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Full Length Article

Overexpression of *AcCMF1*, Onion CCT Family Gene, Promotes Flowering in Transgenic Arabidopsis

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Abstract

Flowering time regulation is essential for horticultural crops. Photoperiod plays an important role in flowering regulation among environmental signals. Onion is a typical biennial plant. The life cycle of onion is strictly regulated by light. Studying genes linked to flowering regulation in onion are meaningful for onion production. The CCT family genes modulate plant flowering in photoperiod flowering pathway. In this study, a novel CCT family gene was isolated from onion. AcCMF1 belonged to the CCT motif family (CMF), containing a CCT domain. The length of *AcCMF1* cDNA was 891bp, encoding a 296-amino acid protein. Subcellular localization analysis revealed AcCMF1 located on cell nucleus. *AcCMF1* was expressed highly in young leaves before bolting. The overexpression of *AcCMF1* promoted the flowering time of Arabidopsis *co* mutant. In conclusion, *AcCMF1* played a positive role in onion flowering regulation under LD. This study provided insight into the molecular mechanisms regulating flowering time in onion, specifically related to photoperiod. Results have practical implications for controlled onion production systems. © 2020 Friends Science Publishers

Keywords: Onion; *AcCMF1*; Flowering regulation; Advanced flowering time

Abbreviations: CO, CONSTANS; COL, CONSTANS-like; COP1, CONSTITUTIVE PHOTOMORPHOGENIC; GI, GIGANTEA; FT, FLOWERING LOCUS T; LD, long day; PPT, Glufosinate ammonium; qRT-PCR, Quantitative real-time Polymerase Chain Reaction; RLs, rosette leaves; SD, short day; TSF, SISTER OF FT; WT, wild-type

Introduction

Control of flowering time is critical for plant development, especially of horticultural crops. Regulation of flowering is a complex network, including both environment factors and internal regulatory signals. Photoperiod is a vital environmental factor in plant flowering regulation. Robson *et al.* (2001) identified numerous genes, which participate in regulation of flowering. The CCT family genes are concerned with photoperiod-induced flowering modulation and light-triggered signaling (Putterill *et al.* 1995; Wenkel *et al.* 2006). The CCT domain initially represented a motif at the C-terminus of CONSTANS (CO), CO-like and TIMING OF CAB1 (TOC1) in Arabidopsis. Previous studies have classified CCT genes into three families: the COL gene family, encoded one or two zinc-finger B-box domains and a CCT domain; the CCT motif family (CMF) with a only CCT domain; the pseudo-response regulator (PRR) gene family with a CCT domain and two conserved regions-pseudo receiver domain (Cockram *et al.* 2012). *AtCO* was the first CCT family gene which consisted of two B-box domains and a CCT domain cloned in Arabidopsis (Robson *et al.* 2001). Surveys on photoperiod pathway showed that the transcription factor CO promoted flowering by increasing the transcripts of FLOWERING LOCUS T (FT) under long day (LD) condition (Putterill *et al.* 1995) . Genetic analyses had uncovered that CO/FT was the core component in photoperiod-mediated flowering control (Nakamichi 2015). *CO* gene integrated the circadian clock and light signals to control plant flowering (Samach *et al.* 2000; Suarez-Lopez *et al.* 2001). *CO-like* (*COL*) genes were downstream component of circadian clock measuring day length. They cooperated with FT and GIGANTEA (GI) , as central functional components in photoperiod pathway (Song *et al.* 2012). In *Arabidopsis*, 17 *COL* genes were identified (Robson *et al.* 2001; Khanna *et al.* 2009). It is reported that *AtCO*, *AtCOL3*, *AtCOL5*, and *AtCOL9* take part in flowering time regulation in Arabidopsis (Putterill *et al.* 1995; Cheng and Wang 2005; Datta *et al.* 2006; Hassidim *et al.* 2009). *AtCO* gene accelerated flowering in

response to long photoperiods in *Arabidopsis*, which repressed photomorphogenesis in darkness (Putterill *et al.* 1995). The overexpression of *AtCOL5* could advance flowering time by raising the transcripts of *FT* (Hassidim *et al.* 2009). *AtCOL9* repressed flowering by decreasing the transcripts of *CO* and *FT*. *AtCOL9* overexpression transgenic lines showed late flowering phenotype in LD condition (Cheng and Wang 2005). The function of *CO* gene was conserved between dicots and monocots in photoperiodic floral induction pathway in *Arabidopsis* and rice (Wenkel *et al.* 2006). *Heading date 1 (Hd1)* was the homologue of *AtCO*, it was revealed that *Hd1* could promote the rice heading in SD condition and inhibiting the rice heading in LD condition (Yano *et al.* 2000). *Ghd7* was a CMF gene, involved in heading date and grains development in rice (Xue *et al.* 2008). *Ghd7* delayed the rice heading by repressing the transcription of Early heading date1 (Ehd1) in the photoperiodic flowering pathway under LD conditions (Xue *et al.* 2008; Nakamichi 2015). Studies in rice showed that such genes were relatively common, they demarcation this group of genes to the *CMF* genes(Cockram *et al.* 2012). *CMF* genes had similarity function with *COL* in plant flowering regulation. *OsCCT1* was a new *CMF* gene repressing the expression of *Ehd1* and *Hd3a* to delay the flowering time (Zhang *et al.* 2015).

Onion (*Allium cepa* L.) is one of the main vegetables with economic production of bulb biennially. In 2018, onion production was 103.3 million tons harvested in 5.3 million hectares throughout the world (http://www.fao.org). The life cycle of onion is strictly regulated by light. There are multiple ecotype of onion dependent on the planting environment, as LD type, SD type and day-neutral. In previous study, an *AcCOL* was obtained, but it did not exhibit discernible circadian expression pattern (Taylor *et al.* 2010). *AcCOL2* showed a circadian expression pattern in common with *AtCO* that possibly regulated the expression of *AcFT1* (Rashid and Thomas 2020). In our previous study, *AcCOL7* was cloned which involved in photoperiod pathway, as well as it likely played a significant role in promoting flowering (Sheng *et al.* 2018).

The CMF genes have not been identified in onion. In this study, a *CMF* gene was isolated from onion named *AcCMF1*. For the purpose of investigating the role of *AcCMF1* in flowering regulation, *AcCMF1* was transformed to *Arabidopsis*. *AcCMF1* played similar roles in flowering regulation. Overexpression *AcCMF1* could partly complement the function of *co* mutant in *Arabidopsis*. These results proposed that CMF gene was involvement in the flowering regulation of onion.

Materials and Methods

Plant materials

A LD type higher-generation inbred onion SA2 was used in this experiment. It was provided by the Onion and Garlic Research Group of Northeast Agricultural University. *Arabidopsis thaliana* wild-type (WT) accessions used were Col-0 and Ler. The *Arabidopsis* mutant *col-5* (SALK_096361C, Col-0), *gi* (CS181, Ler-0*)* and *ga3* (SALK-103671C, Col-0) were obtained from TAIR（http: //www.arabidopsis.org/. Plants were grown on soil in a plant incubator under a 16 h light and 8 h dark period at 22/18^{o}C. Tissue samples were collected from ten-leaf-stage onion on both vegetative and reproductive growth for relative expression of *AcCMF1*. Flowering time was measured by counting the total number of rosette leaves (RLs) and recording the days at bolting. Eight-week-old seedlings were used to measure plant height. Each independent line with three biological replicates was used to measrue number of RLs, flowering days and plant height. There were twenty plants per replication.

Cloning, sequence alignment and phylogenetic analysis of AcCMF1

Sequence of *AcCMF1* was obtained from transcriptome database in our previous study (Yuan *et al.* 2018) . Trizol reagent (Invitrogen, USA) was used to extract total RNA from onion leaves, and cDNA was synthesized using M-MuLV reverse transcriptase (Thermo Scientific, USA). The primers are listed in Table S1.

Simple Modular Architecture Research Tool (SMART) was used to explore the conserved domains of AcCMF1 (http://smart.embl-heidelberg.de/). The CCT family proteins amino acid sequence of *Arabidopsis* and rice were obtained from NCBI. MEGA5 software was used to construct the multiple sequence alignments of AcCMF1 and related CCT family proteins. The phylogenetic tree was constructed through MEGA5 software using the Neighbor-Joining (NJ) method (Saitou and Nei 1987; Tamura *et al.* 2011).

The multiple sequence alignments were drawn using the BoxShade web site (http://www.ch.embnet.org/software/BOX_ form.html).

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from onion using Trizol (Invitrogen, USA). cDNA was synthesized using ReverTra Ace® qPCR RT Master Mix with gDNA Remover (TOYOBO, Shanghai, China). qRT-PCR was carried out using KOD SYBR® qPCR Mix (TOYOBO, Shanghai, China). *Acaction* was the reference gene. Comparative threshold method ($2^{-\triangle\triangle Ct}$) was used to measure relative transcripts levels of genes (Livak and Schmittgen 2001). The primers in this study were listed in Table S1.

Subcellular localization in Arabidopsis mesophyll protoplast

The full length coding sequence (CDS) of *AcCMF1* was

transient expressed in *Arabidopsis* for subcellular localization (Yoo *et al.* 2007). The primers are shown in Table S1. The pG-eGFP vector (with GFP protein driven by CaMV35S promoter) to generate CaMV35S: eGFP-*AcCMF1*. The empty vector was used as control. Fluorescence microscope was used to observe the eGFP-*AcCMF1* subcellular localization.

Ectopic expression of *AcCMF1* in Arabidopsis

The CDS regions of *AcCMF1* was inserted to the pCXSN1250-3301 vector, in which the target genes were controlled by CaMV35S promoter. The recombinant vector was transformed to *Agrobacterium* strain GV3101 and then used to infect *Arabidopsis* (Col-0, Ler, *col-5*, *ga3* and *gi*) via *Agrobacterium*-mediated the floral dip method (Clough and Bent 2010). The transgenic lines were selected on MS medium with Glufosinate ammonium (PPT). PCR was used to select positive transgenic lines. Homozygous transgenic *Arabidopsis* seeds (T_3) were used for further study.

Statistical analysis

The values were obtained from three independent experiments and presented as the mean ± standard errors. Univariate ANOVA analysis was used to represent the significant differences of the data ($P < 0.05$).

Results

Cloning and phylogenetic analysis of onion *AcCMF1*

In this study, a novel CCT family gene was obtained based on the transcriptome database from our previously study (Yuan *et al.* 2018). The gene was identified to contain a CCT domain. It was annotated as *CMF* gene and named *AcCMF1*. According to phylogenetic analysis, CCT family protein from *Arabidopsis* and rice could be classified into four groups (Fig. 1). The members in group I contained two B-box motifs and a CCT domain. The group II members contained a B-box motif and a CCT domain. The group III members included a B-box domain, a diverse B-box domain and a CCT domain. AcCMF1 belonged to group IV without B-box domain had a closer evolutionary relationship with OsGHd7 (Fig. 1). The full length of *AcCMF1* cDNA was 891 bp, encoding 296 amino acids. The sequence alignment of AcCMF1 compared with other members of CCT family was performed (Fig. 2). AcCMF1 showed 15.16 and 17.63% identity with OsGhd7 and ZmGhd7, which were CMF proteins from rice and maize. AcCMF1 had all the conserved amino acids of CCT domain ($RX_5RYX_2KX_2RX_7YX_2RKX_2AX_3PRX_2GRF$) (Fig. 2).

Subcellular localization of AcCMF1

The fusion expression vector used to investigate the intracellular localization of AcCMF1 was constructed as pGII-eGFP-AcCMF1. The empty vector pGII-eGFP was also transformed to *Arabidopsis* as control. *Arabidopsis* protoplasts were extracted and used for observation. We detected strong GFP fluorescence in the nucleus when eGFP-AcCMF1 plasmid was transformed to *Arabidopsis*, while GFP fluorescence was observed in whole *Arabidopsis* protoplast when empty vector pGII-eGFP plasmid transformed (Fig. 3). These results confirmed that AcCMF1 was nuclear-localized protein. CO worked as the transcription factor to promote flowering under LD (Putterill *et al.* 1995). AcCMF1 suggested to be as characteristic transcription factor.

Characterization of *AcCMF1* expression

To characterize the organ specific expression of *AcCMF1*, qRT-PCR was performed in various onion organs at reproductive phase under LD condition (Fig. 4). Although *AcCMF1* expressed throughout the growth cycle of the plant, the transcript level was the highest in the young leaves before bolting, followed by a high expression level in the young flower stems (Fig. 4). A high expression of the gene in young leaves before bolting also indicated that AcCMF1 was an important component receiving optical signal in photoperiod pathway and played an important role in plant flowering regulation.

Results from qRT-PCR showed that *AcCMF1* has double peaks of transcription under both LD and SD conditions. *AcCMF1* was mainly expressed under dark condition. Under LD condition, the expression of *AcCMF1* was peak at 6:00 am and 8:00 pm. The transcripts of *AcCMF1* reached peak at 10:00 am and 8:00 pm under SD condition (Fig. 5).

Role of *AcCMF1* in plant flowering regulation

AcCMF1 was transformed to *Arabidopsis* to investigate its function on flowering regulation. The wild-type (WT) Arabidopsis was bolting with 16 rosette leaves (RLs) on average, at about 32-day-stage on average and the plant height was 37 cm on average. *AcCMF1*-OE-WT lines showed more rosettes, but there was no significantly different on flowering days and plant height between the wild type and *AcCMF1* transgenic Arabidopsis (Supplementary Fig. S1). Compared with the wild-type *A. thaliana*, *co* mutant plants showed dwarf phenotype and their flowering time of was delayed (Fig. 6A). *A. thaliana co* mutant was bolting with 18 RLs and at 38-day-stage on average (Fig. 6BC). *AcCMF1* was overexpressed in *Arabidopsis co* mutant under the control of CaMV35S promoter to further verify the function of *AcCMF1*. Compared to *co* mutant, the flowering time of *AcCMF1*-OE-*co* lines were advanced. The transgenic plants flowered at about 32-day-stage (Fig. 6BC). Plant height of *AcCMF1*-OE-*co* lines was rescued, which was 37 cm on average (Fig. 6D).

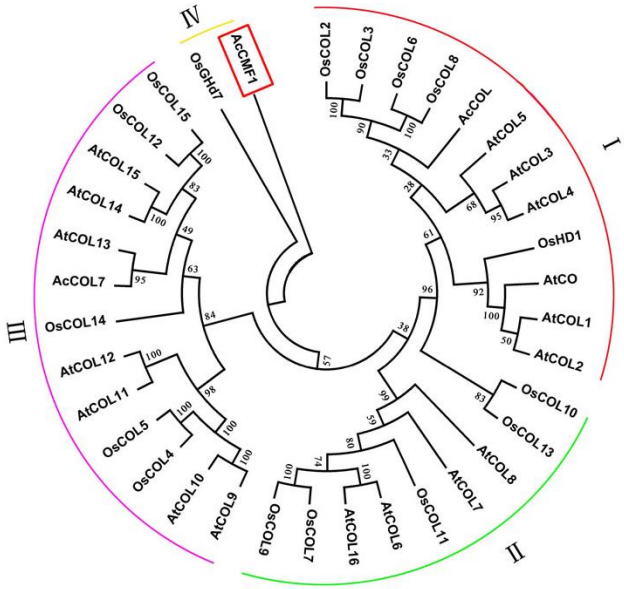

Fig. 1: The phylogenetic relationship and conserved domain analysis of CCT homologs. Neighbor-joining tree of CCT family genes, AcCMF1, AcCOL, AcCOL7, AtCOLs and OsCOLs. Bootstrap values from 1000 replicates were used to assess the robustness of the tree. AcCMF1 from onion was indicated in red boxes

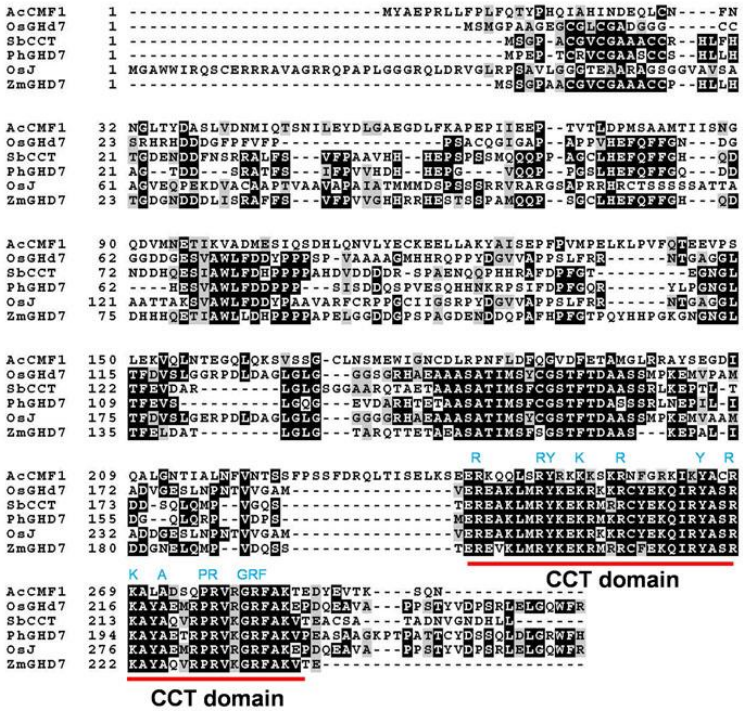

Fig. 2: Conserved protein domains alignment of AcCMF1 with other CMF proteins. The identical and similar residues were shown in black and gray, respectively. The CCT domain was highlighted in red line

The transgenic plants displayed advanced bolting time compared to *co* mutant (Fig. 6). *AcCMF1* not only could promote the plant flowering, but also participated in plants development regulation.

The *gi* is upstream gene of *CO* in the photoperiod pathway of plant flowering regulation. The *gi* mutants showed longer vegetative growth time, thicker stem, longer flowering time and less lateral branches than the wild-type plants. In order to explore the relationship between *CO* genes and other flowering regulation pathways, *AcCMF1*

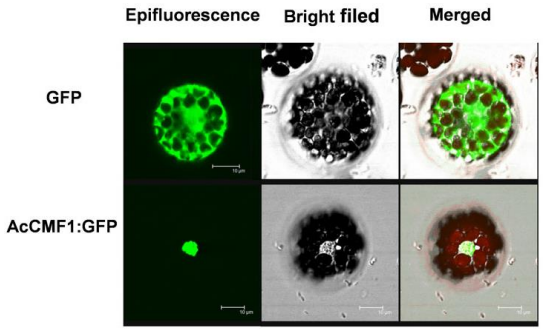

Fig. 3: Subcellular localization of AcCMF1. The left verticals are green fluorescence images, middle verticals are bright-filed images, and right verticals are merged images of bright field and green fluorescence. Scale bars in this figure are 10 μm

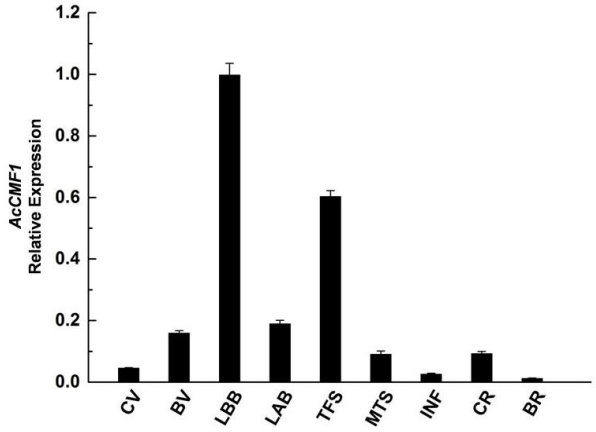

Fig. 4: The expression patterns of *AcCMF1*. The tissue expression patterns of *AcCMF1*. Relative expression levels were determined by qRT-PCR. CV, cauloin in vegetative phase; BV, bulb in vegetative phase; LBB, leaf before bolting; LAB, leaf after bolting; TFS, tender floral stem; MS, mature floral stem; INF, inflorescence; CR, cauloid in reproductive phase; BR, bulb in reproductive phase

was overexpressed in *gi*. The *gi* mutant was bolting at 35-day-stage on average. There was no significant difference in flowering time and plant morphology between *AcCMF1*-OE-*gi* plants and *gi* mutant (Supplementary Fig. S2). *Arabidopsis thaliana ga3* mutant is a GA synthesis blocked mutant. The plant growth of *ga3* was weaker than the wild type. But the flowering time was similar to the wild one. *AcCMF1* overexpressed in *ga3* mutant did not affect the flowering time of *ga3* mutant (Supplementary Fig. S3).

Discussion

Plant flowering is an important developmental process in plant life cycle precisely controlled by various environmental signals especially in commercial crops (Nemoto *et al.* 2003; Miller *et al.* 2008; Jung and Muller 2009; Michaels 2009). CCT family genes exist broadly in

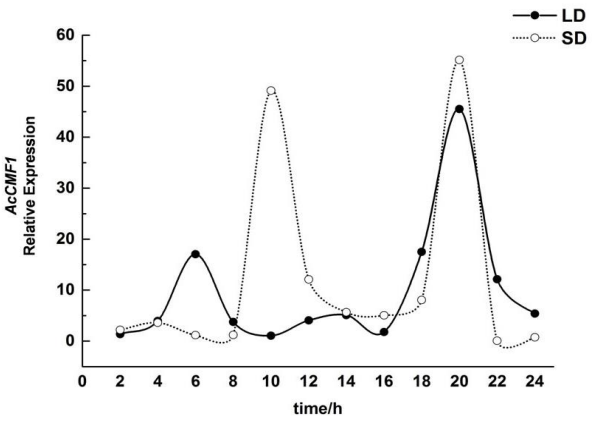

Fig. 5: The diurnal rhythm expression pattern of *AcCMF1* in onion leaves under different photoperiod

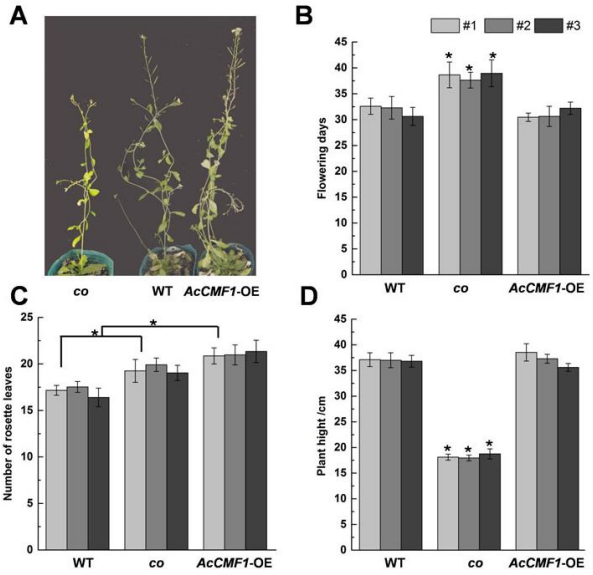

Fig. 6: Overexpression of *AcCMF1* in Arabidopsis *co* mutant. (A) Phenotype (B) Flowering time (C) Number of rosette leaves (D) Plant height of *co* mutant, wild type, *AcCMF1*-OE lines under LD condition. Error bars indicate the standard errors. Asterisks indicate the significant differences ($P < 0.05$)

monocot and dicoty plants. Most members of CCT gene family take effect in plant flowering control. Plant CCT genes were distributed into three categories: COL family, CMF family and PRR family (Cockram *et al.* 2012). COL family and CMF genes were classified into four types. Type I included two normal B-box motifs, such as *AtCO* and *AtCOL1* to *AtCOL5*; *AtCOL6* to *AtCOL8* and *AtCOL16* belonged to type II had a B-box motif and a CCT domain; type III had a B-box motif and a second diverse B-box motif, such as *AtCOL9-AtCOL15*; and CMF genes belonged to type IV with only a CCT domain but no B-box domain (Griffiths *et al.* 2003; Cockram *et al.* 2012; Gangappa and Botto 2014; Wu *et al.* 2017). It has been reported that most type I *COL* homologs played a

positive role in regulation of flowering (Zhang *et al.* 2015; Chaurasia *et al.* 2016). Nevertheless, CCT family genes display multiple functions in flowering regulation. For instance, *AtCOL9* played a negative role in *Arabidopsis* flowering control, repressing *CO* expression (Cheng and Wang 2005). The CMF genes encoded proteins contain a single CCT domain and are critical for domestication and adaptation in cereal crops (Li and Xu 2017). *OsGhd7* was a CMF gene delayed heading under LD conditions but not SD conditions in rice (Xue *et al.* 2008). In this study, a novel CCT family gene, *AcCMF1*, was isolated from onion. AcCMF1 contained a single CCT domain without other structures and taken part in onion flowering regulation (Fig. 1, 2).

CO localized in nucleus, which could bind the promoter of *FT* to trigger its expression to promote flowering (Wenkel *et al.* 2006; Tiwari *et al.* 2010; Nemoto *et al.* 2016). Recent studies suggested that full length of *Phalaenopsis orchid* PaCOL1 protein localized in nucleus. PaCOL1 was still localized in nucleus without B-box domain, but it was localized in cytoplasm and nucleus without CCT motif (Ke *et al.* 2020). In our study, AcCMF1 was localized in nucleus (Fig. 3). This implied that AcCMF might take part in flowering regulation as a transcription factor. Leaf is the most important tissue for plant to intercept light. AtCO was the first certified CCT family gene which control flowering in Arabidopsis as a phloem-specific transcription factor (Robson *et al.* 2001). All *Populus PtCOL* genes were preferentially expressed in leaves (Li *et al.* 2020). In bamboo, *PvCO1* showed abundant transcripts in immature and mature leaves, as well as, *PvCO2* only expressed in bamboo leaves (Xiao *et al.* 2018). *AcCMF1* showed high expression in leaf before bolting (Fig. 4). *CO* was degraded by the ubiquitin ligase CONSTITUTIVE PHOTOMORPHOGENIC 1 (*COP1*) at the posttranscriptional level in dark. In Arabidopsis, the expression of *CO* showed circadian rhythms pattern (Suarez-Lopez *et al.* 2001; Shim *et al.* 2017). Previous study revealed that the transcripts of *CO* was peaking at dawn and dusk in LD condition, which was crucial for the stabilization of CO (Imaizumi *et al.* 2005; Turck *et al.* 2008). Hassidim *et al.* (2009) showed *AtCOL5* overexpression complemented the late flowering phenotype of *co* mutant. *Hd1* was a homolog of *CO* gene in rice. *OsHd1* had a comparable circadian expression pattern with *AtCO*, while *OsHd1* acted as a flowering repressor in SD condition (Takeshi *et al.* 2002). A putative *CO* homolog was cloned and designated *AcCOL* in onion, but *AcCOL* did not display a observable circadian expression pattern (Taylor A *et al.* 2010). *AcCOL2* displayed well diurnal expression pattern in accordance with photoperiod detecting (Rashid and Thomas 2020). In onion, the transcript level of *AcCMF1* showed double peaks 24 h period (Fig. 5). *AcCMF1* might regulat onion flowering by capturing and transforming light signal. The expression of CO was controlled by day and night cycles (Meng *et al.* 2011). *AcCMF1* showed the similar expression pattern with *LfCOL6* in *Lilium× formolong*i, which played positive role in triggering flowering induction under LD (Li *et al.* 2018). This expression pattern was not completely consistent speculating that CCT family genes in onion had different function in flowering regulation and the circadian clock was modulated by different *CCT* genes.

In order to verify the effect of *AcCMF1* family genes on flowering, *AcCMF1* overexpression vector was constructed and transformed to *A. thaliana*. The Arabidopsis *co* mutant performed late flowering phenotype. Nevertheless, overexpressed *AcCMF1* in *Arabidopsis co* mutant could supplement the late flowering phenotype of *co* mutant under LD condition (Fig. 6). *OsGhd7* was an LD-specific repressor played a crucial role in increasing rice yields and controlling heading dates containing only a CCT domain (Xue *et al.* 2008). The *in vivo* investigation indicated *Ghd7* and *Hd1* were interaction to bind the promoter region of *Ehd1* to repress its expression in photoperiod induced flowering pathway (Nemoto *et al.* 2016). *Ghd7* could inhibit the expression of the flowering time pathway genes in conjunction with *Hd1* in *Poaceae* (Nemoto *et al.* 2016). Expression of floral repressor *SbGhd7*, the orthologs of rice *Ghd7*, inhibited *SbCO* transcriptional activity and delayed the flower in sorghum under LD conditions (Yang *et al.* 2014). *AcCMF1* restored the late phenotype of *co* mutant and promote the flowering of onion under LD condition. It is suggestted that *AcCMF1* played positive role in onion flowering regulation and involved in different pathways compared to cereal crops. Further study is essential to gain more knowledge of regulatory mechanism of *AcCMF1* in onion. GIGANTEA (GI) protein modulates the stability of FKF1, which is related to the stabilization of CO in the afternoon of long days (Park *et al.* 1999; Mizoguchi *et al.* 2005; Fowler *et al.* 2014; Hwang *et al.* 2019). It had been reported overexpression of *CO* could restore the late floral phenotype of *gi* mutants under long and short sunshine conditions in Arabidopsis (Ben-Naim *et al.* 2006; Sawa *et al.* 2007). We transformed *AcCMF1* into *gi* mutant of *Arabidopsis*. Overexpressed *AcCMF1* in *gi* mutants had little effect on flowering (Supplementary Fig. S2). There were no significant difference between *gi* mutant and *AcCMF1*-OE lines on flowering time. It was speculated that *gi* did not regulated the accumulation of AcCMF1 and there were other members of onion CCT family involved in the regulation of flowering via *gi*. To verify the interaction between *AcCMF1* and other flowering regulatory genes, *AcCMF1* was overexpressed in *ga3* mutant in Arabidopsis. There were no significant changes between the transgenic plants and *ga3* mutants (Supplementary Fig. S3). It indicated that *AcCMF1* did not take part in the gibberellin pathway of flowering regulation. Previous study mentioned that CCT domain was the essential structure of CO to bind the particular cis-elements of *FT* promoter directly (Tiwari *et al.* 2010). *AcCMF1* might be involved in onion flowering

regulation by adjusting the transcripts of *CO* and *FT*. The mechanism of *AcCMF1* reaction with other CCT or flowering related genes control the onion flowering was unclear and should be explored in further study.

Conclusion

AcCMF1 belonged to onion *CCT* family. The function of *AcCMF1* in plant flowering regulation was revealed by using *AcCMF1* Arabidopsis transgenic lines. *AcCMF1* was expressed highest in young leaves before bolting. *AcCMF1* advanced the flowering time of *co* mutant in Arabidopsis, which also played a positive role in plant flowering.

Acknowledgments

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Author Contributions

Yong Wang designed the experiments, participated in generation of transgenics; Shouyi Ren participated in the cloning experiments and gene expression analysis; Cuicui Zhang participated in qRT-PCR; Yuqi Zhang and Yang Xu participated in sequence alignment and phylogenetic analysis; Jiru Wang participated in subcellular localization; Xiaochen Cong participated in collecting phenotypic data; Lei Qin helped conceiving the study, participated in its coordination and manuscript writing and editing.

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