


MINI-REVIEW



Current progress in orchid flowering/flower development research

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ABSTRACT

Genetic pathways relevant to flowering of *Arabidopsis* are under the control of environmental cues such as day length and temperatures, and endogenous signals including phytohormones and developmental aging. However, genes and even regulatory pathways for flowering identified in crops show divergence from those of *Arabidopsis* and often do not have functional equivalents to *Arabidopsis* and/or existing species- or genus-specific regulators and show modified or novel pathways.

Orchids are the largest, most highly evolved flowering plants, and form an extremely peculiar group of plants. Here, we briefly summarize the flowering pathways of *Arabidopsis*, rice and wheat and present them alongside recent discoveries/progress in orchid flowering and flower developmental processes including our transgenic *Phalaenopsis* orchids for *LEAFY* overexpression. Potential biotechnological applications in flowering/flower development of orchids with potential target genes are also discussed from an interactional and/or comparative viewpoint.

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Importance of floral transition in orchid breeding

Orchids are a valuable floricultural crop and some wild species are under constant threat of extinction due to over-collection for commercial trade.¹ Large and complex polyploid genomes, low transformation efficiency, slow growth and long life cycles mean that it is challenging to overcome the risk of extinction or generate new varieties containing desirable traits with commercial value by either traditional breeding or genetic engineering techniques.^{2,3} For example, it takes more than 2 y for popular orchid cultivars with high commercial value (e.g., *Phalaenopsis* orchids) to switch from the vegetative to the reproductive phase.^{3,4}

The structure of orchid flowers has unique diversification among flowering plants. Moreover, most orchids have defined favorable seasons for floral induction as well as inflorescence and flower development. Although several genes affecting flower development have been identified in orchids, the function of the genes during orchid floral transition still remains to be studied through the establishment of reliable protocols to induce alterations in flowering time of orchids.^{5,6,7} Molecular and genetic studies of orchid flowering are invaluable not only for understanding the molecular mechanisms of flower development including evolutionary trends but also for assisting molecular breeding to produce orchids with desirable traits.

Floral transition in a long-day model plant, *Arabidopsis*

In *Arabidopsis*, photoperiodic information for floral transition is specified through the interaction of the circadian clock and

light. Both of these factors converge to regulate the expression and the activity of the CONSTANS (CO) transcription factor.⁸ CO activates *SUPPRESSOR OF CONSTANS OVEREXPRESSION1* (*SOC1*) and *APETALA1* (*API*) through *FLOWERING LOCUS T* (*FT*) to promote flowering. *FT* encoding a small protein that belongs to the phosphatidyl-ethanolamine binding protein (PEBP) family is expressed in the leaves and the polypeptide moves to the shoot apical meristem and forms a complex with a bZIP transcription factor, FD.^{9,10} Subsequently, the floral meristem identity genes such as *API*, *LEAFY* (*LFY*), *FRUITFULL* (*FUL*) and *CAULIFLOWER* (*CAL*) are induced in the floral meristems emerging on the flanks of the shoot apex.^{10,11}

A MADS-domain protein, *FLOWERING LOCUS C* (*FLC*) represses flowering by preventing the transcription of *FT* in leaves and *SOC1* and *FD* in the shoot apex. *FRIGIDA* (*FRI*) is a positive regulator of *FLC*. However, the mechanisms of cold-mediated repression including epigenetic modifications and antisense transcriptions of *FLC* are not yet fully understood.^{12,13} Other MADS proteins, *AGAMOUS-like19* (*AGL19*) and *AGL24*, closely related to *SOC1* and *SHORT VEGETATIVE PHASE* (*SVP*), respectively, have positive effects on flowering. Of note, their transcripts are accumulated during vernalization and this process is likely to be *FLC*-independent.^{14,15} Summer annual *Arabidopsis* can flower without vernalization but an active repression of flowering takes place at lower ambient temperatures. *FLOWERING LOCUS M* (*FLM*) and *SVP* are central to repression of flowering under the low ambient temperature. In particular, *FLM-β*, an alternatively spliced form of the flowering repressor *FLM*, interacts with *SVP* to respond to ambient temperature changes and *SVP*

delays flowering by repressing *FT* and *SOC1* transcription.¹⁶ Therefore, the decrease of SVP-FLM- β repressor complexes at higher temperatures leads to flowering.¹⁷ The distribution of flowering time genes in *Arabidopsis* is shown in Fig. 1A.

Floral transition in a short-day model plant, rice

As shown in Fig. 1B, in rice, Heading date 3a (Hd3a) and RICE FLOWERING LOCUS T1 (RFT1) are responsible for floral induction under short-day (SD) and long-day (LD) conditions, respectively.¹⁸ The basic leucine-zipper (bZIP) domain transcription factor, OsFD1 interacts with Hd3a through 14–3–3 proteins to form a florigen activation complex (FAC) acting on the

induction of *OsMADS15*, a rice *API* ortholog in the shoot apex during floral induction.^{19,20} *Early heading date 1* (*Ehd1*) acts as a flowering activator by upregulating *Hd3a* and *RFT1* and also controls the inflorescence architecture irrespective of photoperiod.²¹ Interestingly, *Heading date 1* (*Hd1*), a rice ortholog of *Arabidopsis* *CO* has a dual function: it acts as floral activator by activating *Hd3a* under inductive SD conditions but is converted to a repressor of *Hd3a* under long days (LDs).^{18,22} *Grain number, Plant height and Heading date 7* (*Ghd7*) and *Oryza sativa* *CONSTANS-LIKE4* (*OsCOL4*) are upstream suppressors of *Ehd1* whereas *Oryza sativa* *INDETERMINATE1* (*OsID1*)/*Ehd2*/Rice *INDETERMINATE1* (*RID1*) and *OsMADS50*, a homolog of *Arabidopsis* *SOC1* are activators of *Ehd1* (Fig. 1B).²³ Another MADS-box

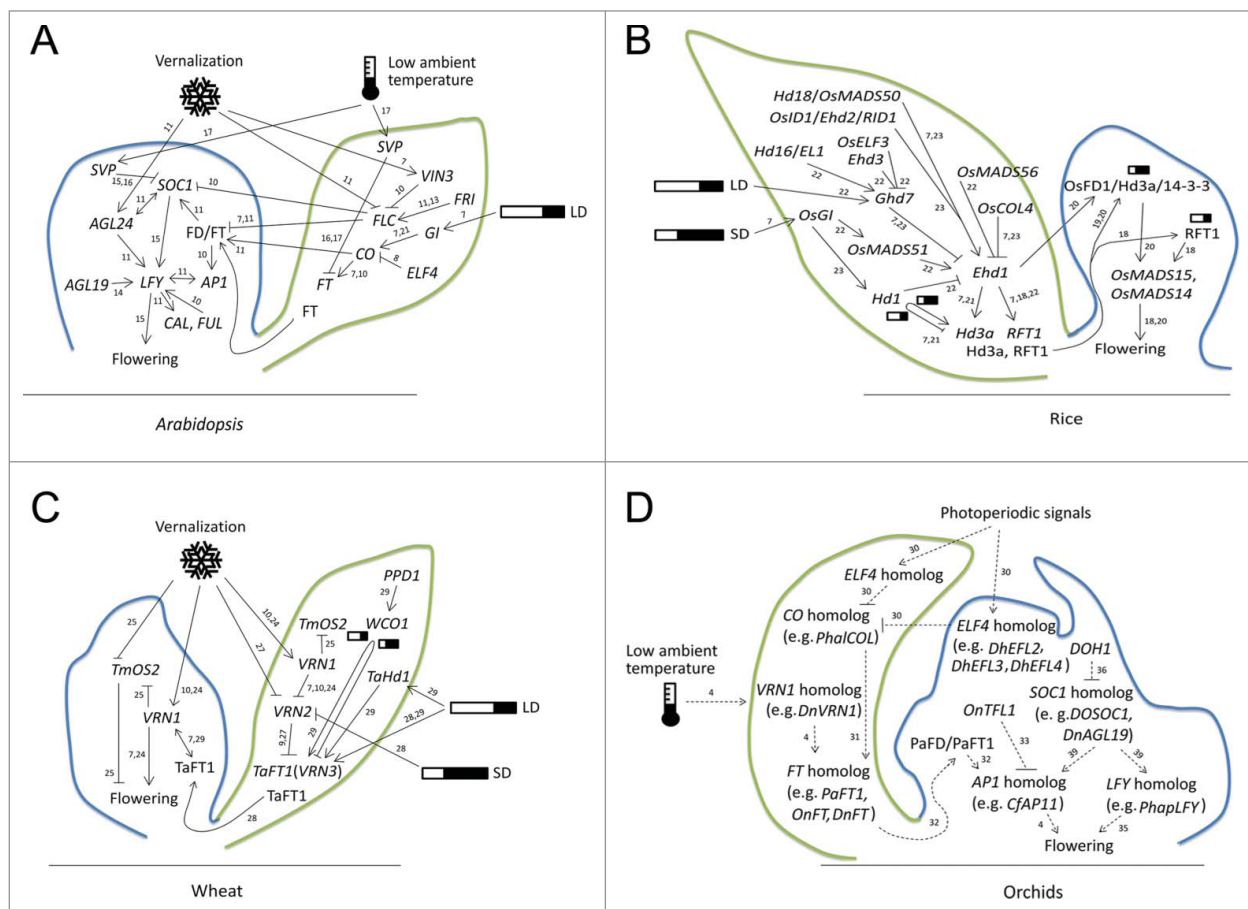


Figure 1. Major flowering genes in the photoperiodic and temperature network in *Arabidopsis*, rice, wheat and orchids. (A) Flowering of *Arabidopsis* is promoted by long days (LDs). The photoperiod signaling cascade involves *G1* and *CO* with modulation by *EARLY FLOWERING4* (*ELF4*). *ELF4* negatively regulates *CO* expression by sequestering *G1* from the *CO* promoter. *CO* positively regulates expression of floral integrator *FT* that mediates *SOC1* activation. The vernalization response in *Arabidopsis* through *VERNALIZATION INSENSITIVE3* (*VIN3*) family proteins represses *FLC* gene family members, which represses *FT* and *SOC1*. In addition, *AGL24* participates in the *FLC*-independent vernalization response to regulate *LFY* expression. The ambient temperature affects flowering time in *Arabidopsis* through SVP-FLM- β repressor complexes that delay flowering by repressing *FT* and *SOC1* transcription at low ambient temperature. (B) In rice, a short day plant, the *OsG1*-*Hd1* (*CO* in *Arabidopsis*)-*Hd3a* (*FT* in *Arabidopsis*) cascade is well conserved. *Hd1* and *Ehd1* positively regulate *Hd3a* and promote flowering under inductive short days (SDs). However, under LDs, *Ghd7* is a major flowering suppressor that represses *Ehd1* and *Hd1* also represses *Hd3a* expression. Instead, *RFT1* is activated through *OsMADS50* and *Ehd1* for eventual flowering under LDs. *OsMADS50* and *OsMADS56* function antagonistically to affect flowering under LDs by controlling expression of *Ehd1*. Rice *API* homologs such as *OsMADS14* and *OsMADS15* are activated by florigens (e.g., *Hd3a* and *RFT1*) in the SAM. (C) In wheat, the *TaFT1/VRN3* gene integrates photoperiod (via *PPD1*-*WCO1*) and vernalization (via *VRN1*-*VRN2*). The photoperiodic signals involving *PPD1* are transmitted to negative regulator *WCO1* and positive regulator *TaHd1* to control *TaFT1/VRN3* expression under SD. *VRN2* prevents flowering before vernalization but later, vernalization induces *VRN1*, which is followed by the downregulation of *VRN2*, thereby releasing *TaFT1/VRN3* which leads to flowering. *ODDSOC2* (*TmOS2/TaAGL33*) functions as a vernalization-regulated flowering repressor. It is downregulated by cold independently of *VRN1*. (D) In orchids, *Phalaenopsis* *PhaCOL* and *PaFT1* were regulated by photoperiod and low ambient temperature, respectively. *Doritaenopsis* *DhEFL2*, 3 and 4, as floral repressors, coordinate floral transition in the photoperiodic flowering pathway. *DnVRN1* and *DnFT* from *Dendrobium nobile* are linked to low temperature (10°C)-induced floral transition. *Dendrobium* Chao Praya Smile *DOSOC1* is expressed specifically in floral meristems during the transition from the vegetative to the reproductive phase and coupled with the upregulation of its *LFY*. *DnAGL19* expression of *D. nobile* repressed by polycomb-group complexes is activated after vernalization reminiscent of *Arabidopsis* *AGL19* pathway to activate *LFY* and *AP1* for flowering. Arrows and T-shaped bars are regulatory links for the promotion and repression of gene transcription, respectively. The numbers next to the arrows and T-shaped bars are references to related studies and the dashed lines indicate predictions of the flowering regulation networks. Prediction of orchids flowering networks should be confirmed by more extensive studies.

gene, *OsMADS51* functioning downstream of *Oryza sativa* *GIGANTEA* (*OsGI*) under short days (SDs) promotes flowering by inducing *Ehd1* expression. Moreover, *Hd16/EARLY FLOWERING1* (*EL1*) acts as a flowering repressor by phosphorylating *Ghd7* resulting in the reduction of *Ehd1* expression. Rice *Ehd3* and *Early flowering3* (*ELF3*) were also identified as repressors of *Ghd7*.²²

Floral transition in a seasonal flowering plant, wheat

In wheat, MADS-domain transcription factor VERNALIZATION1 (*VRN1*), a wheat ortholog of *Arabidopsis* *API*, is a central regulator of vernalization-induced flowering.²⁴ The grass-specific MADS-box gene *ODDSOC2* (*TmOS2/TaAGL33*) and its splice variant *TaAGL22*, the *FLC* orthologs, are downregulated by cold independently of *VRN1*, but *VRN1* represses *ODDSOC2* during the developmental stage under LDs and normal temperature.^{25,26} The *VRN2* containing a zinc-finger motif and a CCT (CONSTANS, CO-like, TOC1) - domain is a flowering repressor downregulated by both vernalization and SDs.²⁷ Cold signals coordinately activate *VRN1* and repress *VRN2* expression during vernalization. Also, *VRN1* downregulates *VRN2* to control the activity of the photoperiodic flowering pathway (Fig. 1C). *VRN2* represses flowering under SDs, and confers the downregulation of *VRN2* and the upregulation of *VRN1* and *VRN3* after the LDs of spring. *VRN3*, an ortholog of *Arabidopsis* *FT*, is a flowering activator and also encodes a mobile protein that is transported from the leaves to the shoot apical meristem (SAM).²⁸ Pseudo-response regulator (PRR) family *PHOTOPERIOD1* (*PPD1*) encodes a CCT-domain protein domain that influences day length sensitivity by altering expression of CO-like genes: *Wheat CO* (*WCO1*) negatively regulates and *Triticum aestivum* *HEADING DATE 1* (*TaHd1*) positively regulates *VRN3* expression associated with late flowering under SD conditions.²⁹

Genes that control flowering in orchids

Not so many functional studies of orchid flowering/flower developmental genes have been reported. Recently, it was reported that expression of *Doritaenopsis* hybrid *EARLY FLOWERING4-like4* (*DhEFL4*) is regulated by photoperiod and overexpression in *Arabidopsis* delays flowering.³⁰ Ectopic expression of *Phalaenopsis* CO-like (*PhalCOL*) encoding a protein with 2 B-box zinc finger motifs and a CCT domain in *Phalaenopsis hybrida* (cv. Wedding Promenade) caused an early-flowering phenotype in tobacco³¹ and *Phalaenopsis aphrodite* *FLOWERING LOCUS T1* (*PaFT1*) that is upregulated under inductive low ambient temperature but is not subject to photoperiodic control showed precocious flowering in *Arabidopsis* and rice when it was ectopically expressed.³² Moreover, phloem-specific expression of *PaFT1* in *Arabidopsis* suppresses the late flowering effect by an active *FRI* allele and *SVP* overexpression. *SVP* forms a complex with *FLM*, a floral repressor of *FT*, by ambient temperature in *Arabidopsis*.¹⁷ A bZIP domain transcription factor *PaFD* has been isolated as a *PaFT1*-interacting protein and was able to partially complement the late flowering phenotype of *Arabidopsis fd-3*.³² *Oncidium* Gower Ramsey *FLOWERING LOCUS T* (*OnFT*) and *TERMINAL*

FLOWER 1 (*OnTFL1*) encoding floral activator *FT* and repressor *TERMINAL FLOWER1* (*TFL1*) homologs, respectively were shown to play opposite roles in *Arabidopsis* flowering.³³ Recently, *Dendrobium nobile* *FLOWERING LOCUS T* (*DnFT*) and *MOTHER OF FT* (*DnMFT*) expressed preferentially in the auxiliary buds and leaves have been reported. Of note, the expression of the 2 genes in the leaves responded to temperature in an opposite manner. The low temperature (10°C) required for flowering of *D. nobile* Lindl: increased *DnFT* expression but decreased *DnMFT* expression.³⁴

The transcript of floral meristem identity gene *Phalaenopsis aphrodite* *LEAFY* (*PhapLFY*) is accumulated in the floral meristem primordia to stimulate flower initiation. It is also believed that *PhapLFY* acts in the early stages of floral organ development.³⁵ Expression of *DENDROBIUM ORCHID HOMEBOX1* (*DOH1*) from *D. Madame Thong-In* is detected in leaf primordia and downregulated in the shoot apex during floral transition as a possible upstream regulator of *DOMADS1* expressed in the transitional shoot apical meristem, advancing the floral transition and flower development (Fig. 1D).³⁶ No homologs of *FLC* have been reported but homologs of *Arabidopsis* *AGL19* were identified in *D. nobile*. In addition, expression of *Oncidium* *MADS1* (*OMADS1*) belonging to the *API/AGL9* group of MADS-box genes is detected in the apical meristem and in the lip and carpel of flowers and *OMADS1* also interacts with *OMADS3* associated with floral initiation of *Oncidium* Gower Ramsey.³⁷ Table 1 provides a summary of the current understanding of orchid flowering genes which are either functional orthologs /homologs of *Arabidopsis*, rice and wheat genes.

Orchid breeding strategies

Generally, orchids grow slowly and have long life cycles. Consequently, it requires a long period of time to generate new cultivars using the traditional breeding process. Transformation technology has been developed for orchids and recently, a few successful methods^{38,39} that use virus-induced gene-silencing (VIGS) approaches have been demonstrated as efficient strategies for functional studies of genes in orchids.⁴⁰ Thus, we may now speed up the orchid breeding process by suppressing the expression of floral repressors resulting in early flowering. Also, virus-induced flowering (VIF) approaches to accelerate the transition to reproductive growth may facilitate research and/or breeding in orchids.⁴¹ For example, either gain of function of *FT* or loss of function of *TFL1* using transient methods such as VIGS/VIF may contribute to a faster orchid breeding process. Using inducible gene expression systems, we may expect to control flowering of orchids with transgenic lines regardless of the ambient environment.³² Specific alleles can be selected for breeding and/or modified by induced mutation (genome editing) to acquire orchid cultivars containing desirable traits when current transformation procedures are stabilized.¹¹ In our recent reports, we have shown that the VIGS of *PaFT1* exhibits delayed spiking of *P. aphrodite* subsp. *formosana* and the VIGS-*PhapLFY* flowers displayed increased chlorophyll content with the aberrant epidermal cell shape.^{32,35} Furthermore, we

Table 1. Key flowering genes in orchids.

Orchids	Gene Name	Accession No.	Homolog ^a	Refs ^b
<i>Phalaenopsis</i>	<i>PaFT1</i>	KJ609179	The PaFT1 protein showed 70%, 76% and 89% identity to Arabidopsis FT (BAA77838), rice Hd3a (BAB61030), and <i>Oncidium</i> orchid OnFT (ACC59806), respectively.	32
<i>Oncidium</i>	<i>OnFT</i>	KJ909968	<i>OnFT</i> cDNA encodes a 176 amino acid protein that shows 70% and 79% identity to Arabidopsis FT (AT1G65480) and rice Hd3a (Os06g0157700), respectively.	33
<i>Cymbidium</i>	<i>CgFT</i>	HM120863	The CgFT protein was 94% with OnFT (EU583502) from <i>Oncidium</i> Gower Ramsey, 79% with Hd3a (BAB61028.1) from <i>Oryza sativa</i> , and 74% with FT (BAA77838.1) from <i>Arabidopsis thaliana</i> .	42
<i>Cypripedium</i>	<i>CfFT</i>	CFTC014733	The partial CfFT align 155 amino acids and showed 82.58% identity to wheat TaFT (ABK32205).	Orch
<i>Phalaenopsis</i>	<i>PaFD</i>	KJ609180	PaFD protein shows 34.7% identity to Arabidopsis FD (AT4G35900).	32
<i>Dendrobium</i>	<i>DnFD</i>	DNTC011756	The partial DnFD align 169 amino acids and showed 65.1% and 32.2% identity to Phalaenopsis FD (KJ609180) and Arabidopsis FD (AT4G35900), respectively.	Vect
<i>Phalaenopsis</i>	<i>PhalCOL</i>	FJ469986	The PhalCOL protein showed 46% and 45% identity to Arabidopsis COL4 (Q940T9.2) and rice Hd1 (ABB17664), respectively.	31
<i>Cymbidium</i>	<i>CeCOL</i>	CETC010739	The CeCOL protein showed 84.5%, 34.9%, and 36.3% identity to Phalaenopsis COL (FJ469986), Arabidopsis CO (AT5G15840) and rice Hd1 (Os06g0275000), respectively.	Vect
<i>Cypripedium</i>	<i>CfFLC</i>	CFTC002458	The partial CfFLC align 145 amino acids and showed 45.5% identity to Arabidopsis FLC (AT5G10140) and 41.2% with wheat TaAGL33 (ABF57950).	Orch Vect
<i>Oncidium</i>	<i>OgFLC</i>	OGTC046040	The partial OgFLC align 145 amino acids and showed 44.8% identity to Arabidopsis FLC (AT5G10140) and 38.6% with wheat TaAGL33 (ABF57950)..	Orch Vect
<i>Cypripedium</i>	<i>CfVRN2</i>	CFTC009002	The partial CfVRN2 align 106 amino acids and showed 50% identity to wheat VRN2 (AAS58481).	Orch
<i>Phalaenopsis</i>	<i>PmVRN2</i>	PMTC002168	The partial PmVRN2 align 142 amino acids and showed 41.55% identity to wheat VRN2 (AAS58481).	Orch
<i>Dendrobium</i>	<i>DOSOC1</i>	KC121576	DOSOC1 shared 52% sequence identity with Arabidopsis SOC1 (AT2G45660), and 57% identity to <i>Oryza sativa</i> OsSOC1 (Os03g0122600).	39
<i>Phalaenopsis</i>	<i>PISOC1</i>	PLTC039163	PISOC1 shared 87.9%, 48.4%, and 54.3% identity to Dendrobium (KC121576), Arabidopsis (AT2G45660) and rice (Os03g0122600), respectively.	Vect
<i>Dendrobium</i>	<i>DnAGL19</i>	KU373056	DnAGL19 protein shows 48% identity to Arabidopsis AGL19 (AT4G22950).	4
<i>Oncidium</i>	<i>OnTFL1</i>	KM233713	OnTFL1 encodes a 173 amino acid protein that shows 71% and 79% identity to Arabidopsis TFL1 (AT5G03840) and rice OsFDR1 (AF159883), respectively.	33
<i>Vanilloideae</i>	<i>VpTFL1</i>	VPTC023176	The partial VpTFL1 align 160 amino acids and showed 81.8%, 69.9%, and 83.6% identity to <i>Oncidium</i> TFL1 (KM233713), Arabidopsis TFL1 (AT5G03840) and rice FDR1 (AF159883), respectively.	Vect
<i>Cymbidium</i>	<i>CfAPI1</i>	JQ031272.1	CfAPI1 shared 45% sequence identity with Arabidopsis AP1 (AT1G69120), and 52% identity to <i>Oryza sativa</i> OsMADS14 (Os03g0752800).	43
<i>Phalaenopsis</i>	<i>PsAP1</i>	PSTC034274	The PsAP1 protein showed 55.8%, 47.6% and 58.3% identity to <i>Cymbidium</i> AP11 (JQ031272.1), Arabidopsis AP1 (AT1G69120) and rice MADS14 (Os03g0752800), respectively.	Vect
<i>Doritaenopsis</i>	<i>DnVRN1</i>	DNTC013820	The partial DnVRN1 align 251 amino acids and showed 61.75% identity to wheat VRN1 (AAZ76881).	Orch
<i>Phalaenopsis</i>	<i>PaVRN1</i>	PATC201550	The partial PaVRN1 align 250 amino acids and showed 60.4% identity to wheat VRN1 (AAZ76881).	Orch
<i>Cymbidium</i>	<i>ChLFY</i>	AGE45851	The ChLFY protein showed 51% and 48% identity to Arabidopsis LFY (AT5G61850) and rice RFL (Os04g0598300), respectively.	Vect
<i>Phalaenopsis</i>	<i>PhalLFY</i>	FJ469985	PhalLFY protein has 60% identity to rice RFL (BAA21547), 54% identity to Arabidopsis LFY (NP_200993), and a high identity with LFY (68% and 67%) from <i>Anacamptis</i> (BAC55081) and <i>Orchis</i> (BAC54955), respectively.	44
<i>Phalaenopsis</i>	<i>PhapLFY</i>	KP893636	PhapLFY protein has 78.5% identity to ChLFY (AGE45851), a <i>Cymbidium</i> orchid LFY homolog, 53.2% identity to RFL (BAA21547), a rice LFY homolog, and 47.0% identity to Arabidopsis LFY (AAM27941).	35
<i>Doritaenopsis</i>	<i>DhEFL2</i>	KP728997	DhEFL homologs showed that DhEFL4 (KP010003) and DhEFL2 (KP728997) are similar with 72% identical amino acids, whereas DhEFL3 (KP728998) is divergent with 72% similarity with DhEFL2 and 68% similarity with DhEFL4.	45
<i>Doritaenopsis</i>	<i>DhEFL3</i>	KP728998	The DhEFL3 protein showed 31.4% identity to Arabidopsis ELF4 (AT2G40080).	Vect
<i>Doritaenopsis</i>	<i>DhEFL4</i>	KP010003	The DhEFL4 protein showed 37% identity to Arabidopsis ELF4(AT2G40080).	Vect
<i>Phalaenopsis</i>	<i>PsEhd1</i>	PSTC018079	The partial PsEhd1 align 266 amino acids and showed 40% identity to rice Ehd1 (Os10g0463400).	Orch
<i>Cymbidium</i>	<i>CsEhd1</i>	CSTC008880	The partial CsEhd1 align 263 amino acids and showed 49.5% identity to rice Ehd1 (Os10g0463400).	Orch

^aThe homology is based on each reference and Orchidstra 2.0, a transcriptome database of orchid species (http://orchidstra2.abrc.sinica.edu.tw/orchidstra2/orchid_blast.php). Amino acid sequences of flowering in *Arabidopsis*, rice and wheat were obtained from GenBank (<http://www.ncbi.nlm.nih.gov/>) and Orchidstra 2.0.

^bOrch, homology gained through Orchidstra 2.0; Vect, homology gained by analysis using Vector NTI program.

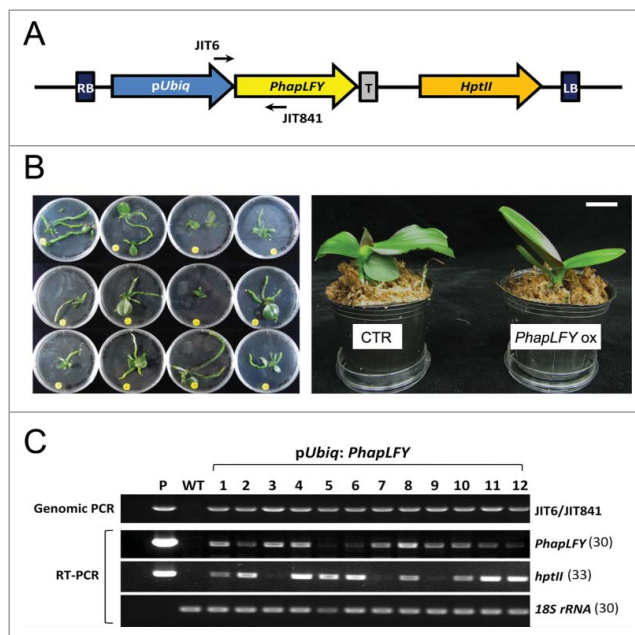


Figure 2. Overexpression of *PhapLFY* in *Phalaenopsis*. (A) Plasmid construction for *PhapLFY* overexpression in *Phalaenopsis* orchids. *pUbiq* means maize ubiquitin promoter and T indicates *nos* terminator. RB and LB indicate right and left T-DNA borders. The *hygromycin phosphotransferase II (hptII)* expressing cassette was used for hygromycin resistance in selection of transgenic orchids. (B) Transgenic *Phalaenopsis* orchids overexpressing *PhapLFY* gene. Each transgenic orchid seedling used for analyses in C (left) and young orchid plantlets. Bar = 2cm. (C) Verification of transgenic *Phalaenopsis* orchids by genomic PCR and RT-PCR. Expression of *PhapLFY* and hygromycin resistance gene (*hptII*) was examined by RT-PCR together with *18s rRNA* in leaves of each plant. The location of primers used for genomic PCR is parked in (A). P indicates plasmid DNA used for transformation as a positive control for PCR and WT indicates a non-transgenic *Phalaenopsis* orchid in the same developmental stage with transgenic orchids. JIT6: 5'TTGTCGATGCTCACCTG3', JIT841: 5'CTAGCCGCTCCTCTGTCTCCGAC3', *PhapLFY*-F: 5'GAGGAGGAGGTGACGATATGATG3', *PhapLFY*-R: 5'GCTGTATTATGTAGCTTGCCTAC3', *hptII*-F: 5'GATTCGGAAAGTCTTGACATG3', *hptII*-R: 5'GCATCAGCTCATCGAGAGCTG3', *18S rRNA*-F: 5'TTAGGCACGGAAGTTTGAGG3', *18S rRNA*-R: 5'ACACTTCACCGACCATTCAA3'.

successfully produced transgenic *Phalaenopsis* for *PhapLFY* overexpression (Fig. 2).

Conclusion and future directions

The *Orchidaceae* is the largest and the most diverse family of flowering plants and most orchids have their own flowering seasons: *D. nobile* requires vernalization for flowering whereas *D. phalaenopsis* flowers at high temperatures. Spiking of *P. aphrodite* subsp *formosana* is significantly inhibited under warm conditions while the natural flowering period of *P. sanderiana* is through the summer in the Philippines. The molecular mechanisms underlying flowering among various orchid species largely remain elusive. Research on flowering to induce early flowering in orchids has been aimed not only at a better understanding molecular and genetic mechanisms of orchid flowering but also at assisting orchid breeding programs.¹ In vitro flowering or floral transition in orchids is affected by various plant growth regulators (PGRs) although the molecular working mechanisms of those PGRs underlying orchid floral induction are unclear. Molecular genetic approaches are required to shed some light on the roles of putative key flowering genes of orchids and will also provide a platform for application of genetic resources to the orchid industry.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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