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ORIGINAL ARTICLE

Physiological controls of chrysanthemum *DgD27* gene expression in regulation of shoot branching

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Abstract

Key message   *DgD27* was cloned from *D. grandiflorum* for the first time and played an important role in shoot branching of chrysanthemum.

Abstract   Shoot branching plays an important role in determining plant architecture. *D27* was previously proven to be involved in the strigolactone biosynthetic pathway in rice, *Arabidopsis*, and *Medicago*. To investigate the role of *D27* in shoot branching of chrysanthemum, we isolated the *D27* homolog *DgD27*. Functional analysis showed that DgD27 was a plastid-localized protein that restored the phenotype of *Arabidopsis d27-1*. Gene expression analysis revealed that *DgD27* was expressed at the highest levels in stem, and was up-regulated by exogenous auxin. Decapitation could down-regulate *DgD27* expression, but this effect could be restored by exogenous auxin. *DgD27* expression was significantly down-regulated by dark treatment in axillary buds. In addition, *DgD27* transcripts produced rapid responses in shoots and roots under conditions of phosphate absence, but only mild variation in responses in buds, stems, and roots with low nitrogen treatment. *DgBRC1* transcripts also showed the same response in buds under low nitrogen conditions. Under phosphate deficiency, indole-3-acetic acid (IAA) levels increased, zeatin riboside levels decreased, and abscisic acid (ABA) levels increased in the shoot, while both IAA and ABA levels increased in the shoot under low nitrogen treatments. Gibberellin acid levels were unaffected by phosphate deficiency and low nitrogen treatments. Taken together, these results demonstrated the diverse roles of *DgD27* in response to physiological controls in chrysanthemum shoot branching.

Abbreviations

CCD	Carotenoid cleavage dioxygenase
LN	Low nitrogen
P	Phosphate
PATS	Polar auxin transport stream
PBS	Phosphate-buffered saline
SL	Strigolactone
ZR	Zeatin riboside

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Introduction

Shoot branching plays an important role in determining plant architecture, and is a highly plastic determinant of plant shape, allowing plants to respond to environmental stresses (Evers et al. 2011). Shoot branching is controlled by complex interactions between genetic, hormonal (e.g., auxins, cytokinins, strigolactones), developmental (bud formation, bud outgrowth), and environmental (e.g., light,

phosphorus, nitrogen) factors (Schmitz and Theres 1999; Beveridge et al. 2003; McSteen and Leyser 2005; Ongaro et al. 2008; Kebrom et al. 2013). Recent studies on plant hormones have focused on how auxins, strigolactones (SLs), and cytokinins (CKs) interact to regulate bud outgrowth and shoot branching (Leyser 2009; Ruyter-Spira et al. 2011; Kebrom et al. 2013; Ward et al. 2013).

A series of increasingly branching mutants in *Arabidopsis thaliana*, *Oryza sativa*, *Petunia hybrida*, and *Pisum sativum* have been identified in which SLs act as endogenous signals, regulating plant development by mediating the inhibition of axillary bud outgrowth (for review, see Al-Babili and Bouwmeester 2015). Through analysis of these mutants, seven genes of either the SL biosynthesis or signaling pathway have been identified. Four components of SL biosynthesis pathway include *D27*, *CCD7*, *CCD8*, and *MAX1*. *D27* encodes a novel chloroplast-located, iron-containing protein, which acts upstream of *MAX1* in the SL biosynthesis pathway, and the *d27* mutant has a relatively highly tillered and dwarf phenotype (Lin et al. 2009; Liu et al. 2011; Waters et al. 2012a); *CCD7* and *CCD8* encode divergent plastidic carotenoid cleavage dioxygenases that function in the chloroplast, while CCD7s cleave different 9-*cis*-carotenes, and CCD8 introduce a series of oxygen molecules (Sorefan et al. 2003; Booker et al. 2004; Snowden et al. 2005; Auldridge et al. 2006; Ledger et al. 2010; Guan et al. 2012; Kohlen et al. 2012; Pasare et al. 2013; Bruno et al. 2014; Lauressergues et al. 2015); *MAX1* encodes a cytochrome P450 monooxygenase, and works downstream of *CCD7* and *CCD8* to catalyze the oxidation of the SL precursor carlactone to the first real SL (Booker et al. 2005; Lazar and Goodman 2006; Challis et al. 2013; Abe et al. 2014; Zhang et al. 2014). Another three components of the SL signaling pathway include *MAX2*, *D14*, and *D53*. *MAX2* encodes an F-box protein, that functions in signaling pathways downstream of SLs and responds to SLs (Stirnberg et al. 2002; Johnson et al. 2006). *D14* encodes an α/β-fold hydrolase, which is proposed to be specific to signaling via *MAX2* that mediates both SL and karrikin signaling (Arite et al. 2009; Hamiaux et al. 2012; Waters et al. 2012b). Finally, *D53* encodes a protein that shares predicted features with the class I Clp ATPase proteins, and acts as a repressor of SL signaling (Jiang et al. 2013; Zhou et al. 2013). In addition, *BRC1* acts downstream of the SLs pathway, locally regulating shoot branching (Minakuchi et al. 2010; Braun et al. 2012; Chen et al. 2013).

Auxins inhibit bud outgrowth and are synthesized in the shoot apex and transported basipetally in the polar auxin transport stream (PATS), while CKs and SLs are transported acropetally in the xylem. Auxins act indirectly on entering the bud through two mechanisms: the auxin transport canalization model and the second messenger

model. The auxin transport canalization model works as follows: if it is assumed that during active growth, buds establish their own PATS into the main stem, then high auxin concentrations in the main stem can prevent bud activation by reducing the sink strength of the main stem for auxins, thereby preventing the canalization of auxin transport out of the bud (Bennett et al. 2006; Prusinkiewicz et al. 2009; Balla et al. 2011). In the second messenger model, auxins in the main stem can regulate the synthesis of CKs or SLs, which act as auxin second messengers within the bud and regulate branching (Tanaka et al. 2006; Brewer et al. 2009, 2015). Auxins can increase the expression of SL biosynthetic genes (Hayward et al. 2009), and can also negatively regulate CK content (Tanaka et al. 2006). CKs are directly transported into axillary buds to regulate outgrowth (Li et al. 1995). SLs regulate xylem sap CK (X-CK) levels through a feedback signal (Beveridge et al. 2000; Morris et al. 2001; Foo et al. 2005). SLs and CKs can also act antagonistically on pea bud growth (Dun et al. 2012). Auxins, CKs, and SLs interact in multiple feedback loops, and provide a robust balance in the regulation of shoot branching (Ferguson and Beveridge 2009; Domagalska and Leyser 2011; Ward et al. 2013).

The biosynthesis of SLs is responsive to environmental conditions, such as light, phosphate (P) and/or nitrogen (N), for regulating plant architecture. Light can affect SL levels or signaling and is particularly relevant to shading responses (Koltai et al. 2011). SLs may act downstream of the *phytochrome B* (*phyB*)-dependent response to both low red: far-red (R:FR) ratios and high R:FR ratios (Finlayson et al. 2010). P or N limitations in plants cause increased SL levels in the roots (Yoneyama et al. 2007a, b; Umehara et al. 2010; Kohlen et al. 2011; Mayzlish-Gati et al. 2012; Yoneyama et al. 2012). The different responses of SL expression to P or N deficiency are related to the nutrient acquisition strategies of plants (Yoneyama et al. 2012). Auxin signaling increases under low P conditions (López-Bucio et al. 2002; Pérez-Torres et al. 2008), while N deficiency decreases cytokinin levels, and the addition of cytokinin can counteract the root response to low phosphate (Martín et al. 2000). In rice, N deficiency increased indole-3-acetic acid (IAA) levels both in shoot and root in a split-root system, while P deficiency had no effect on shoot and root (Yoneyama et al. 2015).

D27 encodes an iron-containing protein in rice, which has homologs in *Arabidopsis* (*AtD27* gene) and in *Medicago truncatula* (*MtD27* gene) (Lin et al. 2009; Liu et al. 2011; Waters et al. 2012a). In rice, *D27* is expressed mainly in the vascular cells of shoots and roots (Lin et al. 2009). In *M. truncatula* and rice, *D27* transcripts are directly regulated by NSP1 and NSP2 (Liu et al. 2011). In-vitro evidence suggests that D27 is a β-carotene isomerase that converts all-*trans*-β-carotene into 9-*cis*-β-carotene (Alder et al.

2012). In *Arabidopsis*, *AtD27* is localized to the plastid, and its transcripts are subject to both local feedback and auxin-dependent signals (Waters et al. 2012a).

Chrysanthemum (*Dendranthema grandiflorum*) is one of the most important commercial cut-flowers, however, most cultivars of cut-flower chrysanthemums produce a number of branches, that must be removed to maintain single cut-flowers, and this practice increases production costs. Therefore, regulating the pattern of shoot branching is a prerequisite for the molecular breeding of chrysanthemum. However, the molecular mechanism of how SLs regulate shoot branching in chrysanthemum is still unclear. In previous studies, SLs directly inhibited bud activity, and the expression of SLs biosynthetic genes were down-regulated by exogenous SL, but overridden by apical auxin application. SLs also down-regulate the biosynthesis of CKs. In addition, SLs contributed to chrysanthemum shoot branching control in response to P starvation conditions in a systemic way. The related genes *DgCCD7*, *DgCCD8*, *DgMAX2*, *DgD14*, and *DgBRC1* have been identified (Liang et al. 2010; Chen et al. 2013; Dong et al. 2013; Wen et al. 2015; Xi et al. 2015).

In this study, we identified *DgD27*, a *D27* orthologous gene of the DUF 4033 superfamily, in chrysanthemum (*D. grandiflorum* 'Jinba'), and found that the expression of *DgD27* was inhibited by decapitation and induced by auxins. Furthermore, *DgD27* could produce a rapid response to light/dark, phosphate free, and low N (LN) treatments. These findings provide new insights into the dynamics of the putative SL biosynthesis component *DgD27* in chrysanthemum.

Materials and methods

Plant materials and growth conditions

Chrysanthemum plantlets were propagated under sterile conditions in jars containing MS agar medium (Murashige and Skoog 1962), and then grown in a tissue culture room at 22–25 °C with a photoperiod of 16/8 h light/dark and a light intensity of 100–120 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Hormone stocks

Naphthaleneacetic acid (NAA; N0640, Sigma-Aldrich, Shanghai, China) was dissolved in 70 % ethanol, and GR24 (LeadGen Labs, Orange CT, USA) was dissolved in acetone. 6-Benzylaminopurine (6-BA; B3408, Sigma-Aldrich, Shanghai, China) was dissolved in 1 M NaOH, while indole-3-acetic acid (IAA; I5148, Sigma-Aldrich, Shanghai, China) and gibberellin acid (GA$_3$; G7645, Sigma-Aldrich, Shanghai, China) were dissolved in ethanol.

Isolation of the full-length coding sequence for *DgD27*

Total RNA was extracted from stems with TRIzol Reagent (15596-026; Life Technologies/Invitrogen) and cDNA synthesis was performed using RevertAid First Strand cDNA Synthesis Kit (#K1621; Thermo Scientific). Primers were designed for DgD27 cloning based on the sequence regions of D27 genes that are conserved among Arabidopsis AtD27, Medicago MtD27, and rice D27 genes. After obtaining a conserved domain fragment using forward primer P_for and reverse primer P_rev, the 3′ fragment of *DgD27* was amplified by the rapid amplification of cDNA Ends (RACE) method, using the 3′ RACE primers 3′-race1 and 3′-race2, and the 5′ fragment of *DgD27* amplified in the same way using the 5′ RACE primers 5′-race1 and 5′-race2. The products, amplified using Prime-STAR HS DNA Polymerase (R010A; TaKaRa, Dalian, China), were cloned into pMD18-T vector (D101A; TaKaRa, Dalian, China) and verified by sequencing (Zhongke Xilin Biotechnology, Beijing, China). Sequence alignment and phylogenic analysis were performed using the ClustalW and ESPript programs (http://www.genome.jp/tools/clustalw/) and the MEGA5.0 program (http://www.megasoftware.net/) respectively. Genomic DNA was extracted from the shoot apex and young leaves using the CTAB method. The primers used were gD27_for and gD27_rev. Primer sequences are listed in Table S1.

Subcellular localization

For construction of the *35S*::*DgD27-GFP* reporter plasmids, the open reading frame (ORF) of *DgD27* was amplified with primers *Sal*I_for and *Sma*I_rev and was cloned into the binary vector pEZS-NL. Plastids are positively identified by co-expression of a protein fusion between the *cRecA* gene from *Arabidopsis* and RFP (Auldridge et al. 2006). Transformation into onion (*Allium cepa*) was performed as described previously (Varagona et al. 1992). After over night incubation in the dark, the GFP signal and RFP signal were examined under a confocal microscope at excitation wavelengths of 488 and 543 nm, respectively. Onion peels were unfolded in water and then viewed under a confocal laser scanning microscope (Nikon); images were acquired using EZ-C1 FreeViewer software (Nikon). Primer sequences are listed in Table S1.

Vector construction and plant transformation

For complementation experiments, the ORF of *DgD27* was amplified with primers *Xba*I_for and *Spe*I_rev and was cloned into vector p-Super1300+. The resulting constructs were transformed into *A. thaliana* mutant *Atd27-1* plants

via *Agrobacterium* using the floral-dip method (Clough and Bent 1998). Independent transformants were screened on MS medium containing 70 mg/L Hygromycin B. Independent homozygous T3 lines with single insertion sites were used for the branching phenotype analysis. Amplification of *Ubiquitin* gene was performed using 30 cycles as a normalization control, and the expression of *DgD27* was performed using 32 cycles. Primer sequences used for semi-quantitative reverse transcription PCR (RT-PCR) are listed in Table S1. Primers used for analysis of *DgD27* or *Ubiquitin* were DgD27_for and DgD27_rev; UBQ_for and UBQ_rev. Primer sequences are listed in Table S1.

Split-plate and two-bud section system

The split-plate system was modified from that described previously for *Arabidopsis* and chrysanthemum (Chatfield et al. 2000; Chen et al. 2013). We removed a 10-mm wide strip of medium from the centre of a plate containing 30 mL solidified MS medium. Using a micro-pipette, we then injected 5 μM NAA or an equal volume of ethanol into the upper side of the media block, and 5 μM GR24 or an equal volume of acetone into the basal side. The plates were then left at 4 °C for the last 72 h to allow the hormone to diffuse evenly throughout the media. Chrysanthemum seedlings grown to 10–12 cm high in sterile condition had two nodes (node *n* and node *n* + 1) and a stem after decapitation or an intact shoot apex. After hormones had diffused evenly throughout the media, two-bud sections were cut from the chrysanthemum seedlings, and then inserted into media. The petri dishes were then held vertically in the culture room. The "control" chrysanthemum seedlings had two nodes and a stem after decapitation; those with an intact shoot apex were named "intact." Topical buds (are equal to node *n*), basal buds (are equal to node *n* + 1), and stems were harvested separately 6 h after treatment for analysis of *DgD27* transcripts. For each sample, 10–12 plants were collected. All experiments were repeated for 3 biological replicates. All samples were snap-frozen in liquid nitrogen, and stored at −80 °C.

Hormone treatments

The chrysanthemum cuttings (7 cm in height) were initially grown in vermiculite for 10 days, then plants were transferred into pots in a greenhouse, and hormone treatments began after 2 weeks (when plants were about 10 cm in height). Once every 2 days, 20 mL solution per 12 plants was sprayed over whole plants including leaves, buds, and stems. The solution contained 50 mg/L IAA, GA, or 6-BA with 0.5 % Tween-20, respectively, and water containing 0.5 % Tween-20 was used as control. The upper one-third section of plant axillary buds and stems was harvested 0, 1, 6, 24, and 48 h after treatments began, each containing tissue originating from 10 to 12 plants. All experiments were repeated for 3 biological replicates. Tissues were snap-frozen in liquid nitrogen, and stored at −80 °C.

Plant decapitation and exogenous auxin expression assay

The chrysanthemum cuttings (7 cm in height) were grown initially in vermiculite for 10 days, then plants were transferred into pots in a greenhouse, and the plant decapitation assay and exogenous auxin assay were performed once the cuttings reached 15 cm in height (about 3.5 weeks). The plant shoot apex was either decapitated or left intact. For the decapitated stems, Eppendorf tubes containing MS agar medium with 5 μM NAA or an equal volume of ethanol were placed over the residual stem after decapitation. Bud 1 was adjacent to the cut site with 2, 3, 4 progressively further away, the stem 1, 2, 3 and 4 mean for internodes, below the numbered bud. Buds and stems were harvested at 0, 1, 6, 24, 48 h after treatments start, snap-frozen in liquid nitrogen, and stored at −80 °C. Three replicates were used for each sample, with 12–15 plants used per replicate.

Light/dark treatments

The chrysanthemum seedlings were grown to 2–3 new leaves in sterile conditions. Two weeks after subculture, the light/dark treatments were initiated. For the light treatment, the seedlings were subjected to a normal 16/8 h light/dark photoperiod, and to a 16/8 h dark/dark photoperiod for the dark treatment. Axillary buds and stems were harvested after 5 days, snap-frozen in liquid nitrogen, and stored at −80 °C. Three replicates were done for each sample, with 10–12 plants used per replicate. Phenotype observation was performed after 5 days, and measurements were recorded for each plant's height, internode length, stem diameter, length of leaf, and width of leaf. The data presented show average values of 10–12 plants.

RNA extraction and quantitative real-time PCR (qRT-PCR) Analysis

Plant samples of 100–150 mg, each containing tissue originating from 10 to 15 plants, were harvested and total RNA was isolated using TRIzol Reagent (Invitrogen, USA). RNA integrity was examined at 260 and 280 nm by NanoDrop 2000 (UV–Vis; Thermo Fisher Scientific Inc., USA) and checked on a gel electrophoresis in 1.5 % agarose gels with 1.5 μL RNA. The RNA (1 μg) of each tissue was reverse transcribed using FastQuant RT Kit

(with gDNase; KR106; Tiangen, China). The resultant cDNA was diluted tenfold and kept at −20 °C for qRT-PCR analysis. qRT-PCR was performed on a StepOne-Plus™ Real-time PCR detection system (Applied Biosystems, Foster City, CA, USA) using KAPA™ SYBR Fast qPCR Master Mix (Microread, Beijing, China). Each reaction consisted of 2 μL of cDNA template, 0.4 μL of each primer, 0.4 μL of ROX High, and 10 μL master mix in a final volume of 20 μL. The reaction procedure was as follows: denaturation at 95 °C for 20 s, followed by 40 cycles of 5 s at 95 °C and 20 s at 58 °C. The chrysanthemum β-Actin gene was used as an internal control for normalization, and the data were analyzed by OneStep-Plus™ software (Applied Biosystems). The relative expression of the detected genes was calculated using the relative $2^{-\Delta\Delta CT}$ method. The experiments were repeated twice with three replicates. Primers used for were DgD27_for and DgD27_rev; DgBRC1_for and DgBRC1_rev; β-Actin_for and β-Actin_rev. Primer sequences are listed in Table S1.

Hydroponic culture

Chrysanthemum seedlings were grown to 8–10 cm high in sterile conditions in 3 weeks. Their roots were then washed free of MS agar medium after opening the caps 1 day, and the plants were transferred to a hydroponic solution consisting of an improved Hoagland's solution. P was supplied as KH_2PO_4 and N was supplied as NH_4NO_3. The pH of all solutions was adjusted to 6.0 with 3 M KOH. The final concentrations in the different solutions were 1.0 mM P and 17.0 mM N (normal solution; that is, the P/N recovery solution), 0 mM P and 17.0 mM N (P absence), and 1.0 mM P and 0.17 mM N (LN). The plants were grown in a growth chamber with a 16/8 h photoperiod at a light intensity of 100–120 μmol m^{-2} s^{-1} at 25/20 °C. The absent P or LN treatments were initiated 12 days after the transfer, then plants were removed to the P/N recovery solution after 2 weeks. The upper one-third section of the plants was regarded as "bud up" compared with the lower one-third of plants, which were regarded as "bud base." Stem has the same status as bud. Leaf, stem up/base, bud up/base, and root tissue were harvested at 0, 1, 10, 24, 48 h after treatments started, snap-frozen in liquid nitrogen, and stored at −80 °C. Three replicates were done for each sample, with 10–12 plants used per replicate.

Hormone quantification

The snap-frozen samples (200 mg) from the light/dark treatments and P absence/LN treatments were ground into fine powder with quartz sand, and dissolved in 3.0 mL of phosphate-buffered saline (PBS) containing 0.1 % (v/v) Tween-20 and 0.1 % (w/v) gelatin (pH 7.5) to quantify free IAA, zeatin riboside (ZR), abscisic acid (ABA), and GA$_3$ by the enzyme-linked immunosorbent assay (ELISA), following the protocol described previously (Zhao et al. 2006). The mouse monoclonal antigen and corresponding antibodies were produced at the Center of Crop Chemical Control, China Agricultural University, China (Weiler et al. 1981; Wang et al. 2012). Calculations of the ELISA data were performed as described by Weiler et al. (1981). Three replicates were done for each sample.

Statistical analysis

All experiments were replicated three times to confirm the results. The data presented are mean ± standard error from a typical single experiment. ANOVA was conducted, followed by a Duncan's test. Different letters indicate significant differences ($P < 0.05$) between different treatments.

Results

Identification of the D27 gene homologue from chrysanthemum

To address the regulatory role of the D27 gene during shoot branching of chrysanthemum, its putative ortholog, DgD27, was isolated, and full-length cDNA of DgD27 was isolated by 5′ and 3′ RACE PCR. The full transcript of DgD27 (accession number KR029722) is 1123 bp in length and contains an 822 bp ORF encoding a predicted protein of 273 amino acids, a 70 bp 5′ untranslated region (UTR), and a 231 bp 3′ UTR. The predicted amino acid sequence of the conserved domain belongs to the DUF 4033 superfamily, a domain of unknown function. The genomic fragment corresponding to the DgD27 gene isolated by PCR comprised 2653 bp, and the DgD27 gene was shown to have a seven-exon structure, comprising 288, 140, 89, 62, 109, 93, and 41 bp in size respectively (Fig. 1a).

Amino acid sequence comparisons between DgD27 and its orthologs from Medicago, Arabidopsis, rice, and maize showed that the predicted DgD27 has a 46.35 % sequence identity to MtD27, 37.91 % identity to AtD27, 40 % identity to OsD27, and 41.82 % identity to ZmD27 (Fig. 1b). To explore the evolutionary relationship among DgD27 genes from various plant species, we performed phylogenetic analysis using MEGA5.0 software. Our analysis showed that DgD27 is more closely related to MtD27 and AtD27, which belong to clade 1 of the DWARF27 clade (Waters et al. 2012a) (Fig. 1c).

Fig. 1 Sequence analysis of the full-length cDNA named *DgD27*. **a** Structure of the *DgD27* gene. **b** Alignment of the predicted amino acid sequences of *DgD27* compared with homologs in *Arabidopsis* (AtD27), corn (ZmD27), rice (OsD27), and *Medicago* (MtD27). The alignment was generated using ClustalW and ESPript (http://espript.ibcp.fr/ESPript/ESPript/). **c** Phylogenetic analysis of response regulators from a range of plant species. The aligned sequences were used to construct a phylogenetic tree using MEGA5.0. Accession numbers are listed in Table S2

Tissue specificity of *DgD27* expression

We used qRT-PCR to determine the expression pattern of *DgD27* in axillary bud, leaf (petiole removed), petiole, stem, shoot apex and root during the vegetative phase of

chrysanthemum. As shown in Fig. 2, the highest expression of *DgD27* was found in stem, followed by in shoot apex, axillary bud, root, and it was only weakly expressed in leaf and petiole. The expression pattern was similar to that reported for another study of *D27* in rice (Lin et al. 2009),

Fig. 2 qRT-PCR analysis of *DgD27* expression in chrysanthemum. All samples were taken from pools of 10 plantlets, and the experiment was repeated three times. Detection of *β-Actin* was used as a normalization control and normalized to axillary bud = 1. *Error bars* show SDs

indicating that *DgD27* expression is strongly correlated with shoot branching development.

DgD27 is localized to the plastid

To further characterize the function of *DgD27*, we investigated the subcellular localization of DgD27. The ORF of *DgD27* was fused to green fluorescent protein (GFP) in the pEZS-NL vector, and the construct *35S::DgD27-GFP* was bombarded into onion epidermal cells. The co-expressed marker was used to mark the plastids as negative controls. We found that the control displayed red fluorescence throughout the plastids (Fig. 3b), while the GFP fluorescence fully overlapped with the red fluorescence from the co-expressed plastid-specific marker (Fig. 3c), demonstrating that DgD27 is located inside the plastids. This is in

accord with plastid localization of OsD27 and AtD27 (Lin et al. 2009; Waters et al. 2012a).

DgD27 complementation of Arabidopsis d27

To confirm the biological function of *DgD27*, a *35S::DgD27* transgene was introduced into the *Arabidopsis d27-1* mutant using a floral dip (Clough and Bent 1998). Three independent transgenic lines were selected for phenotypic appraisal, and the *DgD27* expression level was checked by RT-PCR analysis. As shown in Fig. 4, all three transgenic lines had elevated levels of *DgD27* mRNA expression. Expression of *DgD27* in *d27-1* reduced the mean number of branches, and their branching phenotypes were similar to those of wild-type plants. The expression of *DgD27* in *Atd27-1* plants almost completely restored the phenotype back to that of the wild type. These results confirmed *DgD27* as the functional ortholog of *D27*.

Induction of DgD27 expression by auxin

To investigate auxin regulation of *DgD27*, qRT-PCR analysis was performed on two-bud segments cultured in a split-plate system, with or without apical application of auxin (Fig. 5b). Our results showed that, whether in bud or stem, the *DgD27* expression level dramatically decreased 6 h after decapitation, but this reduction was successfully reversed by apical NAA application, and was most strongly induced in the top bud. These results indicated that auxins can induce *DgD27* expression.

Feedback control of DgD27 expression

We investigated the effects of SLs on *DgD27* expression, using two-bud segments cultured in a split-plate system.

Fig. 3 Subcellular localization of DgD27. **a** Epifluorescence micrographs of onion epidermis transiently transformed with p*35S::DgD27-GFP* plasmid; **b** plastids are positively identified by co-expression of a protein fusion between the *cRecA* gene from *Arabidopsis* and RFP;

c the merged images confirm colocalization of the two fluorescent signals; **d** bright field micrographs of the same field of view to provide the cell outline

Fig. 4 Complementation of *Arabidopsis d27-1* mutant phenotype with *DgD27*. **a** Comparison of phenotypes of wild-type (WT), *d27-1* and *d27-1* transformed with the *35S::DgD27* constructs. **b** The number of secondary rosette branches produced by WT, *d27-1* and three independent homozygous lines transformed carrying *35S::DgD27*. The mean number of rosette branches with a length of at least 5 mm is shown. Data are mean ± SE; $n = 15$. **c** Transcript levels of the overexpressed *DgD27* for the experiment presented in **b** were determined by RT-PCR. Detection of *UBQ* transcript was used as a cDNA normalization control

As shown in Fig. 5a, *DgD27* expression was greatly reduced in all samples upon decapitation. In the bottom bud, *DgD27* expression was down-regulated by GR24 in intact plants compared with untreated controls, but this down-regulation was less than that observed upon decapitation. However, in the top bud, there was an opposite effect on *DgD27* expression between GR24 treatment and decapitation: GR24 increased *DgD27* expression in intact plants compared with untreated controls, and *DgD27* was down-regulated upon decapitation. As shown in Fig. 5b, there was a significant difference

between control and decapitated plants treated with NAA or GR24 in all samples, except the top bud with basal GR24. In the bottom bud, *DgD27* expression was up-regulated by apical NAA or/with basal GR24 compared with untreated controls. Furthermore, the level of up-regulation in decapitated plants treated with both apical NAA and basal GR24 was lower than that of the decapitated plants treated with apical NAA, but higher than the decapitated plants treated with basal GR24. However in the top bud, basal GR24 had no effect on *DgD27* expression, while there was a significant increase in the decapitated plants treated with both apical NAA and basal GR24 or only with apical NAA. These results indicated the presence of different feedback controls for *DgD27* expression between the top and bottom buds in chrysanthemum and also a relative competitiveness between these two buds.

Expression of *DgD27* with hormone treatments

We used qRT-PCR analysis to determine the expression patterns of *DgD27* in intact plants given hormone treatment. As shown in Fig. 6, the results demonstrated that the expression patterns differed for each type of hormone treatment. The expression level of *DgD27* was induced significantly by GA and 6-BA in buds but was induced only weakly by IAA, while it increased significantly in stems at 24 h with 6-BA treatment, and at 48 h with GA treatment. However, the expression level of *DgD27* was recovered to pre-treatment levels in buds at 48 h with GA treatment, while levels remained high with a 6-BA treatment. Taken together, these data suggested that *DgD27* responded to GA and CKs in both buds and stems, and exhibited different expression patterns.

Effects of apical dominance on *DgD27* expression

Chrysanthemum has strong apical dominance: the bud in the upper part of the plant can rapidly grow out after decapitation. To determine the effects of apical dominance on *DgD27* expression levels, a classic decapitation assay was conducted. As shown in Fig. 7a, the transcript levels of *DgD27* dramatically decreased in both buds 1 h after decapitation while remaining at a low level in buds 1 and 2, and increasing in buds 3 and 4 6 h after decapitation. By 48 h after decapitation, levels were high in buds 1 and 2 and had recovered almost to pre-decapitation levels in buds 3 and 4. As shown in Fig. 7b, the transcript levels of *DgD27* dramatically decreased in stems 1 and 2 1 h after decapitation, but dramatically decreased in stems 3 and 4 at 6 h, and then increased 24 h after decapitation; by 48 h after decapitation, levels had recovered almost to pre-decapitation levels in all stems.

Fig. 5 *DgD27* expression response to decapitation, auxin, and GR24. **a** Plantlets were treated with 0 μM or 5 μM GR24 to the intact basal side, and an equal volume of acetone as a control. **b** Decapitated plants were treated with 0 μM or 5 μM NAA to the upper side (with an equal volume of ethanol as a control), and with 0 μM or 5 μM GR24 to the basal side (with an equal volume of acetone as a control). *Dash* vertical control, *multi symbol* none treated. Top buds, bottom buds and stems were collected 6 h after treatment. Detection of *β-Actin* was used as a normalization control. Results are means of three biological replicates analyzed by qRT-PCR, with 10–12 plants for each replicate; *letters* indicate significant differences ($P < 0.05$) between different treatments. ANOVA followed by a Duncan's test. Error bars shown

Fig. 6 Expression patterns of *DgD27* in response of buds and stems to hormone treatments in chrysanthemum. **a** *DgD27* expression was induced by treatment with 50 mg/L IAA, GA, or 6-BA in buds, respectively. **b** *DgD27* expression was induced by treatment with 50 mg/L IAA, GA, or 6-BA in stems, respectively. Each bud and stem sample at 0 h were set to equal 1. Results are the means of three biological replicates with 10–12 plants for each replicate. Error bars shown

Substitution of the decapitated apex with exogenous auxin resulted in a 2- to 20-fold increase in the expression of *DgD27* in all buds and stems (Fig. 7c, d). The closer the bud is located to the exogenous auxin, the higher the expression was induced. This result indicated that induction of *DgD27* expression by exogenous auxin after decapitation has a position effect, and expression consistent with the known positive effect of auxins on gene transcription in two-bud segments cultured experimentally. Our results suggested that *DgD27* transcription was down-regulated rapidly by decapitation, and increased by exogenous auxin, which plays a role in the apical dominance.

Expression of *DgD27* with light/dark treatments

To examine whether *DgD27* can respond to light, we assessed the expression levels of *DgD27* in intact plants with light/dark treatments after 5 days. As shown in Fig. 8 and Table 1, a dark treatment negatively affected plant growth, increased the height and length of internodes, and reduced the stem's diameter and both the length and width of leaves. As shown in Fig. 9, *DgD27* expression was significantly down-regulated by dark treatment in axillary buds, though it was not significantly up-regulated in stems.

We measured the hormone levels in plants exposed to light/dark treatments after 5 days. As shown in Table 2,

Fig. 7 Transcript levels of *DgD27* after decapitation and decapitation + NAA. **a** *DgD27* transcript levels in buds 1–4 were analyzed 0, 1, 6, 24 and 48 h after decapitation. **b** *DgD27* transcript levels in stems 1–4 were analyzed 0, 1, 6, 24 and 48 h after decapitation. **c** *DgD27* transcript levels in buds 1–4 were analyzed 6 h after decapitation and after decapitation + NAA treatment. **d** *DgD27* transcript levels in stems 1–4 were analyzed 6 h after decapitation and

decapitation + NAA. Bud position was recorded basipetally. Stem position was below the numbered bud. Each bud and stem sample at 0 h were set to equal 1. Results are means of three biological replicates with 12–15 plants for each replicate. *Asterisks* indicate statistically significant differences (*P* < 0.05) calculated by Duncan's test between the intact and decapitated plants. Error bars shown

Fig. 8 Phenotype of plants exposed to light and dark conditions after 5 days. **b** Leaves were removed. *Bar* = 1 cm

Table 1 Plant growth was measured 5 days after light or dark conditions

	Height (cm)	Stem diameter (mm)	Length of leaf (cm)	Width of leaf (cm)	Length of internode (cm)
Light	2.21 ± 0.23[b]	1.90 ± 0.16[a]	2.31 ± 0.33[a]	2.31 ± 0.44[a]	0.36 ± 0.11[b]
Dark	3.43 ± 0.39[a]	1.61 ± 0.17[b]	1.92 ± 0.18[b]	1.77 ± 0.29[b]	1.01 ± 0.26[a]

Values are the mean ± SD (*n* = 10–12). Different superscript letters indicate significant differences (*P* < 0.05) calculated by a Duncan's test

four hormone levels showed significant changes 5 days after dark treatments. ABA and GA levels significantly decreased both in axillary bud and stems, while the IAA level only significantly decreased in axillary buds. In contrast, the ZR level only significantly decreased in stems. These results showed that *DgD27* expression in

Fig. 9 Expression patterns of *DgD27* in response of axillary buds and stems to light/dark treatments in chrysanthemum. Results are means of three biological replicates with 10–12 plants for each replicate. *Different letters* above the *bars* indicate significant differences ($P < 0.05$) calculated by a Duncan's test

shoots has a correlation with auxins under light/dark conditions.

Response of *DgD27* in shoot and root under conditions of phosphate absence

To examine whether *DgD27* can respond to lack of phosphate, we assessed the expression levels of *DgD27* in a hydroponic solution with no phosphate and after 2 weeks in a normal phosphate solution. As shown in Fig. 10, the level of *DgD27* expression was significantly up-regulated by phosphate absence both in shoots and roots. In particular it was significantly increased at 1 h and attained higher levels in basal buds 48 h after phosphate was removed. There was a more dramatic response to phosphate absence in buds than in stems, and in addition, the level of *DgD27* expression increased more rapidly in the upper buds than in the basal buds. When plantlets were treated with normal phosphate solution after a 2 weeks absence of phosphate, *DgD27* expression then showed an obvious change both in shoots and roots but not in upper buds. By 48 h after the normal phosphate solution treatment, *DgD27* expression

levels were high in basal buds and basal stems, while they had recovered to almost T0 levels in upper bud, upper stem, and root. There was a complementary mechanism between *DgD27* expression response to phosphate deficiency and recovery conditions in shoots, but not in roots. These results indicated that the response of chrysanthemum to absence of phosphate correlates with *DgD27* transcript levels.

Response of *DgD27* in shoot and root under low N conditions

We investigated whether the effects of N treatment on *DgD27* expression were the same as for phosphate by growing plants in a hydroponic solution with low N, and after 2 weeks transferring them to a normal N recovery solution. We found that the *DgD27* expression level was highest at 1 h in leaves, but there was a mild variation up in buds, stems, and roots with a low N treatment. In addition, after 48 h of LN conditions, there was no increase in *DgD27* expression in shoot, but a slight increase in root (Fig. 11). After transfer to the normal nitrogen solution treatment, *DgD27* expression then showed the opposite response with low N treatment. Levels obviously increased in bud, leaf, and root, but not in stem at 48 h after the normal nitrogen solution treatment. There was also a complementary mechanism between *DgD27* expression response to low N treatment and normal nitrogen solution treatment in both shoot and root. Thus, the expression of *DgD27* under low N conditions was not the same as that observed under conditions of absent phosphate. These results suggested that, unlike phosphate, N does not affect *DgD27* expression in buds and stems of chrysanthemum.

Response of *DgBRC1* in bud under low N conditions

BRC1, a bud-specific transcription factor was previously proven to act downstream of the SL pathway (Dun et al. 2012, 2013). In chrysanthemum, P starvation induced increased endogenous SL analog levels in all parts of the

Table 2 Hormone levels in axillary buds and stems under light/dark conditions

Sample	Treatment	IAA level (ng g^{-1} FW)	ZR level (ng g^{-1} FW)	ABA level (ng g^{-1} FW)	GA level (ng g^{-1} FW)
Axillary bud	Light	68.06 ± 3.70[a]	7.82 ± 0.29[a]	81.60 ± 3.77[a]	7.02 ± 0.15[a]
	Dark	43.87 ± 1.28[b]	7.33 ± 1.28[a]	60.04 ± 8.41[b]	5.41 ± 0.6[b]
Stem	Light	58.33 ± 3.47[a]	9.32 ± 0.34[a]	108.75 ± 2.73[a]	7.79 ± 0.19[a]
	Dark	53.67 ± 3.3[a]	6.83 ± 0.55[b]	41.28 ± 4.85[b]	4.27 ± 0.32[b]

IAA levels, ZR levels, ABA levels, and GA levels in axillary buds and stems were analyzed 5 d after light/dark treatments. Data are mean ± SE. Results are means of three biological replicates with 10–12 plants for each replicate. Different superscript letters indicate significant differences ($P < 0.05$) calculated by a Duncan's test

FW fresh weight

Fig. 10 *DgD27* gene expression under phosphate deficiency/recovery conditions in shoot and root. **a** *DgD27* transcript levels in bud up were analyzed 0, 1, 10, 24 and 48 h after phosphate deficiency/recovery conditions. **b** *DgD27* transcript levels in bud base were analyzed 0, 1, 10, 24 and 48 h after phosphate deficiency/recovery conditions. The data presented are typical of three independent biological replicates with 10–12 plants for each replicate. Error bars shown

plant, and *DgBRC1* transcripts produced rapid responses in nodes under conditions of P starvation (Xi et al. 2015). To examine whether *DgBRC1* can respond to low N conditions, we assessed the expression levels of *DgBRC1* in a hydroponic solution with low N and after 2 weeks in a normal N recovery solution. As shown in Fig. 12, *DgBRC1* expression showed a slight increase in buds after 1 h of LN conditions, and a slight drease after a 48 h treatment. After transfer to the normal nitrogen solution treatment, *DgBRC1* expression then showed the opposite response with low N treatment. The expression levels of *DgBRC1* obviously increased 48 h after the normal nitrogen solution treatment. Thus, the expression of *DgBRC1* under low N conditions was the same as that of *DgD27*, and the results indicated that the response of chrysanthemum to low N does not correlate with *DgBRC1* transcript levels.

Effects of phosphate deficiency/low N on shoot hormone contents

The effects of phosphate deficiency/low N on shoot hormone content were examined in hydroponically grown chrysanthemum plants after 2 weeks. As shown in Table 3, IAA levels increased in upper buds and ABA levels increased in basal buds both under phosphate deficiency and low N treatments. ZR levels decreased in basal buds only under phosphate deficiency treatments. By contrast, neither phosphate deficiency nor low N treatments affected

Fig. 11 *DgD27* gene expression under low nitrogen/nitrogen recovery conditions in shoot and root. *LN* low nitrogen, *NR* nitrogen recovery. **a** *DgD27* transcript levels in bud were analyzed 0, 1, 10, 24 and 48 h after low nitrogen/nitrogen recovery. **b** *DgD27* transcript levels in stem were analyzed 0, 1, 10, 24 and 48 h after nitrogen recovery/nitrogen recovery. **c** *DgD27* transcript levels in leaf were analyzed 0, 1, 10, 24 and 48 h after low nitrogen/nitrogen recovery. **d** *DgD27* transcript levels in root were analyzed 0, 1, 10, 24 and 48 h after nitrogen recovery/nitrogen recovery. The data presented are typical of three independent biological replicates with 10–12 plants for each replicate. Error bars shown

Fig. 12 *DgDBRC1* gene expression under low nitrogen/nitrogen recovery conditions in bud. *LN* low nitrogen, *NR* nitrogen recovery. *DgBRC1* transcript levels in bud were analyzed 0, 1, 10, 24 and 48 h after low nitrogen/nitrogen recovery. The data presented are typical of three independent biological replicates with 10–12 plants for each replicate. Error bars shown

GA levels in upper or basal buds. As shown in Table S3, ABA levels increased in upper stems under phosphate deficiency, while IAA levels and ZR levels increased under low N treatments. ZR levels decreased in basal stems only under phosphate deficiency treatments. As in buds, neither phosphate deficiency nor low N treatments affected GA levels in upper or basal stems. These results showed that multiple hormones respond to phosphate deficiency and/or low N in different parts of shoot. Thus, shoot branching was regulated by different hormonal balance responses to phosphate deficiency or low N.

Discussion

The eukaryotic sequences of *D27* broadly grouped into three distinct clades. Clades 1 and 2 only contain members from land plants, while the clade 3 also contains members from chlorophyte algae and diatoms (Waters et al. 2012a). In the present study, we only isolated a *D27* ortholog gene, named *DgD27*, which belongs to the D27 clade specific to land plants (Waters et al. 2012a). With functional complementation of *d27-1* mutants, a conserved function of *DgD27* was supported. The biological function of the D27-like protein family is unknown, with no known link to SLs (Delaux et al. 2012; Waters et al. 2012a).

The interactions between auxins and SLs in controlling shoot branching have been studied previously (Foo et al. 2005; Johnson et al. 2006; Crawford et al. 2010; Liang

Table 3 Hormone levels in upper buds and basal buds under phosphate deficiency/low N conditions

	Treatment	IAA level (ng g^{-1} FW)	ZR level (ng g^{-1} FW)	ABA level (ng g^{-1} FW)	GA level (ng g^{-1} FW)
Upper bud	Control	42.49 ± 5.73[b]	5.18 ± 0.31[a]	92.82 ± 2.95[b]	7.52 ± 0.49[a]
	−P	61.29 ± 3.79[a]	6.13 ± 0.87[a]	88.01 ± 2.96[b]	7.32 ± 0.69[a]
	LN	56.61 ± 4.10[a]	6.65 ± 3.21[a]	99.51 ± 2.46[a]	7.46 ± 1.50[a]
Basal bud	Control	59.61 ± 10.71[b]	9.76 ± 1.97[a]	56.01 ± 3.37[b]	4.71 ± 0.43[a]
	−P	83.48 ± 7.56[a]	5.84 ± 0.47[b]	85.43 ± 4.91[a]	5.74 ± 1.25[a]
	LN	71.18 ± 6.26[ab]	11.45 ± 1.70[a]	94.53 ± 8.56[a]	5.63 ± 1.01[a]

IAA levels, ZR levels, ABA levels, and GA levels in axillary buds and stems were analyzed 14 d after phosphate deficiency/recovery conditions. Data are mean ± SE. Results are means of three biological replicates with 10–12 plants for each replicate. Different superscript letters indicate significant differences ($P < 0.05$) calculated by a Duncan's test

Control normal solution, *−P* phosphate deficiency, *LN* low nitrogen

et al. 2010; Ward et al. 2013). In this study, we found that different feedback controls for *DgD27* expression between the top and bottom buds were present. Similar patterns of *DgD27* response to NAA/GR24 were also observed in *DgCCD8* and *DgD14* (Liang et al. 2010; Wen et al. 2015). However, the obvious difference was that an apical NAA treatment together with basal GR24 was not more effective at increasing *DgD27* expression in the top bud. Auxins were transported basipetally down the main stem in PATS, whereas SLs were transported acropetally. *DgD27* was upstream of the SLs biosynthesis pathway. Differences in apical and basal bud responses may be caused by transport over the stem segment 6 h after treatments, and this also suggested that there was relative competitiveness existing between different buds in chrysanthemum. Additionally, ABA acted downstream of the main shoot PATS and the SL pathway, while regulated bud outgrowth responses to the R:FR also suppressed lower bud outgrowth and elongation (Reddy et al. 2013; Yao and Finlayson 2015). Different ABA levels and light signaling in different buds may be another important reason leading to altered *DgD27* expression patterns. In future studies, identifying the ABA levels and the responses to ABA and light treatments would help to characterize the relationship between apical and basal buds in chrysanthemum.

In this study, the response of *DgD27* to GA and CKs treatments in buds was the same as that of *DgD14* under the same conditions. These results indicated that GA and CKs treatments triggered the response of the SL pathway. However, the expression levels of these two genes were induced only weakly by IAA, and there was no obvious influence on any structure with IAA (Wen et al. 2015). This evidence suggested that the effects of GA and CKs treatments may be caused by the influence of apical dominance or main shoot PATS.

Use of decapitation and auxin application treatment showed that *DgD27* expression responded rapidly to the release of apical dominance resulting from decapitation. Similar results were obtained in *D10, CCD7, CCD8,* and

DgBRC1 (Ferguson and Beveridge 2009; Hayward et al. 2009; Zhang et al. 2010; Chen et al. 2013). Additionally, *DgD27* expression levels in buds and stems were up-regulated after apical application of NAA, and had a positional effect: near the source of NAA, the increase of expression levels was much higher than that of the distant, further demonstrating that *DgD27* as a response regulator is related to auxin regulation of bud outgrowth.

Light can affect SL levels or the signaling response to shading. Auxin signaling was required for the *phytochrome B* (*phyB*) hypobranching phenotype, SL may be acting downstream of the *phyB*-dependent response to both low R:FR and high R:FR (Finlayson et al. 2010). Auxin sensitivity had also been implicated in shade avoidance (Reddy and Finlayson 2014). In the current study, we found that *DgD27* expression was significantly down-regulated by dark treatment in axillary buds, whereas the IAA level significantly decreased. This was consistent with the fact that auxins can induce *DgD27* expression in chrysanthemum. Dark treatment is extreme shading, and differs from low/high R:FR, and it does not act as a feedback control for *DgD27* expression. This may be why *DgD27* expression was not up-regulated by the dark treatment, as expected. Additionally, the fact that the IAA level significantly decreased may be associated with the stage of growth, evidence of which had been provided in the research on *Arabidopsis phyB* (Reddy and Finlayson 2014).

SLs are able to respond to nutrient supply conditions: the expression levels of the SL pathway genes were up-regulated by conditions of P and/or N deficiency (Liu et al. 2011; Bonneau et al. 2013). In chrysanthemum, three different types of SLs were identified and levels of all three SLs showed a strong increase under absent P conditions, implying that absence of P also stimulates SL biosynthesis in chrysanthemum (Xi et al. 2015). In this study, the results showed that the expression of *DgD27* rapidly increased under conditions of absent P, a result which was consistent with earlier findings in other species (Umehara et al. 2010; Liu et al. 2011). As to the expression pattern, similar

patterns of *DgD27* responses to P absence were also observed in *DgCCD7*, *DgCCD8*, *DgMAX2*, *DgD14*, and *DgBRC1* (Wen et al. 2015; Xi et al. 2015). The expression levels of these six genes in buds had both significantly increased after 48 h in a solution without P, and these results suggested that signals triggered by P starvation had comparative continuity and cooperativity.

Meanwhile, our results showed different responses of *DgD27* expression between absent P and low N conditions. The expression levels of *DgD27* in buds showed that almost no difference was observed in a solution with low N. This result was also observed in *DgD14* between absent P and low N conditions (Wen et al. 2015). Recent studies had indicated that the different responses of SL exudation to P or N deficiency have been shown to depend on the P level (Yoneyama et al. 2012; Czarnecki et al. 2013). In chrysanthemum, P levels were found to be significantly reduced both in shoots and roots under absent P conditions, but were not reduced in shoots under low N conditions (Wen et al. 2015). It was supposed that P did not relocate in plants exposed to low N, and this might be the reason why the SL gene was not triggered in chrysanthemum.

DgBRC1 was mainly expressed in dormant axillary buds to suppress the response of bud outgrowth (Chen et al. 2013). Expression of *DgBRC1* rapidly responded to P absence and this suggested that P starvation triggered signals that facilitated bud inhibition in chrysanthemum (Xi et al. 2015). However, the response of *DgBRC1* to low N conditions was the same as that of *DgD27* and *DgD14* under the same conditions. Thus, the present evidence may suggest that N did not regulate shoot branching directly via the SL pathway and/or *DgBRC1* in chrysanthemum.

After examining the shoot hormone contents, there were found to be three reasons that may have caused the difference between the bud (stem) base and upper parts upon P deficiency and/or low N conditions: (1) Developmental status and distance from the shoot apex influence activation of axillary buds. (2) Local control and/or systemic regulation of auxin in the axillary bud. (3) ABA may play a role in regulation shoot branching upon P deficiency and/or low N conditions.

In summary, *DgD27* played diverse roles in chrysanthemum regulating shoot branching by hormonal and environmental factors (e.g., light, P, N). Our results reveal new insights into the elucidation of shoot branching mechanisms via the putative SL biosynthetic component *DgD27* and provide a theoretical basis for breeding cut-chrysanthemum varieties with no (or fewer) branches.

Author contribution statement Chao Wen and Liangjun Zhao conceived and designed the study. Chao Wen, Qingcui Zhao, Jing Nie, Guoqin Liu, Lin Shen, Lin Xi, and

Chenxia Cheng performed the experiments. Chao Wen wrote the paper. Liangjun Zhao, and Nan Ma reviewed and edited the manuscript. All the authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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