average of hydropathy showed that both proteins had a negative value, indicating that they were hydrophilic. In addition, *PmDELLAs* contain no introns in mei, which is consistent with counterparts from other plants (Fig. 1B) (Foster et al., 2007; Tian et al., 2004).

The predicted molecular weights of the two proteins were 64.7 and 70.0 kDa, respectively. PmDELLAs had two signature motifs, DELLA and TVHYNP, which define the DELLA subfamily and are necessary for GA-induced degradation of DELLA proteins (Fig. 1C). The C-termini of the PmDELLAs had five highly conserved motifs (LHR I, VHIID, LHR II, PFYRE, and SAW), which are shared by GRAS proteins. The PmDELLAs were most divergent in their N-termini, and highly homologous over their C-termini. This finding was consistent with previous studies (Foster et al., 2007; Pysh et al., 1999).

COMPARATIVE AND PHYLOGENETIC ANALYSES OF PmDELLAS IN DIFFERENT SPECIES. For comparative genomic analysis, we searched the genomes of 20 species (Fig. 2), and identified 48 DELLA protein sequences. These species represent the major evolutionary lineages of *Prunus* species and were used for the analysis of evolution of DELLA subfamily. Among the 20 species, only 17 species found DELLA proteins, whereas the two protists (green alga and red alga) and one fungus (yeast) searched no DELLA proteins. This observation indicated that DELLA may have expanded after the divergence of the plants from protest and fungi and that these proteins may have important roles in the evolution of plants. This result was consistent with the evolution of GRAS proteins (Song et al., 2014). The previous study showed that the GRAS family belongs to the Rossmann fold methyltransferase superfamily, which first emerged in bacteria; however, most GRAS proteins are likely to lack methyltransferase activity. Nevertheless, the plant GRAS proteins still bind a similar substrate to the bacterial proteins (Zhang et al., 2012a). We believed DELLA subfamily were a group of archaic proteins

Fig. 4. Expression levels of (A) *PmDELLA1* and (B) *PmDELLA2* in different organs of *Prunus mume* were tested by RNA sequencing. The values on the Y-axis represent the reads per kilobase of exon model per million mapped reads (RPKM) values. Roots were harvested from 2-year-old plants. Stem tissue was obtained from a young stem tip. New-sprouting young leaves were collected in the spring. Fruit was obtained after 5 weeks of blooming. Relative expressions of (C) *PmDELLA1* and (D) *PmDELLA2* in different organs and development stages of *P. mume* were tested by quantitative PCR. RNA was isolated from the root, stem and leaf of 2-year-old plants. Seeds were collected from mature fruits. Flowers and fruits were collected at the three developmental stages. Flower A, B, and C correspond to bud, full-bloom stage, and end-bloom stage and fruit A, B, and C correspond to 10, 45, and 90 d after blooming. The relative quantification values were calculated using two reference genes, the protein phosphatase 2A gene (PP2A) and ubiquitin-conjugating enzyme E2 (UBC). The average value with standard errors of the three repetitions was used to draw the figure.

which was differentiated since the divination of Plantae and Ptoistae.

The number of the DELLA in mei was equal to that of grape (2) and exceeded that of strawberry (1), barrel medic (1), tomato (1), rice (1), spikemoss (1), and bryophyte (1). It was, however, less than that of apple (5), cucumber (3), black cottonwood (6), arabidopsis (5), chinese cabbage (5), orange (3), cocoa (3), maize (3), and banana (3). The genome size of mei (280.1 Mb) was greater than that of cucumber (203.0 Mb), barrel medic (257.6 Mb), and arabidopsis (135.0 Mb), but less than that of the other species examined. The number of DELLA had no direct relationship with the genome size and showed no clear pattern between dicotyledons and monocotyledons, which was the same for woody and herbal plants. We also found that apple, black cottonwood, arabidopsis, and chinese cabbage had higher numbers of DELLAs. All four of these genomes underwent a recent whole-genome duplication (WGD) (Song et al., 2014; Tuskan et al., 2006; Velasco et al., 2010). However, mei has not undergone a recent WGD, which is the same as strawberry and grape (Jaillon et al., 2007; Shulaev et al., 2011). Thus, WGD events facilitated the expansion of the DELLA subfamily.

To investigate the phylogenetic relationship between PmDELLAs and other DELLA proteins, 41 DELLA protein sequences (Supplemental Table 1) from 22 species were downloaded from the NCBI to construct a phylogenetic tree (Fig. 3). The phylogenetic tree included four groups. Group I

was the monocotyledonous group that contained six DELLAs from five species. Groups II and III were dicotyledonous groups. PmDELLA1 and PmDELLA2 were clustered into Groups II and III, respectively. PmDELLA1 was grouped with AtGAI and AtRGA, while PmDELLA2 was grouped with AtRGL1–3. Group IV included DELLA-like proteins from moss and gymnosperm species. The phylogenetic tree also showed that PmDELLA1 is closely related to MdRGL1 and MhGAI1, while PmDELLA2 is closely related to MdRGL2 and MhDELLA.

EXPRESSION PATTERNS OF PmDELLAS IN DIFFERENT ORGANS, AND AT VARIOUS FLOWER AND FRUIT DEVELOPMENTAL STAGES. To investigate the roles of *PmDELLAs* in different organs, and in flower and fruit development stages of mei, the expression patterns of *PmDELLA1* and *PmDELLA2* were determined by RNA-seq and quantitative PCR.

The expression of *PmDELLAs* in five mei organs was analyzed using RNA-Seq data. We extracted the transcriptomic data for five organs (root, stem, leaf, bud, and fruit). Reads per kilobase of exon model per million mapped reads (RPKM) values of the genes in the five organs

are shown in Figure 4A and B. Both genes were expressed in all five organs, with the highest expression levels in the stem. The expression level of *PmDELLA1* in the five organs ranged from 48 to 152 (RPKM values), whereas the expression level of *PmDELLA2* ranged from 25 to 281. The different expression profiles of *PmDELLAs* indicated that they may have similar functions in some organs and specific functions in others.

The expressions of the *PmDELLAs* in seeds and during flower and fruit development stages were analyzed by quantitative PCR. As shown in Figure 4C and D, both genes are actively transcribed, although their expression patterns are different. Both genes showed high expression in the stem, which was consistent with the RNA-seq result. We speculated that they may have important functions in stem development. *PmDELLA1* showed very high expression in the seed; whereas, *PmDELLA2* showed very low expression. This indicated that *PmDELLA1* may have more important functions in seed germination than *PmDELLA2*. In addition, the two genes showed the same expressions patterns during floral blooming. They were both down-regulated in flowers at stage B and slightly up-regulated at stage C. This suggested that they have similar functions during floral blooming. By contrast, the two genes showed different patterns during fruit development. The expression level of *PmDELLA1* gradually increased during fruit development, whereas *PmDELLA2* expression decreased stage by stage. This suggested that they play different roles in fruit development.

Fig. 5. Rosette diameters, plant height, and bloomed ratio of (A–C) *PmDELLA1* and (D–F) *PmDELLA2* transgenic *Arabidopsis thaliana* lines (four lines per gene labeling 1–4) and wild-type control plants (CK) are shown. Relative expressions of *PmDELLA1* (G) and *PmDELLA2* (H) transgenic *A. thaliana* lines and CK were determined by quantitative PCR. For measuring rosette diameters and plant height, plants were cultured under long-day conditions (16 h light/8 h dark) for 25 d. For recording the bloomed ratio, plants were cultured under short-day conditions (8 h light/16 h dark) for 60 d. Each treatment was conducted in three repetitions. Data are collected from 20 plants per line for each repetition. Different letters indicate that CK and transgenic lines differ significantly from each other by Student–Newman–Keuls test (alpha = 0.01). Values shown are means with standard errors.

PHENOTYPE ANALYSIS OF TRANSGENIC ARABIDOPSIS OVEREXPRESSING *PmDELLA* S. Binary vectors containing the full-length *PmDELLA1* or *PmDELLA2* cDNAs sequences were transformed into arabidopsis using the floral dip method (Clough and Bent, 1998) for observing the phenotype of transformed arabidopsis. Four independent 35S:*PmDELLA1* and 35S:*PmDELLA2* transgenic lines were generated. Compared with CK, four lines overexpressing *PmDELLA1* showed 27% to 48% smaller in rosette diameter (Fig. 5A), 43% to 56%

shorter in plant height (Fig. 5B), and 65% to 79% decrease in bloomed ratio (Fig. 5C), while four lines overexpressing *PmDELLA2* showed 43% to 50% shorter in plant height (Fig. 5D), 43% to 64% smaller in rosette diameter (Fig. 5E), and 58% to 89% decrease in bloomed ratio (Fig. 5F). Significant analysis showed that CK and transgenic lines had significant difference by Student–Newman–Keuls test (alpha = 0.01) among rosette diameter, plant height and bloomed ratio. Transgene expression levels of *PmDELLA*s in each line were determined by quantitative PCR (Fig. 5G and H).

Transgenic plants overexpression of genes encoding DELLA proteins has been shown to reduce the response to exogenous GA (Ait Ali et al., 2003; Fleck and Harber, 2002; Fu et al., 2001; Itoh et al., 2002). We observed that CK on GA₃ media increased 23% in seedling hypocotyl elongation compared with CK on normal media. Four *PmDELLA1* lines on GA₃ media increased 9% to 25% compared with four lines on normal media, while four *PmDELLA2* lines increased 10% to 15% on GA₃ media compared with them on normal media (Fig. 6A and B). We observed CK and transgenic lines all had response to exogenous GA₃. However, significant analysis (data not shown) showed that the difference values of CK on GA₃ media and that on normal media and the difference values of transgenic lines of *PmDELLA*s on GA₃ media and that on normal media had significant difference by Student–Newman–Keuls test (alpha = 0.01). This indicated that transgenic arabidopsis overexpressing *PmDELLA*s reduced the response to exogenous GA₃.

Discussion

Mei is a woody perennial tree with an intergenerational in a cycle of 3–5 years (Chen, 1996). *Prunus* crops are economically important because of their tasty fruit, which provide unique dietary contributions to consumers, and as ornamental plants, especially as dwarf cultivars as indoor plants. For these valuable characters, breeders have used many ways to control flowering, fruit growth, and canopy architecture. In this study, two *PmDELLA*s encoding negative regulators of the GA signaling pathway were identified in the mei genome. Both genes had two signature motifs, DELLA and TVHYNP, which define the

Fig. 6. Hypocotyl length of (A) *PmDELLA1* and (B) *PmDELLA2* transgenic *Arabidopsis thaliana* lines (four lines per gene) and wild-type control plants (CK) on GA₃ media (oblique lines) and normal media (blank). Seeds were germinated on 0.5× Murashige and Skoog culture medium (MS) in the presence or absence of 10⁻⁴ M GA₃ and grown in culture room (16 h light/8 h dark) for 7 d. Data are collected from 25 seedlings per line for each repetition (totally three repetitions). Mean hypocotyl lengths are shown with standard errors as vertical bars.

DELLA subfamily and are necessary for GA-induced degradation of DELLA proteins (Foster et al., 2007; Pysh et al., 1999). Mutation of either of these domains results in a semidominant, GA-insensitive, dwarfed phenotype in diverse plant species.

With the abundance of genome resources, the information stored in various genomes can be explored to elucidate the mechanisms that regulate the growth and development of species. Comparative analysis showed that the expansion of the DELLA subfamily is related to genome duplication events. As a key subfamily of GRAS proteins, members of the DELLA subfamily are involved in regulating the GA response. GAs are important hormones that regulate plant development. In woody plants, GAs help to reduce bud dominance, promote flowering, and increase fruit setting. We speculated that some DELLA genes have been retained after the genome duplication process to facilitate the adaption of woody plants to complex environments. We believe that the DELLA subfamily plays an important role in higher plant growth and development.

Phylogenetic analysis showed that 41 DELLA protein sequences could be classed into four groups. The monocots

(group I) and dicots (groups II and III) were divided into two independent groups. This indicated that DELLA proteins were diversified after the monocot-dicot split. We could not predict the evolution of DELLAs any earlier, because there is little information for moss and gymnosperms species. We observed that *PmDELLA1* and *PmDELLA2* were clustered into Groups II and III, respectively. A previous study showed that *GAI* and *RGA* function to control cell expansion in the hypocotyl, shoot, and root, while *RGL2* is the major DELLA protein regulating germination. Expression analysis showed that *PmDELLA2* showed very high expression levels in seed. All this findings indicated that *PmDELLA2* probably have different functions with *PmDELLA1*. Although our preliminary analysis of the known DELLA genes supported this hypothesis, the amount of complete genome data for plants is insufficient to adequately measure the evolutionary history of the DELLA gene.

Expression analysis of *PmDELLAs* in different organs showed that both *PmDELLAs* were active in all organs. Previous studies showed that organ specificities of DELLA genes were different among species. *SLR* and *SLN* are preferentially expressed in certain organs (Chandler et al., 2002; Ogawa et al., 2000). By contrast, *RGA* and *GAI* are expressed constitutively in all tissues and appear to be regulated primarily by proteolysis (Fu et al.,

2004; Lee et al., 2002; Tyler et al., 2004). Only *RGL1*, *RGL2*, and *RGL3* appear to be regulated at the transcript level (Lee et al., 2002; Tyler et al., 2004; Wen and Chang, 2002). We observed that both *PmDELLAs* appear to be transcriptionally regulated. However, the expression patterns of *PmDELLAs* in different organs varied. The two *PmDELLAs* showed different expression levels in seeds: very high for *PmDELLA1* and very low for *PmDELLA2*. Previous studies showed that in *arabidopsis*, *RGL1*, *RGL2*, and *RGL3* are expressed at high levels only in germinating seeds, and/or flowers, and siliques (Lee et al., 2002; Tyler et al., 2004). Thus, the expression pattern of *PmDELLA1* was consistent with genes encoding DELLA proteins in *arabidopsis*. In addition, unlike *arabidopsis* seeds, mei seeds require several weeks of low temperature (0–4 °C) to germinate (Chen, 1996). Thus, we speculated that the germination of mei seeds might be regulated more by environmental and physiological factors than by the activity of the *PmDELLAs*.

The expressions of *PmDELLAs* at various flower and fruit development stages showed that both *PmDELLAs* had the same

patterns during floral blooming, but different patterns during fruit development. Both genes were expressed at relatively low levels during flower blooming. Previous studies showed that GA promotes flowering by targeting DELLA proteins for destruction in arabidopsis, which ultimately leads to activation of *LEAFY*, a key floral meristem identity gene (Achard et al., 2004; Greene, 1989). Certain GA responses such as internode elongation, leaf expansion, and seed germination are conserved between species, whereas the relationship between GA and flowering is not. Changes in the relationships between key regulatory proteins can lead to rapid morphological evolution (Doebley and Lukens, 1998; Purugganan, 2000). In woody plants, specific GA species repress flowering, particularly in grape and apple (Greene, 1989). We speculated that *PmDELLAs* might be positive regulators of flowering through releasing the repression of GA during floral blooming.

Transgenic arabidopsis lines expressing *PmDELLAs* showed a reduction in leaf size and stem height, and delayed flowering. Compared with the controls, the transgenic seedlings had shorter hypocotyls and exhibited reduced elongation responses to exogenous GA. Previous studies showed that overexpression of DELLA proteins in arabidopsis, rice, and tobacco (*Nicotiana tabacum*) caused a range of dwarfed phenotypes that varied according to the transgene expression level (Itoh et al., 2002). The dwarfed phenotypes and the increases hypocotyls of transgenic lines of *PmDELLAs* revealed that the two *PmDELLAs* probably function as negative regulators of GA-dependent processes in mei.

In summary, we studied DELLA subfamily in mei and 19 other species, including seven woody plants. We deduced that *PmDELLAs* are functionally conserved in mei as the negative transcription regulator of GA. The identification of *PmDELLAs* will provide a molecular basis for the genetic improvement of mei, especially for producing dwarf cultivars and regulating flowering time in woody plant. Our bioinformatics and functional analysis provides valuable information to improve the economic, agronomic, and ecological properties of mei and other Rosaceae fruit trees.

Literature Cited

- Achard, P., A. Herr, D.C. Baulcombe, and N.P. Harberd. 2004. Modulation of floral development by a gibberellin-regulated micro-RNA. *Development* 131:3357–3365.
- Ait Ali, T., C. Randsand, and N.P. Harberd. 2003. Flexible control of plant architecture and yield via switchable expression of arabidopsis gai. *Plant Biotechnol. J.* 1:337–343.
- Artimo, P., M. Jonnalagedda, K. Arnold, D. Baratin, G. Csardi, E. De Castro, S. Duvaud, V. Flegel, A. Fortier, and E. Gasteiger. 2012. ExPASy: SIB bioinformatics resource portal. *Nucleic Acids Res.* 40: W597–W603.
- Boss, P.K. and M.R. Thomas. 2002. Association of dwarfism and floral induction with a grape 'green revolution' mutation. *Nature* 416:847–850.
- Cao, D., A. Hussain, H. Cheng, and J. Peng. 2005. Loss of function of four *DELLA* genes leads to light and gibberellin-independent seed germination in arabidopsis. *Planta* 223:105–113.
- Chandler, P.M., A. Marion-Poll, M. Ellis, and F. Gubler. 2002. Mutants at the *Slender1* locus of barley cv Himalaya. Molecular and physiological characterization. *Plant Physiol.* 129:181–190.
- Chen, J.Y. 1996. Chinese mei flowers. (in Chinese) Hainan Publ. House, Haikou, China.
- Cheng, H., L. Qin, S. Lee, X. Fu, D.E. Richards, D. Cao, D. Luo, N.P. Harberd, and J. Peng. 2004. Gibberellin regulates arabidopsis floral development via suppression of DELLA protein function. *Development* 131:1055–1064.
- Clough, S.J. and A.F. Bent. 1998. Floral dip: A simplified method for agrobacterium-mediated transformation of *Arabidopsis thaliana*. *Plant J.* 16:735–743.
- D'Hont, A., F. Denoeud, J. Aury, F. Baurens, F. Carreel, O. Garsmeur, B. Noel, S. Bocs, G. Droc, and M. Rouard. 2012. The banana (*Musa acuminata*) genome and the evolution of monocotyledonous plants. *Nature* 488:213–217.
- Davies, P.J. 1995. Plant hormones: Physiology, biochemistry, and molecular biology. Kluwer Academic Publ., Dordrecht, The Netherlands.
- Dill, A. and T. Sun. 2001. Synergistic derepression of gibberellin signaling by removing *RGA* and *GAI* function in *Arabidopsis thaliana*. *Genetics* 159:777–785.
- Doebley, J. and L. Lukens. 1998. Transcriptional regulators and the evolution of plant form. *Plant Cell* 10:1075–1082.
- Finn, R.D., A. Bateman, J. Clements, P. Coggill, R.Y. Eberhardt, S.R. Eddy, A. Heger, K. Hetherington, L. Holm, and J. Mistry. 2013. Pfam: The protein families database. *Nucleic Acids Res.*, doi: 10.1093/nar/gkt1223.
- Fleck, B. and N.P. Harberd. 2002. Evidence that the arabidopsis nuclear gibberellin signaling protein GAI is not destabilized by gibberellin. *Plant J.* 32:935–947.
- Foster, T., C. Kirk, W.T. Jones, A.C. Allan, R. Espley, S. Karunairetnam, and J. Rakonjac. 2007. Characterization of the DELLA subfamily in apple (*Malus x domestica* Borkh.). *Tree Genet. Genomes* 3:187–197.
- Fu, X., D. Sudhakar, J. Peng, D.E. Richards, P. Christou, and N.P. Harberd. 2001. Expression of arabidopsis *GAI* in transgenic rice represses multiple gibberellin responses. *Plant Cell* 13:1791–1802.
- Fu, X., D.E. Richards, B. Fleck, D. Xie, N. Burton, and N.P. Harberd. 2004. The arabidopsis mutant *sleepy1* (*gar2-1*) protein promotes plant growth by increasing the affinity of the SCF SLY1 E3 ubiquitin ligase for DELLA protein substrates. *Plant Cell* 16:1406–1418.
- Gallego-Bartolomé, J., E.G. Minguet, J.A. Marín, S. Prat, M.A. Blázquez, and D. Alabadí. 2010. Transcriptional diversification and functional conservation between DELLA proteins in arabidopsis. *Mol. Biol. Evol.* 27:1247–1256.
- Goodstein, D.M., S. Shu, R. Howson, R. Neupane, R.D. Hayes, J. Fazo, T. Mitros, W. Dirks, U. Hellsten, and N. Putnam. 2012. Phytozome: A comparative platform for green plant genomics. *Nucleic Acids Res.* 40:D1178–D1186.
- Greene, D.W. 1989. Gibberellins A₄₊₇ influence fruit set, fruit quality, and return bloom of apples. *J. Amer. Soc. Hort. Sci.* 6:1238–1240.
- Itoh, H., M. Ueguchi-Tanaka, Y. Sato, M. Ashikari, and M. Matsuoka. 2002. The gibberellin signaling pathway is regulated by the appearance and disappearance of *SLENDER RICE1* in nuclei. *Plant Cell* 14:57–70.
- Jaillon, O., J. Aury, B. Noel, A. Policriti, C. Clepet, A. Casagrande, N. Choinsne, S. Aubourg, N. Vitulo, and C. Jubin. 2007. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature* 449:463–467.
- Jones, P., D. Binns, H. Chang, M. Fraser, W. Li, C. McAnulla, H. McWilliam, J. Maslen, A. Mitchell, and G. Nuka. 2014. InterProScan 5: Genome-scale protein function classification. *Bioinformatics* 30:1236–1240.
- Larkin, M.A., G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, and R. Lopez. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23:2947–2948.
- Lee, S., H. Cheng, K.E. King, W. Wang, Y. He, A. Hussain, J. Lo, N.P. Harberd, and J. Peng. 2002. Gibberellin regulates arabidopsis seed germination via *RGL2*, a GAI/RGA-like gene whose expression is upregulated following imbibition. *Genes Dev.* 16:646–658.
- Locascio, A., M.A. Blázquez, and D. Alabadí. 2013. Genomic analysis of DELLA protein activity. *Plant Cell Physiol.* 54:1229–1237.
- Looney, N.E. 1997. Hormones and horticulture. *HortScience* 32:1014–1018.

- Ogawa, M., T. Kusano, M. Katsumi, and H. Sano. 2000. Rice gibberellin-insensitive gene homolog, *OsGAI*, encodes a nuclear-localized protein capable of gene activation at transcriptional level. *Gene* 245:21–29.
- Peng, J., D.E. Richards, N.M. Hartley, G.P. Murphy, K.M. Devos, J.E. Flintham, J. Beales, L.J. Fish, A.J. Worland, and F. Pelica. 1999. 'Green revolution' genes encode mutant gibberellin response modulators. *Nature* 400:256–261.
- Peng, J., P. Carol, D.E. Richards, K.E. King, R.J. Cowling, G.P. Murphy, and N.P. Harberd. 1997. The arabidopsis *GAI* gene defines a signaling pathway that negatively regulates gibberellin responses. *Genes Dev.* 11:3194–3205.
- Purugganan, M.D. 2000. The molecular population genetics of regulatory genes. *Mol. Ecol.* 9:1451–1461.
- Pysh, L.D., J.W. Wysocka Diller, C. Camilleri, D. Bouchez, and P.N. Benfey. 1999. The GRAS gene family in arabidopsis: Sequence characterization and basic expression analysis of the scarecrow-like genes. *Plant J.* 18:111–119.
- Rademacher, W. 2000. Growth retardants: Effects on gibberellin biosynthesis and other metabolic pathways. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 51:501–531.
- Rhee, S.Y., W. Beavis, T.Z. Berardini, G. Chen, D. Dixon, A. Doyle, M. Garcia-Hernandez, E. Huala, G. Lander, and M. Montoya. 2003. The arabidopsis information resource (TAIR): A model organism database providing a centralized, curated gateway to arabidopsis biology, research materials and community. *Nucleic Acids Res.* 31:224–228.
- Shulaev, V., D.J. Sargent, R.N. Crowhurst, T.C. Mockler, O. Folkerts, A.L. Delcher, P. Jaiswal, K. Mockaitis, A. Liston, and S.P. Mane. 2011. The genome of woodland strawberry (*Fragaria vesca*). *Nat. Genet.* 43:109–116.
- Silverstone, A.L., C.N. Ciampaglio, and T. Sun. 1998. The arabidopsis *RGA* gene encodes a transcriptional regulator repressing the gibberellin signal transduction pathway. *Plant Cell* 10:155–169.
- Song, X., T. Liu, W. Duan, Q. Ma, J. Ren, Z. Wang, Y. Li, and X. Hou. 2014. Genome-wide analysis of the GRAS gene family in chinese cabbage (*Brassica rapa* ssp. *pekinensis*). *Genomics* 103:135–146.
- Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28:2731–2739.
- Tian, C., P. Wan, S. Sun, J. Li, and M. Chen. 2004. Genome-wide analysis of the GRAS gene family in rice and arabidopsis. *Plant Mol. Biol.* 54:519–532.
- Tuskan, G.A., S. Difazio, S. Jansson, J. Bohlmann, I. Grigoriev, U. Hellsten, N. Putnam, S. Ralph, S. Rombauts, and A. Salamov. 2006. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* 313:1596–1604.
- Tyler, L., S.G. Thomas, J. Hu, A. Dill, J.M. Alonso, J.R. Ecker, and T. Sun. 2004. DELLA proteins and gibberellin-regulated seed germination and floral development in arabidopsis. *Plant Physiol.* 135:1008–1019.
- Vandesompele, J., D.P. Katleen, P. Filip, P. Bruce, V.R. Nadine, A.D. Paepe, and S. Frank. 2002. Accurate normalization of real-time quantitative RT-PCR data by genometric averaging of multiple internal control genes. *Gen. Biol.* doi: 10.1186/gb-2002-3-7-research0034.
- Velasco, R., A. Zharkikh, J. Affourtit, A. Dhingra, A. Cestaro, A. Kalyanaraman, P. Fontana, S.K. Bhatnagar, M. Troggio, and D. Pruss. 2010. The genome of the domesticated apple (*Malus × domestica* Borkh.). *Nat. Genet.* 42:833–839.
- Wang, H., J. Chen, Q. Tian, S. Wang, X. Xia, and W. Yin. 2014a. Identification and validation of reference genes for *Populus euphratica* gene expression analysis during abiotic stresses by quantitative real-time PCR. *Physiol. Plant.* 152:529–545.
- Wang, T., R. Hao, H. Pan, T. Cheng, and Q. Zhang. 2014b. Selection of suitable reference genes for quantitative real-time polymerase chain reaction in *Prunus mume* during flowering stages and under different abiotic stress conditions. *J. Amer. Soc. Hort. Sci.* 139:113–122.
- Wen, C. and C. Chang. 2002. Arabidopsis *RGL1* encodes a negative regulator of gibberellin responses. *Plant Cell* 14:87–100.
- Zhang, D., L.M. Iyerand, and L. Aravind. 2012a. Bacterial GRAS domain proteins throw new light on gibberellic acid response mechanisms. *Bioinformatics* 28:2407–2411.
- Zhang, Q., W. Chen, L. Sun, F. Zhao, B. Huang, W. Yang, Y. Tao, J. Wang, Z. Yuan, and G. Fan. 2012b. The genome of *Prunus mume*. *Nat. Commun.* 3:1318.

Supplemental Table 1. Accession numbers of genes from NCBI database used for phylogenetic analyses.

Gene	NCBI accession no.	Species
AtGAI	CAA75492	<i>Arabidopsis thaliana</i>
AtRGA	CAA72177	<i>A. thaliana</i>
AtRGL1	NP_176809	<i>A. thaliana</i>
AtRGL2	NP_186995	<i>A. thaliana</i>
AtRGL3	NP_197251	<i>A. thaliana</i>
BrRGA2	Q5BN23	<i>Brassica rapa</i>
BrRGA1	Q5BN22	<i>B. rapa</i>
CmGAIP	Q6EI06	<i>Cucurbita maxima</i>
CmGAIP-B	Q6EI05	<i>C. maxima</i>
GhGAI	Q84TQ7	<i>Gossypium hirsutum</i>
GhRGA	AAV28970	<i>G. hirsutum</i>
LsDELLA1	BAG71200	<i>Lactuca sativa</i>
LsDELLA2	BAG71201	<i>L. sativa</i>
LeGAI	Q7Y1B6	<i>Solanum lycopersicum</i>
MdRGL1	AAV56752	<i>Malus ×domestica</i>
MdRGL2	AAV56750	<i>M. ×domestica</i>
MhDELLA	ABS50250	<i>Malus hupehensis</i>
MhGAI1	ABL61270	<i>M. hupehensis</i>
OsSLR1	NP_001051032	<i>Oryza sativa</i>
PvDELLA1	BAF62636	<i>Phaseolus vulgaris</i>
PvDELLA2	BAF62637	<i>P. vulgaris</i>
PpDELLA1	XM_001774262	<i>Physcomitrella patens</i>
PpDELLA2	XM_001754038	<i>P. patens</i>
PsCRY	ABI34432	<i>Pisum sativum</i>
PsDELLA	ABI30654	<i>P. sativum</i>
PtDELLA1	XM_002312414	<i>Populus trichocarpa</i>
PtDELLA2	XM_002314763	<i>P. trichocarpa</i>
PtDELLA3	XM_001774262	<i>P. trichocarpa</i>
PtDELLA4	XM_001754038	<i>P. trichocarpa</i>
RcGAI	XP_002534030	<i>Ricinus communis</i>
RcGAIP-B	XP_002527794	<i>R. communis</i>
SoGAI	AAZ08571	<i>Saccharum officinarum</i>
SkDELLA	ABU63412	<i>Selaginella kraussiana</i>
SmDELLA	ABX10758	<i>Selaginella moellendorffii</i>
SbDELLA	XP_002466594	<i>Sorghum bicolor</i>
SpDELLA	ABU63411	<i>Sphagnum palustre</i>
TaRhtD1a	Q9ST59	<i>Triticum aestivum</i>
VvDELLA1	XM_002534030	<i>Vitis vinifera</i>
VvGAI1	XM_002284612	<i>V. vinifera</i>
ZmD8	Q9ST48	<i>Zea mays</i>
ZmD9	ABI84225	<i>Z. mays</i>

Supplemental Table 2. Primers used for clone and quantitative PCR expression.

Type	Gene	Forward primer	Reverse primer
Clone	PmDELLA1	ATGAAAAGAGAGCATCAGATTTATCATCAATATCCC	TCAGTGAGCCATCACCAGATCGCA
	PmDELLA2	ATGAAGAGAGATCACCGGGATACCTG	TCACCGGGTTGACTCAGTCGAAC
Quantitative PCR	PmDELLA1	TTTGGCAGATCTCGAACCCT	ACAAGACCTTTTCAACCGCC
	PmDELLA2	GAGATCAACCCACCTCCCAA	GTTGGACTTGTTGCTGCTGA
	PP2A	ATATAGCTGCTCAGTTCAACC	AAAAACAGTCACCACATTCTT
	UBC	GCAGGTGGAGTGTTCCTTGTG	GGCAGGGCTCCACTGTTCTTT