Functional Divergence of AP1 and FUL Genes Related to Flowering Regulation in Upland Cotton

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Research Article

Keywords: Cotton, GhAP1.1, GhFUL2, Functional divergence, Flowering

Posted Date: June 16th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-482114/v1

Abstract

The AP1/FUL transcription factors are important for floral development, but the underlying molecular mechanisms remain unclear. In this study, we cloned and identified two *AP1*/*FUL*-like genes, *GhAP1.1* and *GhFUL2*, in upland cotton, which is a commonly cultivated economically valuable crop. Sequence alignments and phylogenetic analyses indicated GhAP1.1 and GhFUL2, which are encoded by genes in the *AP1*/*FUL* clade, have conserved N-terminals, but diverse C-terminal domains. A quantitative real-time PCR analysis revealed that *GhAP1.1* and *GhFUL2* were expressed in the flower and root, and had the opposite expression patterns during different shoot apical meristem stages. The upregulated expression of *GhAP1.1* in Arabidopsis and the silencing of *GhAP1.1* did not induce significant changes to the flowering time or floral organs, but the transcript levels of the florigen *FT* gene and the *AP1* homolog *GhMADS42* increased. The overexpression of *GhFUL2* in Arabidopsis delayed flowering and promoted bolting by decreasing the *FT* and *LFY* expression levels. Silencing *GhFUL2* in cotton dramatically increased the expression of *GhFT* and *GhMADS42* and promoted flowering. Additionally, yeast two-hybrid and bimolecular fluorescence complementation assays indicated that GhAP1.1 can interact with the SVP homolog GhSVP1, whereas GhFUL2 can form heterodimers with SEP1, SEP4 homologs, and GhSVP1. Therefore, we proved that the functional divergence of *GhAP1.1* and *GhFUL2*, which involved changes in sequences and expression patterns, influenced the regulation of cotton flower development.

Key Message

GhFUL2 is a negative regulator that interacts with SEP and SVP proteins in cotton to regulate flowering and plant architecture.

Introduction

Gene duplications, which are prevalent events during plant evolution, occur via whole-genome duplications or tandem duplications. Several studies confirmed the high frequency of gene duplications in land plants, including Arabidopsis, cotton, wheat, and tobacco (Flagel and Wendel 2009; Sun et al. 2014; Yang et al. 2018; Vision et al. 2000). Gene duplications during evolution increase the number of genes in the genome and may be associated with functional divergence (Huang et al. 2020; Yuan et al. 2015). The MADS-box transcription factors play important roles in gene regulatory networks controlling floral transitions as well as flower and fruit development (Becker et al. 2003; Becker and Theissen 2003; Yang and Jack 2004; Preston and Kellogg 2006; Li et al. 2009; Nishikawa et al. 2009).

The angiosperm *APETALA1* (*AP1*)/*FRUITFULL* (*FUL*) gene lineage is an important MADS-box clade in which the number of genes increased because of duplication events. During the key gene duplication event, the *AP1*/*FUL* clade was divided into the core eudicots and non-core eudicot species. The core eudicot genes included Arabidopsis *AP1*, Arabidopsis *FUL*, and *Antirrhinum* sp. *SQUAMOSA*, whereas the

non-core eudicot genes were similar to the Arabidopsis *FUL* gene (Litt and Irish 2003). Although the *AP1*/*FUL* sequences are similar, with both encoding the conserved MADS-box and K-box sequences, and are derived from the same paralog, the AP1 and FUL proteins vary regarding the motifs in the C-terminal domain (Zahn et al. 2005; Shan et al. 2007). The functional divergence between AP1 and FUL resulted from changes in important transcriptional regulators and coding sequences, especially unique conserved motifs (McCarthy et al. 2015). Thus, changes to the gene sequences encoding C-terminal domains and other protein regions may lead to functional divergence.

The functions of the *AP1*/*FUL*-like genes diverged during plant evolution. These genes have important functions influencing plant growth and developmental processes, including floral transitions, fruit ripening, and the opening of the apical hook (Wang et al. 2014; Chen et al. 2015; Li et al. 2016; Führer et al. 2020). In Arabidopsis, AP1 affects the positioning of organs as well as sepal and petal identity (Irish and Sussex 1990), whereas FUL inhibits cell division and promotes cell expansion, while also regulating the transcription of cellular differentiation-related genes during fruit development (Gu et al. 1998). Additionally, *AP1* combined with the *ful* mutation leads to a non-flowering phenotype and altered inflorescence architecture (Ferrándiz1 et al. 2000). In rice, the AP1/FUL transcription factor gene *OsMADS18* and its two paralogs *OsMADS14* and *OsMADS15* have different functions related to plant development. Both *OsMADS14* and *OsMADS15* specify the palea and lodicule identities (Wu et al. 2017), whereas *OsMADS18* is involved in delaying seed germination, altering plant architecture, and decreasing the number of tillers (Yin et al. 2019). In *Brachypodium distachyon* (Poaceae species), four *AP1*/*FUL* paralogs have been identified, with only *BdVRN1* expressed normally during a prolonged cold treatment; the ectopic expression of the other three genes reportedly leads to early flowering and severe morphological alterations to floral organs (Li et al. 2016). In tomato, the overexpression of the *AP1*/*FUL* homolog *MBP20* results in simple leaves and modulated leaf development (Burko et al. 2013). Functional analyses of transgenic tomato plants revealed that *FUL2* overexpression might result in fruit and leaf morphological changes, whereas the suppression of *FUL1* and *FUL2* expression can inhibit fruit ripening through ethylene biosynthesis and the regulation of ripening-related gene expression during tomato fruit ripening processes (Wang et al. 2014).

Cotton is an economically important crop cultivated worldwide. The MADS-box family genes have been identified in several cotton species. For example, 11 *AP1*/*FUL* clade genes were identified in tetraploid cotton and were subsequently analyzed in terms of their structures and expression profiles (Nardeli et al. 2018; Jiang et al. 2014). Among the genes in the *AP1*/*FUL* clade, *GhAP1.7* promotes flowering in Arabidopsis and is regulated by GhLFY in specific pathways (Cheng et al. 2021b). A recent study confirmed GhAP1 interacts with GhCAL to form a heterodimer that may regulate *GhAP1* expression, with *GhAP1*-silenced plants exhibiting significantly late flowering (Cheng et al. 2021a). Although a few *AP1* genes have been functionally characterized, other *AP1*/*FUL* homologs and the associated regulatory mechanisms remain unknown. In this study, two *AP1*/*FUL* homologs were cloned from cotton cultivars exhibiting differential maturation. Moreover, the expression of these homologs in shoot meristems at several stages was analyzed. The results of this study may be useful for clarifying the functional divergence of genes in cotton and related species.

Materials And Methods

Plant materials and growth conditions

Cotton cultivars CCRI50 (late maturing with a normal fruit branch) and ZAO1 (early maturing with a clustered fruit branch) were grown at 28 °C in a greenhouse at the Henan Institute of Science and Technology. Different tissues were sampled from CCRI50 plants. Shoot meristems were harvested from both CCRI50 and ZAO1 plants from the four-leaf expansion stage to the seven-leaf expansion stage. For phytohormone treatments, cotton seeds were sown in plastic pots, which were then incubated at 28 °C with a 14-h light/10-h dark cycle until seedlings reached the two-leaf expansion stage (about 4 weeks). The cotton seedlings were treated with 100 μM abscisic acid, gibberellin, or salicylic acid. The leaves were harvested at 1, 3, 6, 12, and 24 h post-treatment, immediately frozen in liquid nitrogen, and stored at −80 °C until analyzed. The wild-type (WT) and *GhAP1.1*- and *GhFUL2*-overexpressing transgenic Arabidopsis lines were grown in a culture room at 22 °C with a 16-h light/8-h dark cycle. Tobacco plants were grown in a greenhouse at 25 °C with a 14-h light/10-h dark cycle.

Phylogenetic and gene sequence analysis

A phylogenetic tree was constructed according to the neighbor-joining method using MEGA5.05. The *GhAP1.1* and *GhFUL2* coding sequences were amplified from the cDNA of CCRI50.

Gene expression profile analysis

Total RNA was extracted from samples using the RNA Pure Plant Plus kit (Tiangen). The purified RNA served as the template for synthesizing first-strand cDNA using the PrimeScript RT reagent Kit with gDNA Eraser (Takara). A quantitative real-time (qRT)-PCR assay was performed in 384-well plates using the ABI Q6 system (ABI) and SYBR Green Premix Ex Taq (Takara). The *GhACTIN* and *AtUBQ7* genes were selected as the internal controls for cotton and Arabidopsis, respectively. The $2^{-\Delta\Delta Ct}$ method was used to calculate relative gene expression levels.

Virus-induced gene silencing (VIGS) assay

For the VIGS assay, a 315-bp fragment of *GhAP1.1* and a 291-bp fragment of *GhFUL2* were amplified from the cDNA of CCRI50 and inserted into the pCLCrVA vector to construct recombinant plasmids. The pCLCrVA-GhAP1.1 and pCLCrVA-GhFUL2 plasmids were inserted into separate *Agrobacterium tumefaciens* GV3101 cells. The GV3101 cells containing pCLCrVA-GhAP1.1, pCLCrVA-GhFUL2, pCLCrVA (negative control), or pCLCrVA-PDS (positive control) were mixed with cells carrying pCLCrVB (1:1 ratio). The cells were cultured until the OD_{600} reached 2.0, after which they were injected into 10-day-old cotton cotyledons. After 8 weeks, the cotton plants were analyzed regarding their gene expression profiles and phenotypes. The inoculated cotton plants were grown in a greenhouse at 22 °C with a 16-h light/8-h dark cycle. The analysis was repeated at least three times.

Yeast two-hybrid (Y2H) assay

The *GhAP1.1* and *GhFUL2* coding sequences were cloned into the pGBKT7 vector according to the In-Fusion cloning method to construct the bait vectors. The coding sequences of eight *GhSEP* genes and four *GhSVP* genes were amplified from cotton cDNA and ligated to pGADT7 to construct the prey vectors. The bait and prey vectors were used to transform Y2HGold yeast cells as described in the yeast transformation system user manual (Clontech). Protein interactions were screened on SD/−Trp/−Leu double dropout medium (DDO) and SD/−Trp/−Leu/−His/−Ade quadruple dropout medium (QDO) supplemented with aureobasidin A (AbA) and X-α-galactosidase (X-α-Gal) (Clontech). The primers used are listed in Supplementary Table S1.

Bimolecular fluorescence complementation (BiFC) assay

To verify protein interactions *in vivo*, the *GhAP1.1* and *GhFUL2* coding sequences were inserted into the pUC-SPYNE vector, whereas partial sequences of the *SEP* and *SVP* homologs were inserted into the pUC-SPYCE vector. The resulting recombinant plasmids were inserted into *A. tumefaciens* GV3101 cells, which were then introduced into *Nicotiana benthamiana* leaves using needleless syringes for the subsequent coexpression analysis. More specifically, the transiently transformed tobacco plants were incubated for 3 days (post-injection) at 22 °C with a 14-h light/10-h dark cycle. The fluorescence in the lower epidermal cells of tobacco leaves was observed 72 h later using a confocal microscope (Zeiss LSM780).

Results

Sequence alignment and phylogenetic analysis of *AP1/FUL* genes in cotton

An analysis of upland cotton genomic data revealed five *AP1* and four *FUL* orthologs of the Arabidopsis *AP1* and *FUL* genes (Fig. 1a). The encoded amino acid sequences included a conserved MADS domain, a K domain, and a FUL motif. With the exception of *GhAP1.2*, the cotton *AP1* orthologs encoded the AP1 motif. In contrast, the four *FUL* genes in cotton encoded the paleoAP1 motif. Additionally, a comparison with the AtAP1 protein sequence indicated the GhAP1.1 and GhAP1.2 were more similar than GhAP1.3 (Fig. 1b). These results suggest the diversity in the C-terminal domain encoded by *MIKC*-type genes may be associated with functional divergence.

Expression profiles of *AP1/FUL* genes at different shoot meristem stages and in response to phytohormone treatments

Cotton cultivars ZAO1 and CCRI50 were used to analyze shoot meristem expression levels from the four-leaf expansion stage to the seven-leaf expansion stage. The data revealed that *GhAP1.1* was more highly expressed in ZAO1 than in CCRI50, whereas the opposite pattern was observed for *GhFUL2* (Fig. 2a and 2c). The *GhAP1.1* and *GhFUL2* expression levels in different cotton tissues and after phytohormone treatments were examined in a qRT-PCR assay. The results indicated *GhAP1.1* was highly expressed in the flower and apical bud, whereas *GhFUL2* was highly expressed in the root and apical bud (Fig. 2b). The three phytohormone treatments upregulated the *GhAP1.1* and *GhFUL2* expression levels. The *GhAP1.1* transcript levels peaked at 3 h, after which they decreased to control levels (Fig. 3a). The *GhFUL2*

expression levels following the abscisic acid and salicylic acid treatments also peaked at 3 h and then rapidly decreased. In contrast, following the gibberellin treatment, *GhFUL2* expression gradually increased and peaked at 24 h (Fig. 3b).

Ectopic expression of *GhAP1.1* and *GhFUL2* in Arabidopsis

To functionally characterize *GhAP1.1* and *GhFUL2*, binary vectors were constructed for the subsequent examination of the constitutive expression of these genes in transgenic Arabidopsis (ecotype Columbia-0). Eleven and nine independent kanamycin-resistant T_1 transgenic plant lines were generated for 35S::*GhAP1.1* and 35S::*GhFUL2*, respectively. Three T_3 *GhAP1.1* lines flowered 2 days earlier and produced 2–3 fewer rosette leaves than the WT plants under long-day conditions, whereas the phenotypes of the other lines did not differ from the WT phenotype (Table 1). However, six *GhFUL2*-overexpressing transgenic plants flowered 5–6 days later and produced 2–3 more rosette leaves than the WT plants under long-day conditions (Fig. 4a-d and Table 1). The expression of *GhAP1.1*, *GhFUL2*, and flowering-related genes in transgenic plants was analyzed by qRT-PCR. The results revealed that *FT*, *AP1*, and *FUL* expression levels were upregulated and the *LFY* expression level was downregulated in the three *GhAP1.1*-overexpressing transgenic Arabidopsis lines that flowered earlier (Fig. 4e). In the *GhFUL2*-overexpressing transgenic Arabidopsis lines, the expression of both *FT* and *LFY* was significantly downregulated compared with the corresponding expression in the WT plants, whereas *FUL* expression was significantly upregulated (Fig. 4f).

Silencing *GhAP1.1* and *GhFUL2* expression promoted cotton flowering

Virus-induced gene silencing assays were completed to verify the GhAP1.1 and GhFUL2 functions. The empty pCLCrVA vector was used as a negative control, whereas pCLCrVA-PDS served as the positive control. The appearance of white leaves and stems indicated the VIGS was successful (Fig. 5a). A qRT-PCR analysis confirmed the *GhAP1.1* and *GhFUL2* expression levels decreased significantly in the VIGS plants (Fig. 5c and 5f). Compared with the control plants, the flowering time was accelerated only for the plants in which *GhFUL2* was silenced (Fig. 5e). According to the qRT-PCR assay data, the *GhAP1.1* and *GhFUL2* expression levels were significantly lower in the VIGS plants than in the negative control plants. Additionally, the silencing of *GhFUL2* and *GhAP1.1* significantly upregulated the expression of both *GhFT* and *GhMADS42* (Fig. 5d and 5g).

Interactions between GhAP1.1/GhFUL2 and GhSVP/GhSEP-like proteins in Y2H and BiFC assays

The AP1/FUL subfamily proteins may interact with SOC1/SVP/SEP to form heterodimers with regulatory roles. The cotton genome contains more of these MADS-box genes than the Arabidopsis genome. Previous research proved that GhAP1/FUL can interact with GhSOC1 *in vivo* and *in vitro* (Zhang et al. 2016). In the current study, eight *SEP*-like genes and four *SVP*-like genes were cloned to verify interactions in Y2H and BiFC assays. The Y2H assay results indicated GhAP1.1 can interact with GhSEP5 and GhSVP1, whereas GhFUL2 can interact with GhSEP2, GhSEP3, GhSEP4, GhSEP5, GhSEP6, GhSEP7, and GhSVP1 (Fig. 6). These interactions were verified by BiFC assays involving 1-month-old tobacco leaves.

The YFP signals detected in the nucleus and/or membranes reflected the interactions of GhAP1.1 with GhSVP1 and of GhFUL2 with GhSEP2, GhSEP3, GhSEP4, GhSEP5, GhSEP6, and GhSVP1 (Fig. 7). These findings demonstrated that both GhAP1.1 and GhFUL2 can form heterodimers with GhSVP1, but only GhFUL2 can form a heterodimer with SEP1 and SEP4.

Discussion

Upland cotton is an economically valuable tetraploid crop. Polyploidization, which has been a major force driving plant evolution, has resulted in gene duplications and the expansion of gene families. Gene duplication events have been accompanied by the development of new gene functions influencing plant development as well as gene functional divergence and redundancy. The MADS-box genes encode important transcription factors modulating reproductive and vegetative developmental processes.

In upland cotton, 106 *MIKC*-type MADS-box genes have been identified, which is three-fold more than the number of corresponding genes in Arabidopsis. Moreover, these genes include eight *AP1/FUL* homologs (Nardeli et al. 2018). In the current study, we investigated the expression patterns and functions of two *AP1/FUL* genes in cotton. In an earlier study on Arabidopsis, FUL, which was negatively regulated by AP1, was undetectable in the young floral primordia, but it accumulated in the walls of the developing carpel (Mandel and Yanofsky 1995). Other studies confirmed that both *AP1* and *FUL* genes encode ABC-class proteins that affect floral transitions, flower organ identity, and fruit development (Pelaz et al. 2001; Ellul et al. 2004; Lin et al. 2009; Shimada et al. 2009; Shulga et al. 2011; Pabon-Mora et al. 2012; Pabon-Mora et al. 2013). Among monocots, *FUL*-like genes in wheat and barley are responsive to vernalization (Trevaskis et al. 2007; Distelfeld et al. 2009), whereas rice *FUL*-like genes, including *OsMADS14*, *OsMADS15*, *OsMADS18*, and *OsMADS20*, have important functions related to inflorescence and floral meristem identity (Kobayashi et al. 2012b; Wu et al. 2017). In cotton, *AP1* and *FUL* genes are reportedly differentially expressed in various tissues (Jiang et al. 2014). Consistent with this earlier observation, *GhAP1.1* was highly expressed in the flower and apical bud, whereas *GhFUL2* was highly expressed in the root and apical bud. The analyses of the shoot meristem transcriptomes of the two cotton cultivars examined in this study revealed differences in the *GhAP1.1* and *GhFUL2* expression profiles. More specifically, *GhAP1.1* expression was upregulated in ZAO1, whereas *GhFUL2* expression was upregulated in CCRI50. These observations reflect the divergence in *AP1/FUL* expression during shoot apical meristem development in tetraploid cotton species.

The upland cotton genome contains homologs of Arabidopsis *AP1*, including *GhMADS42*, which can promote flowering and alter floral organs when expressed in transgenic Arabidopsis plants (Zhang et al. 2016). The expression of another homolog, *GhAP1.7*, can induce precocious flowering. Additionally, LEAFY, which affects cotton floral meristem identity, can bind to the *GhAP1.7* promoter and negatively regulate expression (Cheng et al. 2021b; Li et al. 2013). In a recent study, *GhCAL*, which is another *AP1* homolog in cotton, was identified, and anti-*GhCAL* transgenic cotton plants exhibited delayed floral bud differentiation and flowering (Cheng et al. 2021a). In contrast to these earlier results, we observed that the overexpression of *GhAP1.1* in Arabidopsis did not induce early flowering or alter the floral organ

phenotype. Accordingly, *GhAP1.1* may be a redundant gene in tetraploid cotton. The expression of a *FUL* ortholog, *GhMADS22*, in transgenic Arabidopsis can promote flowering and result in the production of abnormal flowers (Zhang et al. 2013), but the overexpression of *GhFUL2* in Arabidopsis can delay flowering and promote bolting. In Arabidopsis, the function of the *FUL* gene mimics the function of the *SMALL AUXIN UPREGULATED RNA 10* (*SAUR10*) gene in stems and inflorescence branches (Bemer et al. 2017). In rice, the downregulated expression of *OsMADS18* reportedly delays seed germination and young seedling growth, whereas the upregulated expression of *OsMADS18* decreases the number of tillers (Yin et al. 2019). Considering the tillering in wheat is similar to the bolting in Arabidopsis, this result indicates that *GhFUL2* may have functions affecting plant architecture. In our study, the upregulation of *GhFUL2* expression decreased the expression of *FT* and *LFY* genes in Arabidopsis. Furthermore, *FT* expression was significantly upregulated in *GhFUL2*-silenced cotton plants. A previous study demonstrated that rice *AP1*/*FUL*-like genes (*OsMADS14*, *OsMADS15*, and *OsMADS18*) function upstream of the *FT* homologs *Hd3a* and *RFT1* (Kobayashi et al. 2012a). In wheat, the protein encoded by the *AP1*/*FUL*-like gene *VRN1* binds to the CArG-box of the *FT*-like gene *WFT* to upregulate expression (Tanaka et al. 2018). These results imply that FUL MADS-box transcription factors have diverse functions influencing the flowering time, floral organ formation, and the regulation of plant architecture in cotton.

The interactions among MADS-box proteins during floral development have been relatively well elucidated. Previous research proved that AP1 and SEP3 interact to control sepal development (Pelaz et al. 2001). Another study indicated FUL does not interact with SVP, but both AP1 and FUL interact with SOC1 and SEP4 (de Folter et al. 2005). Interestingly, in cotton, GhAP1.1 can interact with the SVP homolog GhSVP1, but not with SEP proteins, whereas GhFUL2 can form heterodimers with SEP1, SEP4 homologs, and GhSVP1. Three FUL proteins in *Platanus acerifolia* can interact strongly with the E-class (SEP-like) proteins (Zhang et al. 2019). The interaction between FUL and SVP has been observed in other plants (van Dijk et al. 2010; Balanz et al. 2014). Considering the interaction of SEP1, SEP4, or GhSVP1 with GhFUL2, we speculate that GhFUL2 may function cooperatively with SEP1, SEP4 homologs, or GhSVP1 to control the flowering time and floral organ formation or the plant architecture.

In this study, we proved that GhFUL2 can function as a transcription factor with important roles related to floral development and plant architecture. In contrast, *GhAP1.1* may be a redundant gene. Future investigations should focus on the SEP and SVP regulatory mechanisms influencing floral organs and plant architecture. Doing so will expand our understanding of GhFUL2 functions mediating cotton development.

Declarations

Author contribution statement: XZ QM and SF conceived and designed research. XZ and GH conducted experiments and wrote the manuscript. HW and ZR revised the manuscript. All authors read and approved the manuscript.

Funding: This work was financially supported by the State Key Laboratory of Cotton Biology (CB2018A08) and National Key project of Science and Technology (2020ZX08009-12B).

Conflicts of interest: The authors have no conflicts of interest to declare.

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Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

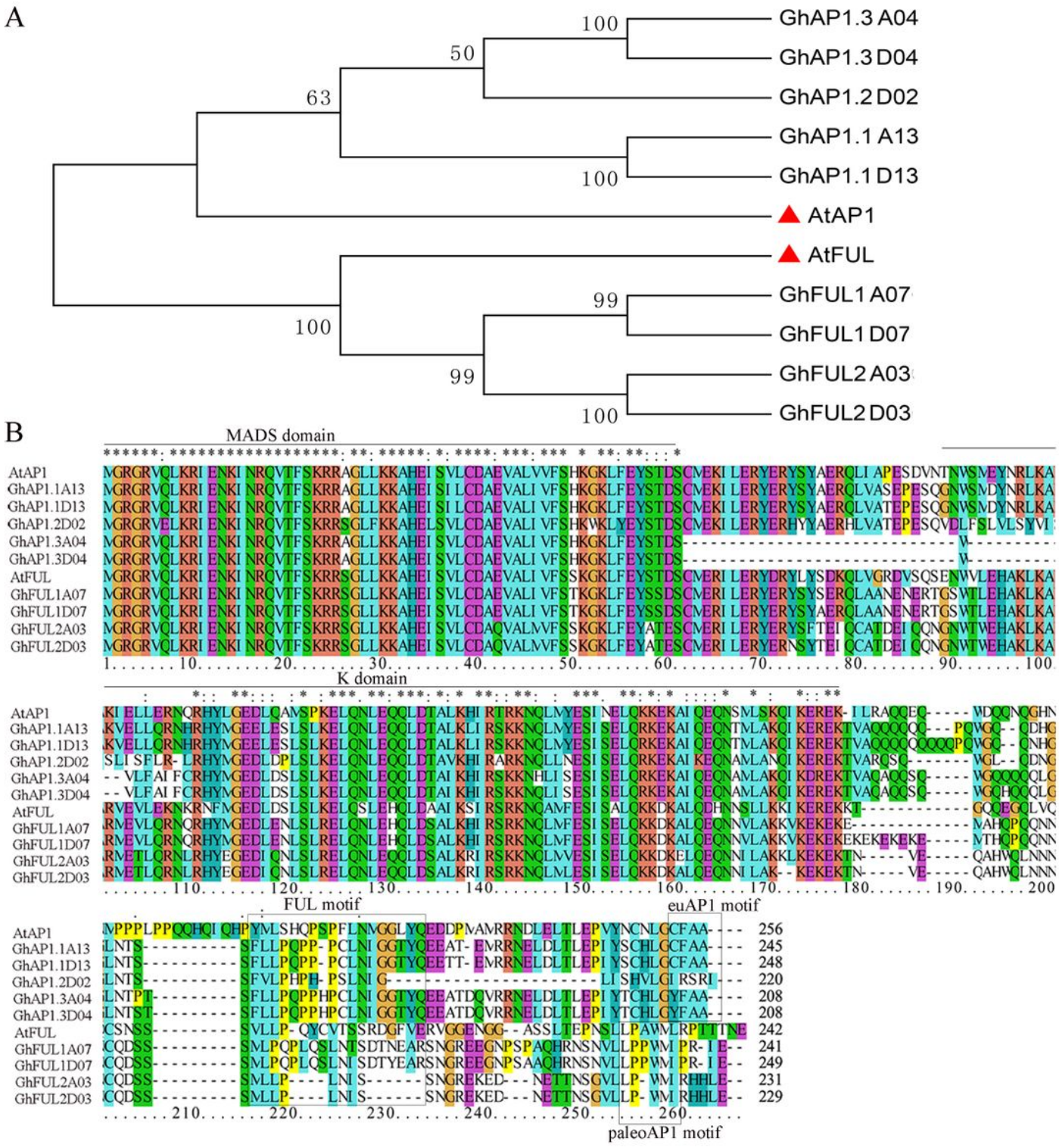

Figure 1

Phylogenetic analysis and analysis of the amino acid sequences encoded by AP1 and FUL in Arabidopsis and cotton. (a) Phylogenetic analysis of Arabidopsis AtAP1 (AT1G69120) and AtFUL (AT5G60910) and their homologs in cotton. (b) Alignment of nine AP1/FUL proteins with their homologs in Arabidopsis (AtAP1 and AtFUL). The N-terminal and the internal amino acid residues (indicated by a line) represent

MADS-box conserved domains containing the MADS and K domains. The C-terminal amino acid residues (in boxes) form the FUL motif, the euAP1 motif, and the paleoAP1 motif

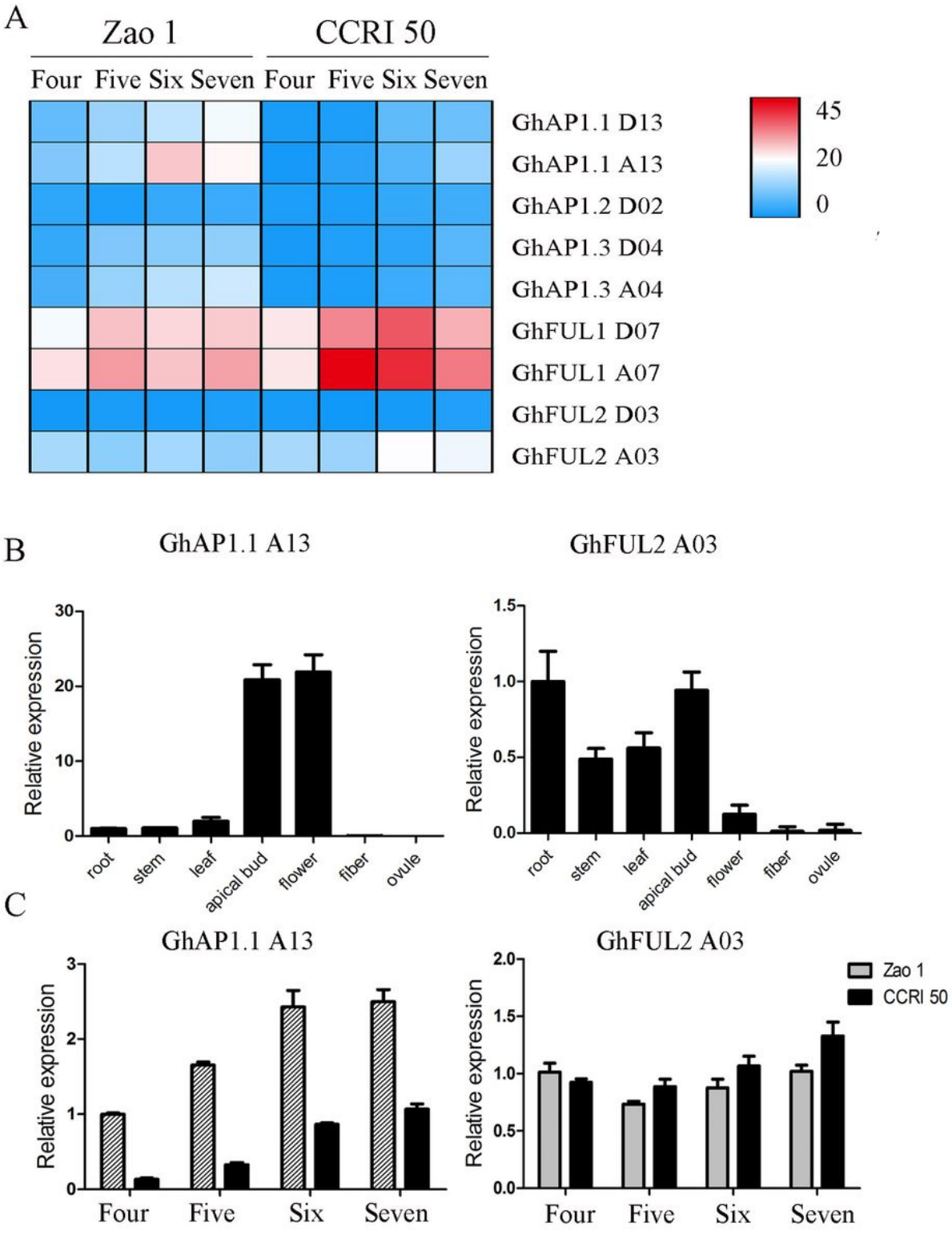

Figure 2

Expression profiles of AP1/FUL homologs in different Gossypium hirsutum tissues and shoot meristem developmental stages. (a) Expression levels are indicated by different colors, with red and deep blue representing the highest and lowest levels, respectively. (b) Tissue-specific expression patterns of

GhAP1.1 and GhFUL2. (c) Expression patterns in the shoot meristem developmental stages of two cotton cultivars. Gene expression levels were determined by quantitative real-time PCR. Data are presented as the mean ± standard error of three biological replicates. An Actin gene was used for data normalization

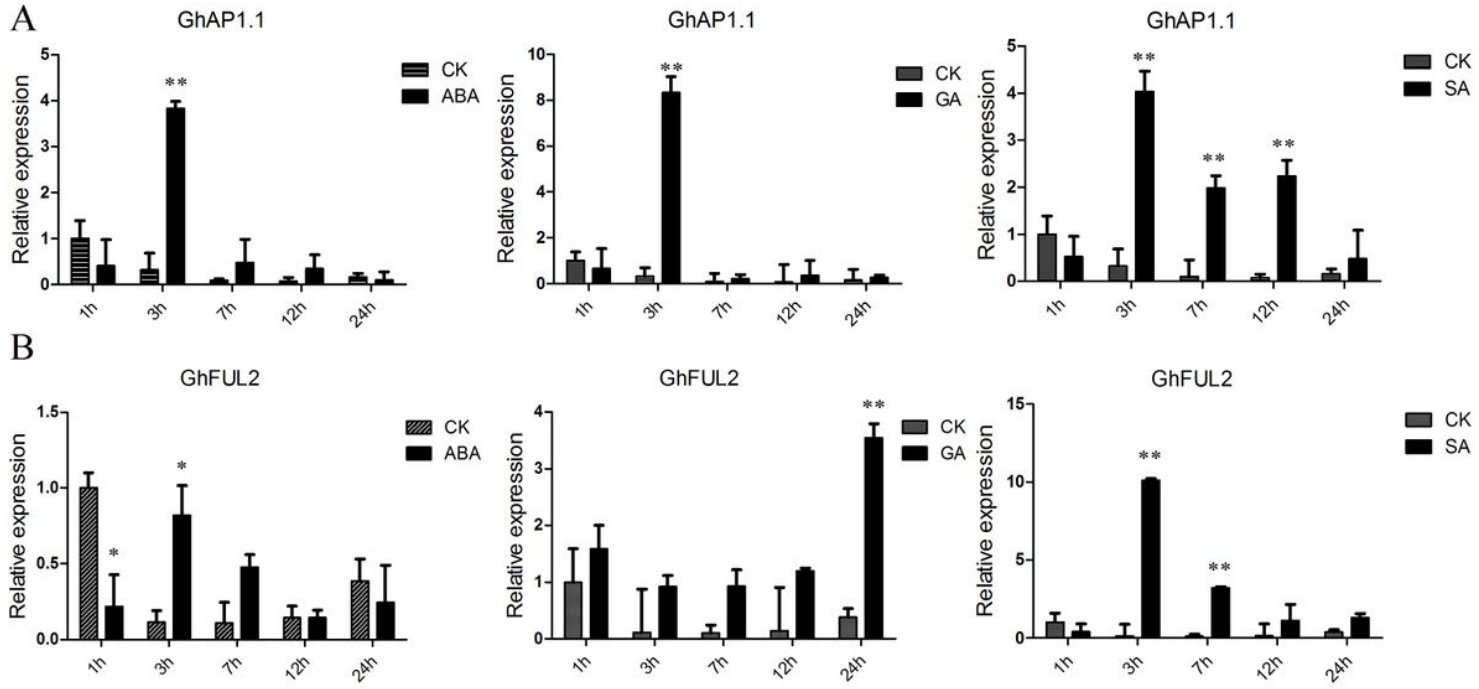

Figure 3

Expression patterns of GhAP1.1 and GhFUL2 in cotton in response to various hormone treatments. Hydroponic cotton seedlings at the two-leaf expansion stage were treated with 100 μM ABA, GA, or SA, after which leaf and stem samples were harvested at different time-points. Total RNA was extracted and a qRT-PCR assay was performed to determine GhAP1.1 and GhFUL2 expression levels, with an Actin gene used as the internal control. (a) Expression profiles of GhAP1.1 following ABA, GA, and SA treatments. (b) Expression profiles of GhFUL2 following ABA, GA, and SA treatments. The significance of the data was determined using Student's t-test (*P < 0.05, **P < 0.01)

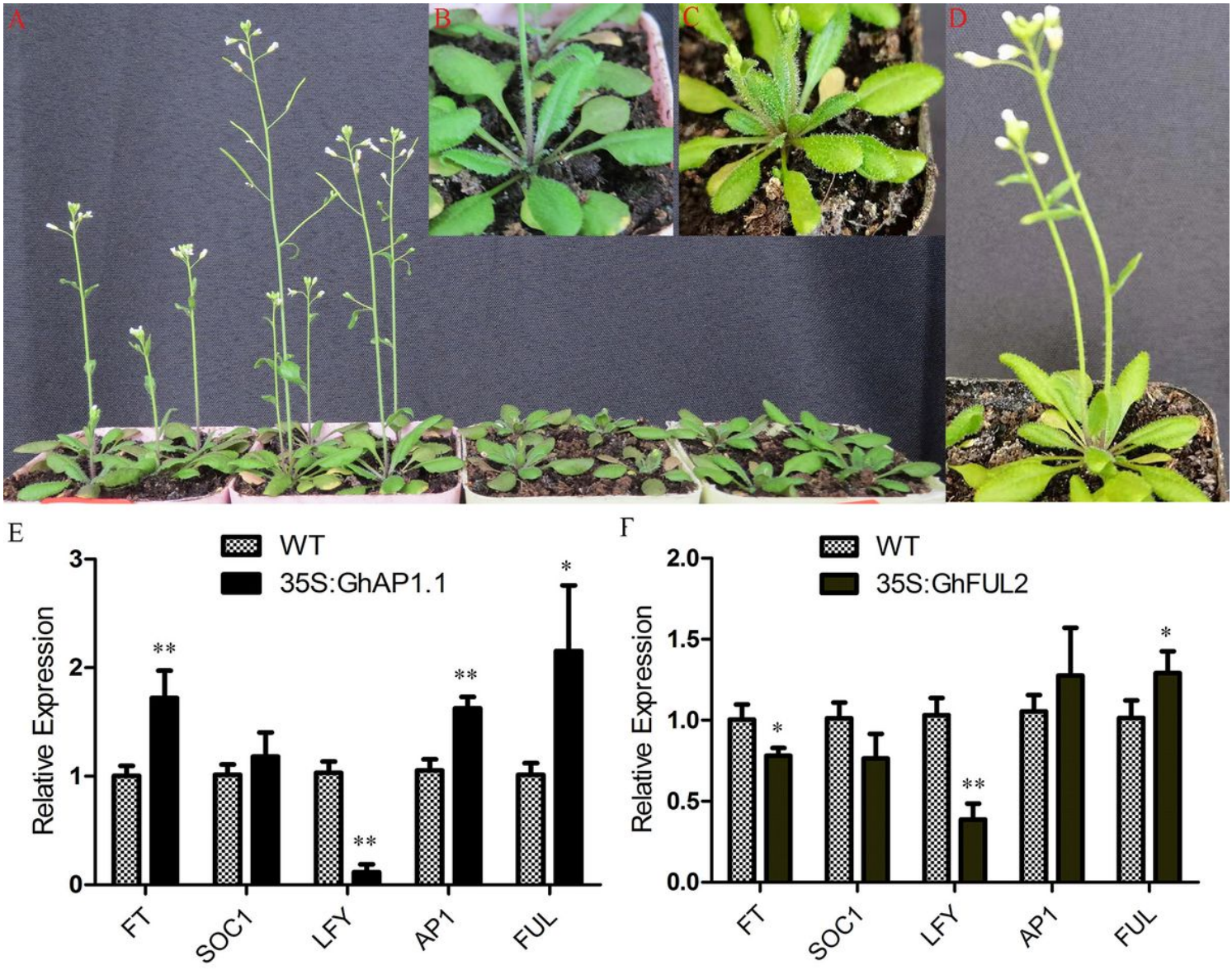

Figure 4

Flowering time and floral phenotypes of Arabidopsis plants and gene expression in WT and GhAP1.1- and GhFUL2-overexpressing transgenic plants. (a) Flowering times of WT and GhAP1.1-overexpressing lines. (b and c) Bolting architecture of WT and GhAP1.1-overexpressing lines. (d) Expression patterns of Arabidopsis flowering-related genes, with UBQ used as an internal control. The significance of the data was determined using Student's t-test (*P < 0.05, **P < 0.01)

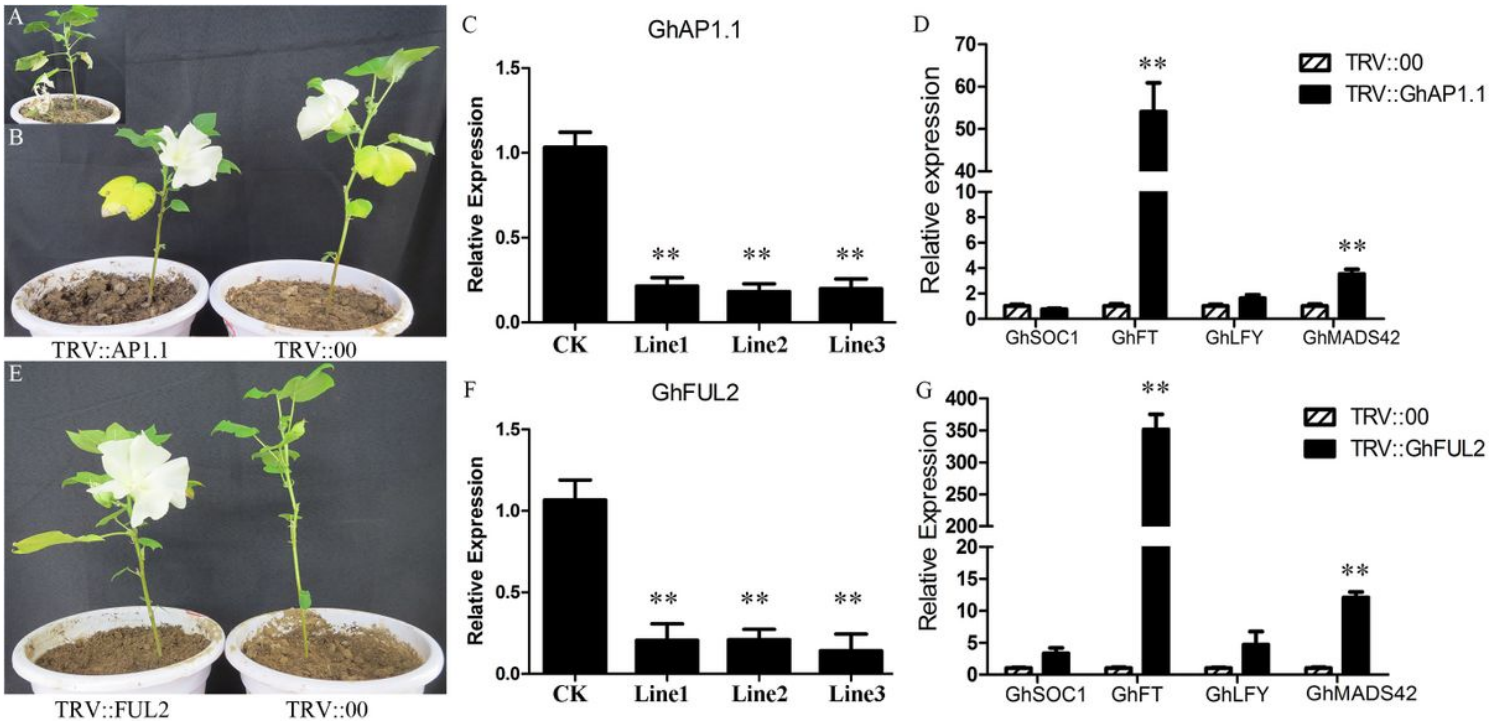

Figure 5

Functional analysis of GhAP1.1 and GhFUL2. (a) VIGS of GhAP1.1 and GhFUL in CCRI50, using pCLCrVA::PDS as a positive control. (b) VIGS of GhAP1.1 in CCRI50, using pCLCrVA::00 as a negative control. (c) Relative expression levels of GhAP1.1 in the control and three VIGS lines. (d) Relative expression levels of GhSOC1, GhFT, GhLFY, and GhMADS42. (e) VIGS of GhFUL in CCRI50, using pCLCrVA::00 as a negative control. (f) Relative expression levels of GhAP1.1 in the control and three VIGS lines. (g) Relative expression levels of GhSOC1, GhFT, GhLFY, and GhMADS42. The significance of the data was determined using Student's t-test (*P < 0.05, **P < 0.01)

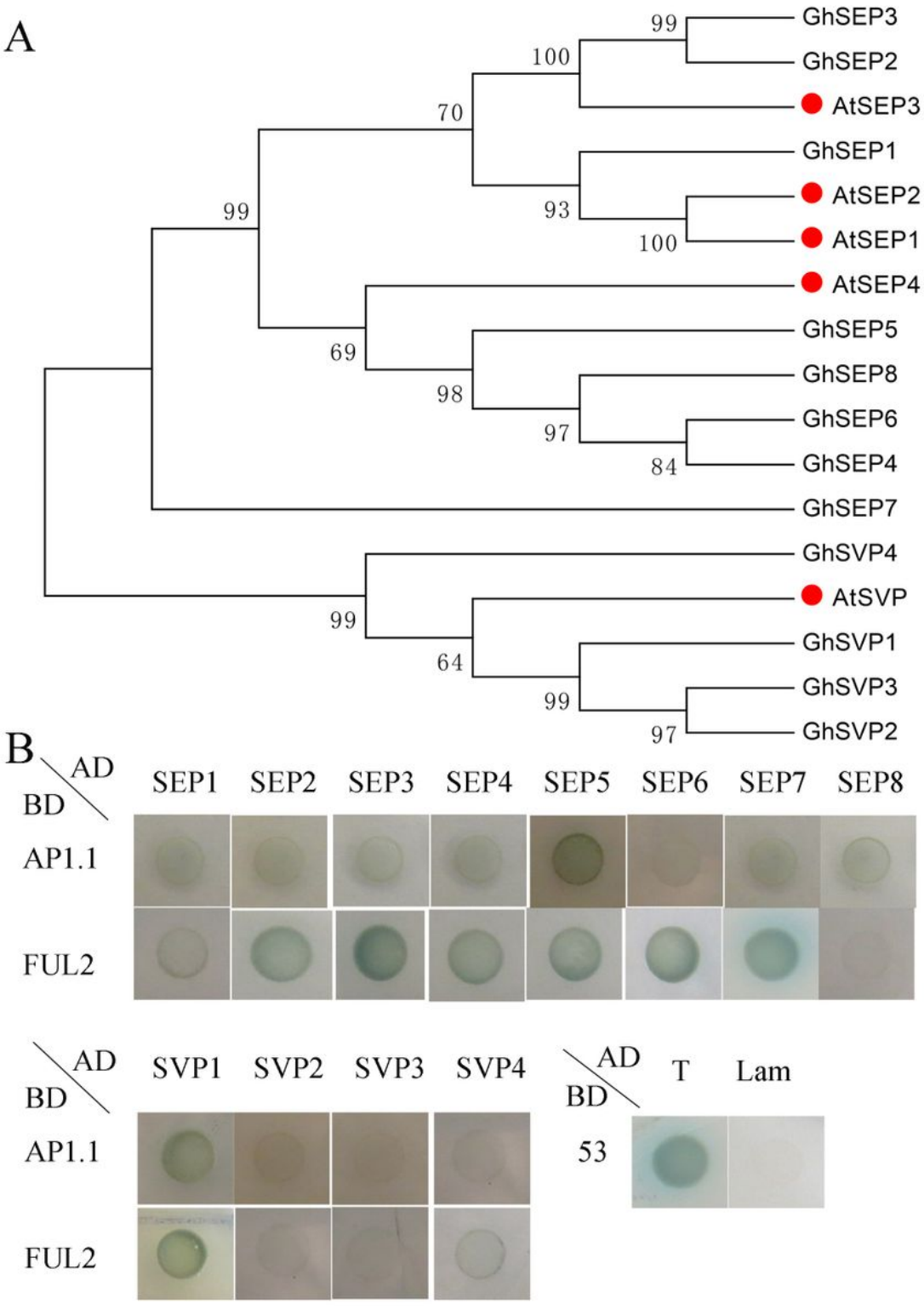

Figure 6

Interaction of GhAP1.1 and GhFUL2 with cotton SEP/SVP-like proteins. (a) Phylogenetic tree of SEP/SVP-like proteins from Arabidopsis and cotton. (b) Interaction of GhAP1.1 and GhFUL2 with cotton SEP/SVP-like proteins in yeast cells. The GhAP1.1 and GhFUL2 genes were fused in-frame to the GAL4 DNA-binding domain (BD)-coding sequence. Eight SEP genes and four SVP genes were fused in-frame to the GAL4 activation domain (AD)-coding sequence. Cell growth on SD/−Trp/−Leu/−His/−Ade quadruple

dropout medium (QDO) supplemented with aureobasidin A and X-α-galactosidase indicated positive interactions. Interactions involving pGBKT7-53 and pGADT7-T/pGADT7-lam were used as positive and negative controls, respectively

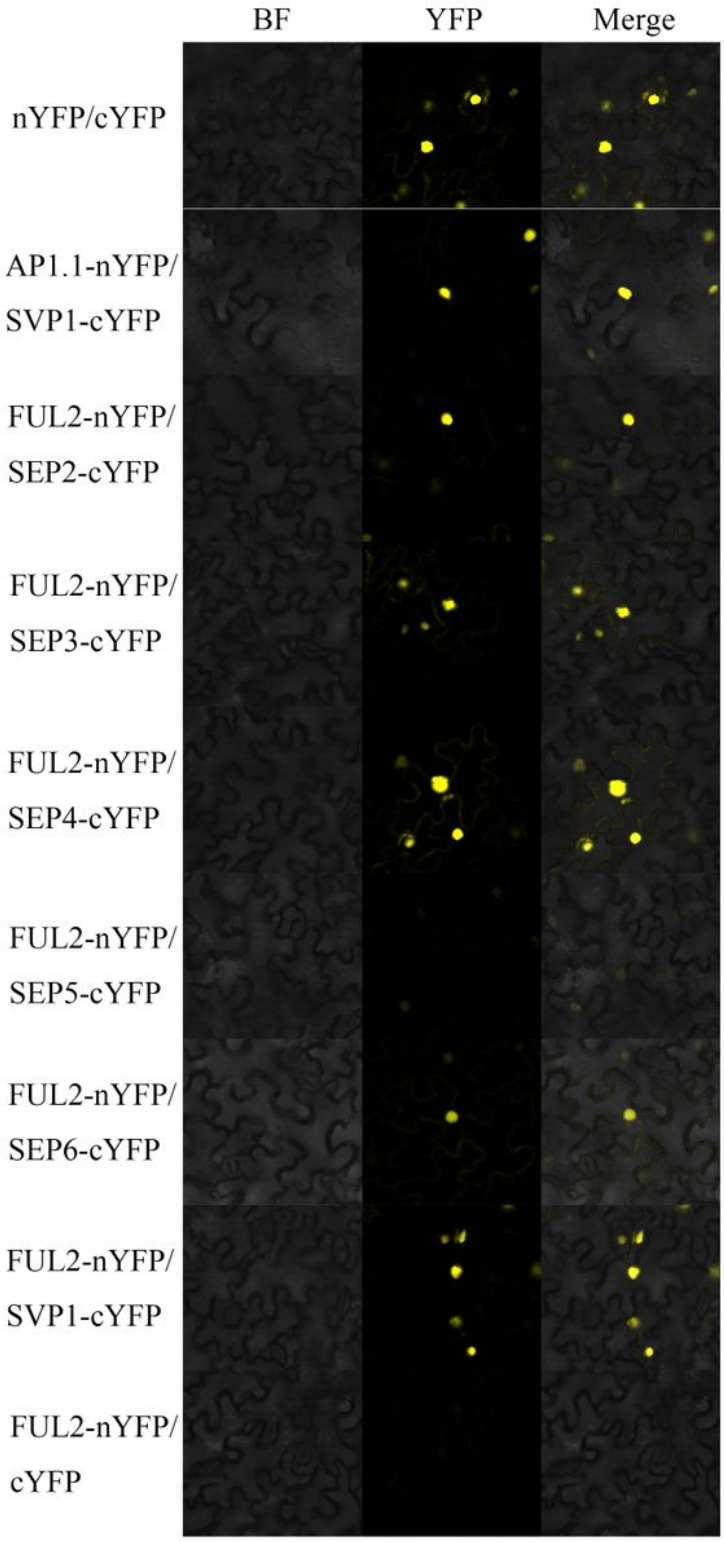

Figure 7

Bimolecular fluorescence complementation (BiFC) assays in tobacco epidermal cells. The GhAP1.1 and GhFUL2 fragments were inserted into the pSPYNE vector, whereas GhSEP2, GhSEP3, GhSEP4, GhSEP5,

GhSEP6, GhSEP7, and GhSVP1 fragments were inserted into the pSPYCE vector. The resulting recombinant pSPYNE and pSPYCE plasmids were transiently co-expressed in tobacco lower epidermal cells for an examination by fluorescence microscopy. The detection of YFP fluorescence indicated a positive interaction. Scale bar, 20 μm

Supplementary Files

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- Fig.S1.tif
- SupplymentTable1.xlsx
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