Plant Molecular Biology Reporter (2018) 36:710–724
https://doi.org/10.1007/s11105-018-1114-y

ORIGINAL PAPER

Ectopic Expression of Two *FOREVER YOUNG FLOWER* Orthologues from *Cattleya* Orchid Suppresses Ethylene Signaling and DELLA Results in Delayed Flower Senescence/Abscission and Reduced Flower Organ Elongation in *Arabidopsis*

Wei-Han Chen [1] · Yung-I Lee [2] · Chang-Hsien Yang [1,3]

Published online: 15 September 2018
© Springer Science+Business Media, LLC, part of Springer Nature 2018

Abstract

Two orthologues of *Arabidopsis FOREVER YOUNG FLOWER* (*FYF*), *CaFYF1* and *CaFYF2*, were identified from *Cattleya intermedia*. To investigate the function of these two genes, we performed ectopic expression of *CaFYF1/2* in *Arabidopsis*. Delayed flower senescence and abscission were observed in 35S::*CaFYF1/2* transgenic *Arabidopsis* plants. Furthermore, once *CaFYF1/2* was fused with the strong repressor domain SRDX, severe delayed flower senescence and abscission were observed in 35S::*CaFYF1/2+SRDX* transgenic *Arabidopsis* plants. In contrast, when 35S::*CaFYF1/2* was converted to a potent activator by fusion with the VP16-AD motif, flower senescence and abscission were promoted in these 35S::*CaFYF1/2+VP16* transgenic dominant-negative mutant *Arabidopsis* plants. These results indicated that similar to *Arabidopsis FYF*, *CaFYF1/2* also act as repressors in controlling floral organ senescence and abscission in transgenic *Arabidopsis* plants. The delayed senescence and abscission of the flower organs in 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX* transgenic *Arabidopsis* plants were unaffected by ethylene treatment. Genes of the ethylene signaling and abscission-associated pathways, such as *EDF1/2/3/4*, *BOP1/2*, and *IDA*, were repressed in 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX* transgenic *Arabidopsis* plants. Furthermore, 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX* transgenic *Arabidopsis* plants showed additional morphological defects, such as short sepals and petals, which were correlated with the upregulation of the *DELLA* genes *RGA*, *GAI*, *RGL1*, and *RGL2*. These results suggested a possible role for *Cattleya* orchid *CaFYF1/2* in controlling floral senescence/abscission by suppressing ethylene signaling and abscission-associated genes as well as controlling flower organ elongation through negative regulation of GA response by activating the expression of the *DELLA* genes during flower development.

Keywords Senescence · Abscission · *Cattleya intermedia* · Ethylene responses · *FOREVER YOUNG FLOWER* · MADS box gene · Repressor

Introduction

Senescence and abscission are the last stages of plant development. Senescence/abscission can participate in plant nutrient reuse and defense against biotic/abiotic stress (Schenk et al. 2005; Avila-Ospina et al. 2014; Koyama 2014). Flower senescence and abscission are regulated by the environment, pollination, and hormones.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11105-018-1114-y) contains supplementary material, which is available to authorized users.

✉ Chang-Hsien Yang
chyang@dragon.nchu.edu.tw

Wei-Han Chen
qqmmyy57@yahoo.com.tw

Yung-I Lee
leeyungi@hotmail.com

[1] Institute of Biotechnology, National Chung Hsing University, Taichung 40227, Taiwan, Republic of China

[2] Botany Department, National Museum of Natural Science, Taichung 40227, Taiwan, Republic of China

[3] Advanced Plant Biotechnology Center, National Chung Hsing University, Taichung 40227, Taiwan, Republic of China

Pollination can induce the synthesis of ethylene, which binds to the ethylene receptor and initiates ethylene signaling, causing flower senescence (Woltering et al. 1995; Sekhon et al. 2012; van Doorn and Kamdee 2014). Flower senescence was delayed in the *ethylene receptor1* (*etr1–1*) and *ethylene insensitive2* (*ein2–1*) ethylene signaling mutant plants (Patterson and Bleecker 2004; Arora 2005). Auxin can also regulate flower senescence since flower senescence was delayed in *auxin response factor1/2* (*arf1/2*) double mutants (Ellis et al. 2005; Kim et al. 2011; Brumos et al. 2014; Shi et al. 2015). The balance between cytokinin and ethylene also affects the senescence/abscission of flowers (Riefler et al. 2006; Hwang et al. 2012; Rogers 2013). Floral organ abscission occurs in the basal region of the silique called the abscission zone (Lewis et al. 2006; Aalen et al. 2013). When flower organs begin to separate from the plant, the abscission zone cells will begin to divide and differentiate. This step is regulated by *BLADE ON PETIOLE1/2* (*BOP1/2*) genes (McKim et al. 2008). Floral organ abscission is deficient in *bop1/2* double mutant plants. After abscission zone cell division/differentiation, digestive enzymes break down the pectin between organs and cause the separation of the floral organs from the plants. This process is controlled by genes such as *INFLORESCENCE DEFICIENT IN ABSCISSION* (*IDA*), and floral organ abscission was significantly deficient in *ida* mutant plants (Jinn et al. 2000; Butenko et al. 2003; Cho et al. 2008; Aalen et al. 2013).

The MADS box genes are plant-specific transcription factors that contain MIKC conserved domains (Theissen 2001). In plants, MADS box genes regulate flower development and transition from the vegetative phase to the reproductive phase. The M domain of MADS box genes can recognize the CArG box and bind to it (Huang et al. 1993). The I and K domains are responsible for protein-protein interactions. The C domain is the functional domain and may contain repressor or activator functions (Theissen et al. 1996). MADS box genes ABCDE have largely been studied in the frame of floral organ initiation. Group A genes regulate sepal and petal development, group B genes regulate petal and stamen development, group C genes regulate stamen and carpal development, group D genes regulate ovule development, and group E proteins interact with the A-D group proteins to perform their functions (Kater et al. 2006).

In addition to regulating floral organ formation, MADS box genes are also involved in the control of various plant developmental processes. For example, the MADS box transcription factor *FOREVER YOUNG FLOWER* (*FYF*) was reported to regulate flower senescence and abscission (Chen et al. 2011a). *FYF* is highly expressed in sepals/petals during early flower development, and its expression significantly decreased when flowers matured. Ectopic expression of *FYF* upregulated *FYF UPREGULATING FACTOR1* (*FUF1*) and repressed downstream genes in ethylene signaling (*EDF1/2/3/4*) and abscission-associated genes (*BOP1/2* and *IDA*), which resulted in delayed flower senescence and abscission (Chen et al. 2011a, 2015). Ectopic expression of the *Oncidium* orchid *FYF* orthologue *OnFYF* could also delay flower senescence/abscission in transgenic *Arabidopsis* plants (Chen et al. 2011b). In addition to *FYF*, there are other MADS box genes that are also involved in the control of floral organ senescence and abscission, such as *AGAMOUS-LIKE 15/18* (*AGL15/18*) and *SHORT VEGETATIVE PHASE* (*SVP*) (Alvarez-Buylla et al. 2000; Fernandez et al. 2000; Adamczyk et al. 2007; Tao et al. 2012; Fernandez et al. 2014; Lee et al. 2014). Ectopic expression of these genes also caused significant delays in flower senescence and abscission. The mechanism for these MADS box genes in regulating flower senescence/abscission is still unclear.

Cattleya orchids are a popular floral crop in the international market since many cultivars with diverse flower shapes, colors, and sizes have been identified. The relatively short shelf life of *Cattleya* orchid flowers has caused a significant reduction of their value in the flower market. Thus, the development of a strategy to elongate the vase life for *Cattleya* orchid flowers is important and should immediately increase their market value. To further explore the potential role of the *FYF* orthologue in the regulation of *Cattleya* orchid floral senescence and abscission, we identified two *FYF* orthologues, *CaFYF1* and *CaFYF2*, from *Cattleya intermedia* and functionally analyzed these genes in this study. We demonstrated that flower senescence and abscission were delayed in 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX* transgenic *Arabidopsis* plants. Our results showed that *CaFYF1/2* also likely act as repressors in controlling floral organ senescence and abscission, similar to the role of *Arabidopsis FYF*. These results provide an applicable future strategy for the control of shelf life of *Cattleya* orchids by manipulation of the *CaFYF1/2* function in this orchid. Furthermore, 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX* also produced short sepals and petals in transgenic *Arabidopsis* plants, which were correlated with the upregulation of *DELLA* genes. Thus, this study showed that *FYF* orthologues could also affect flower organ development by negatively regulating GA response through activation of DELLA expression during flower development.

Materials and Methods

Plant Materials and Growth Conditions

The seeds of Arabidopsis were germinated in murashige and skoog medium (Murashige and Skoog 1962) for 14 days

under long-day conditions (16-h light/8-h dark) before being transferred to soil as described previously (Chen et al. 2015). *Cattleya intermedia* used in this study were maintained in the greenhouse of National Chung-Hsing University, Taichung, Taiwan.

Cloning of the cDNA for *CaFYF1/2* from *Cattleya intermedia*

The cDNA sequences of *CaFYF1/2* were identified from *C. intermedia* through next-generation sequencing (NGS) analysis of *Cattleya* flowers. For cloning cDNA of *CaFYF1/2*, the RNA was extracted from the flower of *C. intermedia* and RT-PCR amplification was performed. The full-length cDNA of *CaFYF1* was amplified by PCR using 5′ primer, CaFYF1-F, and the 3′ primer, CaFYF1-R. The full-length cDNA of *CaFYF2* was amplified by PCR using 5′ primer, CaFYF2-F, and the 3′ primer, CaFYF2-R. Sequences for the primers are listed in the Table S1 in the supplementary material. The amplified cDNA fragments of *CaFYF1/2* were cloned into the linker region in binary vector pEpyon-22K (CHY Lab, Taichung, Taiwan) under the control of cauliflower mosaic virus (CaMV) 35S promoter and used for further plant transformation.

Construction of the *CaFYF1/2+SRDX* and *CaFYF1/2+VP16* Constructs

For the 35S::*CaFYF1/2*+SRDX constructs, the cDNAs for *CaFYF1/2* obtained by PCR amplification using the primers CaFYF1-F and CaFYF1-NS-R were cloned into the pEpyon-2aK plasmid upstream of the SRDX (LDLDLELRLGFA*) sequence, under the control of the CaMV 35S promoter as described previously (Chen et al. 2011a, 2015). For the 35S::*CaFYF1/2*+VP16 constructs, the cDNAs for *CaFYF1/2* obtained by PCR amplification using the primers CaFYF2-F and CaFYF2-NS-R were cloned into the pEpyon-2bK plasmid upstream of the VP16-AD fragment, under the control of the CaMV 35S promoter as described previously (Chen et al. 2011a). Sequences for the primers are listed in the Table S1 in the supplementary material.

Plant Transformation and Transgenic Plant Analysis

Constructs described in this study were constructed and introduced into the *Agrobacterium tumefaciens* strain GV3101 and transformed into *Arabidopsis* plants using the floral dip method as described previously (Clough and Bent 1998). Transformants that survived in medium containing kanamycin (50 μg/ml) were further verified by RT-PCR analyses.

Real-time PCR Analysis

For real-time quantitative RT-PCR, the reaction was performed on a C1000 thermal cycler/Bio-Rad CFX96 touch real-time PCR detection system using SYBER Green Real-time PCR Master Mix (FastStar Universal SYBR Green Master ROX Roche). The amplification condition was 95 °C for 10 min, followed by 40 cycles of amplification (95 °C for 15 s, 55 °C for 15 s, 72 °C for 30 s, and then plate reading) and melted (65–95 °C with plate readings every 1 °C). Sequences for the primers used for real-time quantitative RT-PCR for *CaFYF1*, *CaFYF2*, *IDA*, *BOP1*, *BOP2*, *ETR1*, *ETR2*, *ERS1*, *ERS2*, *EIN4*, *CTR1*, *EIN2*, *EIN3*, *EIL1*, *EDF1*, *EDF2*, *EDF3*, *EDF4*, *RGA*, *GAI*, *RGL1*, and *RGL2* were listed in the Table S1 in the supplementary material. The *Arabidopsis* housekeeping gene *UBQ10* was used as a normalization control with the following primers: RT-UBQ10-F and RT-UBQ10-4-2. The transcript levels for orchid *CaFYF1/2* genes were normalized using reference genes *CaUBQ* (primers RT-CaUBQ-1 and RT-CaUBQ-2) for *Cattleya intermedia*. All experiments were repeated at least twice for reproducibility. Data were analyzed using Gene Expression Macro software (version 1.1, Bio-Rad).

Ethylene Responses

For ethylene sensitivity test, mature wild-type and transgenic *Arabidopsis* plants were sealed in plastic chambers and gassed with air or air containing 10 ppm ethylene for 3 days under long-day conditions (16-h light/8-h dark) as described previously (Chen and Bleecker 1995; Chen et al. 2011a, 2015).

Results

Characterization of *CaFYF1/2* from *Cattleya* Orchids

The *Arabidopsis FYF* gene can delay floral senescence and abscission by upregulation of *FUF1* to repress the ethylene signaling downstream genes *EDF1–4* (Chen et al. 2011a, 2015). Two *FYF* orthologues were identified from *C. intermedia* and named *CaFYF1/2*. *CaFYF1* contains 660 nucleotides encoding 219 amino acids (Supplementary Fig. S1) and shows 44% and 70% identity to *Arabidopsis FYF* and *Oncidium OnFYF*, respectively, with 84% (49/58) and 97% (56/58) of the amino acids identical in the MADS box domain (Fig. 1(a)), respectively. *CaFYF2* contains 675 nucleotides encoding 224 amino acids (Supplementary Fig. S2) and shows 40% and 57% identity to Arabidopsis *FYF* and *Oncidium OnFYF*, respectively, with 83% (48/58) and 93% (54/58) of the

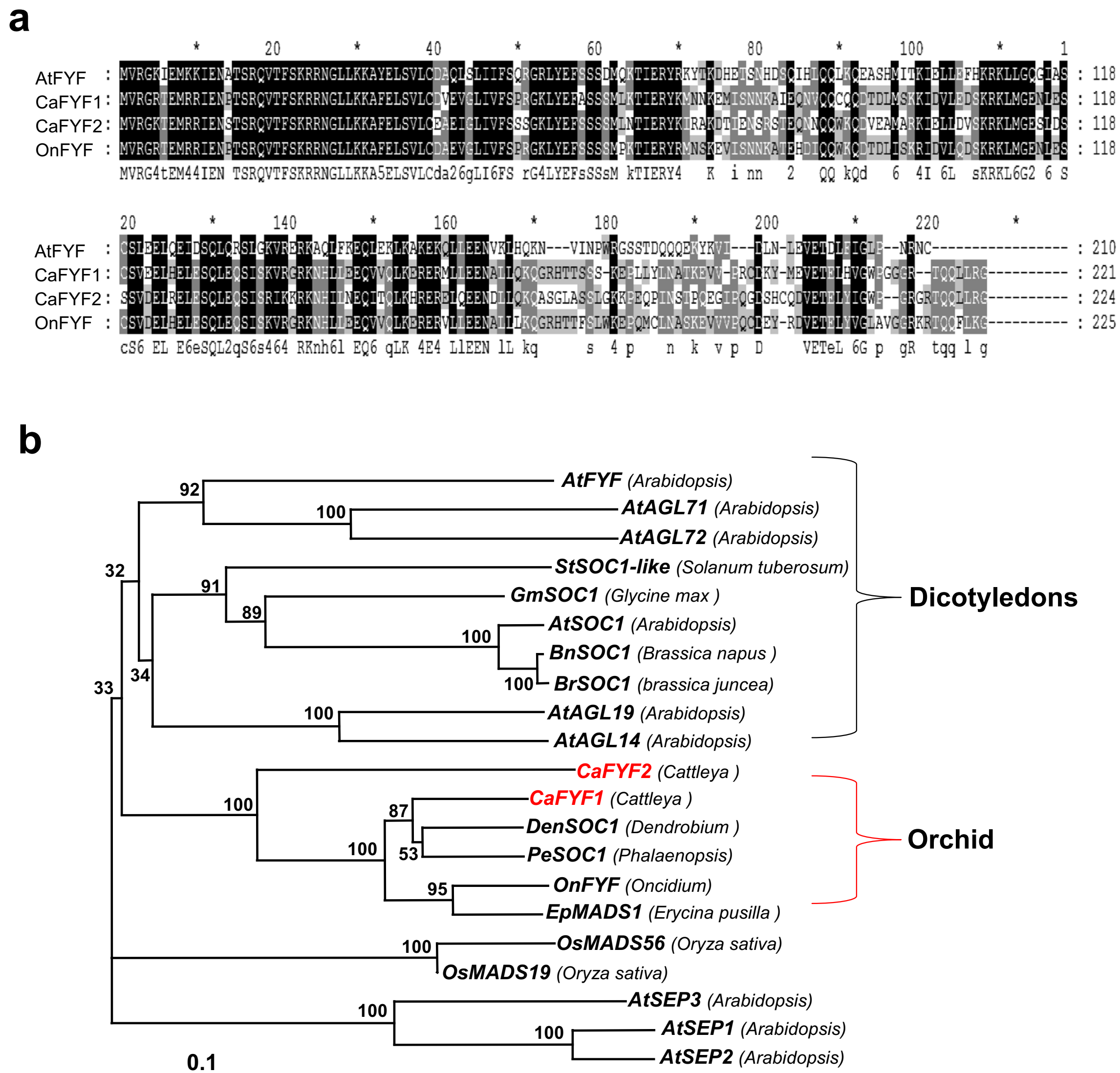
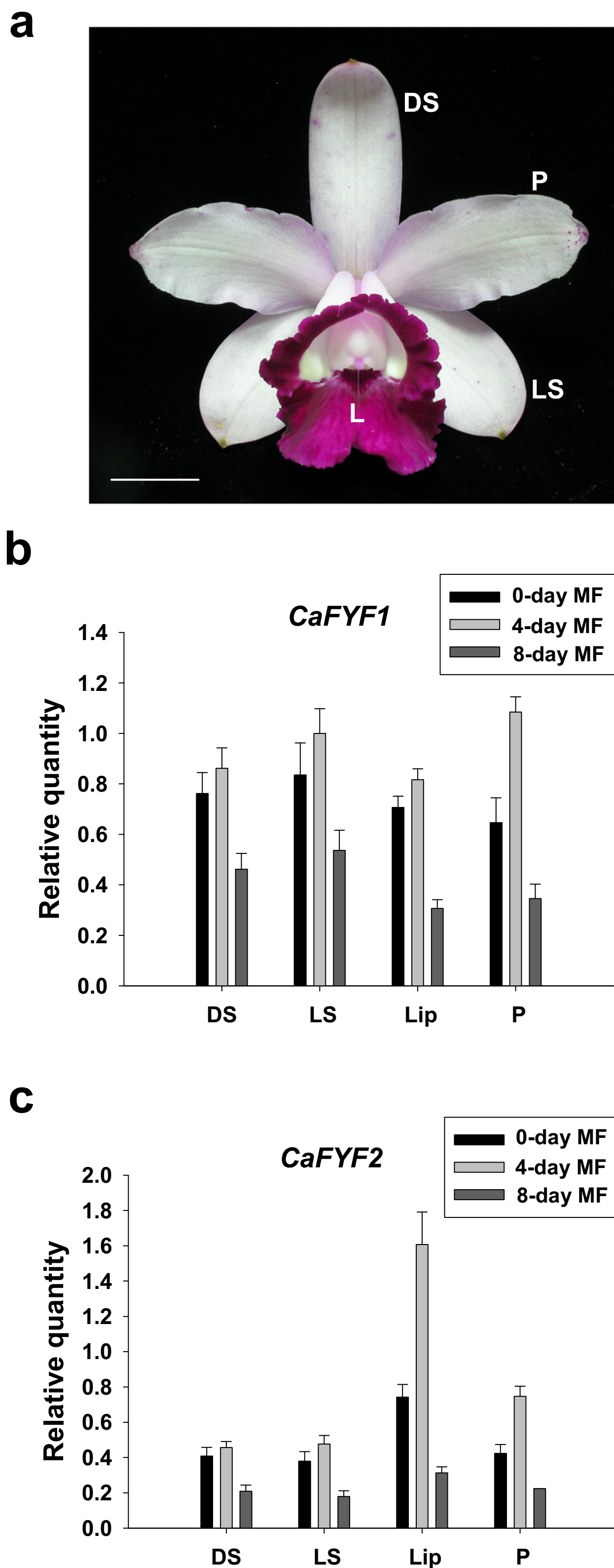


Fig. 1 Comparison of the protein sequences and phylogenetic analysis of FYF proteins in plants. **a** Comparison of the protein sequences of FYF (*Arabidopsis*), OnFYF (*Oncidium*), and CaFYF1/2 (*Cattleya*). The amino acid residues conserved in FYF are highlighted in black; amino acid residues similar to FYF are highlighted in gray, and dashes were introduced into the sequence to improve the alignment. **b** Phylogenetic tree analysis of plant *FYF* genes. Based on the protein sequence, *CaFYF1/2* (in red color) were assigned to the orchid group of *FYF* genes, which are separated from *Arabidopsis FYF* in the dicot group of *FYF* genes. Names of the plant species are listed behind each of the MADS box protein names. Amino acid sequences for protein used in phylogenetic tree analysis were retrieved from the NCBI server (http://www.ncbi.nlm.nih.gov/) and analyzed by DDBJ ClustalW (http://clustalw.ddbj.nig.ac.jp/). The bootstrap phylogenetic tree was shown by using TreeView. Numbers on major branches indicate bootstrap percentages for 100 replicate analyses

amino acids identical in the MADS box domain (Fig. 1(a)), respectively. Based on sequence comparison, CaFYF1 had a higher similarity to the FYF orthologues than CaFYF2. The amino acid sequence alignment for FYF, CaFYF1/2, and OnFYF and the sequences of several other FYF-like genes (Supplementary Fig. S3) from different plants were used to construct a phylogenetic tree (Fig. 1(b)). *CaFYF1/2* were assigned to the orchid clade of *FYF*-like genes, and as expected, *CaFYF1* is closer to the other orchid *FYF* orthologues than *CaFYF2*.

The Expression of *CaFYF1/2* Genes

To analyze the expression pattern of the *CaFYF1/2* genes during *C. intermedia* flower development, mRNA isolated from the organs (dorsal sepal, lateral sepal, petal and lips) of flowers (Fig. 2(a)) at three different developmental stages, 0, 4, and 8 days after flower opening (DAO), was used for real-time quantitative RT-PCR. The results indicated that *CaFYF1* was highly expressed in young (0 DAO) and mature (4 DAO) flowers, and its expression was significantly decreased in old



◀ **Fig. 2** Detection of *CaFYF1/2* expression in flower organs during different developmental stages of *Cattleya*. **a** A *Cattleya intermedia* mature flowers consist of one dorsal sepal (DS), two lateral sepals (LS), two petals (P), and a lip (Lp). Bar = 20 mm. **b**–**c** Detection of *CaFYF1* (**b**) and *CaFYF2* (**c**) expression in the dorsal sepal (DS), lateral sepals (LS), petals (P), and lip (Lip) of mature flowers (MF) at three different developmental stages, 0, 4, and 8 days after flower opening, by using real-time quantitative RT-PCR. The results indicated that *CaFYF1/2* were expressed in all four perianth organs, and the expression was higher in young mature flowers (0- and 4-day MF) and significantly decreased in old mature flowers (8-day MF). The transcript levels of *CaFYF1/2* were determined using two to three replicates and were normalized using *Cattleya UBIQUITIN* (*CaUBQ*). The error bars represent the standard deviation. Each experiment was repeated twice with similar results

flowers (8 DAO) during the late stages of flower development right before senescence occurs (Fig. 2(b)). *CaFYF2* had a similar expression pattern to that of *CaFYF1* in the flowers; it was also detected at a higher level in young and mature flowers than in old flowers (Fig. 2(c)). The expression pattern of higher expression in early than in late flower development for *CaFYF1/2* was similar to that for *Arabidopsis FYF*, which was reported previously (Chen et al. 2011a).

Ectopic Expression of *CaFYF1* and *CaFYF1+SRDX* Delayed Flower Senescence and Abscission in Transgenic *Arabidopsis* Plants

To analyze the function of *CaFYF1*, we constructed 35S::*CaFYF1* and analyzed transgenic *Arabidopsis* plants. Ectopic expression of *CaFYF1* in *Arabidopsis* caused early flowering (Fig. 3(a)), similar to that in the 35S::*FYF* transgenic plants (Chen et al. 2011a). In addition, the delay in flower senescence and abscission was also observed in these 35S::*CaFYF1* plants. Based on the severity of phenotypic alteration, two classes of 35S::*CaFYF1* plants were described. In medium-severe 35S::*CaFYF1* plants, the delay in senescence and abscission of the flower organs was very similar to that observed in 35S::*FYF* flowers described previously (Chen et al. 2011a). The perianth organs of these 35S::*CaFYF1* transgenic flowers did not senesce and abscise even after the maturation of the siliques (Fig. 3(b, c)). The siliques in these medium-severe plants elongated normally (Fig. 3(b, c)), as in wild-type and 35S::*FYF* plants (Chen et al. 2011a). In severe 35S::*CaFYF1* plants, the senescence and abscission of the flower organs were also significantly delayed for longer than that of position 10 throughout the inflorescence (Fig. 3(d, e)). In addition, the flowers of these severe plants produced short sepals and petals (Fig. 3(e–h)), and some failed to open (Fig. 3(e, g)) during the entire flower development. Furthermore, these flowers were mostly male sterile, and the siliques failed to elongate (Fig. 3(d, e)) due to the indehiscent anthers of the stamens (Fig. 3(f, h)). The alteration of flower organ development in addition to the delay in senescence/abscission of the flower organs in these severe

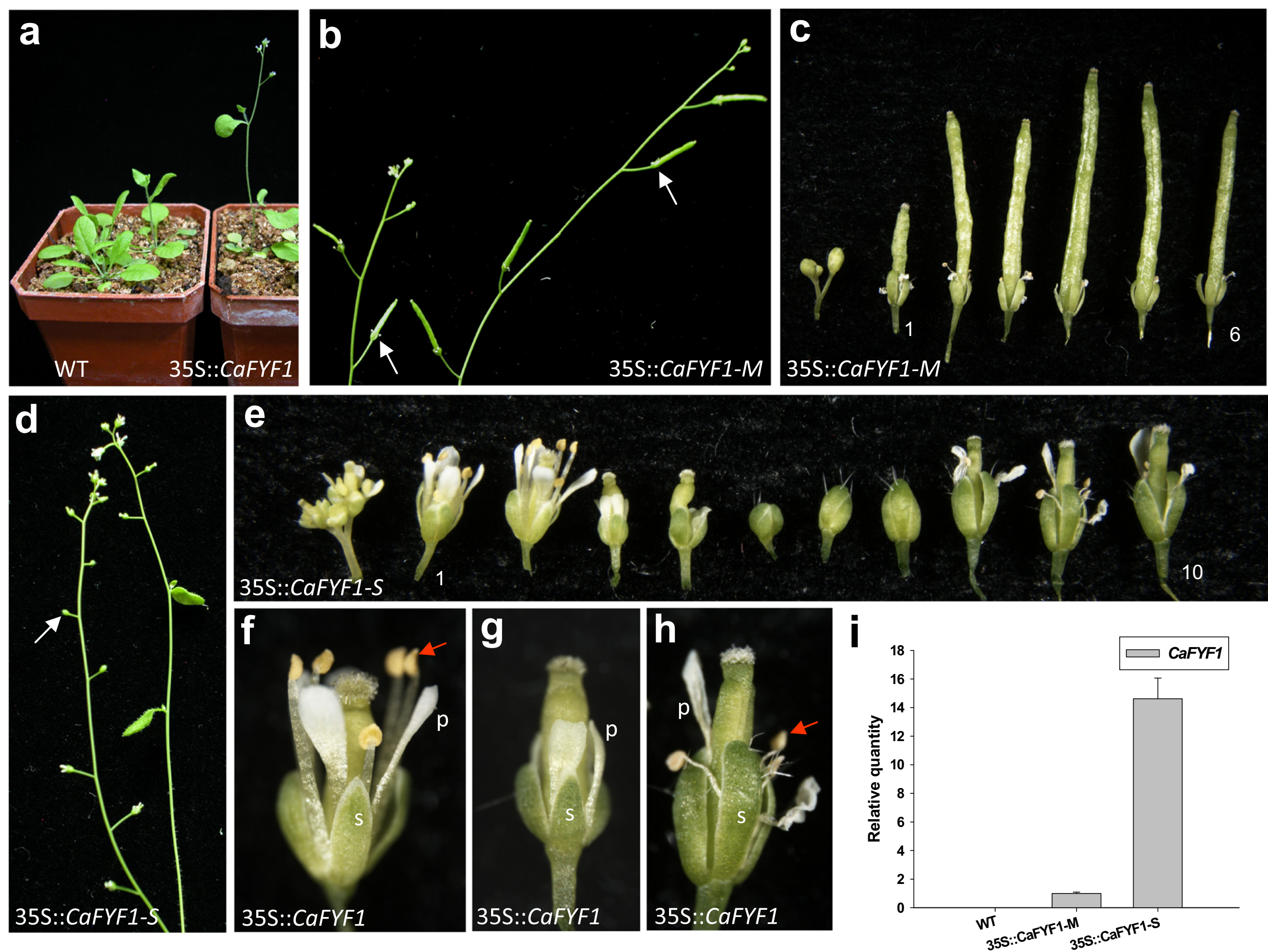


Fig. 3 Ectopic expression of *CaFYF1* delays flower senescence and abscission in transgenic *Arabidopsis* plants. **a** The 35S::*CaFYF1* transgenic *Arabidopsis* plants (right) flowered earlier than wild-type (WT) plants at the same stage (left). **b** Inflorescences of a 35S::*CaFYF1* medium-severe plant (35S::*CaFYF1*-M). The flower organs remain attached to the base of the siliques in these 35S::*CaFYF1* transgenic flowers (arrowed). **c** Flowers along the inflorescence of the 35S::*CaFYF1* medium-severe plant (35S::*CaFYF1*-M) from (**b**). The numbers indicate the position of the flowers. **d** Inflorescences of a 35S::*CaFYF1* severe plant (35S::*CaFYF1*-S) containing unopened flowers (arrowed) with short sepals and petals. The senescence and abscission of the flower organs were also delayed. **e** Flowers along the inflorescence of the 35S::*CaFYF1* severe plant (35S::*CaFYF1*-S) from (**d**). The numbers indicate the position of the flowers. **f**–**h** Close-up of the flowers with short sepals (s) and petals (p) and indehiscent anthers (arrowed) of the stamens from (**e**). **i** Detection of *CaFYF1* expression in one wild-type Columbia plant (WT) and two 35S::*CaFYF1* plants with severe (35S::*CaFYF1*-S) and medium-severe (35S::*CaFYF1*-M) phenotypes. The expression of *CaFYF1* was clearly higher in 35S::*CaFYF1*-S than in 35S::*CaFYF1*-M plants. *CaFYF1* expression was undetectable in untransformed wild-type plants. The transcript levels of *CaFYF1* were determined using two to three replicates and were normalized using *UBIQUITIN10*. The error bars represent the standard deviation. Each experiment was repeated twice with similar results

35S::*CaFYF1* flowers was also observed in the severe 35S::*FYF*+*SRDX* (Chen et al. 2011a) and 35S::*FUF1* flowers (Chen et al. 2015). As shown in Fig. 3(i), relatively higher *CaFYF1* expression was observed in the severe plants compared to the medium-severe plants.

To further analyze the function of *CaFYF1*, we constructed 35S::*CaFYF1*+*SRDX* (C-terminal fused the strong repressor motif SRDX (LDLDLELRLGFA)) and analyzed the transgenic plants. Similar to 35S::*CaFYF1* plants, 35S::*CaFYF1*+*SRDX Arabidopsis* plants also showed early flowering (Fig. 4(a)) and delayed flower senescence and abscission phenotypes (Fig. 4(b, c)). The senescence and abscission of the 35S::*CaFYF1*+*SRDX* flower organs were also significantly delayed for longer than that of position 20 throughout the inflorescence (Fig. 4(b, c)). Similar to the severe 35S::*CaFYF1* plants, the 35S::*CaFYF1*+*SRDX* flowers also produced short sepals and petals (Fig. 4(c, d)), and the siliques failed to elongate throughout development (Fig. 4(b, c)) since male sterility caused by the indehiscence of the anthers was observed

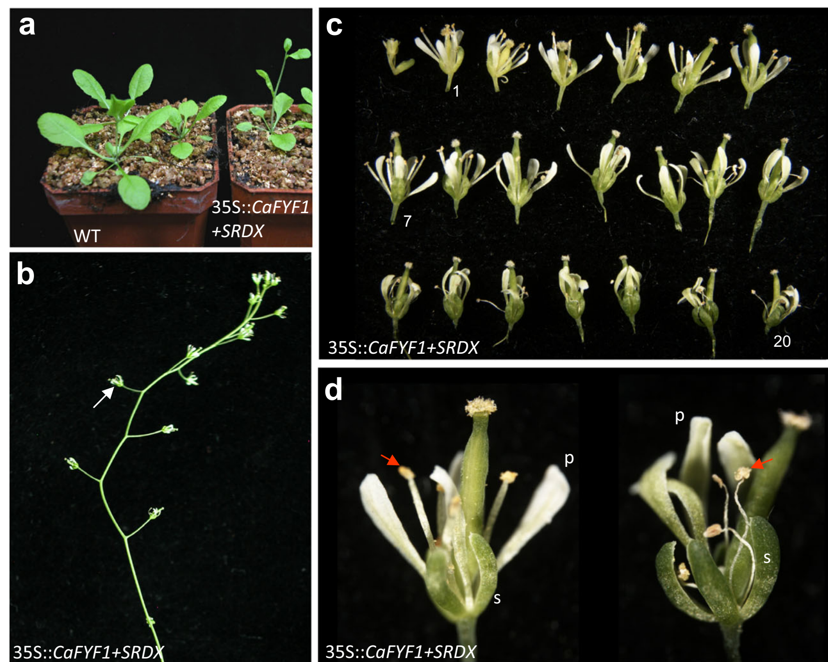


Fig. 4 Ectopic expression of *CaFYF1+SRDX* delays flower senescence and abscission in transgenic *Arabidopsis* plants. **a** The 35S::*CaFYF1+SRDX* transgenic *Arabidopsis* plants (right) flowered earlier than the wild-type (WT) plants at the same stage (left). **b** Inflorescences of a 35S::*CaFYF1+SRDX* plant. The flower organs remain attached to the base of the flowers in the 35S::*CaFYF1+SRDX* transgenic flowers (arrowed). **c** Flowers along the inflorescence of the 35S::*CaFYF1+SRDX* from (**b**) contained short sepals/petals and no further silique development. The senescence and abscission of the flower organs were delayed. The numbers indicate the position of the flowers. **d** Close-up of the flowers with short sepals (s) and petals (p) and indehiscent anthers (arrowed) of the stamens from (**c**)

(Fig. 4(d)). The similar phenotypes observed in 35S::*CaFYF1* and 35S::*CaFYF1+SRDX Arabidopsis* plants indicated that *CaFYF1* acts as a repressor in regulating flower senescence and abscission.

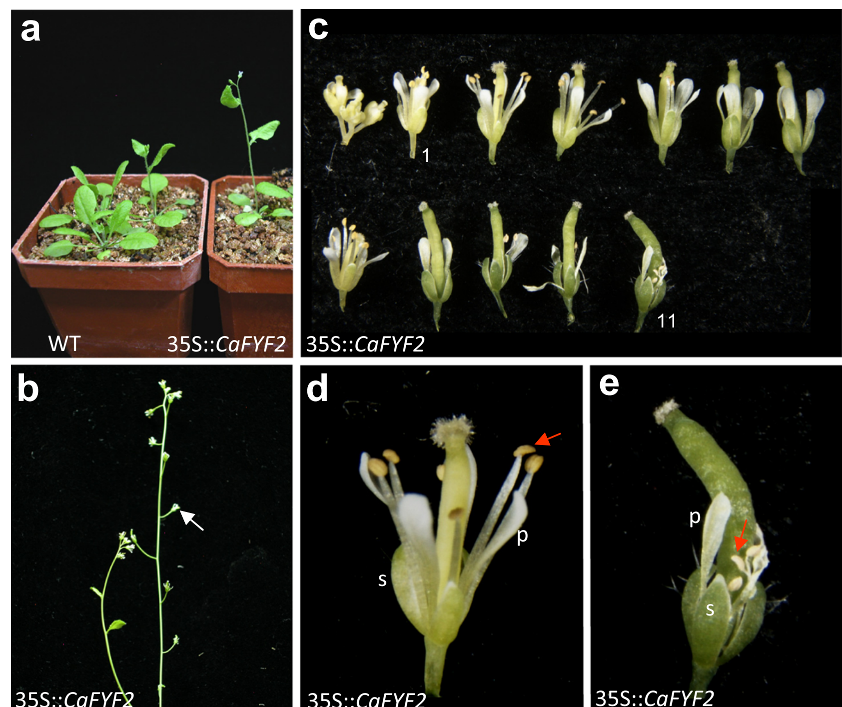


Fig. 5 The 35S::*CaFYF2* transgenic *Arabidopsis* plants showed flower senescence and abscission. **a** The 35S::*CaFYF2* transgenic *Arabidopsis* plants (right) flowered earlier than wild-type (WT) plants at the same stage (left). **b** Inflorescences of a 35S::*CaFYF2* plants contained flowers (arrowed) that showed delayed senescence and abscission of the flower organs. **c** Flowers along the inflorescence of the 35S::*CaFYF2* plant in (**b**) that contained short sepals and petals and no further silique development. The numbers indicate the position of the flowers. **d**–**e** Close-up of the flowers with short sepals (s) and petals (p) and indehiscent anthers (arrowed) of the stamens from (**c**)

Ectopic Expression of *CaFYF2* and *CaFYF2+SRDX* Delayed Flower Senescence and Abscission in Transgenic *Arabidopsis* Plants

With a similar strategy to *CaFYF1*, 35S::*CaFYF2* and 35S::*CaFYF2+SRDX* were also constructed, and the transgenic plants were analyzed to explore the function of *CaFYF2*. Similar to the 35S::*CaFYF1* plants, 35S::*CaFYF2* *Arabidopsis* plants also showed early flowering (Fig. 5(a)) and delayed flower senescence and abscission phenotypes (Fig. 5(b, c)). These 35S::*CaFYF2* flowers also produced short sepals, petals with the indehiscence of the anthers (Fig. 5(c–e)),

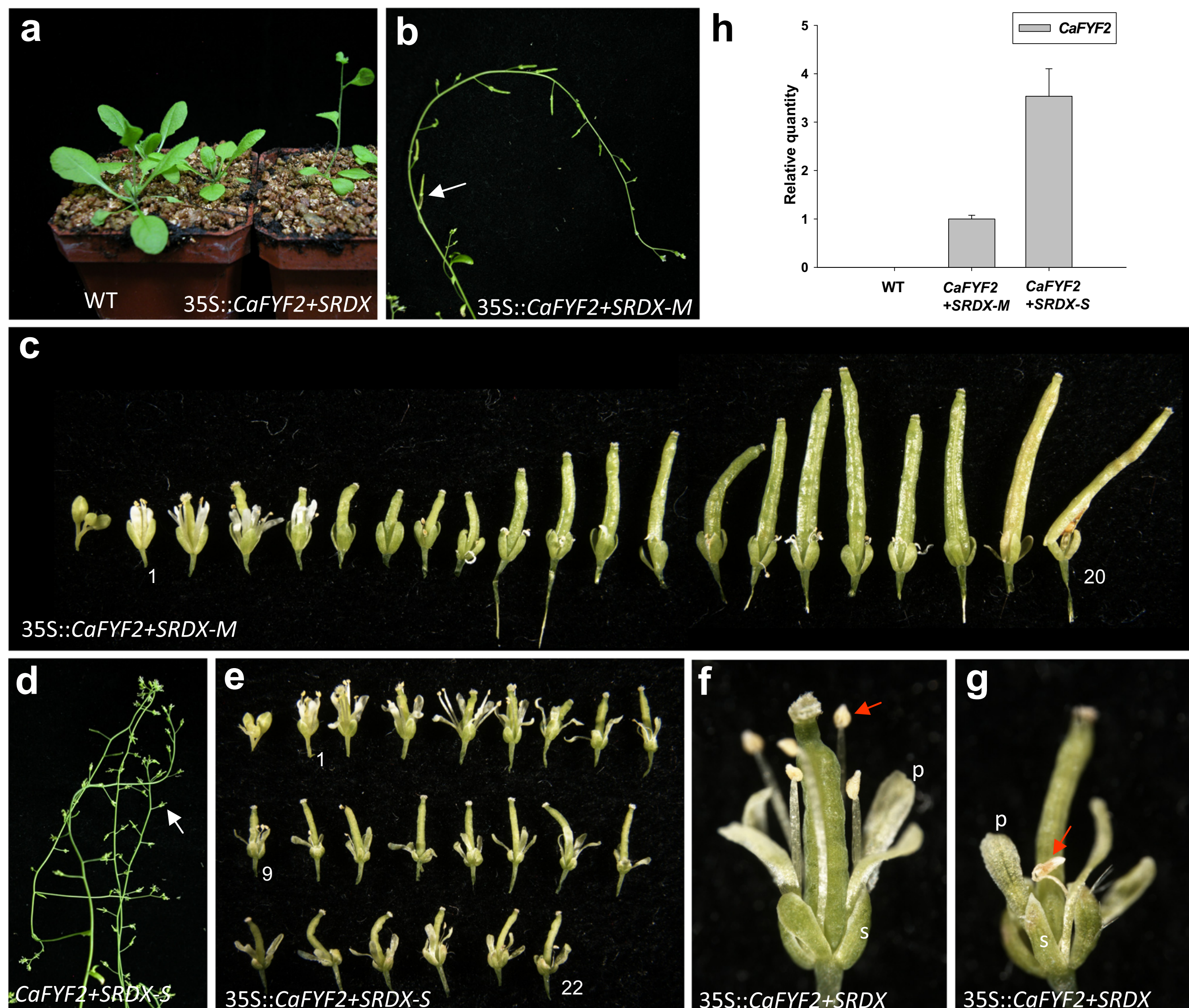


Fig. 6 Ectopic expression of *CaFYF2+SRDX* delays flower senescence and abscission in transgenic *Arabidopsis* plants. **a** The 35S::*CaFYF2+SRDX* transgenic *Arabidopsis* plants (right) flowered earlier than the wild-type (WT) plants at the same stage (left). **b** Inflorescences of a 35S::*CaFYF2+SRDX* medium-severe plant (35S::*CaFYF2+SRDX*-M). The flower organs remain attached to the base of the siliques in the 35S::*CaFYF2+SRDX* transgenic flowers (arrowed). **c** Flowers along the inflorescence of the 35S::*CaFYF2+SRDX* medium-severe plant (35S::*CaFYF2+SRDX*-M) from (**b**). The numbers indicate the position of the flowers. **d** Inflorescences of a 35S::*CaFYF2+SRDX* severe plant (35S::*CaFYF2+SRDX*-S) containing flowers (arrowed) with short sepal/petal and without further silique development. The senescence and abscission of the flower organs for these flowers were delayed. **e** Flowers along the inflorescence of the 35S::*CaFYF2+SRDX* severe plant (35S::*CaFYF2+SRDX*-S) from (**d**). The numbers indicate the position of the flowers. **f**–**g** Close-up of the flowers with short sepals (s) and petals (p) and indehiscent anthers (arrowed) of the stamens from (**e**). **h** Detection of *CaFYF2* expression in one wild-type Columbia plant (WT) and two 35S::*CaFYF2+SRDX* plants with severe (35S::*CaFYF2+SRDX*-S) and medium-severe (35S::*CaFYF2+SRDX*-M) phenotypes. The expression of *CaFYF2* was clearly higher in 35S::*CaFYF2+SRDX*-S than in 35S::*CaFYF2+SRDX*-M plants. In untransformed wild-type plants, *CaFYF2* expression was absent. The transcript levels of *CaFYF2* were determined using two to three replicates and were normalized using *UBIQUITIN10*. The error bars represent the standard deviation. Each experiment was repeated twice with similar results

and failure of silique elongation throughout development (Fig. 5(b, c)). When the 35S::*CaFYF2+SRDX* plants were examined, they showed very similar phenotypes to the 35S::*CaFYF2* plants, with early flowering (Fig. 6(a)) and delayed flower organ senescence and abscission (Fig. 6(b–e)). Both medium-severe 35S::*CaFYF2+SRDX* plants with elongated siliques (Fig. 6(b, c)) and severe 35S::*CaFYF2+SRDX* plants with short sepals/petals, indehiscent anthers, and undeveloped siliques (Fig. 6(d–g)) were observed. As shown in Fig. 6(h), relatively higher *CaFYF2* expression was observed in the severe 35S::*CaFYF2+SRDX* plants than the medium-severe plants. These results indicated that similar to *CaFYF1*, *CaFYF2* also acts as a repressor in regulating flower senescence and abscission.

Ectopic Expression of *CaFYF1+VP16* and *CaFYF2+VP16* Promoted Flower Senescence and Abscission in Transgenic Dominant-Negative *Arabidopsis* Plants

In this study, a dominant-negative form of either *CaFYF1* or *CaFYF2* was generated by fusing the activation domain VP16 to the C-terminal of *CaFYF1* (35S::*CaFYF1+VP16*) or *CaFYF2* (35S::*CaFYF2+VP16*), and the transgenic plants were analyzed. In contrast to the 35S::*CaFYF1* and 35S::*CaFYF1+SRDX Arabidopsis* plants, the 35S::*CaFYF1+VP16* plants showed early senescence and abscission of the flowers (Fig. 7(a, b)). The flower organ senescence and abscission were promoted as early as that at the position 1 flower in these 35S::*CaFYF1+VP16* transgenic *Arabidopsis* plants (Fig. 7(b, c)). In addition, the 35S::*CaFYF1+VP16* flowers failed to open, and no further silique elongation was observed during the entire flower development. Similar to 35S::*CaFYF1+VP16* plants, 35S::*CaFYF2+VP16* plants also showed early senescence and abscission of the flowers (Fig. 7(d, e)). Compared to wild-type flowers, the flowers of the 35S::*CaFYF2+VP16* transgenic *Arabidopsis* plants showed earlier flower organ senescence and abscission at positions 1–2 (Fig. 7(e, f)).

The 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX Arabidopsis* Plants Are Insensitive to Ethylene Treatment

The plant hormone ethylene is the key hormone that controls plant senescence and abscission. Since 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX* all caused similar delayed flower senescence and abscission in transgenic *Arabidopsis*, we thus further investigated whether the *CaFYF1* and *CaFYF2* genes are involved in regulation of the ethylene signaling pathway. When mature wild-type plants were exposed to air containing 10 ppm ethylene in sealed plastic chambers for 3 days, the perianth organs clearly senesced and abscised from the flower as early as

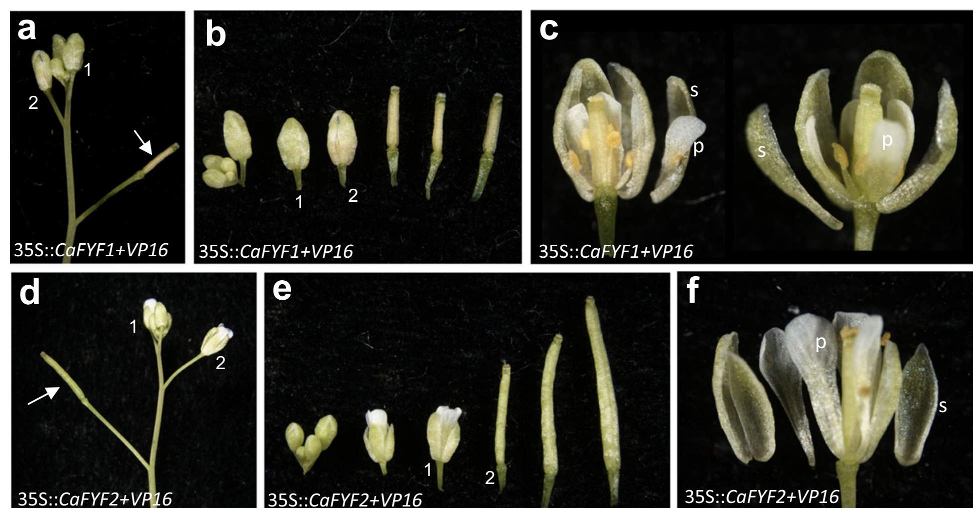


Fig. 7 Ectopic expression *CaFYF1+VP16* and *CaFYF2+VP16* promotes flower senescence and abscission in transgenic *Arabidopsis* plants. **a** Inflorescences of a 35S::*CaFYF1+VP16* plant. The flower organs showed early senescence and abscission (arrowed). **b** Flowers along the inflorescence of the 35S::*CaFYF1+VP16* from (**a**) that showed early senescence and abscission of the flower organs. The numbers indicate the position of the flowers. **c** Close-up of the flowers at positions 1 (left) and 2 (right) from (**b**) that showed early senescence and abscission of the flower organs, which were easily detached by a gentle touch. s, sepals; p, petals. **d** Inflorescences of a 35S::*CaFYF2+VP16* plant. The flower organs showed early senescence and abscission (arrowed). The numbers indicate the position of the flowers. **e** Flowers along the inflorescence of the 35S::*CaFYF2+VP16* from (**d**) that showed early senescence and abscission of the flower organs. The numbers indicate the position of the flowers. **f** Close-up of the flowers at position 2 from (**e**) that showed early senescence and abscission of the sepals (s) and petals (p), which were easily detached by a gentle touch

that at position 1 (Fig. 8(a–c)). In contrast, the ethylene-treated 35S::*CaFYF1* (Fig. 8(d–f)), 35S::*CaFYF1+SRDX* (Fig. 8(g–i)), 35S::*CaFYF2* (Fig. 8(j–l)), and 35S::*CaFYF2+SRDX* (Fig. 8(m–o)) plants were all phenotypically similar to the air-treated control plants without any signs of promotion of flower senescence and abscission. The perianth organs clearly displayed vigor and remained on the flowers in these ethylene-treated 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX* plants (Fig. 8(f,i,l,o)). These results revealed that the delayed senescence and abscission in the 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX Arabidopsis* flowers were unaffected by the ethylene treatment.

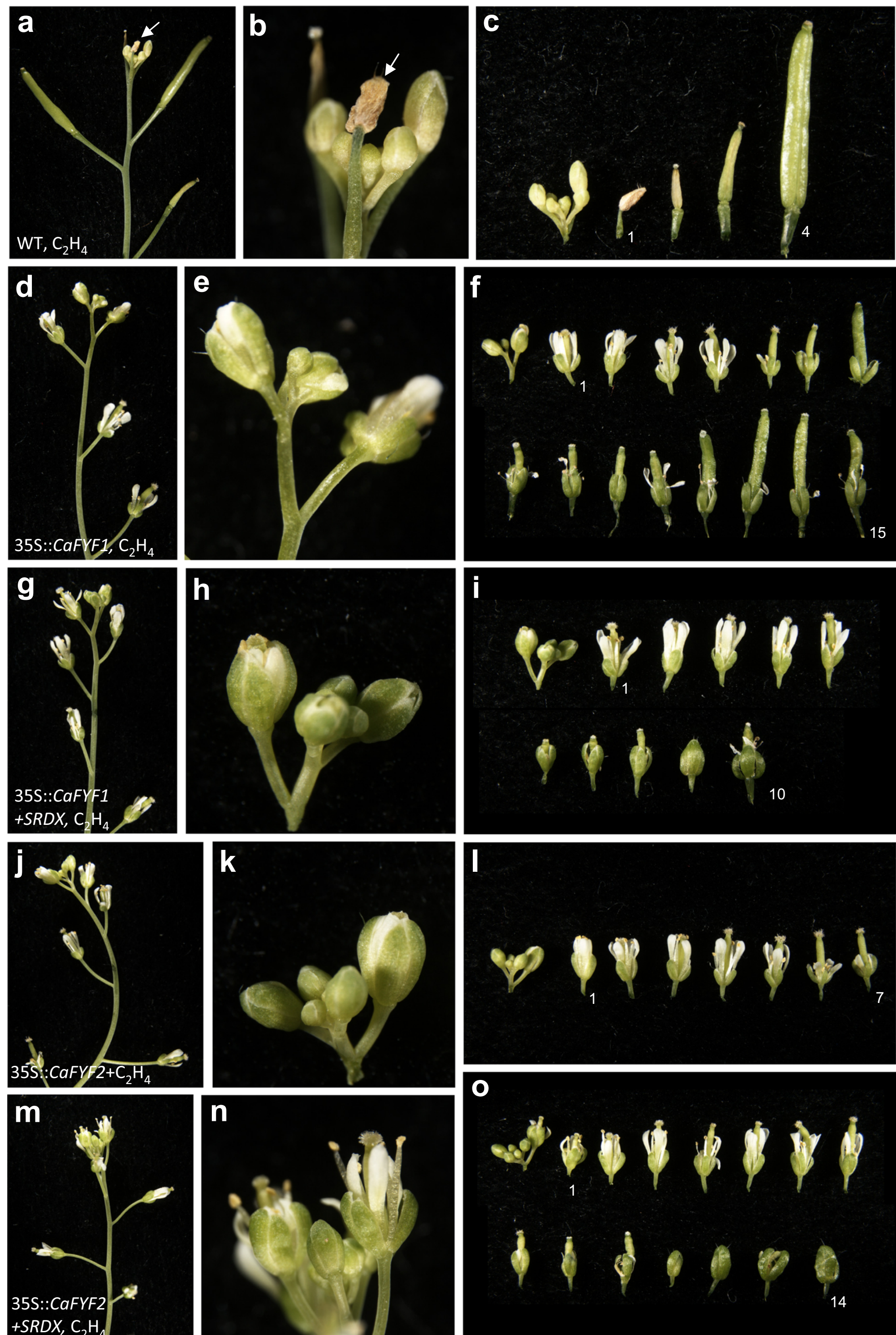


Fig. 8 Analysis of the effect of ethylene on the 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX Arabidopsis* plants. **a** Inflorescences of a wild-type (WT) plant after being exposed to air containing 10 ppm ethylene (C_2H_4) for 3 days. The flowers showed early senescence and abscission (arrowed) without further development. **b** Close-up of the flowers at the top of the inflorescence in (**a**) that showed early senescence and abscission (arrowed). **c** Flowers along the inflorescence in (**a**) that showed early senescence and abscission of the flower organs. The numbers indicate the position of the flowers. **d**, **g**, **j**, **m** Inflorescences of the 35S::*CaFYF1* (**d**), 35S::*CaFYF1+SRDX* (**g**), 35S::*CaFYF2* (**j**), and 35S::*CaFYF2+SRDX* (**m**) plants after being exposed to air containing 10 ppm ethylene (C_2H_4) for 3 days. The flowers were not senescent and abscised after ethylene treatment. **e**, **h**, **k**, **n** Close-up of the flowers at the top of the inflorescence from (**d**, **g**, **j**, **m**), respectively. **f**, **i**, **l**, **o** Flowers along the inflorescence from (**d**, **g**, **j**, **m**), respectively. The numbers indicate the position of the flowers

CaFYF1/2 Inhibit the Downstream Genes *EDFs* in the Ethylene Response

To further investigate the function of the *CaFYF1/2* genes in regulating ethylene signaling, we analyzed the expression of genes in the ethylene signaling pathway in the 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX Arabidopsis* plants. We found similar expression levels for genes upstream of the ethylene signaling pathway (Alonso et al. 2003; Chen et al. 2005; Stepanova and Alonso 2005), such as the ethylene receptors *ETHYLENE RESPONSE 1*,*2* (*ETR1*,*2*), *ETHYLENE RESPONSE SENSOR 1*,*2* (*ERS1*,*2*), and *ETHYLENE INSENSITIVE 4* (*EIN4*); the negative regulator *CONSTITUTIVE TRIPLE RESPONSE 1* (*CTR1*); the positive regulator *EIN2*; and the transcription factors *EIN3* and *ETHYLENE-INSENSITIVE3-LIKE 1* (*EIL1*), in wild-type and 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX Arabidopsis* plants (Fig. 9(a)). In contrast, the expression levels of downstream genes of the ethylene signaling pathway (Alonso et al. 2003; Stepanova and Alonso 2005; Castillejo and Pelaz 2008), such as *EDF1*, *2*, *3*, and *4*, were significantly downregulated in 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX Arabidopsis* plants (Fig. 9(a)). These results clearly indicated that the delayed flower senescence/abscission in the 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX* plants was likely due to suppression of the downstream genes in the ethylene response by the *CaFYF1/2* genes.

In contrast to the gene expression levels detected in the 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX* plants, the expression levels of *EDF1/2/3/4* were clearly upregulated in the early senescent 35S::*CaFYF1/2+VP16* dominant-negative plants (Fig. 9(b)). In addition, the expression of the senescence marker gene *SAG12* (senescence-associated gene 12) (Noh and Amasino 1999) was also significantly upregulated in the 35S::*CaFYF1/2+VP16* plants (Fig. 9(b)).

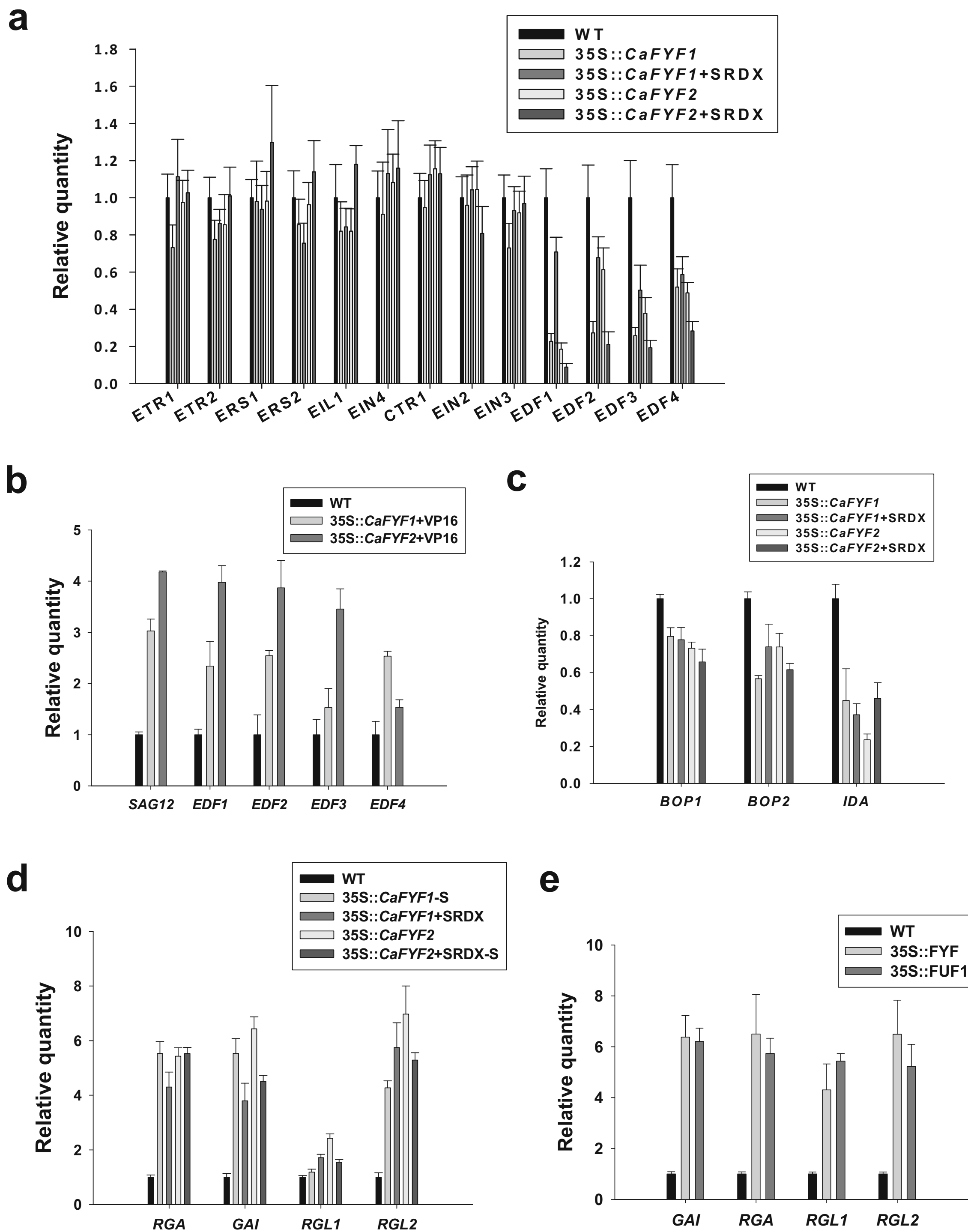
BOP1/2 and *IDA* Expression Is Downregulated in 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX Arabidopsis* Plants

During flower development, the *BOP1/2* genes are required for the formation of the floral AZ (McKim et al. 2008), and the *IDA* gene functions to regulate flower organ abscission (Butenko et al. 2003; Cho et al. 2008; Stenvik et al. 2008). Both *bop1/2* and *ida* mutants caused the defects in flower abscission but not senescence. Since the 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX* plants showed delayed flower organ abscission, we explored the relationships among *BOP1/2*, *IDA*, and *CaFYF1/2*. When the expression of *BOP1/2* was examined, downregulation of these two genes was observed in the 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX* flowers compared to the wild-type flowers (Fig. 9(c)). Similarly, a clear reduction of *IDA* expression was also observed in 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX* flowers (Fig. 9(c)). These results revealed that *CaFYF1/2* could control flower organ abscission by suppressing the abscission-related genes *BOP1/2* and *IDA*.

Expression Levels of the GA-Responsive *DELLA* Genes Are Upregulated in Plants Carrying 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX*

In addition to the delay in flower senescence and abscission, the severe 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX* plants showed additional morphological defects, including short sepals and petals (Figs. 3, 4, 5, and 6). GA was reported to promote the development and elongation of flower organs by suppressing the function of the DELLA proteins (Cheng et al. 2004; Tyler et al. 2004), which contain an N-terminal DELLA domain responsible for the activity of the DELLA proteins in response to GA (Peng et al. 1997; Silverstone et al. 1998; Dill and Sun 2001). In *Arabidopsis*, combinations of DELLA mutants, such as *rga/rgl1/rgl2*, have been reported to rescue the defects of floral organs to different degrees in *ga1–3* mutants (Cheng et al. 2004; Yu et al. 2004; Tyler et al. 2004). By contrast, ectopic expression of a dominant mutant of the DELLA protein, rgl1△17, caused consistent repression of GA responses and underdeveloped flower organs (Wen and Chang 2002), similar to the *ga1–3* mutants (Koornneef and van der Veen 1980).

To further clarify the relationship between *CaFYF1/2* and the GA response during flower organ development, we analyzed the expression of DELLA genes, such as *RGA*, *GAI*, *RGL1*, and *RGL2*, in 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX* plants. The results clearly indicated that all four DELLA genes were upregulated in 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX* plants (Fig. 9(d)). These findings indicated that ectopic expression of *CaFYF1/2* not only caused the delayed senescence/abscission of the flower organs but also affected the genes regulating the GA response and caused the short flower organ phenotype. Since similar alterations of the flower organ development were observed in the 35S::*FYF* (Chen et al. 2011a) and 35S::*FUF1* flowers (Chen et al. 2015), the expression of these DELLA genes was also analyzed in 35S::*FYF* and 35S::*FUF1* plants. Not surprisingly, similar upregulation of these four DELLA genes was observed in the 35S::*FYF* and 35S::*FUF1* plants (Fig. 9(e)). Thus, *FYF* orthologues and the downstream gene *FUF1* may also be involved in the regulation of the GA response during flower development.



Discussion

CaFYF1/2 of *Cattleya* Function in Regulating Flower Senescence and Abscission

We previously reported that *FOREVER YOUNG FLOWER* (*FYF*), a MADS box transcription factor, could regulate flower senescence and abscission in *Arabidopsis* (Chen et al. 2011a). We also found that ectopic expression of the *Oncidium* orchid *FYF* orthologue *OnFYF* could delay flower senescence/abscission in transgenic *Arabidopsis* (Chen et al. 2011b). To develop a strategy to elongate the vase life for *Cattleya* orchid flowers, we identified two putative *FYF* orthologues, *CaFYF1* and *CaFYF2*, from *C. intermedia* and further performed functional analysis of their role in regulating floral senescence and abscission in this study.

◀ **Fig. 9** The gene expression for various transgenic plants. **a** The detection of expression of genes upstream (*ETR1*, *ETR2*, *ERS1*, *ERS2*, *EIL1*, *EIN4*, *CTR1*, *EIN2*, *EIN3*) and downstream (*EDF1*, *2*, *3*, *4*) of the ethylene signaling pathway in wild-type (WT), 35S::*CaFYF1*, 35S::*CaFYF1+SRDX*, 35S::*CaFYF2* and 35S::*CaFYF2+SRDX* flowers by real-time quantitative RT-PCR. **b** The detection of expression of *EDF1*, *2*, *3*, and *4* in wild-type (WT), 35S::*CaFYF1+VP16* and 35S::*CaFYF2+VP16* flowers by real-time quantitative RT-PCR. **c** The expression levels of *BOP1*, *BOP2*, and *IDA* in wild-type (WT) 35S::*CaFYF1*, 35S::*CaFYF1+SRDX*, 35S::*CaFYF2* and 35S::*CaFYF2+SRDX* flowers were detected by real-time quantitative RT-PCR. **d** The expression levels of *RGA*, *GAI*, *RGL1*, and *RGL2* in wild-type (WT) 35S::*CaFYF1-S*, 35S::*CaFYF1+SRDX*, 35S::*CaFYF2* and 35S::*CaFYF2+SRDX-S* flowers were detected by real-time quantitative RT-PCR. **e** The expression levels of *RGA*, *GAI*, *RGL1*, and *RGL2* in wild-type (WT) 35S::*FYF* and 35S::*FUF1* flowers were detected by real-time quantitative RT-PCR. The number of the plants used in real-time quantitative RT-PCR analysis: WT (6), 35S::*CaFYF1* (7), 35S::*CaFYF1-S* (6), 35S::*CaFYF1+SRDX* (8), 35S::*CaFYF1+VP16* (6), 35S::*CaFYF2* (7), 35S::*CaFYF2+SRDX* (8), 35S::*CaFYF2+SRDX-S* (6), 35S::*CaFYF2+VP16* (6), 35S::*FYF* (6) and 35S::*FUF1* (6). In real-time quantitative RT-PCR, transcript levels of these genes were determined using two to three replicates and were normalized using *UBIQUITIN10*. The expression of each gene in various transgenic plants was relative to that in the wild-type plant, which was set at 1. The error bars represent standard deviation

The identity of *CaFYF1* and *CaFYF2* as *FYF* genes in *C. intermedia* was first supported by the sequence identity. *CaFYF1* shows 44% and 70% identity to *Arabidopsis FYF* and *Oncidium OnFYF*, respectively, whereas *CaFYF2* shows 40% and 57% identity to *Arabidopsis FYF* and *Oncidium OnFYF*, respectively. In the MADS box domain, *CaFYF1* and *CaFYF2* showed 97% (568/58) and 93% (54/58) amino acid identity to *Oncidium OnFYF.* The high sequence identity between *CaFYF1/2* and the *FYF* orthologues from various plant species reveals that these two genes are putative *FYF* orthologues of *C. intermedia*.

The second line of evidence demonstrating that *CaFYF1/2* are *FYF* genes is the expression pattern of the *CaFYF1/2* genes during flower development. *CaFYF1/2* showed a very similar expression pattern, with high expression in young (just open) and mature (4 days after open) flowers, which remained vigorous without signs of senescence and abscission. The expression of *CaFYF1/2* was significantly decreased in old flowers (8 days after opening) during the late stages of flower development right before flower senescence occurs. The expression of *CaFYF1/2* was higher in early than late flower development, indicating that their function in preventing senescence/abscission occurred during early flower development and was similar to that for *Arabidopsis FYF*, which was reported previously (Chen et al. 2011a). When *CaFYF1/2* expression decreased during late flower development, this suppression was weakened and resulted in the occurrence of senescence/abscission of the flowers.

The functional similarity of *CaFYF1/2* to *FYF* orthologues in regulating flower senescence/abscission was further demonstrated by transgenic analysis. Flower senescence and abscission were significantly delayed in 35S::*CaFYF1/2* transgenic *Arabidopsis* plants. This result indicated that the function of the *Cattleya CaFYF1/2* genes was related to the regulation of flower senescence and abscission, similar to that for *FYF* of *Arabidopsis* and *OnFYF* of *Oncidium* orchid. Furthermore, similarly delayed flower senescence and abscission in 35S::*CaFYF1/2+SRDX* transgenic *Arabidopsis* plants also suggested that CaFYF1/2 may play a role as a repressor, similar to *Arabidopsis* FYF, in controlling flower senescence and abscission. The delayed floral senescence and abscission of 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX* transgenic *Arabidopsis* plants were strongly correlated with the down-regulation of downstream genes in the ethylene signaling pathway, such as *EDF1/2/3/4* (Alonso et al. 2003; Stepanova and Alonso 2005; Castillejo and Pelaz 2008). In contrast, the expression of *EDF1/2/3/4* was upregulated in the 35S::*CaFYF1/2+VP16* dominant-negative mutants, which showed the promotion of flower senescence and abscission. These results indicated that *CaFYF1/2* is a repressor of the ethylene response in controlling floral senescence and abscission. This assumption was further supported by the insensitivity of 35S::*CaFYF1/2* and 35S::*CaFYF1/2-SRDX* transgenic *Arabidopsis* plants to ethylene treatment. In addition to regulating the ethylene response, *CaFYF1/2* also delayed flower abscission by repressing the abscission-associated genes *BOP1/2* and *IDA* (Hepworth et al. 2005; McKim et al. 2008; Norberg et al. 2005; Butenko et al. 2003). The expression of *BOP1/2* and *IDA* was clearly downregulated in 35S::*CaFYF1/2* and 35S::*CaFYF1/2-SRDX* flowers. Thus, *CaFYF1/2* from *Cattleya* orchids likely controlled flower senescence and abscission by negatively regulating the ethylene response and the abscission-associated genes, similar to other *FYF* orthologues identified from other plant species (Fig. 10).

CaFYF1/2 Regulate Flower Organ Elongation Through Negative Regulation of GA Response

Notably, 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX* plants also showed morphological defects in flowers, such as short sepals and petals. This phenotype was observed previously in *Arabidopsis* plants ectopically expressing *Arabidopsis FYF* (Chen et al. 2011a) or its downstream gene *FUF1* (Chen et al. 2015). However, the mechanism causing this phenotype has not been investigated. DELLA proteins, which were suppressed by GA signaling, were reported to play a critical role in suppressing the development and elongation of flower organs (Cheng et al. 2004; Tyler et al. 2004). Interestingly, when the expression of the DELLA genes *RGA*, *GAI*, *RGL1*, and *RGL2* was analyzed, they were clearly upregulated in 35S::*CaFYF1/2* and 35S::*CaFYF1/2+SRDX* plants. We further confirmed that these four DELLA genes were also activated in 35S::*FYF* and 35S::*FUF1* plants. Thus, this study

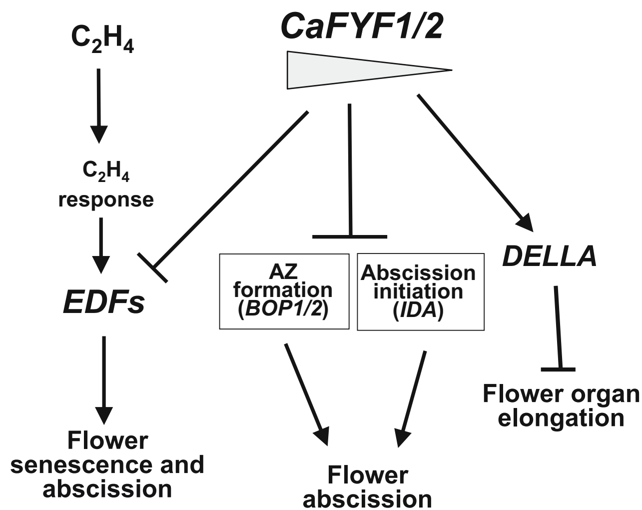


Fig. 10 A model for the function of the Ca*FYF1/2* genes in regulating flower senescence/abscission. In wild-type flowers, *CaFYF1/2* controlled senescence/abscission of the flowers by negatively regulating (sideways "T") the downstream genes *EDF1/2/3/4* in the ethylene signaling pathway. The decrease (gray right-pointing triangle) of *CaFYF1/2* expression during late flower development resulted in the senescence/abscission of the flowers. *CaFYF1/2* also controlled the process of abscission of the flowers by negatively regulating (sideways "T") *BOP1/2* and *IDA*, which are involved in AZ formation and abscission initiation, respectively. In addition to regulating flower senescence/abscission, *CaFYF1/2* and their orthologues also controlled flower organ elongation by negatively regulating the GA response through activation of (black arrows) the expression of the *DELLA* genes during flower development

provided a novel finding that *FYF* orthologues could also affect flower organ development by negatively regulating the GA response through activation of DELLA expression during flower development (Fig. 10). This effect should be reduced during late flower development once *FYF* expression decreased and caused the elongation of the flower organs. The ectopic expression of the *FYF* orthologues (35S::*CaFYF1/2* and 35S::*FYF*) constitutively activated DELLA expression and suppressed the GA response, causing inhibition of flower organ elongation during all stages of flower development as shown in our results.

In summary, this research identified two *FYF* orthologues from *C. intermedia* that may have the same function as *FYF* as a repressor in delaying flower senescence and abscission. *CaFYF1/2* and their orthologues may have additional functions in controlling flower organ elongation by regulating the GA response during flower development. The identification and functional analysis of *CaFYF1/2* in this study provide a useful strategy for the control of shelf life as well as flower shape modification of *Cattleya* orchids in the future.

Funding This work was supported by grants to C-H Y from the Ministry of Science and Technology, Taiwan, ROC, grant numbers MOST 106-2321-B-005-003 and MOST 106-2321-B-005-010. This work was also financially supported (in part) by the Advanced Plant Biotechnology Center from The Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan.

References

Aalen RB, Wildhagen M, Sto IM, Butenko MA (2013) IDA: a peptide ligand regulating cell separation processes in Arabidopsis. J Exp Bot 64:5253–5261

Adamczyk BJ, Lehti-Shiu MD, Fernandez DE (2007) The MADS domain factors AGL15 and AGL18 act redundantly as repressors of the floral transition in *Arabidopsis*. Plant J 50:1007–1019

Alonso JM, Stepanova AN, Leisse TJ, Kim CJ, Chen H, Shinn P, Stevenson DK, Zimmerman J, Barajas P, Cheuk R, Gadrinab C, Heller C, Jeske A, Koesema E, Meyers CC, Parker H, Prednis L, Ansari Y, Choy N, Deen H, Geralt M, Hazari N, Hom E, Karnes M, Mulholland C, Ndubaku R, Schmidt I, Guzman P, Aguilar-Henonin L, Schmid M, Weigel D, Carter DE, Marchand T, Risseeuw E, Brogden D, Zeko A, Crosby WL, Berry CC, Ecker JR (2003) Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. Science 301:653–657

Alvarez-Buylla ER, Liljegren SJ, Pelaz S, Gold SE, Burgeff C, Ditta GS, Vergara-Silva F, Yanofsky MF (2000) MADS-box gene evolution beyond flowers: expression in pollen, endosperm, guard cells, roots and trichomes. Plant J 24:457–466

Arora A (2005) Ethylene receptors and molecular mechanism of ethylene sensitivity in plants. Curr Sci 89:1348–1361

Avila-Ospina L, Moison M, Yoshimoto K, Masclaux-Daubresse C (2014) Autophagy, plant senescence, and nutrient recycling. J Exp Bot 65: 3799–3811

Brumos J, Alonso JM, Stepanova AN (2014) Genetic aspects of auxin biosynthesis and its regulation. Physiol Plant 151:3–12

Butenko MA, Patterson SE, Grini PE, Stenvik GE, Amundsen SS, Mandal A, Aalen RB (2003) Inflorescence deficient in abscission controls floral organ abscission in Arabidopsis and identifies a novel family of putative ligands in plants. Plant Cell 15:2296–2307

Castillejo C, Pelaz S (2008) The balance between CONSTANS and TEMPRANILLO activities determines *FT* expression to trigger flowering. Curr Biol 18:1338–1343

Chen QG, Bleecker AB (1995) Analysis of ethylene signal-transduction kinetics associated with seedling-growth response and chitinase induction in wild-type and mutant *Arabidopsis*. Plant Physiol 108: 597–607

Chen YF, Etheridge N, Schaller GE (2005) Ethylene signal transduction. Ann Bot 95:901–915

Chen MK, Hsu WH, Lee PF, Thiruvengadam M, Chen HI, Yang CH (2011a) The MADS box gene, FOREVER YOUNG FLOWER, acts as a repressor controlling floral organ senescence and abscission in Arabidopsis. Plant J 68:168–185

Chen MK, Lee PF, Yang CH (2011b) Delay of flower senescence and abscission in Arabidopsis transformed with an FOREVER YOUNG FLOWER homolog from Oncidium orchid. Plant Signal Behav 6: 1841–1843

Chen WH, Li PF, Chen MK, Lee YI, Yang CH (2015) FOREVER YOUNG FLOWER negatively regulates ethylene response DNA-binding factors by activating an ethylene-responsive factor to control Arabidopsis floral organ senescence and abscission. Plant Physiol 168:1666–1683

Cheng H, Qin L, Lee S, Fu X, Richards DE, Cao D, Luo D, Harberd NP, Peng J (2004) Gibberellin regulates Arabidopsis floral development via suppression of DELLA protein function. Development 131: 1055–1064

Cho SK, Larue CT, Chevalier D, Wang H, Jinn TL, Zhang S, Walker JC (2008) Regulation of floral organ abscission in Arabidopsis thaliana. Proc Natl Acad Sci U S A 105:15629–15634

Clough SJ, Bent AF (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Plant J 16:735–743

Dill A, Sun T (2001) Synergistic derepression of gibberellin signaling by removing RGA and GAI function in Arabidopsis thaliana. Genetics 159:777–785

Ellis CM, Nagpal P, Young JC, Hagen G, Guilfoyle TJ, Reed JW (2005) *AUXIN RESPONSE FACTOR1* and *AUXIN RESPONSE FACTOR2* regulate senescence and floral organ abscission in *Arabidopsis thaliana*. Development 132:4563–4574

Fernandez DE, Heck GR, Perry SE, Patterson SE, Bleecker AB, Fang SC (2000) The embryo MADS domain factor AGL15 acts postembryonically: inhibition of perianth senescence and abscission via constitutive expression. Plant Cell 12:183–198

Fernandez DE, Wang CT, Zheng Y, Adamczyk BJ, Singhal R, Hall PK, Perry SE (2014) The MADS-domain factors AGAMOUS-LIKE15 and AGAMOUS-LIKE18, along with SHORT VEGETATIVE PHASE and AGAMOUS-LIKE24, are necessary to block floral gene expression during the vegetative phase. Plant Physiol 165: 1591–1603

Hepworth SR, Zhang Y, McKim S, Li X, Haughn GW (2005) *BLADE-ON-PETIOLE*-dependent signaling controls leaf and floral patterning in *Arabidopsis*. Plant Cell 17:1434–1448

Huang H, Mizukami Y, Hu Y, Ma H (1993) Isolation and characterization of the binding sequences for the product of the Arabidopsis floral homeotic gene AGAMOUS. Nucleic Acids Res 21:4769–4776

Hwang I, Sheen J, Muller B (2012) Cytokinin signaling networks. Annu Rev Plant Biol 63:353–380

Jinn TL, Stone JM, Walker JC (2000) HAESA, an Arabidopsis leucine-rich repeat receptor kinase, controls floral organ abscission. Genes Dev 14:108–117

Kater MM, Dreni L, Colombo L (2006) Functional conservation of MADS-box factors controlling floral organ identity in rice and Arabidopsis. J Exp Bot 57:3433–3444

Kim JI, Murphy AS, Baek D, Lee SW, Yun DJ, Bressan RA, Narasimhan ML (2011) YUCCA6 over-expression demonstrates auxin function in delaying leaf senescence in Arabidopsis thaliana. J Exp Bot 62: 3981–3992

Koornneef M, van der Veen JH (1980) Induction and analysis of gibberellin-insensitive mutants in Arabidopsis thaliana (L.) Heynh. Theor Appl Genet 58:257–263

Koyama T (2014) The roles of ethylene and transcription factors in the regulation of onset of leaf senescence. Front Plant Sci 5:650

Lee JH, Chung KS, Kim SK, Ahn JH (2014) Post-translational regulation of short vegetative phase as a major mechanism for thermoregulation of flowering. Plant Signal Behav 9:e28193

Lewis MW, Leslie ME, Liljegren SJ (2006) Plant separation: 50 ways to leave your mother. Curr Opin Plant Biol 9:59–65

McKim SM, Stenvik GE, Butenko MA, Kristiansen W, Cho SK, Hepworth SR, Aalen RB, Haughn GW (2008) The BLADE-ON-PETIOLE genes are essential for abscission zone formation in Arabidopsis. Development 135:1537–1546

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–479

Noh YS, Amasino RM (1999) Identification of a promoter region responsible for the senescence-specific expression of SAG12. Plant Mol Biol 41:181–194

Norberg M, Holmlund M, Nilsson O (2005) The *BLADE ON PETIOLE* genes act redundantly to control the growth and development of lateral organs. Development 132:2203–2213

Patterson SE, Bleecker AB (2004) Ethylene-dependent and -independent processes associated with floral organ abscission in Arabidopsis. Plant Physiol 134:194–203

Peng J, Carol P, Richards DE, King KE, Cowling RJ, Murphy GP, Harberd NP (1997) The Arabidopsis GAI gene defines a signaling pathway that negatively regulates gibberellin responses. Genes Dev 11:3194–3205

Riefler M, Novak O, Strnad M, Schmülling T (2006) Arabidopsis cytokinin receptor mutants reveal functions in shoot growth, leaf senescence, seed size, germination, root development, and cytokinin metabolism. Plant Cell 18:40–54

Rogers HJ (2013) From models to ornamentals: how is flower senescence regulated? Plant Mol Biol 82:563–574

Schenk PM, Kazan K, Rusu AG, Manners JM, Maclean DJ (2005) The SEN1 gene of Arabidopsis is regulated by signals that link plant defence responses and senescence. Plant Physiol Biochem 43: 997–1105

Sekhon RS, Childs KL, Santoro N, Foster CE, Buell CR, de Leon N, Kaeppler SM (2012) Transcriptional and metabolic analysis of senescence induced by preventing pollination in maize. Plant Physiol 159:1730–1744

Shi H, Reiter RJ, Tan DX, Chan Z (2015) INDOLE-3-ACETIC ACID INDUCIBLE 17 positively modulates natural leaf senescence through melatonin-mediated pathway in Arabidopsis. J Pineal Res 58:26–33

Silverstone AL, Ciampaglio CN, Sun T (1998) The Arabidopsis RGA gene encodes a transcriptional regulator repressing the gibberellin signal transduction pathway. Plant Cell 10:155–169

Stenvik GE, Tandstad NM, Guo Y, Shi CL, Kristiansen W, Holmgren A, Clark SE, Aalen RB, Butenko MA (2008) The EPIP peptide of INFLORESCENCE DEFICIENT IN ABSCISSION is sufficient to induce abscission in *Arabidopsis* through the receptor-like kinases HAESA and HAESA-LIKE2. Plant Cell 20:1805–1817

Stepanova AN, Alonso JM (2005) *Arabidopsis* ethylene signaling pathway. Sci STKE 2005:cm4

Tao Z, Shen L, Liu C, Liu L, Yan Y, Yu H (2012) Genome-wide identification of SOC1 and SVP targets during the floral transition in Arabidopsis. Plant J 70:549–561

Theissen G (2001) Development of floral organ identity: stories from the MADS house. Curr Opin Plant Biol 4:75–85

Theissen G, Kim JT, Saedler H (1996) Classification and phylogeny of the MADS-box multigene family suggest defined roles of MADS-box gene subfamilies in the morphological evolution of eukaryotes. J Mol Evol 43:484–516

Tyler L, Thomas SG, Hu J, Dill A, Alonso JM, Ecker JR, Sun TP (2004) DELLA proteins and gibberellin-regulated seed germination and floral development in Arabidopsis. Plant Physiol 135:1008–1019

van Doorn WG, Kamdee C (2014) Flower opening and closure: an update. J Exp Bot 65:5749–5757

Wen CK, Chang C (2002) Arabidopsis RGL1 encodes a negative regulator of gibberellin responses. Plant Cell 14:87–100

Woltering EJ, Somhorst D, Van Der Veer P (1995) The role of ethylene in interorgan signaling during flower senescence. Plant Physiol 109: 1219–1225

Yu H, Ito T, Zhao Y, Peng J, Kumar P, Meyerowitz EM (2004) Floral homeotic genes are targets of gibberellin signaling in flower development. Proc Natl Acad Sci U S A 101:7827–7832