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Research article

Ectopic expression of Chrysanthemum *CDM19* in *Arabidopsis* reveals a novel function in carpel development

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ABSTRACT

Background: APETALA3 (*AP3*) has significant roles in petal and stamen development in accordance with the classical ABC model.
Results: The *AP3* homolog, *CDM19*, from *Chrysanthemum morifolium* cv. Jinba was cloned and sequenced. Sequence and phylogenetic analyses revealed that *CDM19* is of *DEF*/*AP3* lineage possessing the characteristic MIKC-type II structure. Expression analysis showed that *CDM19* was transcribed in petals and stamens of ray and disc florets with weak expression in the carpels. Ectopic expression of *CDM19* in Arabidopsis wild-type background altered carpel development resulting in multi-carpel siliques. *CDM19* could only partially rescue the Arabidopsis *ap3-–3* mutant.
Conclusions: Our results suggest that *CDM19* may partially be involved in petal and stamen development in addition to having novel function in carpel development.
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1. Introduction

Flower development is one of the key pillars in the evolutionary success of angiosperms [1]. In higher angiosperms, the main conserved structure of the flower includes from the periphery to the center: sterile perianth (sepals in the first and petals in the second whorl) and reproductive organs (stamens in the third and carpels in the fourth whorl) [1,2,3,4]. Development of these floral organs is directed by transcription factors (TFs), which are mainly in the MADS-box family. These TFs effect organ formation in a combinatorial manner in the now-so-called ABCDE model [5,6,7]. In Arabidopsis, A-function genes including *AP1* and *AP2* (a non-MADS box gene) which are responsible for sepal development and in combination with B-function genes (*AP3* and *PI*) specify the identity of petals. The B-function genes together with *AGAMOUS* (*AG*), a C-function gene, specify the identity of the stamens while carpel development is determined by C-function genes [8,9]. The largely redundant E-function genes *SEPALLATA* (*SEP1*), *SEP2*, *SEP3* and *SEP4* are essential for the formation of all the four types of floral organs [10,11]. The SEP proteins interact directly with the products of the ABC genes forming higher order complexes that specify floral organ identity [1,4,12]. This finding has led to the postulation of the 'floral quartet model' showing the combinatorial role played by the MADS-box protein tetrameric complexes in specifying floral organs [13,14].

The molecular basis of flower formation has been enhanced by the availability of floral homeotic mutants and isolation of the corresponding genes. Research on *Arabidopsis thaliana*, *Antirrhinum majus*, and *Petunia hybrida* have since led to the characterization of various MADS-box floral homeotic genes [15,16,17,18]. A gene duplication event predating angiosperm diversification gave rise to the B-class proteins APETALA3 (AP3) and PISTILLATA (PI) [19]. The B-class function is highly conserved in both monocots and eudicots with respective mutants exhibiting similar phenotypes for petal and stamen development [20]. *AP3* expression is confined to the 2nd and 3rd whorl at early development stages and expressed at low levels in mature siliques [21].

PI has a similar expression profile in addition to its expression in whorl 4 during early stages of floral development [19,22]. The AP3/PI heterodimer directly controls few transcription factors and a number of genes with basic cellular functions required for petal and stamen morphogenesis [23]. The heterodimer positively and directly regulate *AP3* through binding to the CArG box consensus sequences in the *AP3* promoter [23,24]. Some direct targets of AP3/PI include *APETALA1* (*AP1*), *NAP* (*NAC-LIKE, ACTIVATED BY AP3/PI*), encoding protein involved in the transition between cell division to cell expansion

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during petal and stamen development [25], two genes of GATA proteins involved in chlorophyll biosynthesis, carbon and nitrogen metabolism [26] and *BANQUO* genes involved in light signaling photomorphogenesis and regulation of flowering time [24]. Genome-wide identification of AP3/PI binding sites and target genes in Arabidopsis indicated that the heterodimer controls the expression of more than 460 genes, many of which are transcription factors required in different pathways during petal and stamen development [23,27]. Apetalous and stamen carpelloid phenotypes in *Brassica rapa* and *B. napus* were shown to be due to mutations in *AP3* genes [28]. Similarly, suppression of the ornamental *Tricyrtis sp*. B-function gene was associated with the existence of petaloid tepals. Plants with suppressed B class genes produced sepaloid tepals in whorl 1 and 2 confirming the involvement of the B proteins in imparting petal characteristics in whorl 1 [29].

In the Asteraceae family, the flower head is composed of three different lateral organs including the phyllaries, ray and disc florets. Phyllaries are modified leaves mimicking sepals of the solitary flower [30,31]. The chrysanthemum (*Chrysanthemum morifolium)* capitulum is a specialized flower structure consisting of many florets which are highly compressed thus resembling a solitary flower [32,33]. The morphology of the florets is diverse as a result of their corolla symmetry and fertility consisting of the marginal ray florets (female) and the central tubular disc florets (bisexual) [33]. Studies in gerbera, another member of the Asteraceae family, indicated that floral organ identity regulation was highly conserved based on functional analysis of organ identity proteins [16,34], albeit different from Arabidopsis, owing to the differences in flower structure. AP3 homologs *CDM115* and *CDM19* have been identified in chrysanthemum; however, their function is unknown [35].

The current study focused on determining the function of chrysanthemum B-function protein CDM19. Results from this study suggest that *CDM19* is partly involved in petal and stamen specification in addition to novel functions in carpel development. Ectopic expression of *CDM19* in Arabidopsis wild-type background led to the development of multi-carpels, while *ap3-3* mutant complementation lead to a weak rescue phenotype with staminoid organs and sepaloid petals with white margins.

2. Materials and methods

2.1. Plant materials and growing conditions

Chrysanthemum cultivar 'Jinba' was obtained from the Chrysanthemum Germplasm Resource Preserving Centre, Nanjing Agricultural University, China, where the average day/night temperature is 26/18°C and relative humidity of 70%. Arabidopsis *ap3-3* mutant line (CS3086) ecotype Landsberg *erecta* background was obtained from the Arabidopsis Biological Center, Ohio State University, OH, USA. Plant growth conditions followed the standard regimes.

2.2. Cloning of CDM19 and sequence analysis

Total RNA was isolated from 'Jinba' inflorescence buds at bud break-stage using the RNAiso reagent (TaKaRa, Tokyo, Japan) following the manufacturer's instructions then treated with RNase-free DNase I (TaKaRa, Japan) to eliminate any contaminating genomic DNA. The initial cDNA strand was synthesized from a 1-μg aliquot of total RNA using M-MLV reverse transcriptase (TaKaRa), following the manufacturer's protocol. A *CDM19* complete coding sequence homologous to the 'Parliament' deposited in NCBI database (AY173064.1) was amplified using the specific primer pair (CDM19-F and -R, Table 1). The PCR products were purified and inserted into pMD19-T (TaKaRa) for sequencing to confirm the fidelity of the obtained fragment.

Table 1
Sequences of the PCR primers used.

Name	Sequence (5′-3′)
CDM19- F	ATGGCTAGAGGAAAGATCCA
CDM19- R	ACCAAACAAGCCATAAGT
ap3-3-F	GATCAAGAGGATAGAGAACCAGACAAATCGA
ap3-3-R	CGCATCAAGAATTTAACCAACCAGCG
CDM19-GFP-F	CGGGGTACCGAATGGCTAGAGGAAAGATCCA (*Kpn* I)
CDM19-GFP-R	ATTTGCGGCCGCACCAAACAAGCCATAAGT (*Not* I)
CDM19-RT-F	TGTTTGGAAGATCTTGGCTTTG
CDM19-RT-R	TGTATACATCTTGGGCACTCCT
AtActin-F	CCTTCGTCTTGATCTTGCGG
AtActin-R	AGCGATGGCTGGAACAGAAC
CDM19-test-F	TGTTTGGAAGATCTTGGCTTTG
CDM19-test-R	TGTATACATCTTGGGCACTCCT
CmEF1α-F	TTTTGGTATCTGGTCCTGGAG
CmEF1α-R	CCATTCAAGCGACAGACTCA

2.3. Phylogenetic analysis

The amino acid sequences of CDM19 orthologs were obtained from NCBI database (https://www.ncbi.nlm.nih.gov/) and used to conduct phylogenetic analysis based on the neighbor-joining method in MEGA 5.0 software. Bootstrap values were estimated from 1000 replicates.

2.4. Subcellular localization of CDM19

The open reading frame (ORF) of *CDM19* was amplified with the primer pair CDM19-GFP-F/ R (Table 1) using a Phusion High-Fidelity PCR kit (New England Biolabs, Ipswich, MA, USA). The amplicon and the pENTR™1A dual selection vector (Invitrogen Carlsbad, CA, USA) were double-digested with *Kpn I* and *Not I*. The fragments were then ligated with T4 DNA ligase (TaKaRa) and sequenced to validate the fidelity of the construct. To obtain *CDM19-GFP*-construct, the pENTR™1A-*CDM19* fusion was subjected to LR reaction using Gateway technology (Invitrogen) and the binary vector pMDC43 [35]. The pMDC43-*CDM19* and empty pMDC43 vectors were transiently expressed in onion epidermal cells via particle bombardment with a PDS-1000 device (Bio-Rad, Hercules, CA, USA), following the manufacturer's instructions. To act as a nuclear marker, p*35S::D53-RFP* construct was co-transformed alongside the CDM-GFP construct. Following incubation of the onion cell in the dark at 28°C for 16 h, the GFP signal activity was monitored by confocal laser scanning microscopy (Leica TCS SP5).

2.5. Transactivation assay

The *CDM19* ORF was introduced into the *EcoR1*/*Pst1* cloning site of the pGBKT7 vector (Clontech, Mountain View, CA, USA) and transformed into *Saccharomyces cerevisiae* strain Y2HGold (Clontech) to test the transactivation activity. Selection for transformants carrying either *pGBKT7-CDM19* or *pGBKT7* employed an SD/-Trp medium, whereas pCL1 was selected on SD/-Leu medium.

2.6. Yeast two-hybrid assay

The *pGBKT7-CDM19* construct was transformed into the yeast strain *AH109* for screening the chrysanthemum cDNA libraries. Full lengths of positive clones were ligated into the *pGADT7* vector (Clontech) at the *EcoRI* and *XhoI* cloning sites. The recombined constructs were co-transformed with *pGBKT7-CDM19* to *AH109* yeast cells for further validation. Similarly, proteins including A-function protein CmAP1 (BAN19222.1), B-function protein CDM86 (AAO22986.1), a PI-orthologue and E-function protein CmSEP3 were also tested for interaction with CDM19 in both directions as either bait or prey

constructs. All protocols were performed following the manufacturer's instructions (Clontech).

2.7. Transcription profiling by qRT-PCR

Total RNA was extracted from various chrysanthemum tissues including leaf, ray petal, ray pistil, disc petal, stamen, and pistil using the RNAiso reagent (TaKaRa) following the manufacturer's instructions. The RNA was treated with RNase-free DNase I (TaKaRa) to remove genomic DNA contamination. The first cDNA strand was synthesized from 1-μg total RNA using M-MLV reverse transcriptase (TaKaRa) following the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) using SYBR Premix Ex Taq™ II (TaKaRa) was used to detect the expression of *CDM19*. Each 20-μL qRT-PCR reaction contained 10-μL SYBR Green PCR master mix, 1 μL of each primer, 3-μL H_2O, and 5-μL cDNA template. The PCR cycling regime comprised an initial denaturation (95°C for 120 s), followed by 40 cycles of 95°C for 10 s, 55°C for 15 s, and 72°C for 20 s. Three independent biological replicates were performed. The resulting data were represented by the mean ± SD of three biological replicates. The primer pair *CDM19*-RT-F/R was used and *CmEF1α* (KF305681) as an endogenous control in chrysanthemum. The relative expression levels of related genes were calculated using the $2^{-\Delta\Delta Ct}$ method [36]. All primer pairs for expression analysis are listed in Table 1.

2.8. Binary vector construction and Arabidopsis transformation

The coding region of *CDM19* with or without a stop codon (overexpression and repression, respectively) was cloned into entry

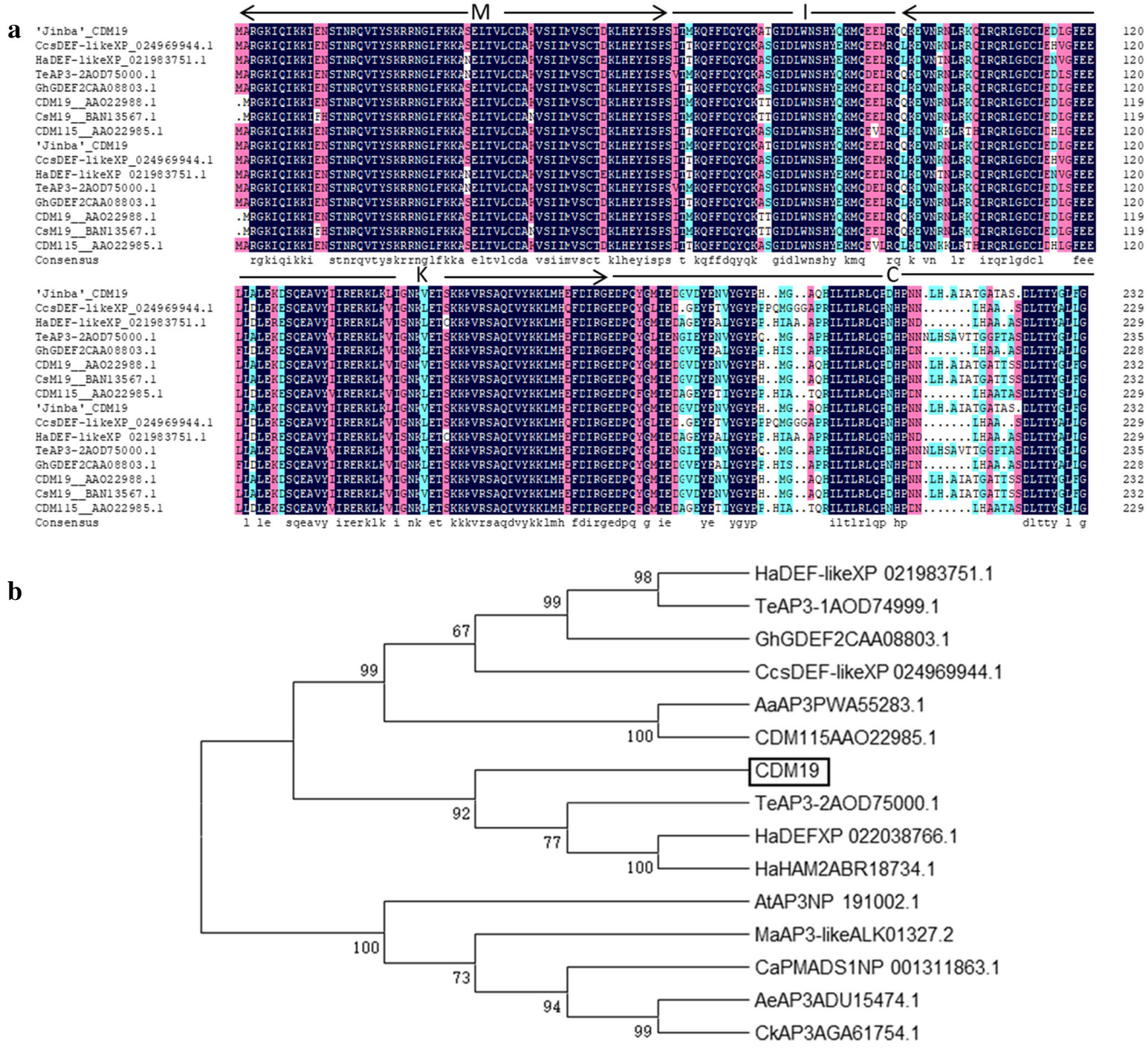

Fig. 1. Putative peptide sequences of CDM19 and phylogeny with other DEF/AP3 proteins. (**a**) Alignment of CDM19 amino acid sequence with other homologous proteins. The MIKC domains are highlighted. (**b**) Phylogenetic tree showing homologs of CDM19 from other species. ClustalW was used to align the amino acid sequences, and the neighbor- joining method was used to build the phylogenetic tree with MEGA5.0. The accession numbers for the sequences are listed below: Ccs DEF-like (*Cynara cardunculus* var. scolymus, XP_024976425.1), HaDEF-like (*Helianthus annuus*, XP_022038766.1), TeAP3–2 (*Tagetes erecta*, AOD75000.1), HaHAM2 (*Helianthus annuus*, ABR18734.1), GhDEF2 (*Gerbera hybrida*, CAA08803.1), AaAP3 (*Artemisia annua*, PWA55283.1), CDM115 (*Chrysanthemum x morifolium*, AAO22985.1), CcsDEF-like (*Cynara cardunculus* var. scolymus, XP_024969944.1), HaDEF-like (*Helianthus annuus*, XP_021983751.1), TeAP3–1(*Tagetes erecta*, AOD74999.1), MaAP3-like (*Mercurialis annua*, ALK01327.2), AeAP3 (*Actinidia eriantha*, ADU15474.1), CaPMADS 1 (*Capsicum annuum*, NP_001311863.1), CkAP3 (*Cornus kousa*, AGA61754.1). CDM19 (*C. morifolium* cv. Jinba,) is boxed.

clone, pENTR™1A using *Not1*/*Kpn1* restriction enzymes, confirmed to be in frame through sequencing and then shuttled into plant expression vector *35AA-SRDX_BCKH* under the control of the cauliflower mosaic virus 35S promoter in the sense orientation to generate the plasmid *35S::CDM19*. The constructs were individually transformed into *A. tumefaciens* strain *EHA105* using the freeze–thaw transformation method. The recombinant vector, in which the stop codon had been added, was transformed into heterozygous *ap3-3 A. thaliana* plants using the floral dip method as suggested by Clough and Bent [37]. Transformed progenies were selected by culturing the seeds on 0.5× MS medium agar plates containing 20 μg mL^{-1} hygromycin B and transplanted to potting mixture while at 4-leaf stage and grown under long-day conditions (16-h light/8-h dark). The presence of the transgene was checked by PCR analysis and the expression level monitored using RT-PCR based on transgene-specific primers and *A. thaliana* β-actin as the internal control. Homozygous *ap3-3* transformants were isolated using a dCAPS marker designed with the dCAPS finder program [38]. The same transformational procedure was followed in transforming wild type (Columbia ecotype) for ectopic expression of *CDM19* (with stop codon) or as a chimeric repressor (without the stop codon). Transformed progenies were advanced by self-pollination to obtain the T3 generation. Transgene zygosity was checked using RT-PCR based on the transgene-specific primer pair CDM19-test-F/R.

3. Results

3.1. Cloning and sequence analysis of Chrysanthemum CDM19

The MADS-box gene *CDM19* was previously identified in *Chrysanthemum morifolium* cv. Parliament [39], and the complete coding sequence of the gene 696-bp long is available in the NCBI databank (AY173064.1). We compared the data for this sequence, the *Chrysanthemum seticuspe* genome (BDUE01042532.1) and the highly homologous to the *CDM19* genes, *Helianthus annuus* HaDEF-like (XP_022038766.1) and HaHAM2 (ABR18734.1) and *Tagetes erecta* TeAP3-2 (AOD75000.1), and developed primers for cloning a CDM19-homolog from *C. morifolium* cv. Jinba (Table 1). CDS of 'Jinba' *CDM19* was cloned and sequenced. Compared to the 'Parliament' *CDM19*, it was one triplet shorter (699 vs. 696 bp), has non-synonymous SNPs, and encoded a protein of 233 amino acid residues, consisting of highly conserved MADS and Keratin-like (K) domains, intervening (I) region connecting the domains, and variable C-domain (Fig. 1a).

The phylogenetic analysis showed that CDM19 forms a clade with HaHam2 and HaDEF from *Helianthus annuus* and TeAP3-2 from *Tagetes erecta* (Fig. 1b), indicating that these proteins may share conserved function in Asteraceae species; whereas, CDM19 forms diverse clades with AtAP3 suggesting that CDM19 may function differently in chrysanthemum.

3.2. Subcellular localization, transcription profiling and transcriptional activation of CDM19

Fused protein CDM19::GFP was shown to be localized throughout the cell including cytoplasm and nuclear (Fig. 2a). In yeast-based transcriptional activation assay cells harboring *pDEST-GBKT7-CDM19*, in which CDM19 had been fused in frame to the sequence encoding GAL4 DNA binding domain showed no growth on SD/-His-Ade medium indicating that CDM19 is not functional as a transcriptional activator, at least in the heterologous system tested (Fig. 2b).

A qRT-PCR analysis indicated that *CDM19* was expressed in various floral tissues with high expression in ray petals, disc petals and stamens of chrysanthemum cultivar Jinba (Fig. 3a).

Transcripts of *CDM19* were also detected in ray and disc pistils, although at lower levels (Fig. 3a). In developing inflorescences, transcription of *CDM19* increased as the buds grow (Fig. 3b).

3.3. Protein–protein interactions between CDM19 and other proteins

Using the yeast GAL4 two-hybrid system, CDM19 has been shown to interact with chrysanthemum CDM86, suggesting that CDM19 protein–protein interactions are characteristic of plant B-class MADS-domain proteins. However there was no direct interaction between CDM19 and CmAP1 or CmSEP3 (Fig. 3c).

3.4. Ectopic expression of CDM19 in Arabidopsis

Arabidopsis wild-type plants that ectopically expressed *CDM19* did not show any homeotic conversion despite the high expression levels in these lines (Fig. 3d). However, carpel development was altered in

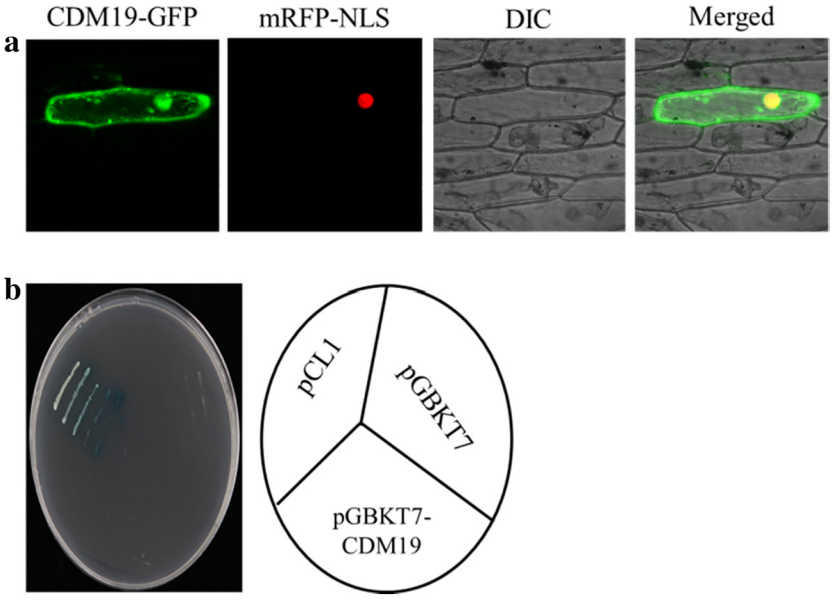

Fig. 2. Analysis of sub-cellular localization and transactivation of CDM19. (**a**) Transient expression in onion epidermal cells. Bar: 100 μm. (**b**) Analysis of the ability of CDM19 to activate gene transcription in vivo in a yeast cell. Cells harboring pCL1 (positive control) were able to grow on SD/-His-Ade medium (SD, Synthetic Dropout Media), while those containing pGBKT7 (negative control) or pDEST-GBKT7-*CDM19* showed no growth. Inoculation pattern is shown on the right.

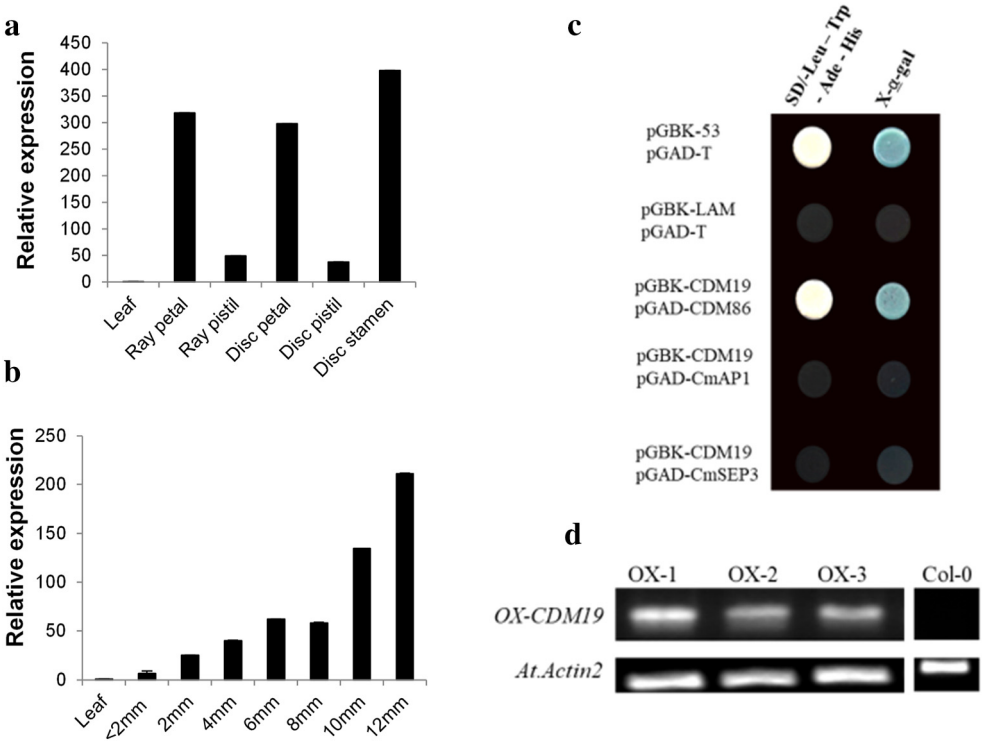

Fig. 3. *CDM19* transcriptional pattern and interaction of CDM19 with other proteins. (**a**) Expression in various chrysanthemum floral organs and (**b**) inflorescence buds of different sizes as assayed by qRT-PCR. (**c**) Interaction of CDM19 with either CDM86 (AAO22986.1), CmAP1(BAN19222.1) or CmSEP3 in yeast two-hybrid analysis. (**d**) Transcripts abundance of *CDM19* in three transgenic overexpression lines and WT plants.

the overexpression lines (Fig. 4). The severity of deformation of the carpels varied with most inflorescences having coiled, bumpy and multi-carpel siliques. The resulting phenotypes suggest that ectopic accumulation of CDM19 in the meristem region corresponding to the pistil disrupts the traditional protein–protein interactions of the carpel-specific MADS-domain transcription factors and, as a result, changes the development of the carpels.

Expression of the *35S::CDM19-SRDX* in wild-type Arabidopsis plants showed a possible role of CDM19 in regulating petal and stamen development (Fig. 5). These *SRDX* repression lines were characterized by short petals and stamen filaments. Due to the inability of the stamens to reach the stigma for pollination, the flowers of the repression lines were unable to set fertile seeds in the developing siliques [40,41].

To assess the potential ability of CDM19 to perform the function of AP3 and replace AP3 in protein–protein interactions, we fused the CDM19 protein with the SRDX-domain, derived from the EAR-motif, a repression domain in plants. The use of this construct should prevent the expression of possible target genes of the multimeric transcriptional complex, where CDM19 can replace AP3. In the case of strong competence between CDM19 and AP3, the resulting plants should have the ap3 loss-of-function phenotype [40,41].

The resulting 35S::CDM19-SRDX Arabidopsis flowers were characterized by shortened petals and filaments compared with wild-type Arabidopsis (Fig. 5). Moreover, due to the inability of the stamens to reach stigma for pollination, the flowers of the repression lines were unable to fertilize the ovules and set seeds in the developing siliques.

Thus, we assume that either CDM19 is capable of only slightly replacing AP3 in protein complexes, or the competitive complexes formed with CDM19 are not fully functional, however, the observed effect indicates the possible role of CDM19 in regulating the development of petals and stamens.

In the *35S::CDM19* Arabidopsis *ap3-3* mutant complementation lines, *CDM19* showed varying degrees of stamen and petals rescue (Fig. 6). A number of independent transformants in the *ap3-3* homozygous background were recovered, which showed a range of phenotypes. For ease of describing the phenotypes, the degree of rescue was classified as either 'weak' or 'moderate'. Flowers in the weak rescue category had mosaic organs in whorl three consisting of carpelloid characteristics (flat or tubular organs with ovules and stigmatic tissue) with 1 or 2 filament-like organs and the four sepaloid petals in whorl two (Fig. 6b–d, Fig. 6g–i, Fig. 6l–n). In the flowers of the 'moderate' rescue lines, the third whorl was formed by filamentous structures, and the second by sepaloid pale-green petals with petal-like margins (Fig. 6e, Fig. 6j, Fig. 6o). However, in all the lines assessed, none of them developed functional stamens or petals resembling those in the wild-type indicating that *CDM19* could only partially rescue the loss-of-function *ap3-3* mutant.

4. Discussion

Numerous studies of genes that provide ABCDE activities in many plant species have demonstrated the role of the B-class MADS-box genes in determining the identity of petals and stamens [13,29,33,42,43,44,45,46]. However, functional information about these important genes in Asteraceae species is limited to several reports of the three *Gerbera hybrida* genes, whose cosupression affects petal and stamen identity [33], five *Tagetes erecta* genes, among which only the overexpression of PI homolog changed the floral morphology of transgenic tobacco plants [47], six genes in *Helianthus annuus* [48,49,50], and four genes in *Chrysanthemum morifolium*, which, when overexpressed, differently influenced the morphology of tobacco flowers [34,39,48,51].

Arabidopsis thaliana and *Antirrhinum majus* have two B class genes *PI* and *AP3* and *GLOBOSA* (*GLO*) and *DEFICIENS* (*DEF*) respectively [52,53]. B class proteins form an obligate heterodimer (AP3/PI, DEF/GLO) that is required for their function in DNA binding [45] and for nuclear localization of the proteins [54]. The observed localization of the CDM19 protein in both the cytoplasm and the nucleus is not consistent with data from McGonigle and colleagues [54], who found

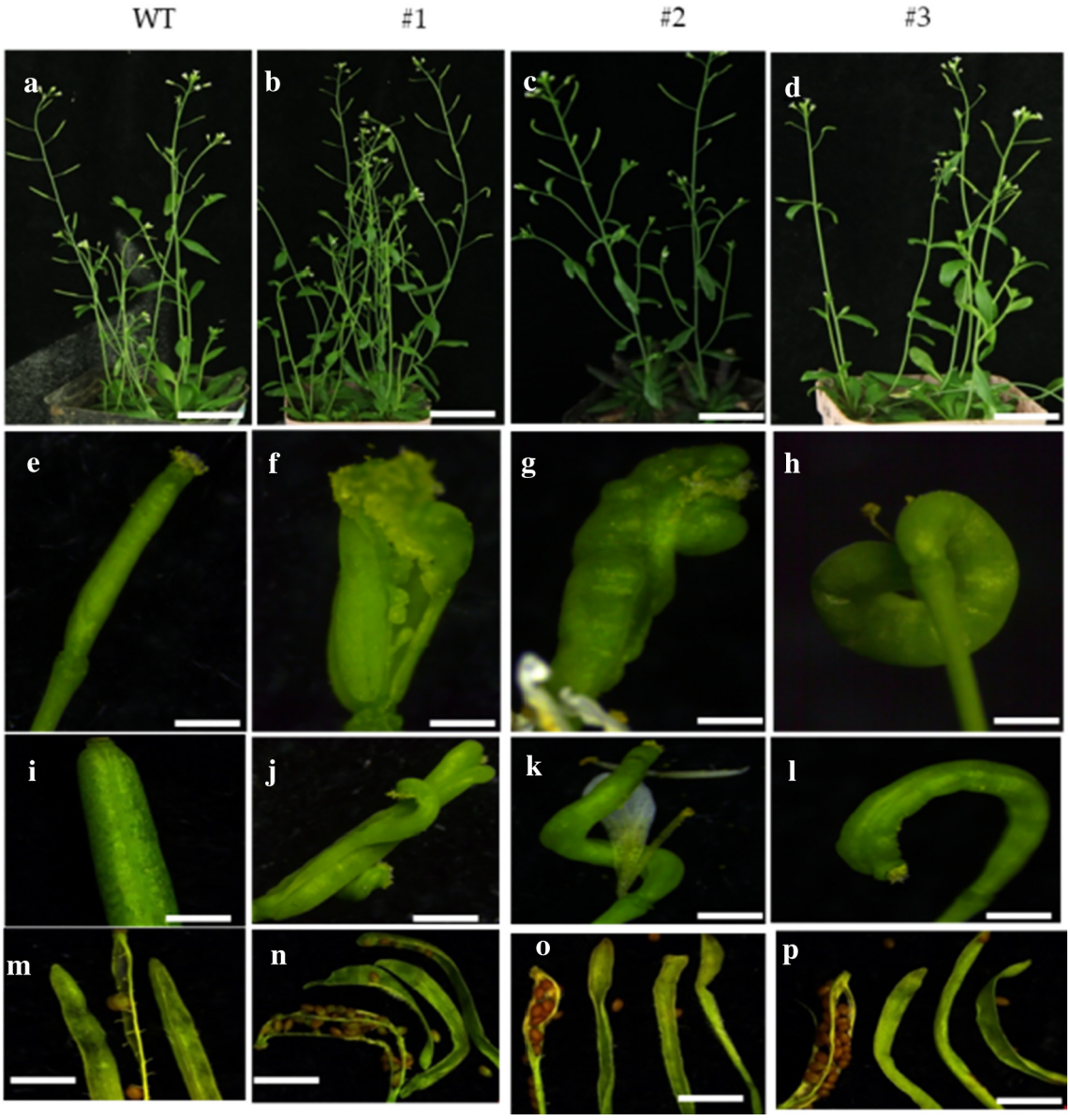

Fig. 4. Flower phenotypes in Arabidopsis 35S::CDM19 lines (wild-type background). An inflorescence (a), developing carpels (e, i) and mature siliques (m) from wild-type (Col) plant. Transgenic lines (b, c, d) with corresponding developing carpels (f–h and j–l) and mature siliques (n, o, p). Bars a–d = 1 cm; and e–p = 0.5 mm

that the localization of AP3 and PI in the nucleus occurs only when these proteins are simultaneously translated in the cell. However, our data are in agreement with the results of Immink and colleagues [55], who showed that AP3 alone can be present in the nucleus and in the cytoplasm.

The present study showed that the expression of *C. morifolium* cv. Jinba *CDM19* gene in the petals and pistils of ray and disc florets and in the stamens of disc florets is consistent with the previously analyzed expression of the B-class MADS-box genes in other Asteraceae. For instance, *T. erecta* genes *TeAP3-1* and *TeAP3-2* were strongly expressed in the perianth of disk and ray florets, stamens of disk florets and weakly in pistils [47]. Given that *TeAP3-1* transcripts were also detected in the ovaries [47], we assume that 'Jinba' *CDM19* expression may also be found there. Thus, the analyzed *CDM19* gene demonstrated the petal/stamen-specific expression pattern characteristic of B-class *AP3*-like genes, and an additional pistil-specific pattern that is observed for *AP3*-like genes in Asteraceae species [47], but only for PI-like genes in other species [56,57,58]. The pistil-specific expression suggests the possible involvement of CDM19 in pistil and ovary development, and may be the cause of carpel conversions in 35S::CDM19 Arabidopsis plants shown in the current study. The high expression level of *CDM19*, shown in the petals and stamens, is consistent with the expression of orthologous genes in Arabidopsis [10], lilies [29], Torenia [59], and other plant species. The CDM19 protein is highly homologous to the AP3 transcription factors in Asteraceae species, including *Tagetes*, *Gerbera* and *Helianthus* AP3, which have a typical type II MIKC structure [60,61]. Moreover, similar to AP3 proteins from other plant species, including 'Parliament' CDM19 [27,34,48,62], 'Jinba' CDM19 lacks the ability to activate transcription alone and forms a heterodimer with the PI-homolog, CDM86. Considering that the MADS-domain proteins function as quaternary complexes [63], we assume that the CDM19/CDM86 heterodimer is an important part of the quartets specifying the identity of petals, stamens and, probably, pistils or ovaries. The *CDM19* overexpression effect in Arabidopsis observed in this study confirms this assumption, since transgenic 35S::CDM19 plants did not have homeotic conversions in sepals, as in the case of other orthologous genes [21,64,65], but pistils consisting of more than two carpels with sometimes modified shape.

In addition, phenotypes observed in the Arabidopsis *ap3-3* mutant with *CDM19* overexpression, as well as in the 35S::CDM19-SRDX lines,

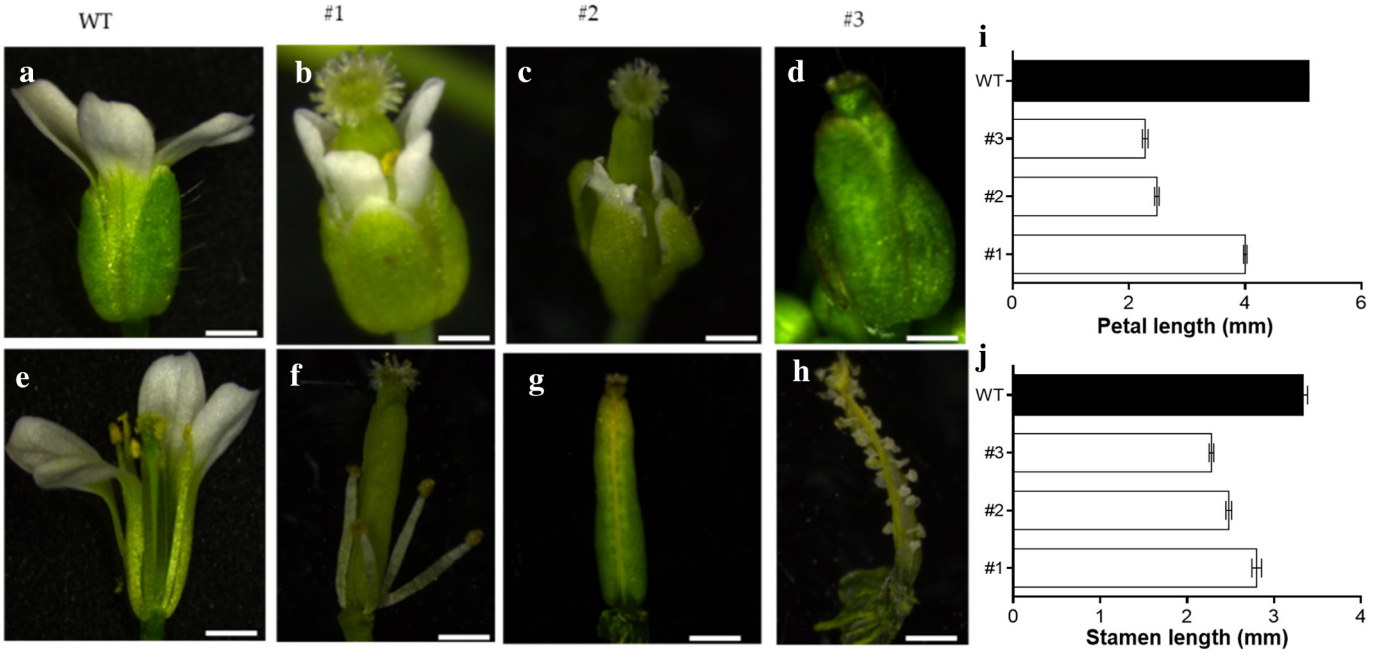

Fig. 5. Flower phenotypes in Arabidopsis 35S::CDM19-SRDX lines (wild-type background). A mature flower from wild-type (Col) plant (a), with sepals removed to expose the petals and the stamens (e). Flowers from the transgenic SRDX repression lines characterized by shortened petals (b, c, d), shortened filaments (f), and siliques (g) carrying shriveled undeveloped ovules (h). Bars = 0.5 mm. (i) and (j) changes in petal and stamen length. Error bars is ± s.e. value, $n > 100$, $p \leq 0.01$.

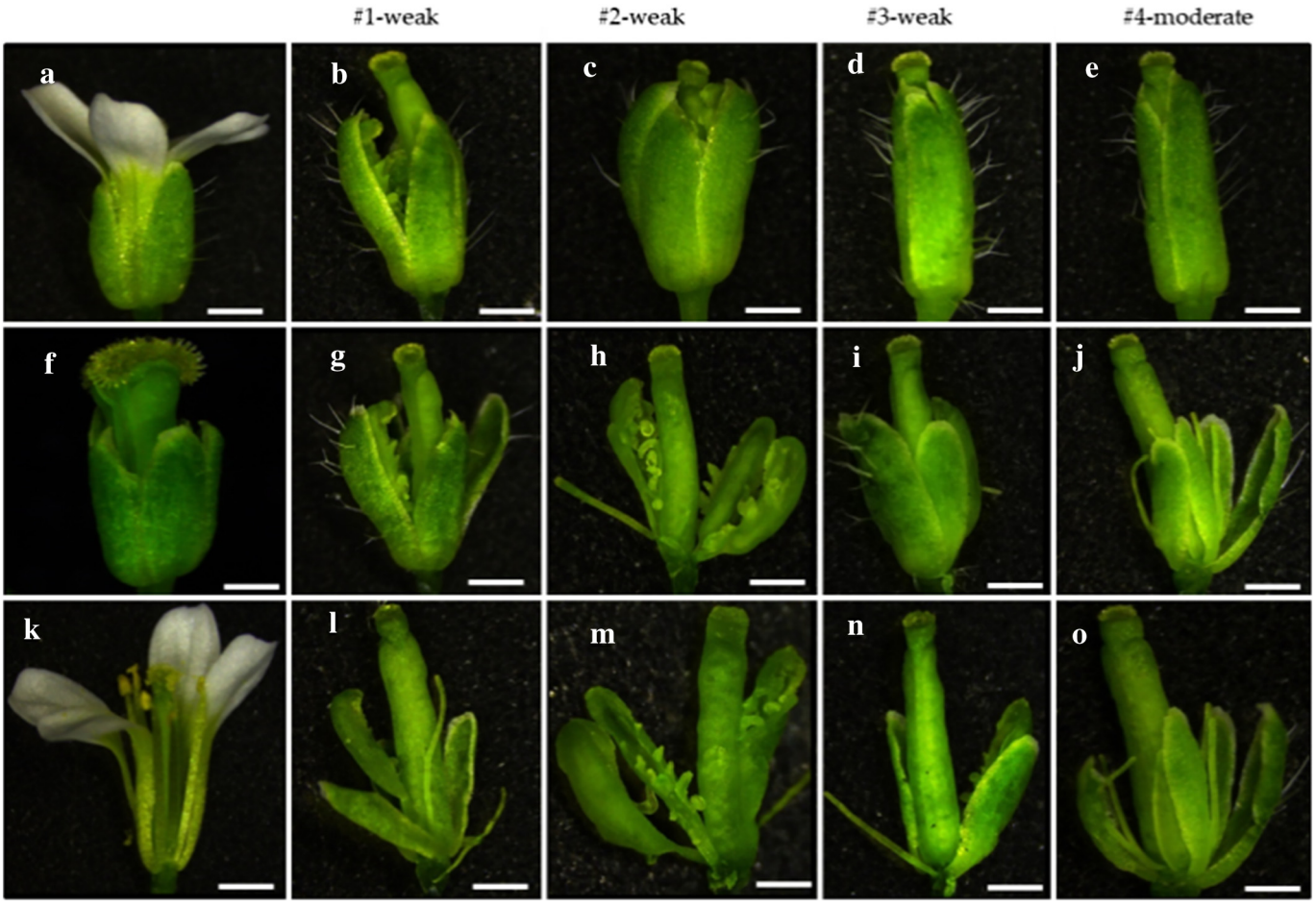

Fig. 6. Flower phenotypes of 35S::CDM19 Arabidopsis lines (*ap3-3* mutant background). A mature wild-type (Ler) flower (a), with sepals removed to expose the petals and the stamens (k); ap3–3 flower (f). Transgenic flower 35S::CDM19 on homozygous *ap3-3* background (b, c, d, e) with stamens restored to various degrees to filamentous or carpel-like organs (g-j and l-o); moderate rescue accompanied by the development of filamentous stamens and sepaloid petals with white margins (j, o). Bars = 0.5 mm.

indicate a possible role of CDM19 in petal and stamen development and its ability to partially replace AP3. We suggest that the inability of CDM19 to replace AP3 may be caused by the presence of another AP3 homolog in chrysanthemum, CDM115, the ectopic expression of which in *Nicotiana tabacum* leads to an increase in the number of flowers and seed capsules and a decrease in seed mass from one capsule, but does not affect the identity of the petals and stamens [48]. Taken together, the data obtained suggest that CDM19 contributes to the AP3-function in chrysanthemum redundantly with CDM115, and may additionally affect pistil, ovary, or ovule identity.

Conflicts of interest

The authors declare no conflict of interest.

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