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Original Article

Identification and Characterization of a *SEPALLATA*-like MADS-box Gene from Cucumber (*Cucumis sativus* L.)

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Abstract

SEPALLATA (*SEP*) genes encode the E class MADS-box transcription factors that play vital roles in various aspects of plant growth and development. In this study, we isolated a *SEP*-like gene from cucumber (*Cucumis sativus* L.), which was previously named as *CsMADS01*. *CsMADS01* had a coding sequence (CDS) of 741 bp, and coded a deduced protein of 246 amino acid residues that was predicted to be located in the nucleus. The putative CsMADS01 protein was typically characterized by the MIKC-type MADS (including MADS, I, K, and C domains) and shared high homology with other SEP-like proteins. Phylogenetic analysis of CsMADS01 and SEP proteins from other plants revealed that CsMADS01 was a member of the SEP1/2 clade of SEP proteins and was the most closely related to ZjMADS48 from Chinese jujube. Promoter analysis showed that several *cis*-elements related to stress response and hormones were present in the promoter region of *CsMADS01*. Expression analysis indicated that *CsMADS01* was highly expressed during the development of male and female flowers, and the expression increased gradually along with fruit development. Ectopic expression of *CsMADS01* in *Arabidopsis* resulted in a phyllody-like phenotype and the transgenic plants never flowered. These results suggest that *CsMADS01* plays an important role in the growth and development of cucumber.

Keywords: cucumber; gene expression; MADS-box; SEPALLATA (SEP); transgenic *Arabidopsis*

Introduction

MADS-box family genes encode transcription factors characterized by the presence of 58-60 highly conserved N-terminal DNA binding domain (termed as the MADS domain), which is conserved across a wide range of organisms including plants, fungi, and mammals (Riechmann and Meyerowitz, 1997; Xu *et al*., 2014; Nardeli *et al*., 2018). According to the phylogenetic analysis, the MADS-box genes can be divided into two functional types named as type I and type II (Alvarez-Buylla *et al*., 2000; Masiero *et al*., 2011), and the type II members are also termed as MIKC-type MADS for the presence of three additional domains from N-terminal to C-terminal: intervening (I) domain, keratin (K) domain, and C-terminus (C) domain, when compared with the type I members (Kaufmann *et al*., 2005; Ren *et al*., 2017; Zhou *et al*., 2019b). The MIKC-type MADS genes are well-known for their roles in floral organ development. Except for *APETALA2* (*AP2*), all A, B, C, D, and E class genes belong to the MIKC-type MADS-box genes according to the hypothesis of floral organ ABCDE model, and these genes specify the formation of floral organs in a combinatorial way (Weigel and Meyerowitz, 1994; Gutierrez-Cortines and Davies, 2000; Zahn *et al*., 2006; Theissen *et al*., 2016).

SEPALLATA (*SEP*) genes are the E class genes that are preferentially expressed in flowers and fruits for plant floral organ development (Honma and Goto, 2001; Theissen and Saedler, 2001). The *SEP* genes were first described in tomato and petunia, and inhibition of their expression would result in highly aberrant flowers (Angenent *et al*., 1994; Pnueli *et al*., 1994). *Arabidopsis* contains four *SEP*

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genes (*SEP1-4*), which are expressed in all four whorls of floral organs and play redundant roles in flower meristem identity and organ identity. In *sep1 sep2 sep3* triple-mutants, flowers possessed only sepals, while all floral organs were replaced by leaf-like organs in *sep1 sep2 sep3 sep4* quadruple mutants (Pelaz *et al*., 2000; Ditta *et al*., 2004; Malcomber and Kellogg, 2005). In addition, the *sep1-sep2-sep4* triple mutants displayed a phenotype similar to that of wild-type (WT) plants in floral organ development, revealing that *SEP3* is much more critical for flower development than other *SEP* genes in *Arabidopsis* (Ditta *et al*., 2004). Rice contains five floral homeotic genes (*OsMADS1*, *5*, *7*, *8* and *34*), all of which displayed both functional conservation (E function) and diversification (Cui *et al*., 2010; Gao *et al*., 2010; Kobayashi *et al*., 2012; Khanday *et al*., 2013; Lin *et al*., 2014; Meng *et al*., 2017; Wu *et al*., 2018). In addition, the SEP proteins can form MADS-box protein complexes with proteins of other classes for floral organ development. For example, OsMADS5 and OsMADS34 can physically interact with candidate class A, B, C, D, E, and AGL6-like floral homeotic proteins to control inflorescence and spikelet morphogenesis (Kobayashi *et al*., 2012; Hu *et al*., 2015; Meng *et al*., 2017; Wu *et al*., 2018). Besides floral organ development, *SEP* genes are also involved in many other aspects of growth and development in plants, such as fruit development (Ito *et al*., 2017; Li *et al*., 2017), stress response (Chen *et al*., 2019), bud growth and dormancy (Zhang *et al*., 2017b). These findings reveal that the E class *SEP* genes play essential roles in many aspects of plant growth and development with both redundancy and specificity (Soza *et al*., 2016; Zhang *et al*., 2017b).

Our previous report has indicated that there are four *SEP* genes (*CsMADS01–CsMADS04*) in cucumber genome (Hu and Liu, 2012), while only *CsMADS02* was cloned and functionally characterized (Zhou *et al*., 2019a). In the present study, another *SEP* gene (*CsMADS01*) was isolated from cucumber and its expression profile during flower and fruit development was examined. In addition, a phyllody-like phenotype was observed in the *CsMADS01*-overexpressing *Arabidopsis* plants. These findings suggest that *CsMADS01* plays an important role in the growth and development of cucumber.

Materials and Methods

Plant materials and growth conditions

Cucumber (*Cucumis sativus* var. *sativus* line 9930) and *Arabidopsis thaliana* ecotypes Columbia-0 (Col-0) were used in this study. Cucumber plants were planted in the field of Jiangxi Agricultural University, Nanchang, China. Different developmental stages of female flowers (MF) and male flowers (FF) were collected for expression profile analysis according to our previous study (Zhou *et al*., 2019b). The wild-type (WT) and transgenic *Arabidopsis* seeds were germinated on one-half Murashige and Skoog medium (1/2 MS) and the seedlings were grown on a plant growth chamber in a long-day photoperiod (16-h light/8-h dark cycle) at 22-24 °C.

RNA extraction and cloning of the CsMADS01 gene

Total RNA was extracted from cucumber female flowers with the TransZol Reagent kit (TransGen, China) following the manufacturer's protocol. After checking the integrity with a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), the first-strand cDNA was synthesized with TransScript All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal) (TransGen, China). The semi-quantitative reverse transcription PCR (RT-PCR) was carried out to amplify the coding sequence (CDS) of the *CsMADS01* gene by using the specific primers CsMADS01-1F (5'-aaaaCCATGGATGGGAAGAGGAAGAGTAG-3') and CsMADS01-1R (5'-aaaaAGATCTTCAAAGCATCCAACCAGGGAG-3') based on the sequence of *CsMADS01* (Gene ID: Csa004117) (Hu and Liu, 2012), with the procedure described in our previous study (Zhou *et al*., 2019b). PCR products were cloned into the pMD18-T vector (TaKaRa, Japan) and sequenced (Shanghai Sangon, China).

Bioinformatics analysis

The putative CsMADS01 protein sequence was uploaded to ProtParam (http:// web. expasy. org/ protparam/) to calculate the theoretical molecular weight (MW), isoelectric point (pI), and grand average of hydropathy index (GRAVY). The SOPMA tool (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page = npsa_sopma.html) was used to analyze the secondary structure of CsMADS01. The subcellular localization of CsMADS01 was predicted using the online servers ProtComp 9.0 (http://linux1.softberry.com/berry.phtml), and WoLF PSORT (https:// www.genscript. com/tools/ wolf-psort). The amino acid sequences of CsMADS01 and other selected MADS-box proteins from various plant species were aligned by Clustal Omega (https:// www.ebi.ac.uk/Tools/msa/clustalo/), and the alignments were used to create a neighbor-joining (NJ) phylogenetic tree with the MEGA 7.0 software. The bootstrap was set as 1000 replicates. To predict *cis*-elements in promoter of *CsMADS01*, the 1,500 bp regions upstream of the initiation codon (ATG) of *CsMADS01* was examined by the PlantCARE database (http:// bioinformatics. psb. ugent. be/webtools/plantcare/html/).

Gene expression analysis

RT-PCR was performed to examine the expression of *CsMADS01* in different developmental stages of MF and FF using the reaction procedure previously described (Zhou *et al*., 2019a). The primer sequences are listed in Table S1. For expression analysis of *CsMADS01* during fruit development, the RPKM values of *CsMADS01* were obtained from the fruitENCODE database (http://www.epigenome.cuhk.edu.hk/ZhongWeb/cucumber/cucumber_index.jsp) as described previously (Lü *et al*., 2018).

Vector construction, Arabidopsis transformation and phenotypic analysis

The CDS of *CsMADS01* in pMD18-T vector was

digested with the restriction sites *Nco* I and *Bgl* II, and ligated into the pCAMBIA1301 vector to generate the overexpression construct pCAMBIA1301-CsMADS01. Then, the resulting plasmid was introduced into *Agrobacterium tumefaciens GV3101*, and *GV3101* containing pCAMBIA1301-CsMADS01 was transformed into Col-0 via the floral dip method (Clough and Bent, 1998). The transgenic *Arabidopsis* seeds were screened by growing on 1/2 MS medium supplemented with 50 mg/L hygromycin, and transferred to soil. The transcription levels of *CsMADS01* were examined in the transgenic plants by RT-PCR, and the *AtTubulin4* gene was used as the internal control, with the procedure described previously (Zhou *et al*., 2018). The primer sequences are listed in Table S1. T_3 homozygous offspring were employed for phenotypic analysis.

Results and Discussion

Cloning and sequence analysis of the CsMADS01 gene

RT-PCR was used to amplify the ORF of *CsMADS01* gene using specific primers based on the sequence of the *CsMADS01* gene (Gene ID: Csa004117) in our previous study (Hu and Liu, 2012). The gene contained the sequence of the complete CDS (741 bp), which encoded a putative protein of 246 amino acids (Fig. 1A). The SMART analysis showed that CsMADS01 possessed the representative MADS and K-box domains, which were located in the positions of 1-60 and 80-172 amino acids, respectively (Fig. 1A). The secondary structure analysis by SOPMA revealed that CsMADS01 possessed 50.41% alpha helices, 9.35% extended strands, 5.28% beta turns, and 34.96% random coils, respectively (Fig. 1B). In addition, ProtParam analysis showed that CsMADS01 had a theoretical MW of 28.22 kDa, a pI of 9.20, and a GRAVY value of -0.628, suggesting that it is a hydrophilic and basic protein. Finally, ProtComp and WoLF PSORT results revealed that CsMADS01 was localized to the nucleus. These results indicated that *CsMADS01* encodes a MADS-box transcription factor.

Characterization of CsMADS01

BLAST search showed that CsMADS01 had the highest homology to Csa4G126990 in cucumber. It should be noted that the amino acid sequence of Csa4G126990 was identical to the positions of 63-246 amino acids of CsMADS01, and the positions of 1-60 of CsMADS01 was the MADS-box domain (Figs. 1 and 2), which is an indispensable domain for SEP members. Coincidentally, the amino acid sequence of CsMADS01 was identical to the corrected sequence of Csa4G126990, which was formally named as CsSEP2 (Wang *et al*., 2016). In addition, the CDS and gDNA sequences of *CsMADS01* were also identical to the full sequences of *CsSEP2* (Table S2) (Wang

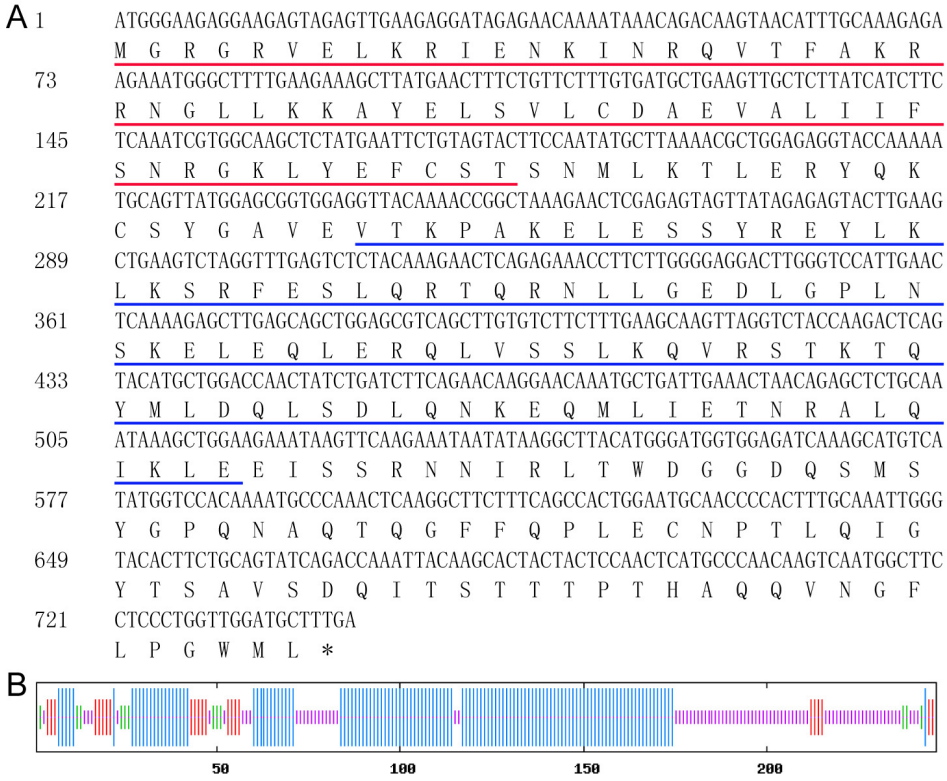

Fig. 1. Sequence analysis of *CsMADS01* and its deduced protein. (A) The CDS and deduced amino acid sequences of *CsMADS01*. *Asterisk* represents stop codon. The MADS and K-box domains are underlined with red and blue, respectively. (B) Secondary structure analysis of CsMADS01 by SOPMA. The alpha helix, extended strand, beta turn, and random coil residues are presented by blue, red, green, and purple vertical lines, respectively

et al., 2016). These results demonstrated that CsMADS01 is identical to CsSEP2, which may be a functional SEP in cucumber.

Our previous study has shown that CsMADS01 is a member of SEP subfamily (Hu and Liu, 2012). We then compared the amino acid sequence of CsMADS01 with that of other SEP proteins in literature references by using Clustal Omega. The amino acid sequence of CsMADS01 showed 53.68-85.19% identity with these SEP proteins, such as OsMADS34 in rice (53.68% identity) (Gao *et al*., 2010; Kobayashi *et al*., 2010), GbSEP in *Ginkgo biloba* (55.00% identity) (Cheng *et al*., 2016), CaMADS in cucumber (60.68% identity) (Chen *et al*., 2019), CsMADS02 in cucumber (69.70% identity) (Zhou *et al*., 2019a), AtSEP2 in *Arabidopsis* (74.07% identity) (Pelaz *et al*., 2000), BolMADS5 in *Brassica oleracea* (76.33% identity) (Sheng *et al*., 2019), AtSEP1 in *Arabidopsis* (76.45% identity) (Pelaz *et al*., 2001), FaMADS9 in strawberry (80.74% identity) (Seymour *et al*., 2011), MdMADS8 in apple (81.48% identity) (Ireland *et al*., 2013), ZjMADS48 in Chinese jujube (82.32% identity) (Zhang *et al*., 2017a), and PrupeSEP1/PrpMADS7 in peach (85.19% identity) (Xu *et al*., 2008; Li *et al*., 2017). In addition, all the SEP proteins contained a highly conserved MADS domain, followed by less conversed I and K domains, and a highly variable C domain (Fig. 2), which is the characteristic of the MIKC^{C}-group of Type II MADS-box proteins (Ren *et al*., 2017; Zhou *et al*., 2017). Moreover, two additional conserved motifs, SEP I and SEP II, were present in the C-terminus of these proteins (Fig. 2), which is typical for SEPs and was also found in other SEP proteins, such as RanSEP3 (Soza *et al*., 2016), and HrSEP-2 (Mitoma and Kanno, 2018).

Phylogenetic analysis of CsMADS01 and other SEP subfamily members

Several gene duplications might have occurred in the most recent common ancestor of angiosperms and gymnosperms, resulting in the division of the SEP-like genes of angiosperms into two major clades, the *LOFSEP* clade (also called *SEP1/2/4* clade or *AGL2/3/4* clade) and the *SEP3* clade, within the SEP phylogeny (Malcomber and Kellogg, 2005; Zahn *et al*., 2005). To study the phylogenetic relationships of CsMADS01 and other SEP subfamily members, a phylogenetic tree was created based on the amino acid sequences of CsMADS01, CsMADS02 (Zhou *et al*., 2019a), and SEP proteins from *Arabidopsis* (Pelaz *et al*., 2000), rice (Arora *et al*., 2007), Chinese jujube (Zhang *et al*., 2017a), and *Brassica oleracea* (Sheng *et al*., 2019). These SEP proteins can be clustered into three clades, namely SEP1/2, SEP3, and SEP4 (Fig. 3), which is consistent with the evolution lines reported in previous studies (Xu *et al*., 2008; Ireland *et al*., 2013; Zhou *et al*., 2017; Zhou *et al*., 2019a). CsMADS01 shared high genetic homology with ZjMADS48, AtSEP1, AtSEP2, and BolMADS5 in the SEP1/2 clade (Fig. 3), suggesting that they may have similar functions.

Cis-element analysis of the CsMADS01 promoter

To investigate the possible regulatory functions of the *CsMADS01* gene, the 1,500 bp region upstream of the translational initiation codon of *CsMADS01* was analyzed. As a result, three kinds of hormone-related *cis*-elements, such as CGTCA-motif, ABRE, and TCA-element, which are involved in response to methyl jasmonate (MeJA), abscisic acid (ABA), and salicylic acid (SA), respectively, were identified in the *CsMADS01* promoter. In addition, four kinds of stress-related *cis*-elements were present in the *CsMADS01* promoter, such as W-box response to fungal elicitor, MBS response to drought and salt stress, ARE response to anaerobic induction. These results indicated that *CsMADS01* plays possible roles in hormone and stress responses. Recently, a *SEP* gene in pepper, *CaMADS*, was found to act as a positive stress-responsive regulator in the cold, salt, and osmotic stress signaling pathways (Chen *et al*., 2019).

Expression analysis of CsMADS01 during flower and fruit development

Previous results have shown that *CsMADS01* is primarily expressed in flowers (Hu and Liu, 2012; Wang *et al*., 2016). To investigate the possible function of *CsMADS01* in flower development, RT-PCR was used to examine the expression of *CsMADS01* in five different developmental stages of MF and FF as described in a previous study (Zhou *et al*., 2019b). As shown in Fig. 5A and Fig. 5B, *CsMADS01* gene was continuously and highly expressed during the development of male and female

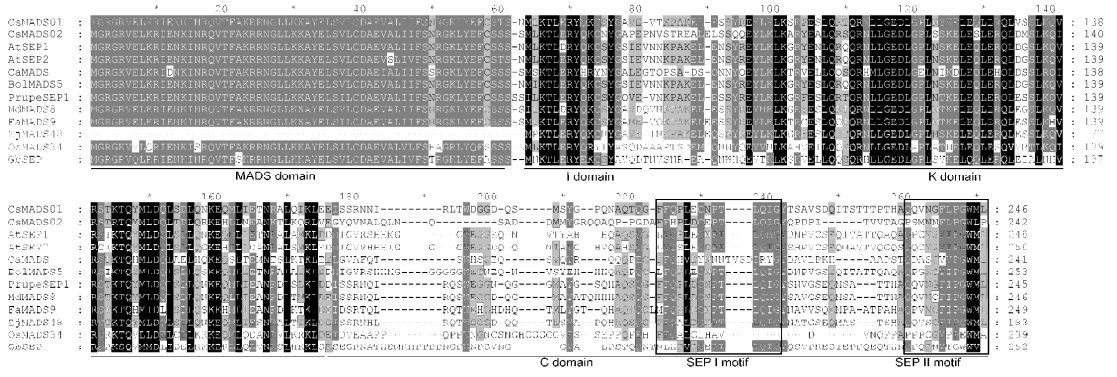

Fig. 2. Multiple sequence alignment of CsMADS01 with SEP proteins from other plant species. The MADS, I, K, and C domains are underlined. Two conserved motifs, SEP I and SEP II, are boxed

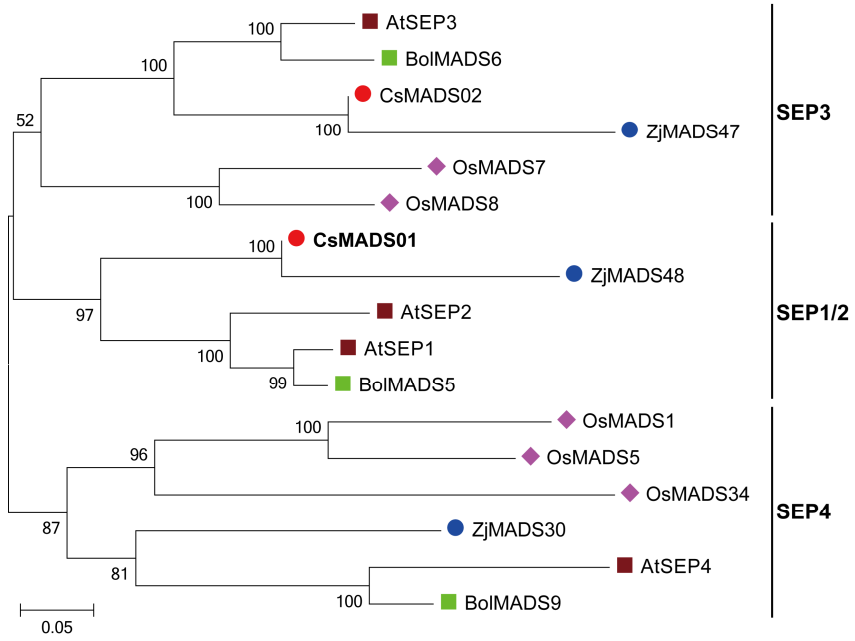

Fig. 3. Phylogenetic analysis of CsMADS01 and other SEP subfamily members from different plant species. Different SEP proteins from cucumber (red round, Cs), *Arabidopsis* (brown square, At), rice (purple rhombus, Os), and *Brassica oleracea* (green square, Bol) were aligned with Clustal Omega and the phylogenetic tree was created using the NJ method in MEGA 7.0 by bootstrap analysis with 1000 replicates. CsMADS01 is bolded

Fig. 4. Putative cis-element analysis of the CsMADS01 promoter

flowers, suggesting that it may play an essential role in flower development of cucumber. Similarly, all three SEP subfamily genes in Chinese jujube (*ZjMADS30*, *ZjMADS47* and *ZjMADS48*) were highly expressed in the sepal, petal and pistil (Zhang *et al*., 2017a). In mulberry (*Morus notabilis*), all four SEP subfamily genes also displayed floral organ specific expression (Luo *et al*., 2018). Two SEP subfamily genes in *B. oleracea*, *BolMADS5* and *BolMADS6*, also exhibited relatively high expression in four whorls of the flower including the sepal, petal, stamen, and pistil, while another SEP subfamily gene (*BolMADS9*) was rarely expressed in these tissues (Sheng *et al*., 2019). Our previous study also revealed that *CsMADS02*, another *SEP* gene in cucumber, also displayed the highest expression in flowers, especially female flowers (Zhou *et al*., 2019a). However, the expression pattern of *CsMADS02* during female and male flower development was different from that of *CsMADS01* (Figs. 5A and 5B). These results indicated that *CsMADS01* and *CsMADS02* may have different functions during flower development in cucumber. This phenomenon may due to the different evolution lines of the two *SEP* genes: *CsMADS01* and *CsMADS02* are members of the SEP1/2 and SEP3 evolution lines, respectively (Fig. 3). We also examined the expression of *CsMADS01* during fruit development using the transcriptome data from the fruitENCODE database. As shown in Fig. 5C, the expression of *CsMADS01* increased gradually from 10 DPA to 40 DPA during fruit development, while no expression was detected in the leaves. An exon skipping in the *CsMADS01/CsSEP2* gene resulted in defective fruits and enlarged sepals in both female and male flowers (Wang *et al*., 2016). These results indicated that *CsMADS01* may also function in fruit ripening. Similarly, two apple SEP1/2 evolution line genes *MdMADS8* and *MdMADS9* exhibited higher expression during fruit maturation, and play important roles in both fleshy fruit development and fruit ripening (Ireland *et al*., 2013). PrupeSEP1 was also found to be involved in the regulation of ripening and softening of melting flesh peach (Li *et al*., 2017). A tomato *SEP* gene, *MADS-RIN* (or *RIN*), plays vital roles in regulating various aspects of fruit development and ripening through controlling many important ripening genes (Ito *et al*., 2017; Li *et al*., 2018; Li *et al*., 2019).

Overexpression of CsMADS01 caused a phyllody-like phenotype of transgenic Arabidopsis

To elucidate the potential functions of *CsMADS01*, the gene was overexpressed in *Arabidopsis* under the control of the cauliflower mosaic virus (CaMV) 35S promoter. After transformation of *Arabidopsis*, most transgenic plants displayed significantly altered phenotypes, and two independent transgenic lines with higher expression of *CsMADS01*, named as OE1 and OE2 (Fig. 6A), were used for phenotypic analysis. The transgenic plants displayed increased numbers of leaves coupled with increased formation of branches, leading to a leafy phenotype (Fig. 6B and 6C). Besides, the phenotype of high-expression transgenic line (OE2) was more severe than that of low-expression transgenic line (OE1), suggesting that the leafy phenotype was due to the ectopic expression of *CsMADS01* in *Arabidopsis*. Similarly, altered leaf phenotypes were also reported for the ectopic expression of *SEP* genes in *Arabidopsis*, such as *LMADS3* (Tzeng *et al*., 2003), *TaMADS1* (Zhao *et al*., 2006), and *CsMADS02* (Zhou *et al*., 2019a). In addition, the transgenic plants never flowered, while the WT plants could flower normally (Fig. 6D), indicating the flower organs were replaced by leaves in the transgenic plants, resulted in a phyllody-like phenotype. In *Arabidopsis*, all the four *SEP* genes play vital roles in specifying the identities of four whorls of the flower and determine floral meristem identity (Pelaz *et al*., 2000; Ditta *et al*., 2004; Malcomber and Kellogg, 2005). Single *SEP* gene mutations resulted in only subtle or no changes in phenotypes when compared with the WT, while floral organs were replaced by leaf-like organs in the *sep1 sep2 sep3 sep4* quadruple mutant (Ditta *et al*., 2004). A phytoplasmal secreted protein PHYL1 interacts with and degrades the floral homeotic MADS domain proteins APETALA1 (AP1) and SEP1-4 proteins, resulting in the transformation of flower organs into leaf-like structures (Maejima *et al*., 2014; Maejima *et al*., 2015). In rice, *OsMADS34* is also involved in the transition from vegetative to reproductive growth via the specification of inflorescence meristem identity (Gao *et al*., 2010), and can act as a transcription

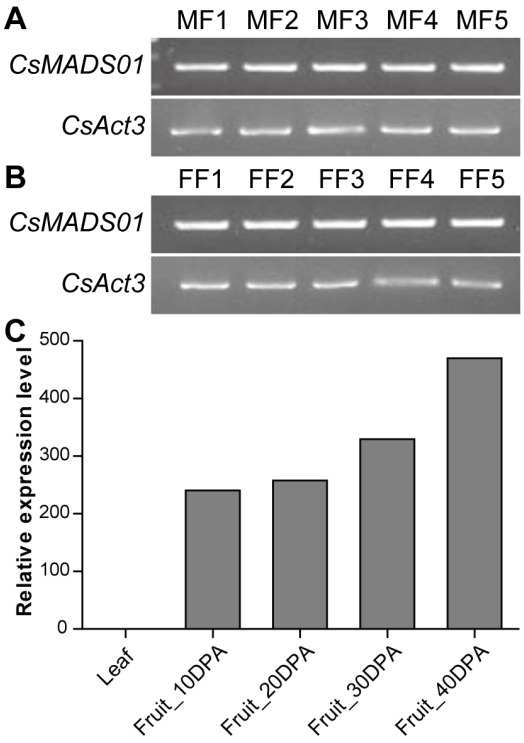

Fig. 5. Expression analysis of *CsMADS01* during flower and fruit development in cucumber. (A and B) RT-PCR analysis of *CsMADS01* in five different developmental stages (MF1 to MF5, and FF1 to FF5) in cucumber. (C) In silico expression analysis of *CsMADS01* during fruit development in cucumber. The expression of *CsMADS01* is presented as RPKM values.

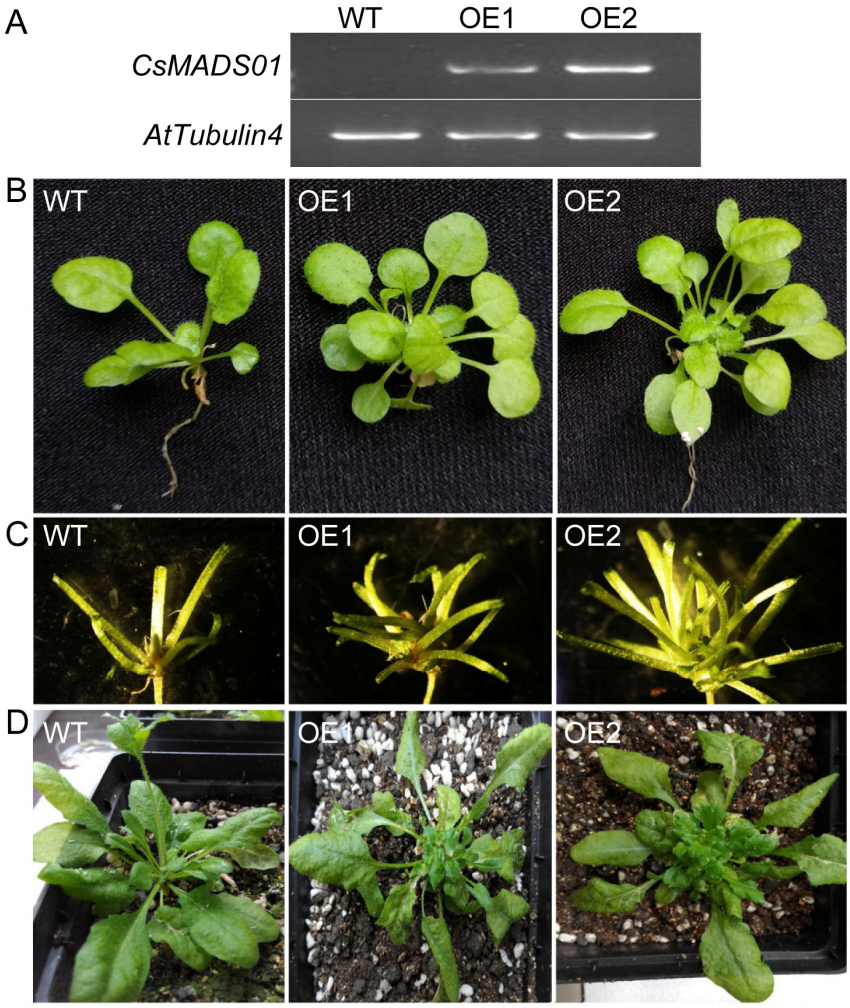

Fig. 6. Overexpression of *CsMADS01* in *Arabidopsis*. (A) RT-PCR analysis of *CsMADS01* in WT and transgenic lines (OE1 and OE2). (B and C) Phenotypes of *CsMADS01* transgenic lines (OE1 and OE2) and WT plants at 22 days after sowing. (B) Overexpression of *CsMADS01* caused a leafy phenotype. (C) The branches of transgenic *Arabidopsis* and WT plants were shown. (D) Phenotypes of *CsMADS01* transgenic lines (OE1 and OE2) and WT plants at 36 days after sowing

repressor to influence grain yield by suppressing the transcription of related genes (Ren *et al*., 2016). The sterile lemmas, lemmas, paleas, and the inner floral organs were converted into leaf-like structures in the *osmads1-z osmads5-3 osmads34-1* triple mutant (Wu *et al*., 2018). A recent study showed that compared with *Rosa chinensis* cv. Old Blush, *R. chinensis* cv. Viridiflora displayed a phyllody phenotype, in which flower organs were converted into leaf-like organs, and this phenomenon is associated with the modified expression of the genes related to flowering and flower development, including *RcSEP1* and *RcSEP3* (Yan *et al*., 2016). Therefore, it can be speculated that the phyllody-like phenotype of *CsMADS01*-overexpressing transgenic *Arabidopsis* plants may be due to the misexpression of *Arabidopsis* endogenic *SEPs* and other flower organ identity genes. Similarly, ectopic expression of a wheat *SEP*-like gene, *TaMADS1*, caused early flowering and abnormal development of all floral organs in *Arabidopsis*, including the conversion of sepals into leaf-like structures, which may be due to the regulation of some flower meristem identity genes (Zhao *et al*., 2006).

Conclusions

In summary, we performed the isolation and functional characterization of *CsMADS01* from cucumber. This gene was previously named as *CsSEP2* by a previous study (Wang *et al*., 2016), and is involved in floral organ and fruit development. The deduced CsMADS01 protein contains the typical MADS, I, K, and C domains, as well as two conserved motifs (SEP I and SEP II) specific for SEP proteins. Overexpression of *CsMADS01* in *Arabidopsis* led to a phyllody-like phenotype and the transgenic plants never flowered. These results lay a foundation for further revealing the regulatory mechanisms of flower and fruit development in cucumber.

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Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

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Table S1. RT-PCR primers used for gene expression analysis in this study

Gene	Forward primer	Reverse primer
CsMADS01	AGAAACCTTCTTGGGGAGGA	GAAGCCATTGACTTGTTGGG
CsAct3	GACATTCAATGTGCCTGCTATG	CATACCGATGAGAGATGGCTG
AtTubulin4	GCGAACAGTTCACAGCTATGTTCA	GAGGGAGCCATTGACAACATCTT