Isolation and Functional Analysis of *CmMAX1* from Chrysanthemum

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ADDITIONAL INDEX WORDS. shoot branching, strigolactone, auxin, cytokinin, expression analysis

ABSTRACT. *More axillary buds 1 (MAX1)*, initially identified in arabidopsis (*Arabidopsis thaliana*), is a key regulatory gene in strigolactone synthesis. *CmMAX1*, an ortholog of *MAX1* was cloned from chrysanthemum (*Chrysanthemum morifolium* cv. Jinba). It had an open reading frame of 1611 bp and encoded 536 amino acid of P450 protein, with a conserved heme-binding motif of PFG×GPR×C×G, as well as PERF and KEXR motifs. The predicted amino acid sequence of *CmMAX1* was most closely related to the *MAX1* ortholog identified in lotus (*Nelumbo nucifera*), NnMAX1, with 55.33% amino acid sequence similarity. Expression analysis revealed there was no significant difference of *CmMAX1* expression among various tissues. Phosphorus (P) deficiency significantly improved the expression levels of *CmMAX1*. Strigolactone, auxin, and cytokinin negatively regulated the expression of *CmMAX1*. Overexpression of *CmMAX1* reduced the branch numbers of arabidopsis *max1-1*. These results suggest that *CmMAX1* may be a candidate gene for reducing the shoot branching of chrysanthemum.

Cytochrome P450s (CYPs), one of the biggest gene super-families in plant genomes (Nelson and Reichhart, 2011), participate in all major plant biosynthetic pathways, including biosynthesis of pigments, defense-related compounds, ultraviolet protectants, fatty acids, and hormones (Guttikonda et al., 2010). CYP711 was designated as a new clan of CYPs, with MAX1 identified as the first member (Booker et al., 2005).

MAX1, is involved in the synthesis of strigolactone, which has been defined as a novel phytohormone that can inhibit shoot branching (Al-Babili and Bouwmeester, 2015; Gomez-Roldan et al., 2008; Umehara et al., 2008). Strigolactones are derived from the carotenoids (Lopez-Raez et al., 2008; Matusova et al., 2005), which were cleaved sequentially by carotenoid cleavage dioxygenase (CCD) 7 (encoded by *MAX3/RMS5/DAD3/D17*) and CCD8 (encoded by *MAX4/RMS1/DAD1/D10*) (Xie et al., 2010). D27, encoding an iron-containing protein, acted after CCD7 and CCD8 (Lin et al., 2009). MAX1 acted downstream of D27 and converted carlactone (CL) to carlactonoic acid (CLA) (Abe et al., 2014; Waters et al., 2012). Studies in rice (*Oryza sativa*) showed that MAX1s could be classified into three types according to the products: A1 type, converting CL to CLA; A2 type, converting CL to 4-deoxyorobanchol (4DO) via CLA; and A3 type, converting CL to CLA and 4DO to orobanchol (Yoneyama et al., 2018).

MAX1 orthologs were discovered in a wide number of species, although absent from the moss *Physcomitrella patens* (Proust et al., 2011), which led to the hypothesis that *MAX1* was incorporated into the MAX pathway after the divergence of the moss and tracheophyte lineages (Hepworth, 2012). Furthermore, several orthologs of *MAX1* were present in monocots, such as five in rice (Umehara et al., 2010) and three in maize [*Zea mays* (Yoneyama et al., 2018)], and are generally present as a single copy in eudicots. Nevertheless, orthologs of

MAX2, *MAX3*, and *MAX4* were present in all plants searched (Hepworth, 2012), which made *MAX1* more confusing.

Chrysanthemum is one of the most important flowers in the world; however, the reduction of branching is required in the cultivation of cut chrysanthemum to ensure the quality of the single flower. Existing cultivars generally produce lots of lateral branches, which increases cultivation costs by one third as a result of the manual pinching and bud picking. Therefore, new chrysanthemum cultivars with less or no branches are needed.

Previous complementation experiments showed that *MAX1* was present and active in some species (Hepworth, 2012). Do they have conserved function in chrysanthemum? With the aim of answering this question and use of the gene for regulating shoot branching, we isolated an ortholog of *MAX1* from chrysanthemum cultivar Jinba, analyzed its expression in different tissues and treatments, and investigated the function of *CmMAX1* by complementation experiments. This study lays a foundation for further research into shoot branching in chrysanthemum, and also provides gene resources for breeding new cultivars with fewer branches.

Materials and Methods

PLANT MATERIALS AND GROWTH CONDITIONS. Chrysanthemum cultivar Jinba, arabidopsis Columbia wild type (WT), *max1-1*, and transgenic plants were all grown in a tissue culture room at 23 ± 2 °C, with a photoperiod of 16/8 h (light/dark) and a light intensity of 100 μmol·m⁻²·s⁻¹.

ISOLATION OF THE *CmMAX1* GENE. Total RNA was extracted from stems of chrysanthemum plants using a Trizol reagent kit (Invitrogen, Shanghai, China) and was digested with RNase-free DNase (Takara, Dalian, China). First-strand complementary (cDNA) was synthesized using Superscript III reverse transcriptase (Invitrogen). Two sets of polymerase chain reaction (PCR) primers were designed for the cloning of *CmMAX1* based on the conserved sequence of arabidopsis *MAX1*, *Medicago MtMAX1*, *Populus PtMAX1*, and rice *OsMAX1*. After obtaining a partial fragment by PCR using forward primer (5'-ATTTTCAGRTTTCABTGRAG-3') and

Received for publication 13 Apr. 2018. Accepted for publication 4 Sept. 2018. This work was supported by The Natural Science Research Project of Colleges and Universities (KJ2017A154).

We thank Professor Ottoline Leyser for providing the arabidopsis *max1-1* mutant.

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A	CmMAX1	MTDSLGL.....QLSSPSLYSSSSFFSILALVAGILCYFYAPLVNVRKVPGLALP	49
	MAX1		0
	MtMAX1	.MLFIS.....VILNVPLASTIFILVTLMGGLVGYLYWPFWKLRRKVPGPSLP	47
	PhMAX1	.MEFLSTNIQHSIVDTVEVLTRPPTSTICTILALLATVLVYFYGYWRVRKVPGPSFP	59
	Consensus		
	CmMAX1	FVGHLHLLALHGPVEVSILAKRYGPVFRFHNGROPLVIIADAELCREVGIIKKYKDFQHR	109
	MAX1	MGROPLIIIIAELCREVGIIKKYKDFLNP	30
	MtMAX1	LVGHLPLLAKYGPVSVLAKQYGPYRFHMGROPLIIIIADAELCKEYGIKKYKEIPNRS	107
	PhMAX1	LVGHLPLMARYGPVSVLAKQYGPYRFHMGROPLIVIVADAELCREVGIRKKEKIDPNRS	119
	Consensus	grqpl i a aelc e gi k k rs	
	CmMAX1	IPSPINASPIHLKSLAWMRDPRWTSNRNTVLPMFQPSHLTKLIEMMOSSIETAKYLP.I	168
	MAX1	IPSPISASPLHKKGLFFTRDKRWSKMRNTILSLYQPSHLTSLIPTMHSEITSATHNLD.S	89
	MtMAX1	IPSPISASPLHKKGLFFTRNSQWSTMRNTILSVYQPSHLANLVPMOSFIESATQNDDT	167
	PhMAX1	IPSPIAASPLHKKGLFFTRDSRWSTMRNTILSVYQPSYLAQLVPMOSFIESATKNLD..	177
	Consensus	ipspl asp h k l r w rnt l qps l l p m s i l	
	CmMAX1	DGKDVSFSLSLKMTLDVLGDAVFCVFEGLLNALTHDHNDKKDFGIINPHTHDHDKD...	225
	MAX1	KPRDIVFSNLFLKLTTDIIGQAAFGVDFGLSGK.....KPIKDVEVTD...	132
	MtMAX1	SKEDIIFSLSLRLATDVIGDAAGVNFGLSKPH.....SICESMNNVEQSSANS	217
	PhMAX1	SEGDLTFSLSLKLATDVIGDAAGVDFGLSKP.....ITDKMNHQEKDS...	222
	Consensus	d fs l l d g a fgv fgl	
	CmMAX1	.EIESFIKOHLSISKLNDSGSGFSTIIGLVFPIVOEPFRRILCRIPYTMOWMFTINK	284
	MAX1	FINQHVYSTTOLKMDLSGSLIILGLLPILOEPERQVLKRIPTMDWRVEKTNA	187
	MtMAX1	DEVSIIFINQHIYSTTOLKMDLSGSGFSTIIGLIAPILQEPTRQILKRIPTMDWKMECTNK	277
	PhMAX1	.EVQEFINQHNYSTTOLKMDLSGSGFSTIIGLVFPILOEPFRQILKRIPTIDWKVETNK	281
	Consensus	fi qh s lk d sgs s i gl pi qep r l rip t dw e n	
	CmMAX1	SLTSKLDKIVLKRKMDNRSRKKDFLSLIINASESETASSKLFTPDYIRALTYEQLLAGST	344
	MAX1	RLSGQLNEIVSKRAKEAETDSKDFLSLILKARESDPFAKNIFTSDYISAVTYEHLLAGSA	247
	MtMAX1	NLTGRLLDVIKKRMEDKSRTSKNFLSLIINTRESKSVSENVFSFDYISAVTYEHLLAGSA	337
	PhMAX1	NLSRRLDEIVAKRMEEKYGSSKDFLSLILQARESEKLAKNVFTSDYISAVTYEHLLAGSA	341
	Consensus	l l iv kr k flslil es f dyi a tye llags	
	CmMAX1	STSTLSVAIYLVSRYPEVEKLLQEIADFGLDQVETABDLESKFPPYLDQVYKEVLRFY	404
	MAX1	TTAFTLSSVLYLVSGHLDVEKRLQEIIDGFGNRLDIAHDLQHKFPPYLDQVYKEAMRFY	307
	MtMAX1	TTSFTLSSIVLVAGHPNVEKLLQEIIDGFGPHDKIENAKDLNESFPYLDQVYKEAMRIY	397
	PhMAX1	TTSFTLSSIIYLVAGHPKVEQKLAIEIDAFGLDQVETABDLESKFPPYLDQVYKEAMRCY	401
	Consensus	t ftls ylv ve l eid fg d p a dl f yldqv ke r y	
	CmMAX1	TASPLVAREAVKEVEVGGYVLPKGTWVWVALGVLAKNPKDFPEPEMFIPERIDPNCNEEK	464
	MAX1	MVSPLVARETAKEVEIIGGYLLPKGTWVWVALGVLAKNPKDFPEPEMFIPERIDPNGEEK	367
	MtMAX1	TVSPLVARETSNEVEIIGGYLLPKGTWVWVALGVLAKNPKDFPEPEMFIPERIDPCKGEMK	457
	PhMAX1	TVSPLVARETSAEVEIIGGYLLPKGTWVWVALGVLAKNPKDFPEPEMFIPERIDPNCNEEK	461
	Consensus	splvare eve ggy lpkgtwvw al vlak epe fperidp e k	
	CmMAX1	HRHPYANIPFGIGPRMCLGQFALQEIKLALYLYQRYVFRHSNNFNPLEFEYSIILNF	524
	MAX1	HRHPYAFIPFGIGPRACVCGQFALQEIKLTLHLRYNYIFRHSLEMEIPLQLDYGIILSF	427
	MtMAX1	HRHPYAFIPFGIGPRACVCGQFALQEIKLTLHLRYNYIFRHSLEMEIPLQLDYGIILNF	517
	PhMAX1	QRHPYANIPFGIGPRACVCGQFALQEIKLTLHLRYNYIFRHSPLMEKELELEYGIVLNY	521
	Consensus	rhpya ipfgigpr c qg f qeikl l ly y frhs p y l	
	CmMAX1	KYGVKVIATKR	535
	MAX1	KNGVKLRITKR	438
	MtMAX1	KHGIKLRVTKR	528
	PhMAX1	KHGVKVCATKR	532
	Consensus	k g k ikr	

Fig. 1. Sequence alignments and phylogenetic analysis of CmMAX1 in *Chrysanthemum morifolium*. (A) Alignments of the predicted amino acid sequences of CmMAX1 compared with *Arabidopsis thaliana* (MAX1), *Medicago truncatula* (MtMAX1), and *Petunia hybrida* (PhMAX1). Three conserved functional domains—P (proline) F (phenylalanine) G (glycine) × GPR (arginine) × C (cysteine) × G, PE (glutamic acid) RF, and K (lysine) ExxR—are marked by a black frame. (B) Phylogenetic analysis. The phylogenetic tree was constructed using the maximum likelihood method. The accession numbers of selected MAX1s are as follows: *Manihot esculenta* MeMAX1 (XP_021598065.1), *Hevea brasiliensis* HbMAX1 (XP_021681567.1), *Jatropha curcas* JcMAX1 (XP_012077261.1), *Populus euphratica* PeMAX1 (XP_011019422.1), *Gossypium hirsutum* GhMAX1 (XP_016689695.1), *Durio zibethinus* DzMAX1 (XP_022731995.1), *Theobroma cacao* TcMAX1 (XP_007012311.1), *Gossypium arboreum* GaMAX1 (XP_017619614.1), *Ziziphus jujuba* ZjMAX1 (XP_015884519), *Prunus avium* PaMAX1 (XP_021823617.1), *Rosa chinensis* RcMAX1 (XP_024165263.1), *Juglans regia* JrMAX1 (XP_018844671.1), *A. thaliana* MAX1 (NP_565617.2), *Citrus sinensis* CsMAX1 (XP_006475901.1), *Ipomoea nil* InMAX1 (XP_019190529.1), *Sesamum indicum* SiMAX1 (XP_020550592.1), *Erythranthe guttatus* EgMAX1 (XP_012857912.1), *Capsicum annuum* CaMAX1 (XP_016537773.1), *P. hybrida* PhMAX1 (AEB97383.1), *Nicotiana attenuata* NaMAX1 (XP_019250188.1), *Vitis vinifera* VvMAX1 (XP_002279086.3), *Nelumbo nucifera* NnMAX1 (XP_010262061.1), *Punica granatum* PgMAX1 (PK170646.1), *Salvia miltiorrhiza* SmMAX1 (AJD25243.1), and *M. truncatula* MtMAX1 (AGI65360.1).

Fig. 1. (Continued)

reverse primer (5'-AAGTCCATCTTVAGTTGHGTTGT-3'), the 3' and 5' ends of *CmMAX1* were amplified using the 3' RACE System for Rapid Amplification of cDNA Ends (Invitrogen) and the 5'-full RACE core set (Takara), respectively. The *CmMAX1* full-length cDNA was isolated by combining the three parts of the fragments. The products amplified using PrimeStar HS DNA Polymerase (Takara) were cloned into the *PEASY*-Blunt Simple Cloning Vector (Trans, Beijing, China) to determine the sequence of *CmMAX1*. The protein molecular formula was determined using the ProtParam analysis tool on an ExPASy server (Gasteiger et al., 2005). Alignment of multiple sequences was carried out using DNAMAN software (version 5.2.2; Lynnon Biosoft, San Ramon, CA). Phylogenetic analysis was conducted using the MEGA 5.05 program (Tamura et al., 2011) by the maximum likelihood method.

QUANTITATIVE REAL-TIME PCR (qRT-PCR) AND SEMIQUANTITATIVE REVERSE TRANSCRIPTION ANALYSIS. For the experiment of *CmMAX1* expression in different tissues, roots, stems, leaves, axil, and apex were collected from 30-d-old chrysanthemum seedlings. The P deficiency experiment was conducted by treating the roots of the chrysanthemum seedlings with P-free nutrient solution for 12 h, then the roots were harvested for the further experiments. For the hormone treatment experiments, 5 μ L of 2 μ M GR24, 6-benzylamino adenine (6-BA; 50 μ M), or indole-3-acetic acid (IAA; 100 μ M) solutions were applied to node 4 (the fourth bud from the top

Fig. 2. Quantitative real-time polymerase chain reaction analysis of total *CmMAX1* expression in *Chrysanthemum morifolium*. All samples were taken from three pools of 15 plantlets, and the experiment was repeated three times. Detection of *Tublin* was used as a complementary DNA normalization control. Vertical bars indicate SE. Lowercase letters a and b indicate significant differences.

Fig. 3. Expression of *CmMAX1* in *Chrysanthemum morifolium* treated under normal conditions (watered with nutrient solution) and under a phosphorus (P) deficiency (watered with P-free nutrient solution for 12 h). Each value represents the means of three biologic replicates. Vertical bars indicate SE. Lowercase letters a and b indicate significant differences.

Fig. 4. Expression of *CmMAX1* in *Chrysanthemum morifolium* treated under normal (without treatments) and 5 μ L of 2 μ M GR24 (a synthetic analog of strigolactone), 50 μ M 6-benzylamino adenine (6-BA), or 100 μ M indole-3-acetic acid (IAA) for 2 h. Vertical bars indicate SE. Lowercase letters a and b indicate significant differences.

downward) of 30-d-old chrysanthemum seedlings. After 2 h, the buds treated with different hormones were collected. RNA extraction, qRT-PCR, semiquantitative reverse transcription,

Fig. 5. Overexpression of *CmMAX1* in *Chrysanthemum morifolium* partially rescued the phenotype of *Arabidopsis thaliana max1-1* mutant. (A) Comparison of phenotypes of wild type (WT), empty vector control (EVC), *max1-1*, and *max1-1* transformed with the 35S::CmMAX1 construct. (B) Transcripts were assayed by reverse transcriptase polymerase chain reaction from total RNA of leaves. Detection of the *UBQ* transcript was used as a complementary DNA normalization control. (C) The number of secondary rosette branches produced by WT, EVC, *max1-1*, and four independent homozygous lines carrying 35S::CmMAX1. (D) The height of the main branches of WT, EVC, *max1-1*, and four independent transgenic lines are shown. Error bars represented the sd of the means. Lowercase letters a, b, and c indicate significant differences.

and analysis were performed as described by Dong et al. (2013). To analyze the transcription from the transgenes in 35S::CmMAX1 *max1* lines, primers 5'-GAGTTGAAGTTGGAGGT-TACG-3' (forward) and 5'-GACCAAGGCACATTCGAGG-3' (reverse) were used. *Ubiquitin (UBQ)* was used as a normalization control with primers 5'-AACCTTGAGGTTGAATCATC-3' (forward) and 5'-GTCCTTCTTTCTGGTAAACGT-3' (reverse) (Liang et al., 2010).

DEVELOPMENT OF CONSTRUCT, ARABIDOPSIS TRANSFORMATION, AND PHENOTYPE ANALYSIS. The full length of the *CmMAX1* cDNA sequence was amplified with 5'-ATGAGCACTTCTTTCT-TAGTTTA-3' (forward) and 5'-TTACATTCGTTTAATGGC-TATGAC-3' (reverse). Plasmid 35S::CmMAX1 was made by ligating the open reading frame (ORF) of *CmMAX1* into the binary vector PBI121 at the XbaI and SacI sites. The construct was transformed into arabidopsis mutant *max1-1* plants via *Agrobacterium tumefaciens* strain LBA4404 by the floral-dip method (Clough and Bent, 1998). Transformants were screened using a Murashige and Skoog medium containing 50 mg·L⁻¹ kanamycin. Homozygous T₃ lines were identified and used in this study. The number of rosette branches was scored and the height of the main stem was measured for phenotype analysis.

STATISTICAL ANALYSIS. All data were repeated at least three times. Tukey's test at $P < 0.05$ was used to evaluate the degree of rescue of the *max1* phenotype by the *CmMAX1* gene.

Results

CLONING OF CmMAX1 AND SEQUENCE ANALYSIS OF CmMAX1. *CmMAX1* cDNA included a complete ORF of 1611 bp, which encoded proteins of 536 amino acids. The deduced amino acid sequence contained a heme-binding motif (PFG × GPR × C × G), PERF, and a K-helix motif (KExxR), which were the most highly

conserved regions of P450 (Poulos et al., 1987), with a calculated molecular mass of 61.29 kDa and an isoelectric point of 8.92. It was speculated that the molecular formula of the CmMAX1 protein was C₂₈₂₂H₄₃₇₈N₇₂₀O₇₇₁S₁₇.

PHYLOGENETIC ANALYSIS OF CmMAX1 HOMEODOMAIN PROTEINS. Sequence alignment of the deduced amino acid sequence of the chrysanthemum MAX1 ortholog with other known MAX1 proteins was performed (Fig. 1A). CmMAX1 shared 60.7%, 60.7%, and 57.9% similarity with PhMAX1, MtMAX1, and MAX1 proteins, respectively. To investigate the evolutionary relationships among the predicted MAX1 proteins, a phylogenetic tree was constructed by an alignment of MAX1 proteins from a taxonomically diverse set of species (Fig. 1B). The phylogenetic tree revealed that CmMAX1 was closest to NnMAX1.

TISSUE SPECIFICITY OF CmMAX1 EXPRESSION. To identify the tissues in which *CmMAX1* is most highly

expressed, the level of *CmMAX1* transcripts in different tissues was examined. As shown in Fig. 2, the *CmMAX1* transcript was detected in all tissues examined. The difference of *CmMAX1* expression in roots, stems, leaves, and axil was not significant, and the expression level of *CmMAX1* in apex was lowest.

P DEFICIENCY IMPROVED EXPRESSIONS OF CmMAX1. To study the relationship between *CmMAX1* expression and P deficiency, we analyzed the expression of *CmMAX1* in chrysanthemum seedlings treated with a P deficiency. Our results showed that the expression level of *CmMAX1* in P-deficient plants increased about six times compared with the control (Fig. 3).

EXPRESSION OF CmMAX1 WAS REPRESSED BY DIFFERENT PHYTOHORMONES. To study the regulation of *CmMAX1* by strigolactone, auxin, and cytokinin, we detected the expression of *CmMAX1* after 2 h of treatment with GR24, IAA, or 6-BA. The results showed that all three hormones repressed the expression of *CmMAX1*, and the expression level of *CmMAX1* decreased to about 0.21, 0.27, and 0.32 of the control, respectively (Fig. 4).

FUNCTIONAL ANALYSIS OF CmMAX1. The *CmMAX1* ORF driven by cauliflower mosaic virus 35S promoter (35S::CmMAX1) was expressed in the arabidopsis *max1-1* mutant background. Four independent lines—line 1, line 3, line 6, and line 7—were selected for further analysis. As expected, all four lines displayed high expression levels of the *CmMAX1* gene. The number of rosette branches with a length of at least 5 mm was scored to quantify the degree of rescue of the *max1-1* mutant phenotype by the *CmMAX1* gene overexpression. *CmMAX1* expression reduced the mean number of branches from 8.2 in *max1-1* to 3.25, 3.05, 4.2, and 3.5, respectively (Tukey's test at $P < 0.05$; Fig. 5A and B), improving the height of plants from 202 mm in *max1* to 266.8, 270.3, 255.5, and 263 mm (Tukey's test at $P < 0.05$; Fig. 5A and C).

Discussion

MAX1 encodes a cytochrome P450 family member that acts downstream of D27 to produce a carotenoid-derived branch-inhibiting hormone (Waters et al., 2012). Mutation of *MAX1* led to the bushy phenotype in arabidopsis (Stirnberg et al., 2002). However, *MAX1* orthologs were absent from *Physcomitrella*, present in monocots and eudicots as multiple copies and as a single copy, respectively, which indicated that *MAX1* had a different pattern compared with the other genes. Thus, studies on *MAX1* have important implications for understanding the evolution of the MAX pathway. In addition, expression and functional analysis of *MAX1* orthologs will provide valuable information for molecular breeding.

In this study, another ortholog of *MAX1* was cloned from chrysanthemum cultivar Jinba, then submitted to Nelson's P450 databases, and named *CYP711A34* (Nelson et al., 1993). We found only one copy existed in chrysanthemum, which was consistent with the hypothesis that *MAX1* is generally present as a single copy in eudicots (Hepworth, 2012). Sequence analysis revealed that *CmMAX1* had deduced an amino acid sequence that contains heme-binding motif PFG × GPR × C × G, the PERF motif, and a K-helix motif KExxR. All three motifs were known to be involved in heme-binding or thought to stabilize the heme-binding pocket (Paquette et al., 2009). A phylogenetic tree based on the amino acid sequences was constructed. It showed that all the CYP711 members originated from the same ancestral origin, but diverged subsequently at different phases of evolution. *CmMAX1* was closely related to *NnMAX1* from lotus. It shared 55.33% amino acid similarity with *NnMAX1*, demonstrating that *CmMAX1* was definitely a member of CYP711 according to the theory that P450 proteins with more than 55% similarity were grouped into the same subfamily (Mizutani and Ohta, 2010).

During the transition phase from vegetative growth to reproductive growth in plants, the hormone levels will be altered, which might lead to changes in gene expression. Given this, we tested the expression of *CmMAX1* during the vegetative growth stage. Expression analysis showed that *CmMAX1* in detected tissues varied little. This result was different from the previous report in petunia, in which *PhMAX1* was expressed highest in the low-stem samples (Drummond et al., 2012).

Studies in multiple species have shown that the biosynthesis of strigolactones is increased during P deficiency (Wang et al., 2015; Xi et al., 2015; Yoneyama et al., 2007). P starvation upregulated the expression level of *DgCCD7* and *DgCCD8* in chrysanthemum (Xi et al., 2015). In our study, the expression levels of *CmMAX1* increased significantly compared with normal condition, indicating P deficiency can induce the expression of *CmMAX1*.

Strigolactone, auxins, and cytokinin are the most important hormones regulating shoot branching. Strigolactone and cytokinin were thought to be the second messengers of auxin to inhibit or promote directly the growth of axillary buds (Domagalska and Leyser, 2011). *CmMAX1* decreased significantly after 2 h of GR24 treatment, which indicated there was a feedback regulation in the synthesis pathway of strigolactone. The application of 6-BA caused a decrease in the expression of *CmMAX1*, suggesting that cytokinins may regulate the synthesis of strigolactone by inhibiting the expression of *CmMAX1*. Studies in arabidopsis showed that *MAX3* and *MAX4* transcripts are regulated positively by auxin (Hayward et al., 2009). However, IAA treatment led to

the significant decrease of *CmMAX1* expression (Fig. 4), suggesting the complex regulation of the strigolactone synthesis pathway by auxin.

The overexpression of *CmMAX1* in arabidopsis not only reduced *max1-1* branching, but also increased plant height (Fig. 3C). This result demonstrates that *CmMAX1* is a functional gene. Overexpression of *PgMAX1* (*Picea glauca*) in arabidopsis was found to be capable of complete rescue of *max1-1*, and the overexpression of *SmMAX1* (*Selaginella moellendorffii*) obviously reduced branching, although *SmMAX1* only shares 38.7% amino acid similarity with *MAX1* (Hepworth, 2012). These results indicated the partial conservation of *MAX1* function across a wide evolutionary range.

We obtained the full length of *CmMAX1* cDNA from chrysanthemum cultivar Jinba, and detected the expression of tissues and different treatments. Moreover, we confirmed the function of the *CmMAX1* gene in vivo by the complementation experiment. Our results presented here reveal that the *MAX1* ortholog was present and active in chrysanthemum, and also that *CmMAX1* might be a candidate gene for the regulation of shoot branching.

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