DOAP1 Promotes Flowering in the Orchid Dendrobium Chao Praya Smile

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APETALA1 (AP1) encodes a key MADS-box transcription factor that specifies the floral meristem identity on the flank of the inflorescence meristem, and determines the identity of perianth floral organs in the model plant Arabidopsis thaliana. Orchids are members of the Orchidaceae, one of the largest families of angiosperms. Although the expression patterns of a few AP1-like genes in orchids have been reported, their actual functions in orchid reproductive development are so far largely unknown. In this study, we isolated and characterized an AP1 ortholog, DOAP1, from Dendrobium Chao Praya Smile. DOAP1 was highly expressed in reproductive tissues, including inflorescence apices and flowers at various developmental stages. Overexpression of DOAP1 resulted in early flowering in Arabidopsis, and was able to rescue the floral organ defects of Arabidopsis ap1 mutants. Moreover, we successfully created transgenic Dendrobium Chao Praya Smile orchids overexpressing DOAP1, which displayed earlier flowering and earlier termination of inflorescence meristems into floral meristems than wild-type orchids. Our results demonstrate that DOAP1 plays an evolutionarily conserved role in promoting flowering and floral meristem specification in the Orchidaceae family.

Keywords: DOAP1, MADS-box gene, flowering time, flower development, orchid, Arabidopsis

INTRODUCTION

During the floral transition in flowering plants, the vegetative shoot apical meristem that produces leaves is transformed into the inflorescence meristem that produces flowers. This process is controlled by flowering regulatory networks in response to various environmental and endogenous signals, and the underlying molecular mechanisms have been intensively studied in the model plant Arabidopsis thaliana. In Arabidopsis, environmental and endogenous flowering signals perceived by various genetic pathways mediate transcriptional regulation of two major floral pathway integrators, FLOWERING LOCUS T (FT) and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1), which in turn activate two floral meristem identity genes, APETALA1 (API) and LEAFY (LFY), to regulate the specification and formation of floral meristems on the flank of the shoot apical meristem (Kardailsky et al., 1999; Kobayashi et al., 1999; Blazquez and Weigel, 2000; Lee et al., 2000; Samach et al., 2000; Liu et al., 2009).

API encodes a MADS-box transcription factor that not only specifies the floral meristem identity, but also contributes to determination of the identity of perianth floral organs in Arabidopsis (Irish and Sussex, 1990; Mandel et al., 1992; Bowman et al., 1993). When the activity...
of API is lost, floral meristems that would normally develop into flowers are partially converted into inflorescence meristems. In ap1 mutants, flowers exhibited transformation of sepals into leaf-like organs and failure of petal development. In contrast, overexpression of API results in early flowering and transformation of inflorescence meristems into floral meristems (Mandel and Yanofsky, 1995). During the floral transition, API expression is directly activated by LFY and a protein complex consisting of FT and FD (Wagner et al., 1999; Abe et al., 2005; Wigge et al., 2005). In emerging floral meristems, API plays dual functions as an activator and a repressor. It activates B class homeotic genes to mediate the specification of petals (Hill et al., 1998; Ng and Yanofsky, 2001), while it also suppresses a group of flowering time genes to prevent the reversion of floral meristems into inflorescence meristems (Yu et al., 2004; Liu et al., 2007; Kaufmann et al., 2010). Moreover, API directly regulates the homeostasis of cytokinin by suppressing its biosynthesis and activating its degradation to establish determinate floral meristems (Han et al., 2014).

So far API orthologs have been isolated in a wide range of plant species, such as pea (Pisum sativum) (Berbel et al., 2001), apple (Malus domestica) (Kotoda et al., 2002), common wheat (Triticum aestivum) (Adam et al., 2007), moth orchid (Phalaenopsis ‘Hatsuyuki’) (Song et al., 2011), longan (Dimocarpus longan) (Winterhagen et al., 2013), trifoliate orange (Poncirus trifoliata L. Raf.) (Sun et al., 2014), Birch (Betula platyphylla) (Huang et al., 2014) and poplar (Populus tomentosa) (Chen et al., 2015). Through mainly examining their expression patterns and their effects on heterologous systems, most of these genes have been suggested to affect either the flowering process or floral organ formation.

Dendrobium is one of the largest genera in the family Orchidaceae, which is one of the largest families of angiosperms (Teixeira da Silva et al., 2014). The Dendrobium genus comprises more than 1200 species, and its members have attractive ornamental and medicinal values (Yu and Goh, 2001; Teixeira da Silva et al., 2014). The high demand of Dendrobium orchids has enabled Dendrobium growers to create many varieties and hybrids with various floral traits. However, the long vegetative phase and low survival rate of orchid seedlings under natural environmental conditions make it difficult to propagate and breed Dendrobium orchids (Yu and Goh, 2001; Chai and Yu, 2007; Wang et al., 2009). Therefore, it is important to elucidate the molecular mechanisms underlying the floral transition in orchids so as to identify appropriate regulators for targeted manipulation or screening of orchid traits. Recent studies have isolated and characterized several orthologs of Arabidopsis floral pathway integrators in orchids. Overexpression of three orthologs of FT, OnFT, DnFT and CgFT from Oncidium, Dendrobium, and Cymbidium orchids, respectively, causes early flowering in transgenic Arabidopsis or tobacco plants (Hou and Yang, 2009; Li et al., 2012; Xiang et al., 2012). In addition, overexpression of DOSOCI, an ortholog of the Arabidopsis SOCI, promotes flowering in both transgenic Arabidopsis and Dendrobium orchids (Ding et al., 2013).

In this study, we isolated an API ortholog, DOAPI, from Dendrobium Chao Praya Smile, and characterized its function through examining its expression patterns and investigating its biological effects in both transgenic Arabidopsis and orchid plants. Our results suggest that DOAPI may play a conserved role in promoting flowering and floral meristem development in the Orchidaceae family.

**MATERIALS AND METHODS**

**Plant Material and Growth Conditions**

*Dendrobium* Chao Praya Smile (a hybrid of *Dendrobium* Pinky and *Dendrobium* Kiyomi Beauty) plants were grown in pots in the greenhouse at 28 ± 4°C under natural lighting conditions. In our *in vitro* culture system for *Dendrobium* Chao Praya Smile, protocorms developing from seeds were used as the starting materials. They were cultured in modified liquid Knudson C (KC) medium supplemented with 2% (w/v) sucrose, 15% (v/v) coconut water and 4.4 μM benzyladenine (BA) at 24°C under a 16-h photoperiod on rotary shakers at 120 rpm (Yu and Goh, 2000a; Ding et al., 2013). Plantlets at the floral transitional stage were transferred to the two-layer modified KC medium as previously described (Hee et al., 2007; Sim et al., 2007). Wild-type Arabidopsis thaliana ecotype Columbia (Col-0) and 35S:DOAPI transgenic plants in both wild-type and api-10 Col-0 backgrounds were grown under long-day conditions (16 h light/8 h dark) at 23 ± 2°C.

**Isolation of DOAPI**

Total RNA was extracted from inflorescences of *Dendrobium* Chao Praya Smile using the RNeasy® Plant Mini Kit (QIAGEN). To isolate the putative API orthologs, two degenerate primers, 5′-CAGCTGARGCGRATMGAGAAC-3′ and 5′-GCKMAGCATCCAWGGYGG-3′, were manually designed based on the conserved regions among API orthologs in various plant species. Based on the partial cDNA sequence obtained, the full-length cDNA of DOAPI was further amplified using the SMARTER® RACE cDNA Amplification Kit (BD Biosciences Clontech).

**Sequence Analysis**

Protein sequences of API-like genes were retrieved from the National Center for Biotechnology Information (NCBI) database. Alignment of amino acid sequences was performed using the Clustal Omega multiple sequence alignment program1 and BOXSHADE 3.212. The phylogenetic tree was generated by the neighbor-joining algorithm using the MEGA6 software3.

**Expression Analysis**

Total RNA was extracted from orchids or Arabidopsis plants using the RNeasy® Plant Mini Kit (QIAGEN), and reverse-transcribed using the SuperScript™ II Reverse Transcriptase

1 http://www.ebi.ac.uk/Tools/msa/clustalo/
2 http://www.ch.embnet.org/software/BOX_form.html
3 http://www.megasoftware.net/
FIGURE 1 | Alignment of the amino acid sequences of DOAP1 and its orthologs from other plant species. Conserved residues are shown in black, while similar residues are shown in gray. Dashes indicate gaps to maximize alignment. The species names are shown behind their corresponding protein names as follows: DOAP1, Dendrobium Chao Praya Smile; CeAP1, Cymbidium ensifolium; PjAP1, Phalaenopsis japonica; ZmAP1, Zea mays; OsAP1, Oryza sativa; LpAP1, Lolium perenne; TaAP1, Triticum aestivum; AP1, Arabidopsis thaliana; MdAP1, Malus domestica; CsAP1, Citrus sinensis; PtAP1, Populus trichocarpa; PaAP1, Paeonia suffruticosa; VuAP1, Vigna unguiculata; ZjAP1, Ziziphus jujube; DlAP1, Dimocarpus longan; VvAP1, Vitis vinifera; NnAP1, Nelumbo nucifera; PaAP1, Persea Americana; MgAP1, Magnolia grandiflora. Non-AP1-like proteins that are encoded by other MADS-box subfamily genes were used as outliers in this alignment as follows: DEFICIENS-AGAMOUS-LIKE2 (DAL2) of Picea abies, and two SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1)-like proteins from Rosa hybrid cultivar (RosaSOC1) and Picea abies (PaSOC1).

Kit (Invitrogen) according to the manufacturer’s instructions. Real-time PCR reaction was performed in triplicates on the CFX384 Real-Time PCR Detection System (Bio-Rad) with the SYBR Green Master Mix (Toyobo). UBIQUITIN (DOubi) in Dendrobium Chao Praya Smile was used as a reference gene (Ding et al., 2013). Calculation of relative gene expression levels was performed as previously reported (Liu et al., 2007). Semi-quantitative reverse transcription PCR (RT-PCR) was carried out as previously reported (Xu et al., 2006) using either Arabidopsis β-TUBLIN2 (TUB2) or orchid ACTIN (DOActin) as a reference gene.

Arabidopsis Transformation
The full-length DOAP1 cDNA fragment was cloned into pGreen0229-35S under the control of two CaMV 35S promoters (Yu et al., 2004). The resulting construct was introduced into Agrobacterium tumefaciens GV3101 competent cells by electroporation. 35S:DOAP1 transgenic Arabidopsis lines were...
created by *Agrobacterium tumefaciens*-mediated transformation and selected by 0.3 g/L Basta on soil.

**Orchid Transformation by Agrobacterium-Mediated Transformation**

Genetic transformation of *Dendrobium* Chao Praya Smile was performed according to the reported 1-methionine sulfoximine (MSO) selection system with minor modifications (Yu et al., 2001; Chai et al., 2007; Ding et al., 2013). *Dendrobium* Chao Praya Smile calli were cut into small pieces 3–5 mm in diameter and then cultured in modified KC liquid medium supplemented with 2% (w/v) sucrose, 15% (v/v) coconut water and 4.4 µM BA. *Agrobacterium* pellet was re-suspended in KC liquid medium to be co-cultivated with prepared orchid calli. Acetosyringone (100 µM) was added to induce transformation. After co-cultivation for 2 h, calli were placed on solid KC medium in the dark for three nights. *Agrobacterium*-infected calli were rinsed by water containing 200 mg/L cefotaxime to remove the bacteria, and grown on solid KC medium containing 0.5 µM MSO as a selection agent. Surviving calli were subcultured onto fresh solid medium every 20 days. After four rounds of MSO selection, green calli were transferred to modified KC solid medium containing

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**FIGURE 2 | Phylogenetic tree of AP1/AGL9-like MADS-box proteins.** Amino acid sequences of AP1/AGL9-like MADS box genes were obtained from the NCBI database. DOAP1 investigated in this study is highlighted in blue. The multiple sequence alignment was performed via Clustal Omega. The tree was constructed with MEGA6 using the neighbor-joining method. Genus names of respective species are given in the parentheses behind the corresponding protein names. Bootstrap values (>50%) in 1,000 replicates are shown below the nodes.
the conserved amino acid sequences of AP1. Smile, we designed a pair of degenerate primers based on To isolate AP1 Chao Praya Smile.

RESULTS

Isolation of DOAP1 from Dendrobium Chao Praya Smile

To isolate AP1-like genes from Dendrobium Chao Praya Smile, we designed a pair of degenerate primers based on the conserved amino acid sequences of AP1 orthologs in various plant species. A partial cDNA fragment was obtained by reverse transcription PCR using RNA extracted from inflorescence apices of Dendrobium Chao Praya Smile. Since this fragment showed high sequence similarity with other AP1-like genes, we further designed primers based on this fragment and obtained the corresponding full-length cDNA sequence, designated DOAP1 (GenBank accession No. KY471451), using the rapid amplification of cDNA ends (RACE) method.

DOAP1 cDNA is 897 bp in length with a 639 bp coding region. The deduced DOAP1 amino acid sequence contains a highly conserved MADS domain and a less conserved K domain, and a diverse C terminal region as found in other AP1-like genes from various plant species (Figure 1). Multiple sequence alignment showed that DOAP1 shared the highest sequence identity with other orchid AP1 orthologs, such as CeAP1 (Cymbidium ensifolium; 69.95% identity) and PjAP1 (Phalaenopsis japonica; 62.56% identity). DOAP1 also had higher sequence identity with monocot AP1 orthologs, such as TaAP1 (Triticum aestivum; 58% identity), OsAP1 (Oryza sativa; 56.72% identity) and ZmAP1 (Zea mays; 54.59% identity), than Arabidopsis AP1 (53.3% identity).

To determine the evolutionary relationship between DOAP1 and other AP1-like proteins from other plant species, we constructed a phylogenetic tree based on the analysis of amino acid sequences of MIK regions (Figure 2). The tree showed that DOAP1 was clustered together with other orchid AP1-like proteins, such as OMADS10 (Chang et al., 2009) and DOMADS2 (Yu and Goh, 2000b), in the monocotyledonous subgroup of SQUA that includes AP1-like proteins isolated from monocots (Purugganan et al., 1995; Theissen et al., 1996).

DOAP1 Expression Pattern in Dendrobium Chao Praya Smile

To study the expression pattern of DOAP1 in Dendrobium Chao Praya Smile, cdNA was synthesized from RNA extracted from various orchid tissues, including leaves, roots, inflorescence apices, floral buds at different stages (stages 1–3, at which buds were 0.5–1 cm, 1–2 cm, and over 2 cm in length, respectively), half-bloomed flowers and fully bloomed flowers (Figure 3A). Quantitative real-time PCR analysis showed that DOAP1 transcripts were detected at the highest level in inflorescence apices and at the lowest level in roots (Figure 3B). Notably, DOAP1 expression was significantly higher in inflorescence apices than in vegetative shoot apices. Moreover, DOAP1 expression gradually increased in developing floral buds at various stages and half-bloomed flowers, but decreased afterward in fully bloomed flowers (Figure 3B). These expression patterns indicate that DOAP1 function may be closely associated with flowering time control and flower development in Dendrobium Chao Praya Smile.

DOAP1 Promotes Flowering and Partially Complements ap1 Mutants in Arabidopsis

In order to investigate the biological function of DOAP1, we firstly generated transgenic Arabidopsis plants harboring the

Southern Blot

Genomic DNA isolated from wild-type and 35S:DOAP1 transgenic orchids was digested by EcoRI for 16 h, resolved on 1.2% (w/v) agarose gels, and then transferred onto nylon membranes. Blots were hybridized overnight with the specific digoxigenin-labeled DNA, washed and detected in accordance with the manufacturer’s instructions (DIG Application Manual for Filter Hybridization, Roche) as previously reported (Yu and Goh, 2000b).

2 µM MSO for lethal selection. After three rounds of selection, putative surviving transgenic lines were cultured on modified KC solid medium for further investigation.

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DOAP1 coding sequence driven by the CaMV 35S promoter. Among 50 independent 35S:DOAP1 transgenic lines created at the T1 generation, 46 lines showed an early flowering phenotype typically producing only 5–7 rosette leaves as compared to wild-type plants, which developed 9–12 rosette leaves under long day conditions (Figures 4A,B). Semi-quantitative RT-PCR using DOAP1-specific primers confirmed that DOAP1 was overexpressed in a representative 35S:DOAP1 transgenic Arabidopsis line with a single T-DNA insertion (Figure 4C), implying that the early flowering phenotype of 35S:DOAP1 is associated with overexpression of DOAP1. In addition to the defect in flowering time, the inflorescence meristems of 35S:DOAP1 transgenic lines usually terminated as flowers after producing only a few flowers, while these flowers produced floral organs indistinguishable from those of wild-type flowers (Figures 4D,E), suggesting that overexpression of DOAP1 promotes the formation of floral meristems, but does not affect floral organ identity in Arabidopsis.

We further performed Agrobacterium-mediated transformation to introduce 35S:DOAP1 into ap1-10 mutants, which were generated from ethyl methanesulfonate-mutagenized populations of Arabidopsis ecotype Columbia (Schultz and Haughn, 1993), to examine whether DOAP1 could complement the loss function of AP1. While ap1-10 exhibited comparable flowering time to wild-type plants, most of the ap1-10 35S:DOAP1 lines flowered much earlier than wild-type and ap1-10 plants, a flowering pattern resembling 35S:DOAP1 in the wild-type background (Figure 4A). We further identified several ap1-10 35S:DOAP1 transgenic lines with single T-DNA insertion, which consistently displayed the similar early flowering phenotype at various generations, and selected one representative line for further investigations. Like other ap1 mutants, ap1-10 showed typical defects in the identity of perianth organs, such as homeotic transformation of petals into stamens or stamen-petal chimeric structures (Figure 4F). In contrast, petal formation was restored in ap1-10 35S:DOAP1 (Figure 4G), demonstrating that DOAP1 plays the same role as AP1 in regulating petal formation in Arabidopsis. Similarly, semi-quantitative RT-PCR detected high expression of DOAP1 in the representative ap1-10 35S:DOAP1 line (Figure 4C), substantiating a causal link between the observable phenotypes and overexpression of DOAP1 in ap1-10 35S:DOAP1.
**DOAP1 Promotes Early Formation of Inflorescences in *Dendrobium Chao Praya Smile***

To understand the endogenous function of *DOAP1* in *Dendrobium Chao Praya Smile*, we created transgenic orchids bearing 35S:*DOAP1* in a pGreen vector (Figure 5A) using an integrated orchid gene transformation and *in vitro* orchid culture system (Yu et al., 2001; Chai et al., 2007; Ding et al., 2013).

After *Agrobacterium*-mediated transformation of *Dendrobium* Chao Praya Smile calli, the transformed materials were selected on the medium containing 0.5 and 2 µM MSO as a selection agent for initial and lethal selection (Figure 5B), respectively. After a total of seven rounds of selection, most of the calli turned necrotic and eventually died, while some putative transformants survived and proliferated into protocorm-like bodies, which further developed into young plantlets. We screened out nine independent 35S:*DOAP1* transgenic *Dendrobium* Chao Praya Smile lines, and confirmed the presence of the 35S:*DOAP1* transgene in these lines by PCR genotyping using the specific primers from 35S and *DOAP1* (Figure 5C).

We then compared the growth status of wild-type and 35S:*DOAP1* *Dendrobium* Chao Praya Smile plants using our established *in vitro* culture system (Ding et al., 2013), which allows rapid *in vitro* development of orchid plants from the vegetative to reproductive phase. Under our growth conditions, it took wild-type orchid plants 25–30 weeks to grow from protocorm-like bodies to plantlets with the first visible inflorescence stalks (Figure 6A), whereas 8 out of 9 35S:*DOAP1* transgenic lines displayed the first visible inflorescences at 10–17 weeks of culture (Figures 6A,B). These phenotypes indicate a role of *DOAP1* in promoting the transition of vegetative shoot apical meristems into inflorescence meristems. As compared to wild-type inflorescences (Ding et al., 2013), the inflorescence apices of these 35S:*DOAP1* transgenic plants were usually terminated as floral buds immediately after producing only 1–2 floral buds or even without generating any other floral structure (Figure 6C), suggesting a quick transformation of inflorescence meristems into floral meristems in 35S:*DOAP1*. 

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**FIGURE 5 | Generation of 35S:*DOAP1* transgenic orchids. (A)** Schematic diagram of the T-DNA region of the pGreen0229-35S:*DOAP1* construct. LB, left border; Tnos, nopaline synthase terminator sequence; bar, bialaphos resistance gene; Pnos, nopaline synthase promoter sequence; 35S, Cauliflower mosaic virus 35S promoter sequence; TCaMV, Cauliflower mosaic virus terminator sequence; RB, right border. (B) Genetic transformation of *Dendrobium* Chao Praya Smile using the MSO selection system. After lethal section on the solid medium containing 2 µM MSO (left panel), green surviving calli were further subcultured onto fresh medium every 20 days (right panel). (C) PCR genotyping of *DOAP1* transgenic orchids shows the presence of 35S:*DOAP1* transgene in nine independent lines of *Dendrobium* Chao Praya Smile. The specific primers from the 35S promoter and *DOAP1*, respectively, were designed for genotyping. The 35S:*DOAP1* plasmid and the genomic DNA of wild-type orchids were used as a positive control (+ve) and a negative control (WT), respectively.
In addition, Southern blot hybridization revealed the presence of the T-DNA region containing the bialaphos resistance (bar) gene in the genome of 35S:DOAP1 #1 and #2 lines (Figure 6D), which substantiated the results showing the presence of the 35S:DOAP1 transgene in the 35S:DOAP1 genome (Figure 5C). As expected, semi-quantitative RT PCR showed that DOAP1 was overexpressed in inflorescence apices of 35S:DOAP1 transgenic orchids compared to wild-type orchids (Figure 6E). These results imply that the phenotypes of 35S:DOAP1 transgenic orchids are associated with overexpression of DOAP1 in these plants.

DISCUSSION

Although the Orchidaceae is one of the largest and most widespread families of flowering plants, the molecular mechanism underlying the flowering process of the members in this family remain largely unknown. The difficulty in performing molecular genetic studies on orchids is partially due to their long vegetative phase and recalcitrant nature for gene transformation. In this study, we have taken advantage of an established Agrobacterium-mediated gene transformation system based on MSO selection coupled with in vitro orchid culture system (Yu et al., 2001; Hee et al., 2007; Sim et al., 2007; Ding et al., 2013) to study the biological function of a newly isolated AP1 ortholog, DOAP1, from Dendrobium Chao Praya Smile.

Our findings have provided several pieces of evidence to support that DOAP1 plays a conserved role in promoting the floral transition and specifying the identity of perianth floral organs. First, in addition to the conserved MADS and K domains found among AP1-like proteins, phylogenetic analysis has revealed that DOAP1 and other orchid AP1-like proteins are assigned to the same clade in the monocotyledonous subgroup of SQUA, which contains AP1/SQUA-like proteins from monocots (Purugganan et al., 1995; Theissen et al., 1996). Second, DOAP1 is highly expressed in inflorescence apices and flowers at various developmental stages, which is similar to the patterns exhibited by most of AP1-like genes studied (Becker and Theissen, 2003; Litt and Irish, 2003). Notably, DOAP1 is also expressed in vegetative shoot apices and upregulated in inflorescence apices, suggesting that it could be one of the earliest regulatory genes that promote the floral transition in...
Abe, M., Kobayashi, Y., Yamamoto, S., Daimon, Y., Yamaguchi, A., Ikeda, Y., et al. (2005). FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. Science 309, 1052–1056. doi: 10.1126/science.1115983

Adam, H., Ouellet, F., Kane, N. A., Agharbaoui, Z., Major, G., Tominaga, Y., et al. (2007). Overexpression of TaVRN1 in Arabidopsis promotes early flowering and alters development. Plant Cell Physiol. 48, 1192–1206. doi: 10.1093/pcp/pcm089

Becker, A., and Theissen, G. (2003). The major clades of MADS-box genes and their role in the development and evolution of flowering plants. Mol. Phylogenet. Evol. 29, 464–489. doi: 10.1016/S1055-7903(03)00207-0

Berbel, A., Navarro, C., Ferrandiz, C., Canas, L. A., Madueno, F., and Beltran, J. P. (2001). Analysis of PEAM4, the pea AP1 functional homologue, supports a model for AP1-like genes controlling both floral meristem and floral organ identity in different plant species. Plant J. 25, 441–451. doi: 10.1046/j.1365-313x.2001.00997.x

Blázquez, M. A., and Weigel, D. (2000). Integration of floral inductive signals in Arabidopsis. Nature 404, 889–892. doi: 10.1038/35009125

Bowman, J. L., Alvarez, J., Weigel, D., Meyerowitz, E., and Smyth, D. R. (1993). Control of flower development in Arabidopsis thaliana by APETALA1 and interacting genes. Development 119, 721–743.

Chai, D., Lee, S. M., Ng, J. H., and Yu, H. (2007). L-methionine sulfoximine as a novel selection agent for genetic transformation of orchids. J. Biotechnol. 131, 466–472. doi: 10.1016/j.jbiotec.2007.07.951

Chai, D., and Yu, H. (2007). Recent advances in transgenic orchid production. Orchid Sci. Biotechnol. 1, 34–39.

Chang, Y.-Y., Chiu, Y.-F., Wu, J.-W., and Yang, C.-H. (2009). Four orchid (Oncidium Gower Ramsey) AP1/AGL9-like MADS box genes show novel expression patterns and cause different effects on floral transition and formation in Arabidopsis thaliana. Plant Cell Physiol. 50, 1425–1438. doi: 10.1093/pcp/pcp087

Chen, Z., Yang, X., Su, X., Rao, P., Gao, K., Lei, B., et al. (2015). Identification and expression analysis of APETALA1 homologues in poplar. Acta Physiol. Plant. 37, 1–10. doi: 10.1007/s11738-015-1805-z

Ding, L., Wang, Y., and Yu, H. (2013). Overexpression of DOSOC1, an ortholog of Arabidopsis SOC1, promotes flowering in the orchid Dendrobium Chao Parya Smile. Plant Cell Physiol. 54, 595–608. doi: 10.1093/pcp/pcp026

Han, Y., Zhang, C., Yang, H., and Jiao, Y. (2014). Cytokinin pathway mediates APETALA1 function in the establishment of determinate floral meristems in Arabidopsis. Proc. Natl. Acad. Sci. U.S.A. 111, 6840–6845. doi: 10.1073/pnas.1318532111

REFERENCES

Abe, M., Kobayashi, Y., Yamamoto, S., Daimon, Y., Yamaguchi, A., Ikeda, Y., et al. (2005). FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. Science 309, 1052–1056. doi: 10.1126/science.1115983

Adam, H., Ouellet, F., Kane, N. A., Agharbaoui, Z., Major, G., Tominaga, Y., et al. (2007). Overexpression of TaVRN1 in Arabidopsis promotes early flowering and alters development. Plant Cell Physiol. 48, 1192–1206. doi: 10.1093/pcp/pcm089

Becker, A., and Theissen, G. (2003). The major clades of MADS-box genes and their role in the development and evolution of flowering plants. Mol. Phylogenet. Evol. 29, 464–489. doi: 10.1016/S1055-7903(03)00207-0

Berbel, A., Navarro, C., Ferrandiz, C., Canas, L. A., Madueno, F., and Beltran, J. P. (2001). Analysis of PEAM4, the pea AP1 functional homologue, supports a model for AP1-like genes controlling both floral meristem and floral organ identity in different plant species. Plant J. 25, 441–451. doi: 10.1046/j.1365-313x.2001.00997.x

Blázquez, M. A., and Weigel, D. (2000). Integration of floral inductive signals in Arabidopsis. Nature 404, 889–892. doi: 10.1038/35009125

Bowman, J. L., Alvarez, J., Weigel, D., Meyerowitz, E., and Smyth, D. R. (1993). Control of flower development in Arabidopsis thaliana by APETALA1 and interacting genes. Development 119, 721–743.

organ specification. For example, another orchid AP1-like gene, DOMADS2, isolated from Dendrobium Madame Thong-In is not only highly expressed in the shoot apical meristem during the floral transition, but also expressed in orchid reproductive organs, such as column (gynostemium, a fused structure of stigmas, styles and stamens) and ovary (Yu and Goh, 2000b), indicating that DOMADS2 may also be involved in reproductive organ development. Similarly, another AP1-like gene, EpMADS12, in Erycina pusilla is also detectable in multiple floral organs, such as lips, column, and pollinarium (Lin et al., 2016). These diverse expression patterns of AP1-like genes indicate that AP1-like genes may evolve with multiple functions in regulating flowering and flower development in orchids. Further investigation of these orchid AP1-like genes through knocking down or knocking out their expression in orchids will shed light on their endogenous functions in orchid reproductive development.

AUTHOR CONTRIBUTIONS

NS, YW, LS, and HY conceived and designed the study. NS and SB performed the experiments. NS, SB, YW, LS, and HY analyzed data. NS, YW, LS, and HY wrote the paper. All authors read and approved the manuscript.

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Kardailsky, I., Shukla, V. K., Ahn, J. H., Dagenais, N., Christensen, S. K., Nguyen, C. J., and Yang, C. H. (2009). Functional analysis of FT and TFL1.

Liu, C., Zhou, J., Bracha-Drori, K., Yavalov, S., Ito, T., and Yu, H. (2007). Expression analysis of an APETALA1/FRUITFULL-like gene in Phalaenopsis sp. "Hatsuyuki" (Orchidaceae).

Mandel, M. A., and Yanofsky, M. F. (1995). Molecular evolution of flower development: diversification of the plant MADS-box regulatory gene family. Genetics 140, 345–356.

Ng, M., and Yanofsky, M. F. (2001). Activation of the Arabidopsis B class homeotic genes by APETALA1. Plant Cell 13, 739–753. doi:10.1105/tpc.13.4.739

Purugganan, M. D., Rounsley, S. D., Schmidt, R. J., and Yanofsky, M. F. (1995). Molecular evolution of flower development: diversification of the plant MADS-box regulatory gene family. Genetics 140, 345–356.

Sim, G. E., Loh, C. S., and Goh, C. J. (2007). High frequency early in vitro flowering of Dendrobium Madame Thong-In (Orchidaceae). Plant Cell Rep. 26, 383–393. doi:10.1007/s00299-006-0242-2

Song, I.-J., Fukuda, T., Ko, S.-M., Ito, T., Yokoyama, J., Ichikawa, H., et al. (2011). Expression analysis of an APETALA1/FRUITFULL-like gene in Phalaenopsis sp. "Hatsuyuki" (Orchidaceae). Hortic. Environ. Biotechnol. 52, 183–195. doi:10.13580-11-0199-0

Sun, L.-M., Zhang, J.-Z., Mei, L., and Hu, C.-G. (2014). Molecular cloning, promoter analysis and functional characterization of APETALA1-like gene from precocious trifoliate orange (Poncirus trifoliate L. Raf.). Sci. Hortic. 178, 95–105. doi:10.1016/j.scienta.2014.08.015

Teixeira da Silva, J. A., Aceto, S., Liu, W., Yu, H., and Kanno, A. (2014). Genetic control of flower development, color and senescence of Dendrobium orchids. Sci. Hortic. 175, 74–86. doi:10.1016/j.scienta.2014.05.008

Theissen, G., Kim, J. T., and Saddler, 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. doi:10.1007/p1000610

Wagner, D., Sablowski, R. W., and Meyerowitz, E. M. (1999). Transcriptional activation of APETALA1 by LEAFY. Science 285, 582–584. doi:10.1126/science.285.5427.582

Wang, Z., Wang, L., and Ye, Q. (2009). High frequency early flowering from in vitro seedlings of Dendrobium nobile. Sci. Hortic. 122, 328–331. doi:10.1016/j.scienta.2009.05.018

Wigge, P. A., Kim, M. C., Jaeger, K. E., Busch, W., Schmid, M., Lohmann, J. U., et al. (2005). Integration of spatial and temporal information during floral induction in Arabidopsis. Science 309, 1056–1059. doi:10.1126/science.1114358

Winterhagen, P., Tiyayon, P., Samach, A., Hegele, M., and Wünsche, J. N. (2013). Isolation and characterization of FLOWERING LOCUS T subforms and APETALA1 of the subtropical fruit tree Dimocarpus longan. Plant Physiol. Biochem. 71, 184–190. doi:10.1016/j.plaphy.2013.07.013

Xiang, L., Li, X., Qin, D., Guo, F., Wu, C., Miao, L., et al. (2012). Functional analysis of FLOWERING LOCUS T orthologs from spring orchid (Cymbidium goeringii Rchb. f.) that regulates the vegetative to reproductive transition. Plant Physiol. Biochem. 58, 98–105. doi:10.1016/j.plaphy.2012.06.011

Xu, Y., Teo, L. L., Zhou, J., Kumar, P. P., and Yu, H. (2006). Floral organ identity genes in the orchid Dendrobium crumenatum. Plant J. 46, 54–68. doi:10.1111/j.1365-313X.2006.02669.x

Yu, H., and Goh, C. J. (2000a). Differential gene expression during floral transition in an orchid hybrid Dendrobium Madame Thong In. Plant Cell Rep. 19, 926–931. doi:10.1007/s002990000227

Yu, H., and Goh, C. J. (2000b). Identification and characterization of three orchid MADS-box genes of the AP1/AGL9 subfamily during floral transition. Plant Physiol. 123, 1325–1336. doi:10.1104/pp.123.4.1325

Yu, H., and Goh, C. J. (2001). Molecular genetics of reproductive biology in orchids. Plant Physiol. 127, 1390–1393. doi:10.1104/pp.010676

Yu, H., Ito, T., Wellmer, F., and Meyerowitz, E. M. (2004). Repression of AGAMOUS-LIKE 24 is a crucial step in promoting flower development. Nat. Genet. 36, 157–161. doi:10.1038/ng1286

Yu, H., Yang, S., and Goh, C. (2001). Agrobacterium-mediated transformation of a Dendrobium orchid with the class 1 knox gene DOHI. Plant Cell Rep. 20, 301–305. doi:10.1007/s0029900100334

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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