Scientia Horticulturae 175 (2014) 74–86

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Review

Genetic control of flower development, color and senescence of *Dendrobium* orchids

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ARTICLE INFO

Article history:
Received 22 December 2013
Received in revised form 10 March 2014
Accepted 14 May 2014

Keywords:
Dendrobium
Floral transition
Floral traits
MADS-box genes
'Orchid code'

ABSTRACT

For ornamental plants, in particular orchids, the flower is central to their beauty and commercial value. The ability to manipulate the floral transition from the vegetative to the reproductive phase and floral traits requires an understanding of the underlying molecular genetic mechanisms and robust transgenic protocols. Using *Dendrobium* species and hybrids, this review explores the advances that have been made in the genetics of flower development, color and senescence. Although the homologs of several MADS-box genes are still to be found, those that have already been cloned and analyzed bring promise to what has yet to be unraveled about the control of flower color and development. Recent advances in orchid transformation and the introduction of *Dendrobium* homologs into *Arabidopsis thaliana* have shed new light on the complexities of flower color and the ABCDE model of flower development in orchids, or the 'orchid code'.

Contents

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http://dx.doi.org/10.1016/j.scienta.2014.05.008
0304-4238/

1. The importance of *Dendrobium* in the context of the Orchidaceae

The Orchidaceae is one of the largest families of angiosperms and is distributed throughout the world. Orchid flowers are very attractive because of the great diversity in flower color and morphology. The perianth of orchids consists of two whorls of petaloid organs termed tepals. While the organs in the outer tepal whorl are usually referred to as sepals (see Teixeira da Silva et al., 2014b), they are petaloid in many species. The inner tepal whorl usually consists of two lateral petals and a median labellum or lip. The lip is usually a large and colorful organ used as an insect trap for pollination. Male and female reproductive organs, which are inner whorls of floral organs, are fused into a single unit called the column. The name "*Dendrobium*" is from the Greek *dendron* ("tree") and *bios* ("life"), it means "one who lives on trees", or, essentially, "epiphyte". This genus of epiphytic, or occasionally lithophytic orchids have adapted to a wide variety of habitats that develop pseudobulbs and axillary inflorescences, which vary in length from insignificant to 1 m long, and can carry from a few (1–4) (*e.g. Dendrobium nobile*) to as many as 100 (*e.g. Dendrobium speciosum*) flowers. Deciduous species carry their leaves for one to two years then typically flower on leafless canes, while canes of evergreen species usually flower in the second year and can continue to flower for a number of years (*e.g. Dendrobium densiflorum*) (Lavarack et al., 2000). The flowers of *Dendrobium* last from a single day (*Dendrobium crumenatum*) (Yap et al., 2008) to 10 months (*Dendrobium cuthbertsonii*) (Cribb et al., 1985), and mostly for several weeks.

Dendrobium is one of the largest genera of the Orchidaceae and comprises more than 1200 species (Adams, 2011), many of which are important commercial orchids, commanding second place among potted flowering plants in the USA and accounting for around 20% of the total orchid sales (USDA, 2012). Besides some *Dendrobium* species which are grown as medical plants (Ng et al., 2012), commercially planted *Dendrobium* falls in two categories, *nobile*-type and *phalaenopsis*-type, in horticulture, and usually they are sold as potted and cut flowers respectively (Baker and Baker, 1996). The great demand by *Dendrobium* lovers has resulted in numerous varieties and hybrids, which have greatly extended colors and flowering period (Lavarack et al., 2000). Members of this genus have a wide geographic range (Pridgeon and Morrison, 2006). Novel colors and plant, leaf or floral architecture, or additional value-added traits such as disease resistance, or modified floral fragrance, would be desired for establishing new markets. *Dendrobium* cut flowers have a long flowering life, year-round availability, and a well established *in vitro* regeneration system (Teixeira da Silva, unpublished), including *in vitro* flowering (Teixeira da Silva et al., 2014a), making this genus a candidate model orchid for development, cryopreservation (Teixeira da Silva et al., 2014c) and molecular studies.

The ability to control flowering time and flower color would give *Dendrobium* breeders a greater ability to expand their germplasm repertoire (Chai and Yu, 2007). For example, in *Arabidopsis thaliana*, *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*) encodes a MADS (*MCMI* of *Saccharomyces cerevisiae*, *AG* of *A. thaliana*, *DEF* of *Antirrhinum majus* and *SRF* of *Homo sapiens*)-box protein (Sommer et al., 1990; Moon et al., 2003), which is essential in integrating multiple flowering signals to regulate the transition from vegetative to reproductive development (Moon et al., 2003; Lee and Lee, 2010). Ding et al. (2013) isolated a *SOC1*-like gene, *DOSOC1*, from *D.* 'Chao Praya Smile'. This gene was strongly expressed in reproductive organs, including inflorescence apices, pedicels, floral buds and open flowers, and its expression increased significantly in whole plantlets during the transition from vegetative to reproductive development. Transgenic *Dendrobium* orchid lines overexpressing *DOSOC1* consistently exhibited earlier flowering than wild-type (WT) orchids.

This review aims to bring integrated insight of the published literature into what is known about the genetics of *Dendrobium* flower color, flowering, including senescence, and flower development. Only a handful of papers have been published in the English literature on flower scent in *Dendrobium* and the topic of *Dendrobium* biotechnology, including tissue culture, micropropagation and *in vitro* flowering, is covered in detail in a separate review (Teixeira da Silva et al. unpublished). *In vitro* flowering of *Dendrobium*, for example, as has been achieved for *D.* 'Chao Praya Smile' (Hee et al., 2007) or for *D.* 'Madame Thong-In' (Sim et al., 2007), would allow for the standardized production of flowers *in vitro*. This technology would permit the mechanisms of flower color, flowering and flower development to be studied in a shorter period of time, which might be applicable to many orchids (Teixeira da Silva et al., 2014a).

2. The genetics of flower induction and development in *Dendrobium*

In flowering plants, including orchids, the floral transition from vegetative to reproductive growth takes places in response to endogenous signals or exogenous environmental cues, such as hormones, photoperiod or temperature (Boss et al., 2004; Hou and Yang, 2009).

Floral initiation is induced by a period of moderately low temperatures (vernalization) in Nobile-type and a few other *Dendrobium* cultivars, while in some others, it is regulated by photoperiod (Rotor, 1952, 1959). Homologs of the important *Arabidopsis* genes involved in the photoperiodic pathway were identified from *Oncidium* (Chang et al., 2011), *Cymbidium* (Zhang et al., 2013), *Erycina* (Chou et al., 2013) and *Phalaenopsis* (Zhang et al., 2011), but not from *Dendrobium*. Using an *in vitro* flowering system of *D.* 'Madam Thong-in', Yu and Goh (2000b) identified 53 cDNA clones differentially expressed in vegetative shoot apical meristems (VSAM) and 16 cDNA fragments specifically expressed in transitional shoot apical meristems (TSAM). A MADS-box gene (*otg7*) was further characterized, and found to be expressed early in bract primordia and also highly in mature flowers. Using *otg7* as a probe, they isolated three MADS-box genes of the AP1/AGL9 subfamily (*DOMADS1-3*) from the TSAM cDNA library. Further expression studies indicated that these genes might play important roles during the floral transition (Yu and Goh, 2000a). Besides, the only reported orchid *KNOX*-like gene, *DOH1*, was isolated from *D.* 'Madam Thong-in' (Yu and Goh, 2000a). They found that *DOH1* was required for the floral transition and was a possible upstream regulator of *DOMADS1* (Yu et al., 2000).

Three additional *AP1*/*FUL*-like MADS-box genes isolated from *Dendrobium thyrsiflorum* (Reichb. f.) might play a role in floral development after the floral transition (Skipper et al., 2005). Ding et al. (2013) isolated an orchid homolog (*DOSOC1*) of *SOC1*, a floral pathway integrator in *Arabidopsis*, from *D.* 'Chao Praya Smile'. Overexpression of *DOSOC1* in wild-type *Arabidopsis* plants resulted in early flowering, which was coupled with upregulation of two other flowering promoters, *AGAMOUS-LIKE 24* and *LEAFY*.

With respect to *Dendrobium* orchids that flower in response to vernalization, just as reported in other monocots, there are no *Dendrobium* analogs/homologs of *FLC*, which regulates the vernalization pathway in *Arabidopsis*. But the homologs of cereal *VRN1* (*AP1*/*FUL*-like gene) and *VRN3* (*FT*-like gene), and *Arabidopsis AGL19* were identified from *D. nobile* (Liang et al., 2012). The expression of all the three genes in auxiliary buds was regulated by vernalization. A *SEP*-like gene (*DnSEP3-like*) was also cloned and characterized in *D. nobile*, and its expression was upregulated as the duration of

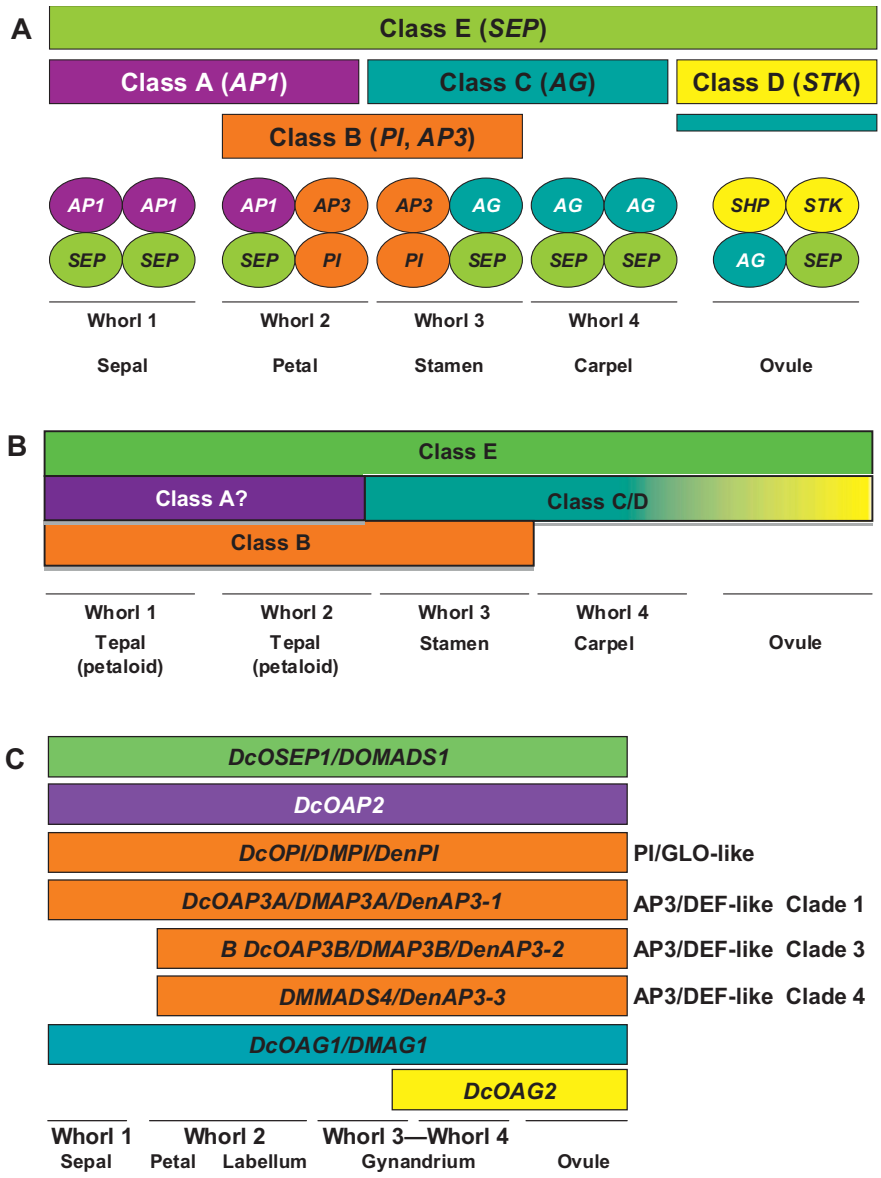

Fig. 1. Simplified ABCDE models for flower development in *Arabidopsis* (A), *Tulipa* (B) and *Dendrobium* orchids (C). The modified ABCDE model for *Dendrobium* (C) is incorporated with the "orchid code", which explains the unique floral structures found in orchids. The scheme is based on, but modified from, Aceto and Gaudio (2011). *AG*, *AGAMOUS*; *AP1*, *APETALA1*; *AP3*, *APETALA3*; *PI*, *PISTILLATA*; *SEP*, *SEPALLATA*; *SHP*, *SHATTERPROOF*; *STK*, *SEEDSTICK*.

vernalization increased (Chen et al., 2013). Similarly, the expression of an *FT* homolog (*DnFT*) in *D. nobile* was also affected by vernalization (Li et al., 2012), and *35S:DnFT* transgenic *Arabidopsis* flowered earlier than wild-type plants. These results imply that the regulatory networks governing the floral transition are partly conserved in *Dendrobium*, temperate cereals, and *Arabidopsis*.

Orchid floral morphology, structure, and physiological properties are fascinating. Reproductive development in orchid flowering shoots occurs through the transition of dormant meristems from producing vegetative structures to producing inflorescence branches, floral bracts, and finally flowers (Hsiao et al., 2011). Orchids possess several reliable floral morphological synapomorphies, including the presence of a gynostemium, also called column, fused by the style and at least part of the androecium, and a highly evolved petal called the lip. Since all expected whorls in flowers are present in orchids, such a highly sophisticated flower organization offers an opportunity to discover new variant genes and different levels of complexity within morphogenetic networks. Therefore, the Orchidaceae can be used to validate the ABCDE model of flower development, derived from the ABC model (Coen and Meyerowitz, 1991) in monocots and study how MADS-box genes, which are a major group of regulators that contribute to flower development, are involved in defining the different, highly specialized structures in orchid flowers (Krizek and Fletcher, 2005; Tsai et al., 2008; Fernando, 2010; Aceto and Gaudio, 2011). According to the ABCDE model, the organ identity in each whorl is determined by a unique combination of the activities conferred by five classes of floral organ identity genes (FOIGs), A, B, C, D and E (Fig. 1A). Generally, the expression of class A genes alone specifies sepal formation in the first whorl of a flower. Class A and B genes specify petal development in the second whorl, while the combination of class B and C genes controls stamen formation in the third whorl. The expression of the class C genes alone determines the carpel development in the fourth whorl. The functions of class A and C genes are mutually repressive or antagonistic, while class D genes specify ovule development (Pinyopich et al., 2003; Teixeira da Silva and Nhut, 2003).

Table 1
List of floral organ identity genes (FOIGs) isolated from genus *Dendrobium* (modified from Tsai and Chen, 2006, Aceto and Gaudio, 2011, and Abdullakasim and Handa, 2012).

Class** (in ABCDE model)	Subfamily	Gene name	*Dendrobium* species	GenBank AN	Reference
A	*AP1/SQUA*-like	*DOMADS2*	*D.* Madame Thong-In	AF198175	Yu and Goh (2000a)
		DthyrFL1	*D. thyrsiflorum*	AY927236	Skipper et al. (2005)
		DthyrFL2	*D. thyrsiflorum*	AY927237	Skipper et al. (2005)
		DthyrFL3	*D. thyrsiflorum*	AY927238	Skipper et al. (2005)
	AP2-like	*DcOAP2*	*D. crumenatum*	DQ119837	Xu et al. (2006)
B	*AP3/DEF*-like	*DcOAP3A*	*D. crumenatum*	DQ119838	Xu et al. (2006)
		DcOAP3B	*D. crumenatum*	DQ119839	Xu et al. (2006)
		DMAP3A	*D. moniliforme*	EU056327	Sirisawat et al. (2010)
		DMAP3B	*D. moniliforme*	EU056328	Sirisawat et al. (2010)
		DMMADS4	*D. moniliforme*	GU132995	Sirisawat et al. (2009)
		DdAP3	*D. devonianum*	GU126414	Chen et al. (2010)
		DenAP3-1	*D.* Spring Jewel	EU444025	Pan et al. (2011)
		DenAP3-2	*D.* Spring Jewel	EU444026	Pan et al. (2011)
		DenAP3-3	*D.* Spring Jewel	EU444027	Pan et al. (2011)
	PI/GLO-like	*DenPI*	*D.* Spring Jewel	EU444028	Pan et al. (2011)
		DcOPI	*D. crumenatum*	Not available	Xu et al. (2006)
		DMPI	*D. moniliforme*	EU056326	Sirisawat et al. (2010)
C	*AG*-like	*DthyrAG1*	*D. thyrsiflorum*	DQ017702	Skipper et al. (2006)
		DcOAG1	*D. crumenatum*	DQ119840	Xu et al. (2006)
D	*STK*-like	*DthyrAG2*	*D. thyrsiflorum*	DQ017703	Skipper et al. (2006)
		DcOAG2	*D. crumenatum*	DQ119841	Xu et al. (2006)
		DNMADS2	*D. nobile*	EF535599	Wen et al. (unpublished)
E	*SEP*-like	*DcOSEP1*	*D. crumenatum*	DQ119842	Xu et al. (2006)
		DOMADS1	*D.* Madame Thong-In	AF198174	Yu and Goh (2000a)
		DOMADS3	*D.* Madame Thong-In	AF198176	Yu and Goh (2000a)
		DnSEP3	*D. nobile*	HQ388352	Chen et al. (2013)

AN, GenBank accession number.
AG, *AGAMOUS*; *AP*, *APETALA*; *DEF*, *DEFICIENS*; *FUL*, *FRUITFUL*; *GLO*, *GLOBOSA*; *PI*, *PISTILLATA*; *SEP*, *SEPALLATA*; *STK*, *SEEDSTICK*.

Class E genes control development of all floral organs, including sepals, petals, stamens, or carpels (Ditta et al., 2004) (Fig. 1A). The identity of perianth organs in orchids is regulated by two lineages of class B genes, *AP3*/*DEF*-like and *PI*/*GLO*-like genes, and class E genes (Xu et al., 2006). A deeper functional and evolutionary interpretation of the genes in the orchid ABCDE model is provided by Aceto and Gaudio (2011). So far at least 25 FOIGs have been isolated from several *Dendrobium* species (Table 1; Fig. 2), including *D.* 'Madame Thong-In', *D. thyrsiflorum* (Reichb. f.), *D. crumenatum* and *D. moniliforme*, *D.* 'Spring Jewel' and *D. nobile* (Yu and Goh, 2000a, 2000b; Skipper et al., 2005, 2006; Xu et al., 2006; Sirisawat et al., 2009, 2010; Pan et al., 2011; Xu et al., 2010; Chen et al., 2013; Wen et al. unpublished). These genes are discussed in the following sections.

2.1. *Dendrobium* MADS-box genes of the AP1/AGL9 group

The class A FOIGs are necessary for the proper development of sepals and petals normally found in the first and second whorls of a flower. Class A and C activities are mutually antagonistic. MADS-box genes *DOMADS1*, *DOMADS2* and *DOMADS3* from *D.* 'Madame Thong-In', which are homologous to *SEP1*, *AP1*/*SQUA* and *SEP3*, respectively, were successively activated during the floral transition and their expression was persistent even in various floral organs (Yu and Goh, 2000a, 2000b; Yu et al., 2002). The *SEP*-like subfamily (*SEPALLATA* genes in *A. thaliana*) is divided into *SEP3* and *SEP1/2/4* clades (previously known as *AGL9* and *AGL2/3/4* clades, respectively) (Theissen, 2001; Malcomber and Kellogg, 2005; Zahn et al., 2005). The third clade, *AGL6*-like genes, also belongs to the *AP1*/*AGL9* group (Purugganan, 1997), and *Petunia AGL6*-like gene (*PhAGL6*) was functionally characterized as a *SEP*-like gene (Rijpkema et al., 2009). In addition to their role in determining floral organs, almost all members of the *AP1*/*AGL9* group are also involved in the initiation and development of the floral meristem (Purugganan et al., 1995), suggesting that *AP1*/*AGL9* group genes may play general roles in mediating other regulators involved in various aspects of flower development (Yu and Goh, 2000a; Kaufmann et al., 2009; Deng et al., 2011). This is exemplified by the formation of DcOAP3A-DcOPI-DcOSEP1 and DcOAP3B-DcOPI-DcOSEP1, higher order complexes detected by a yeast three-hybrid system (Xu et al., 2006). In *D.* 'Madame Thong-In', *DOMADS1* and its ortholog, *DcOSEP1*, from *D. crumenatum* were uniformly expressed in the inflorescence meristem and floral primordia, and later in all of the floral organs (Yu and Goh, 2000a). The expression pattern of *DOMADS1* in mature flowers coincided with that of *DcOSEP1* in *D. crumenatum*, as with their *Arabidopsis* orthologs (Pelaz et al., 2000; Xu et al., 2006). The onset of *DOMADS3* transcription was in early shoot apical meristem (SAM) just before the generation of the first flower primordium, and can later only be detected in pedicels (Yu and Goh, 2000a). *DOMADS3* may function not only in the floral transition, but also in pedicel development. *DOMADS1* were expressed in the inflorescence meristem, floral primordia and all floral organs (Xu et al., 2006). *DOMADS2* was expressed early in the shoot apical meristem and throughout the floral transition, but later restricted to the column. *DOMADS3* was expressed before flower primordia differentiate, and later only in pedicels. Multiple *cis*-acting elements, including six CArG-box sequences and five DNA-binding sites of the class 1 *knox* gene *DOH1* (a negative regulator), exist in the promoter region of the *DOMADS1* gene, and contribute to the regulation of *DOMADS1* expression in reproductive organs and the stem of *D.* 'Madame Thong-In' (Yu et al., 2000, 2002). Three other MADS-box paleo*AP1*-like genes from *D. thyrsiflorum*, *DthyrFL1-3*, belong to the *AP1*/*AGL9* group, and were transcribed at low levels in vegetative tissues including roots and leaves, at higher levels in ovules, and at much higher levels in inflorescences (Skipper et al., 2005). Expression of *DthyrFL1* and *DthyrFL2* increased from small to large floral buds.

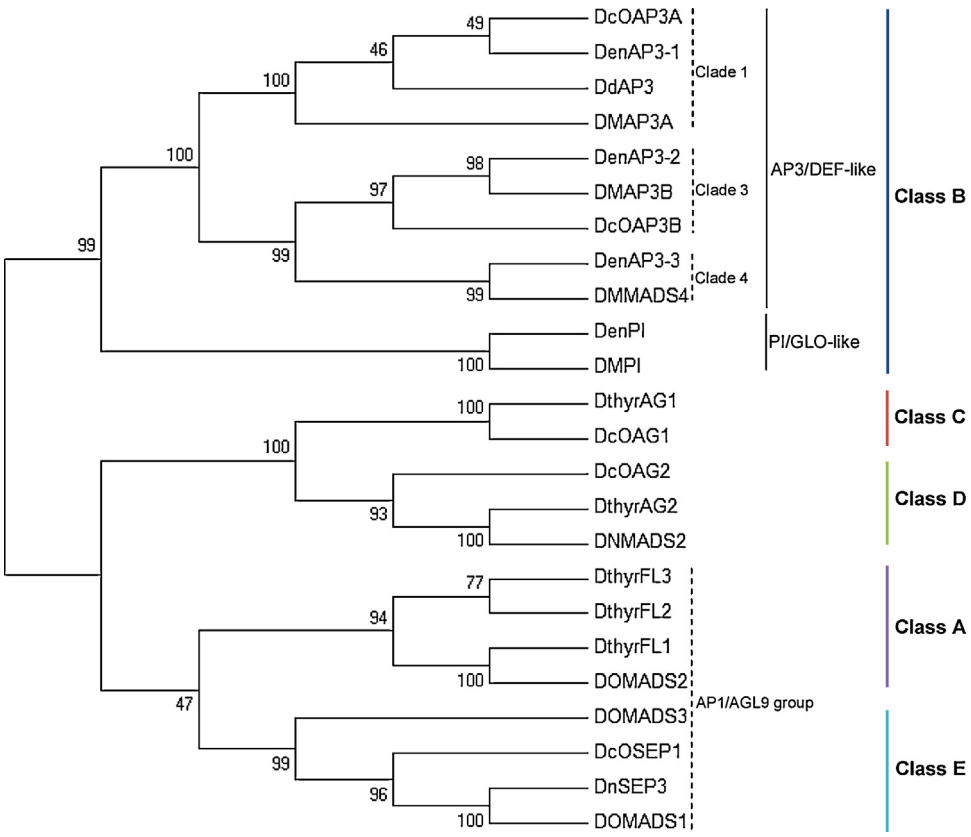

Fig. 2. Neighbor-joining (NJ) tree obtained from the alignment of the amino acid sequences of the virtual translation of the *Dendrobium* MADS-box genes (Table 2), outlining the various functional classes. The amino acid alignment was performed using T-COFFEE and the NJ tree was constructed using the MEGA 5 software, with 1000 bootstrap replicates. Numbers indicate bootstrap percentage.

2.2. *Dendrobium* Class B MADS-box genes

Class B MADS-box genes are required of the specification of petals and stamens. They include two major lineages: *AP3/DEF*-like genes (*APETALA3* and *DEFICIENS* genes in *A. thaliana* and *A. majus*, respectively) and *PI/GLO*-like genes (*PISTILLATA* and *GLOBOSA* genes in *A. thaliana* and *A. majus*, respectively) (Kramer et al., 1998; Zahn et al., 2005). Many class B MADS-box orchid genes share a common feature. In addition to their normal expression in the second whorl, their expression is mostly expanded into the first whorl, which may be responsible for the development of petaloid sepals in orchids (Aceto and Gaudio, 2011). In orchids, there are four types (clades 1–4) of *DEF*-like genes and generally one type of *GLO*-like genes. To date, three types of *DEF*-like genes belonging to clades 1, 3, and 4 were isolated in *Dendrobium*. The clade 1 *DEF*-like genes, including *DcOAP3A* (*D. crumenatum*), *DMAP3A* (*D. moniliforme*) and *DenAP3-1* (*D.* 'Spring Jewel'), together with *GLO*-like genes, including *DcOPI* (*D. crumenatum*), *DMPI* (*D. moniliforme*) and *DenPI* (*D.* 'Spring Jewel'), were expressed in all floral organs. Both types of class B genes (*DEF*-like and *GLO*-like genes) are expressed in whorl 1 of *Dendrobium* flowers, which explains the specification of petaloid sepals in whorl 1 in the modified ABCDE model. However, *DMAP3A* and *DMPI* were also expressed in immature ovaries and leaves, although *DMAP3B* was not expressed in those organs (Sirisawat et al., 2010). This expression pattern does not fit the modified ABCDE model. Further analysis is needed to clarify the function of these genes in the ovary development. The different expression pattern of *DEF*-like genes is described in the next section about "the orchid code".

2.3. *Dendrobium* Class C and D MADS-box genes

Class C genes regulate carpel development, and they also act together with class B genes to determine stamen development. Class D genes are primarily involved in ovule development. To date, two class C and three class D genes were isolated from *Dendrobium* species (Table 1). At the C terminal region, these genes have AG motifs I and II, which are common to all class C and D gene products and may determine their interaction with specific protein partners (Kramer et al., 2004). *DthyrAG2* (*D. thyrsiflorum*) and *DNMADS2* (*D. nobile*) have an MD-motif YET/AKA/DDXX, which is typical of monocot D lineage genes (Yun et al., 2004), although *DcOAG2* (*D. crumenatum*) does not have. *DcOAG1* and *DcOAG2* genes belong to class C and D MADS-box genes, respectively (Xu et al., 2006). *DcOAG1* is an ortholog of *AG* of *A. thaliana*, and contains an N-terminal extension preceding the MADS domain, which is typical of class C genes, and an intron 8, which is common in several *AG*-like genes (Kramer et al., 2004). *DcOAG2*, a homolog of *A. thaliana SEEDSTICK* (*STK*), was expressed in ovaries and anthers. *DcOAG1* was expressed in all floral organs, but not exclusively, as observed for some basal angiosperms (Kim et al., 2005; Xu et al., 2006). *DthyrAG1* and *DthyrAG2* are class C and D genes from *D. thyrsiflorum* (Skipper et al., 2006). These genes have some characteristics: (1) *DthyrAG1* has intron 8 before the stop codon; (2) both genes were expressed during ovule and flower development, specifically in the rostellum, stigma and stylar canal; (3) *DthyrAG1* was only expressed early, but *DthyrAG2* was expressed throughout ovule development, suggesting the role of *DthyrAG2* in late ovule development.

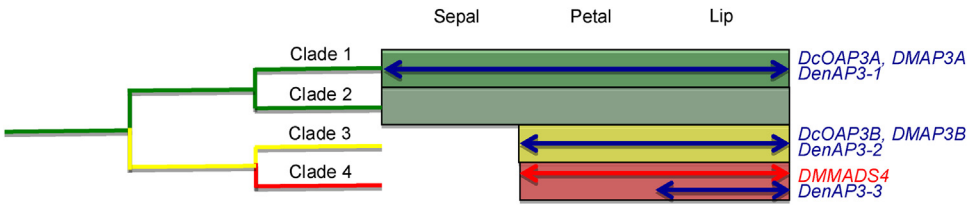

Fig. 3. Phylogenetic relationship and expression patterns of four types of *DEF*-like genes in *Dendrobium* species. Colors indicate different clades of *DEF*-like genes in orchids based on the orchid code (Mondragón-Palomino and Theissen, 2008) and the homeotic orchid tepal model (Pan et al., 2011).

3. The '*Dendrobium* code', part of the 'orchid code', a derivative ABCDE model for orchid MADS-box genes

Unlike other eudicots, which have different morphology for sepals (whorl 1) and petals (whorl 2), flowers of monocots (such as tulips or agapanths) have phenotypically similar petaloid organs (tepals) in the outer whorls 1 and 2. Consequently, the ABCDE model was modified (Fig. 3) to extend the expression of class B genes into whorl 1, in addition to their expression in whorls 2 and 3 (Kanno et al., 2003; Nakamura et al., 2005).

In orchids, the lip of the inner tepals is morphologically distinguishable from other tepals, which cannot be satisfactorily explained by the modified ABCDE model. Mondragón-Palomino and Theissen (2008, 2009, 2011) devised the 'orchid code' to explain the formation of the orchid perianth. This code suggests that the class B *AP3/DEF*-like genes play a key role in determining the identity of tepal (sepal and petal) and lip, while the function of the class B *PI/GLO*-like genes is similar to other B class genes in eudicots. In this orchid code, there are four clades of *DEF*-like and a clade of *GLO*-like lineage (Table 2). Clade 1 contains *PeMADS2*-like genes, including *PeMADS2* (*Phalaenopsis equestris*), *VaplaDEF1* (*Vanilla planifolia*) and *OMADS5* (*Oncidium* Gower Ramsey). Clade 2 consists of *OMADS3*-like genes, including *OMADS3* (*O.* Gower Ramsey) and *PeMADS5* (*P. equestris*). Clade 3 contains *PeMADS3*-like genes, including *PeMADS3* (*P. equestris*), *GogalDEF3* (*Gongora galeata*) and *HrDEF* (*Habenaria radiata*). Clade 4 contains *PeMADS4*-like genes such as *PeMADS4* (*P. equestris*) and *SpodoDEF3* (*Spiranthes odorata*). Clades 1 and 2 are sister clades with gene expression in both outer and inner tepals, while clades 3 and 4, also sister clades, specify only inner tepal development. Only one *GLO*-like gene was

Table 2
Expression patterns of *AP1/SQUA*-like, *AP2*-like, *AP3/DEF*-like, *PI/GLO*-like, *AG*-like, *STK*-like and *SEP*-like sub-family genes (i.e., FOIGs) in various floral organs of different *Dendrobium* orchids.

Sub-family gene	Species or variety	Gene	Floral organ					Reference
			Sepal	Petal	Lip	Column	Ovary	
AP1/SQUA-like								
	D. Madame Thong-In	*DOMADS2*	–	–	–	+++[a]	+++[a]	Yu and Goh (2000a)
	D. thyrsiflorum	*DthyrFL1*	NA[b]	NA[b]	NA[b]	NA[b]	+	Skipper et al. (2005)
	D. thyrsiflorum	*DthyrFL2*	NA[b]	NA[b]	NA[b]	NA[b]	++	Skipper et al. (2005)
	D. thyrsiflorum	*DthyrFL3*	NA[b]	NA[b]	NA[b]	NA[b]	++	Skipper et al. (2005)
AP2-like								
	D. crumenatum	*DcOAP2*	+++	+++	+++	+++	+++	Xu et al. (2006)
AP3/DEF-like								
Clade 1	*D. crumenatum*	*DcOAP3A*	+++	+++	+++	+++	NA	Xu et al. (2006)
	D. moniliforme	*DMAP3A*	+++	+++	+++	+++	++	Sirisawat et al. (2010)
	D. Spring Jewel	*DenAP3-1*	+++	+++	+++	+++	NA	Pan et al. (2011)
Clade 3	*D. crumenatum*	*DcOAP3B*	–	+++	+++	+++	NA	Xu et al. (2006)
	D. moniliforme	*DMAP3B*	–	+++	+++	+++	–	Sirisawat et al. (2010)
	D. Spring Jewel	*DenAP3-2*	–	+++	+++	+++	NA	Pan et al. (2011)
Clade 4	*D. moniliforme*	*DMMADS4*	–	+++	+++	+++	+++	Sirisawat et al. (2009)
	D. Spring Jewel	*DenAP3-3*	–	–	+++	–	NA	Pan et al. (2011)
PI/GLO-like								
	D. crumenatum	*DcOPI*	+++	+++	+++	+++	NA	Xu et al. (2006)
	D. moniliforme	*DMPI*	+++	+++	+++	+++	+++	Sirisawat et al. (2010)
	D. Spring Jewel	*DenPI*	+++	+++	+++	+++	NA	Pan et al. (2011)
AG-like *(C-lineage)*								
	D. thyrsiflorum	*DthyrAG1*	–	–	–	+++	+++	Skipper et al. (2006)
	D. crumenatum	*DcOAG1*	+++	+++	+++	+++	+++	Xu et al. (2006)
	D. moniliforme	*DMAG1*	–	–	–	+++	NA	Sirisawat et al. (2008)
STK-like *(D-lineage)*								
	D. thyrsiflorum	*DthyrAG2*	–	–	–	+++	+++	Skipper et al. (2006)
	D. crumenatum	*DcOAG2*	–	–	–	++	+++	Xu et al. (2006)
SEP-like[c]								
	D. crumenatum	*DcOSEP1*	+++	+++	+++	+++	+++	Xu et al. (2006)
	D. Madame Thong-In	*DOMADS1*	+++	+++	NA	+++[a]	+++[a]	Yu and Goh (2000a)
	D. Madame Thong-In	*DOMADS3*	–	–	NA	–	–	Yu and Goh (2000a)

FOIG, floral organ identity gene; NA = not analyzed.
– = no expression; + = weak expression; ++ = moderate expression; +++ = strong expression.
[a] Gene expression was detected in the mixture of column and ovary/ovule.
[b] Gene expression analyzed in flower buds but not separately in each organ.
[c] Organ-specific expression was not reported by Chen et al. (2013) and is thus not reported in the table.

found in the *GLO*-like lineage of most orchids, such as *PeMADS6* (*P. equestris*) although *H. radiata* and *Orchis italica* have two *GLO*-like genes (*HrGLO1/2* and *OrcPI/2*, respectively) (Kim et al., 2007; Cantone et al., 2011; Salemme et al., 2011). The genetic basis underlying floral color and development in orchids, focusing primarily on *Phalaenopsis*, was reviewed by Hsiao et al. (2011) and Tsai et al. (2014).

Experimental information on MADS-box genes has indicated the specific function of *Dendrobium* floral organ identity genes. Class C and D genes (*DthyrAG1/2*, *DcOAG* and *DMAG1*) were expressed in the column and ovary/ovule, while class E genes (*DcOSEP1* and *DOMADS1*) were expressed in all floral organs (Table 2 and Fig. 3). Clade 1 *DEF*-like genes (*DcOAP3A*, *DMAP3A* and *DenAP3-1*) were expressed in all floral organs together with *GLO*-like genes, *DcOPI*, *DMPI* and *DenPI* (Table 2). The expression pattern of these genes fits the modified ABCDE model nicely and the expansion of function B genes in *Dendrobium* species may be responsible for the specification of petaloid sepals in the first whorl. However, one of the class C genes, *DcOAG1*, from *D. crumenatum* were expressed in all floral organs, although the expression of other *Dendrobium* class C genes are limited in the column and ovary/ovule (Table 2 and Fig. 1C). Also, the class A gene, *DcOAP2* was expressed in all floral organs, while *DOMADS2*, from *D.* 'Madame Thong-In' was absent from the first whorl and expressed in the column and ovaries (Fig. 1C; Yu and Goh, 2000a). The expression pattern of two clade 4 *DEF*-like genes are not the same which may be the reason why different developmental stages were used. Further molecular analyses are needed to clarify the precise expression pattern and the function of these genes.

According to the "orchid code", clade 1 and 2 *DEF*-like genes mediate the development of sepals, petals and lips, clade 3 *DEF*-like genes mediate the development of petals and lips, and the clade 4 *DEF*-like genes regulate lip development (Fig. 3). The HOT model is somewhat similar to the "orchid code" (Mondragón-Palomino and Theissen, 2008), but more precisely points out the effect of *PeMADS4* (*AP3A2* clade, clade 4) in determining lip morphogenesis at a relevantly later floral development stage (Hsiao et al., 2011; Pan et al., 2011). The expression patterns of clade 1 and clade 3 *DEF*-like genes from *Dendrobium* support this model as shown in Fig. 3. Although the expression of a clade 3 *DEF*-like gene, *DenAP3-3* from *D.* Spring Jewel, was limited in the lip, *DMMADS4* from *D. moniliforme* was expressed in the lip, petals, and the column (Fig. 3; Sirisawat et al., 2009). Limited expression of clade 3 *DEF*-like genes in the lip was also found in *Phaius tankervilleae* (*PtAP3-3*), whereas the other clade 3 genes from *Paphiopedilum* 'Macabre' (*PaphAP3-2*), *Oncidium* 'Gower Ramsey' (now *Oncidesa*) (*OncAP3-3*) and *Brassavola nodosa* (*BnAP3-1*), show various expression patterns (Pan et al., 2011).

The function of most orchid class B MADS-box genes has been clarified by ectopic expression in *Arabidopsis* or by the rescue of *Arabidopsis* mutant phenotypes. Overexpression of paleo *AP3*-like genes from *D. crumenatum* (*DcOAP3A*) and *D. moniliforme* (*DMAP3B*) in *Arabidopsis* did not affect floral phenotypes because in most orchids, most *AP3/DEF*-like genes are of the paleo *AP3*-type and their sequences are vastly different from *Arabidopsis AP3* (Xu et al., 2006; Sirisawat et al., 2010). Unlike the *AP3/DEF* lineage, the *PI/GLO*-like genes are conserved between *Arabidopsis* and orchids since ectopic expression of *PI*-like genes from *D. crumenatum* (*DcOPI*) and *D. moniliforme* (*DMPI*) caused the partial transformation of sepals to petal-like organs in the first floral whorl (Xu et al., 2006; Sirisawat et al., 2010). Sirisawat et al. (2010) and Abdullakasim and Handa (2012) overexpressed *DMMADS4*, an *AP3/DEF*-like gene from *D. moniliforme*, in *Arabidopsis* to verify whether it could induce a heterodimeric interaction to regulate flower organ identity with DMPI, a PI/GLO-like protein in *D. moniliforme*. 35S::*DMMADS4* plants had the same floral characteristics to wild-type *Arabidopsis*, while F_1 progenies generated by crossing 35S::*DMMADS4* with 35S::*DMPI* exhibited the phenotype that resembled *Arabidopsis* plants overexpressing *AP3/PI* (Krizek and Meyerowitz, 1996) in which sepals in whorl 1 were converted into petal-like organs. These results indicate that *DMMADS4* and *DMPI* may interact to form a heterodimer in regulating petal development.

4. Flower color in *Dendrobium*

Anthocyanins and colored flavonoid glycosides give fruit and flowers their characteristic colors as a result of accumulation in vacuoles. Chalcone synthase (CHS) and dihydroflavonol 4-reductase (DFR) are two key enzymes of the anthocyanin biosynthetic pathway. CHS is represented by a gene family, while DFR is often represented by a single gene in many plant families (To and Wang, 2006). DFR reduces the 4 carbonyl of dihydroflavonol to give leucoanthocyanidin and then to individual pigments (cyanidin, pelargonidin, delphinidin) (Davies et al., 2003; Nie et al., 2005; To and Wang, 2006; Tanaka et al., 2010) (Fig. 4).

Saito et al. (1994) found an acylated cyanidin glycoside to be the main pigment in red-purple flowers of *D.* 'Pramot'. Kuehnle et al. (1997) found in several species and hybrids that 3′-hydroxylated cyanidin was the major pigment, whereas two aglycones, pelargonidin and delphinidin, were either rare or absent. Mudalige-Jayawickrama et al. (2005) identified a rare hybrid *D.* X Icy Pink 'Sakura' (K1224) with 98% pelargonidin and 2% cyanidin. In this hybrid, kaempferol derivatives were the main flavonol with no 3′-hydroxylated quercetin derivatives, while *DFR* expression occurred only in flower buds. The nucleotide sequence of the K1224 *DFR* clone was identical to that of a typical cyanidin accumulating line *D.* X Jacquelyn Thomas 'Uniwai Prince' (UH503), even though flower color was different in both hybrids. RT-PCR analysis showed that *DFR* was expressed in open flowers and closed buds of both lines, but not in any vegetative tissues. Unlike *DFR*, *CHS* expression was detected in all vegetative tissues except for pseudobulbs. *DFR* and *CHS* expression in both lines peaked in floral stages 3 and 4 (Table 2), and almost disappeared as flowers opened. Two partial *CHS* clones from UH503, *CHS-6* and *CHS-9*, had significant similarity with a *Phalaenopsis* Blume 'True Lady' homologue (Kuehnle and Champagne unpublished). The *CHS* gene was characterized by Pitakdantham et al. (2010) in *D.* Sonia 'Earsakul'. Expression of the *CHS* gene was high in unpigmented young flower buds, but its expression decreased sharply when pigments accumulated in young flower buds. *CHS* gene expression was maintained at the same level until flowers were fully opened. Transformation of *D.* 'Sanya' protocorm-like bodies (PLBs) with the *CHS* gene by sonication-assisted *Agrobacterium*-mediated transformation (SAAT) resulted in 5 transgenic lines, but the effects on flowering and senescence were not tested (Zheng et al., 2011).

Mudalige-Jayawickrama et al. (2005) found one full-length cDNA clone *Den-CHS-4* from UH503, to be highly conserved (80–94% similarity) across 25 genera. Regulatory genes such as flower-specific bHLH, Myb and WD40 transcription factors activate the transcription of anthocyanin biosynthetic genes in maize, snapdragon and petunia (Mol et al., 1998). *MYB* genes comprise a large family of transcription regulators that are involved in the regulation of many aspects of plant development, including hormone signaling, metabolism, seed coat development, defense pathways, lignin biosynthesis and regulation of secondary metabolism, such as the phenylpropanoid biosynthetic pathway (Petroni and Tonelli, 2011). The authors claimed that the high levels of pelargonidin in K1224 could be due to a mutation in the flavonoid 3′-hydroxylase (F3′H) gene, promoting DFR to accept dihydrokaempferol (DHK) as the substrate rather than dihydroquercetin (DHQ) or dihydromyricetin

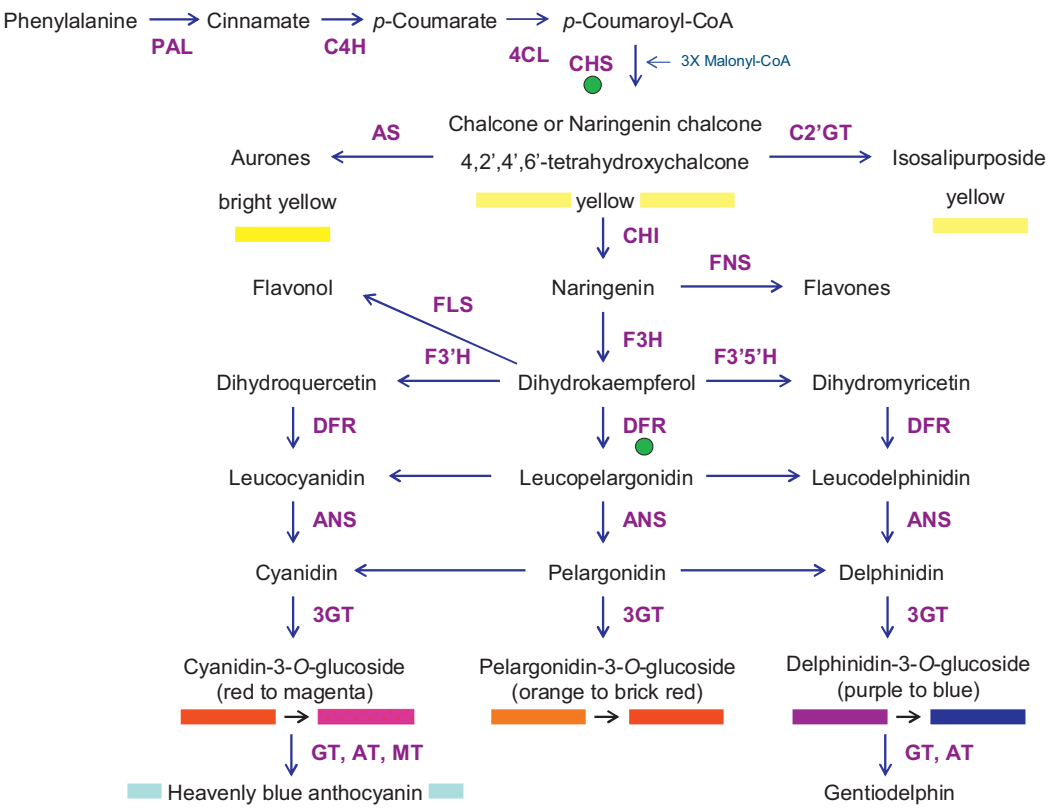

Fig. 4. Flavonoid biosynthetic pathway relative to flower color. Based on To and Wang (2006), Chandler and Tanaka (2007). Purple names indicate enzymes. 3GT, UDP-glucose: anthocyanidin 3-*O*-glucosyltransferase; 4CL, 4-coumarate:CoA ligase; ANS, anthocyanidin synthase; AS, aureusidin synthase; AT, acyl CoA-dependent acyltransferases; C2′GT (syn. THC2′GT), UDP-glucose: tetrahydroxychalcone 2′-*O*-glucosyltransferase; C4′GT (syn. THC4′GT), UDP-glucose: tetrahydroxychalcone 4′-*O*-glucosyltransferase; C4H, cinnamate 4-hydroxylase; CHI, chalcone isomerase; CHS, chalcone synthase; DFR, dihydroflavonol 4-reductase; FLS, flavonol synthase; FNS, flavone synthase; F3H, flavanone 3β-hydroxylase; F3′H, flavonoid 3′-hydroxylase; F3′5′H, flavonoid 3′, 5′-hydroxylase; GT, UDP-glucose-dependent glucosyltransferases; MT, S-adenosylmethionine-dependent methyltransferase; PAL, phenylalanine ammonia-lyase. Bright green dot (●): enzymes for which genes have been identified or cloned in *Dendrobium* spp. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(DHM), which would allow orange flowers to be bred. Finally, *Den-DFR-1* showed highly specific expression in floral tissues, and its promoter could serve as a perianth-specific promoter in flower color genetic transformation. A study by Pitakdantham et al. (2011) showed 99% similarity between the *DFR* amino acid sequences of *D.* Sonia 'Earsakul', *D.* 'Red Bull', *D.* hybrid 'Uniwai Prince' and *D.* hybrid 'Greeting Fragrance'. These authors also found that *DFR* gene expression could be detected in young flower buds of purple *D.* Sonia 'Earsakul' and dark purple *D.* 'Red Bull' at an early stage of pigmentation, *i.e.*, when medium-sized flower buds had slight pigmentation, and peaked when the flower was nearly open with slight pigmentation in petals and the lip. *DFR* gene expression decreased sharply when half-open flowers were fully pigmented. Even though Pitakdantham et al. (2011) found strong similarity between the *Dendrobium DFR* gene and that of other orchids, namely *Bromheadia* and *Oncidium*, in the latter two orchids, *DFR* gene expression occurred throughout the life time of the flower (Liew et al., 1998; Hieber et al., 2006). The expression profiles of the *DFR* gene during flower development of *D.* Sonia 'Earsakul' were separately examined by Piluk and Ratanasut (2012) in sepals and petals in 7 growth stages: stage 1, flower bud length <2 cm; stage 2, flower bud length 2.0–2.3 cm; stage 3, flower bud length 2.8–3.0 cm; stage 4, flower bud length 3.3–3.5 cm; stage 5, flower bud length 3.8–4.0 cm; stage 6, opening flower; stage 7, fully-opened flower. *DFR* was expressed evenly in stages 2–5 in sepals and from stages 1–6 in petals, but weakly in stages 1 and 6 and strongly in stages 4 and 5, particularly in purple tissues. In their study, 7 stages were defined, whereas Mudalige-Jayawickrama et al. (2005) described 10 stages for *D.* X Jacquelyn Thomas 'Uniwai Prince' (Table 3).

Whang et al. (2011) isolated *DFR*, *CHS*, and flavonoid 3′,5′-hydroxylase (*F3′5′H*) genes from *D. moniliforme*. While *F3′5′H* transcripts accumulated to high levels at the base of the column relative to levels in perianths, there was no significant difference in *DFR* and *CHS* expression among all floral organs. When white organs of the perianth were transformed by particle bombardment, transient expression of the *F3′5′H* gene, but not *DFR* and *CHS* genes, produced reddish-purple pigmentation, suggesting that lack

Table 3
Description of stages of development of buds and flower of a typical cyanidin accumulating line *Dendrobium* X Jacquelyn Thomas 'Uniwai Prince' (UH503) (modified from Mudalige-Jayawickrama et al., 2005).

Stage	Description
1	1.3–1.5 cm long, almost all are immature buds with a light green adaxial surface and some purple on the abaxial surface
2	1.5–1.7 cm long, buds are small, adaxial surface green but abaxial surface turned purple
3	1.7–1.9 cm long, adaxial surface turning dark purple
4	1.9–2.1 cm long, dark purple perianth on both surfaces
5	2.1–2.3 cm long, buds remain unopened but have turned dark purple
6	2.4–2.8 cm long, most buds are mature with dark purple perianth on adaxial and abaxial surfaces
7	Flowers half-opened
8	Flowers fully opened, dark purple perianth, one position on the raceme below stage 7 flower
9	As for stage 8, but two flowers below stage 7 on the raceme
10	As for stage 9, but three flowers below stage 7 on the raceme

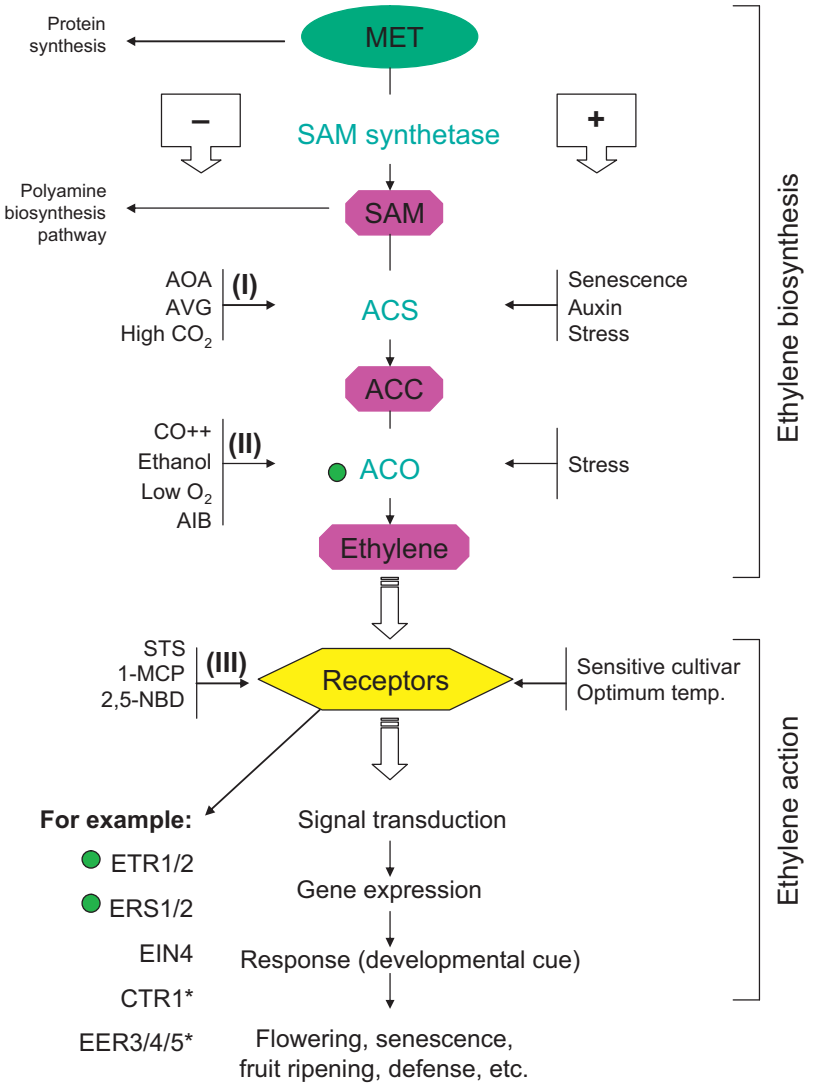

Fig. 5. Ethylene biosynthetic pathway and factors affecting senescence. In the pathway, two steps involving ACS (I) (inhibited by (minus sign) AOA (amino-oxyacetic acid) and AVG (aminoethoxyvinyl glycine) and stimulated by (plus sign) senescence) and ACO (II) (inhibited by AIB (aminoisobutyric acid) and ethanol and stimulated by Mn^{2+} and stress) are rate-limiting for ethylene production. Stresses may include pathogen infection, wounding, ozone, UV, *etc.* Chemicals (STS (silver thiosulphate), 1-MCP (1-methylcyclopropene), 2,5-NBD (2,5-norbornadiene), *etc.*) are also effective at the receptor level (III) and prevent the binding of ethylene. 2,5-NBD blocks ethylene action by competing with ethylene for binding sites. Based on Teixeira da Silva (2006), Ebrahimzadeh et al. (2008), Yoo et al. (2009). Blue text indicates enzymes: SAM (syn. *S*-AdoMet), *S*-adenosylmethionine; ACC, 1-aminocyclopropane-1-carboxylic acid; ACS, ACC synthase; ACO, ACC oxidase. Receptors: EIN, ETHYLENE-INSENSITIVE; ERS, ETHYLENE SENSOR; ETR, ETHYLENE-RESISTANT. Ethylene signaling genes*: CTR, CONSTITUTIVE TRIPLE RESPONSE; EER, ENHANCED ETHYLENE RESPONSE. Bright green dot (●): enzymes for which genes have been identified or cloned in *Dendrobium* spp. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of color in the *D. moniliforme* perianth is at least due to transcriptional control of *F3′5′H*.

5. Flower senescence in *Dendrobium*

Ethylene in higher plants is involved in flower senescence and abscission, which can affect flower color, and quality and longevity of cut flowers. Senescence, broadly the combination of events that lead to the death of cells, tissues or organs, is mediated by a series of highly coordinated physiological and biochemical changes, including increased activity of hydrolytic enzymes, degradation of macromolecules, loss of cellular compartmentalization and increase in respiratory activity. Understanding these processes would allow researchers to slow down senescence. The biosynthetic pathway of ethylene involves two conversions (van Doorn and Woltering, 2008). The first conversion from S-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) is catalyzed by ACC synthase (ACS), while the second one from ACC to ethylene is catalyzed by ACC oxidase (ACO) (Teixeira da Silva, 2006; Ebrahimzadeh et al., 2008; Fig. 5). ACO gene families tend to be expressed in a tissue-specific manner in several plants (Yoon and Kiebler, 2013). After ethylene is synthesized, it is perceived and triggers the downstream signaling pathway that modulates relevant gene expression and biological responses (Jones, 2013). Five ethylene receptors exist in *Arabidopsis* (ETR1, ETR2, ERS1, ERS2, and EIN4; EIN = ETHYLENE-INSENSITIVE; ERS = ETHYLENE SENSOR; ETR = ETHYLENE-RESISTANT), and the detailed information on these receptors has been discussed elsewhere (*e.g.*, Wang et al., 2002; Yoo et al., 2009; Liu and Wen, 2012; Shakeel et al., 2013).

Using *ACO*-specific primers designed from *ACO* sequences of three other *Dendrobium* hybrids, namely ‘Pompadour’

(Genbank accession number (AN): EF487342), 'Karen' (Genbank AN: EF487343) and 'Sonia' (Genbank AN: EF061081), Nagtong et al. (2009) isolated an *ACO* gene from different floral tissues and vegetative organs of a relatively ethylene-insensitive cultivar 'Anna'. An open reading frame encoding the entire amino acid sequence of the *ACO* gene was designated as *DenACO*, which contained 942 bp and encoded a protein of 313 amino acids (Genbank AN: GQ332400). 'Anna' *DenACO* was 98% similar to *ACOs* of *D.* 'Pompadour', 'Karen', 'Sonia' and 'Missteen' (Genbank AN: EU151724). *DenACO* expression peaked in stage 2 of flower development and could be detected in all flower tissues tested (petals, sepals, pedicels, lip, and stigma) as well as in vegetative tissues (leaves and roots). Similarly, they found that *DenACS* transcripts accumulated in flower stages 2 and 3, approximately 3–4 times higher than those in stage 1 (Nagtong et al., 2010). In a related study, Razali et al. (2010) isolated and characterized 4 homologs of ethylene receptors, ETR1, ERS1, ETR2 and ERS2, in *D.* 'Pompadour', while *Den-ERS1* had also been cloned from *D.* 'Pompadour' a year earlier by another group (Thongkum et al., 2009).

6. Other related advances in *Dendrobium*

Differentially expressed genes (DEGs) have been identified in *Dendrobium* species at various developmental stages (Yu and Goh, 2000b; Yang et al., 2003a, 2003b; Faridah et al., 2009). In *D. crumenatum*, 9 DEGs were detected in various floral organs. Three of these genes that were highly expressed in the column shared high sequence similarity to the small heat shock protein of tobacco (*Nicotiana tabacum*) (DEG3-8), pectin methylesterase of willow (*Salix gilgiana*) (DEG6-1) and the 14-3-3 protein of apple (*Malus* x *domestica*) (DEG9-9) during fruit ripening.

A putative cytokinin oxidase (CKX) gene from *D.* 'Sonia' *DSCKX1* (*Dendrobium* Sonia cytokinin oxidase), was cloned after exposure of shoot tips to 6-benzyladenine (BA) (Yang et al., 2003a, 2003b). CKX is involved in cytokinin metabolism. To investigate the biological function of cytokinins and CKX in orchids, Yang et al. (2003a, 2003b) generated transgenic *D.* 'Sonia' plants constitutively over- or underexpressing the *DSCKX1* gene. The former showed enhanced CKX activity and a reduction in cytokinin contents, *i.e.*, the overproduced DSCKX1 protein was a functional enzyme responsible for the oxidation of cytokinins. *35S::DSCKX1* transformants showed stunting, reduced leaf size, shoot proliferation from callus, and longer and more prolific roots than wild-type in response to lower levels of cytokinins. In another study, differential screening of thidiazuron (TDZ)-induced floral initiation in *D. nobile* identified MADS-box gene and a *Sos* (*Son of evenless*) homologue, indicating that TDZ could induce flowering *in vitro* (Wen et al., 2013a). Interestingly, work by the same authors showed that application of TDZ to *D. nobile* protocorm-like bodies *in vitro* induced a *VRN1*-like gene, *DnVRN* (GenBank accession number EF53559) (Wen et al., 2013b). Following 40 days of vernalization treatment of *D. nobile* axillary buds, the exposure to low temperature (15/10 °C; day/night), revealed overrepresentation of genes related to stress responses from a collection of 15017 expressed sequence tags (ESTs).

Only a few papers have been published in the English literature on flower scent in *Dendrobium*. Initial GC–MS studies by Flath and Ohinata (1982) on *Dendrobium superbum* identified 25 volatile components, mainly methyl ketones and 2-alkyl acetates. Another floral volatile, (*Z*)-11-eicosen-1-ol, discovered in *Dendrobium sinense*, serves to attract pollinators (Brodmann et al., 2009).

Feng et al. (2013) recently constructed two preliminary genetic linkage maps using 90 F_1 progeny individuals derived from an interspecific cross between *D. nobile* and *D. moniliforme* using random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR). These maps would allow for more in-depth genetic studies, for mapping medicinal and horticultural traits and for marker-assisted selection in *Dendrobium* breeding programs.

7. Conclusions and future objectives

There is an intimate link between orchid transgenics (Teixeira da Silva et al., 2011) and orchid biotechnology (Chandler and Tanaka, 2007; Hossain et al., 2013; Teixeira da Silva, 2013). As described by Abdullakasim and Handa (2012), *Dendrobium* has some floral homeotic mutants. These mutants are very helpful for analyzing the function of MADS-box genes by comparing the expression pattern and/or gene structure between WT and mutants. In addition, the orchid orthologues of key flowering time genes, *FT* (*FLOWERING LOCUS T*) and *MFT* (*MOTHER OF FT AND TFL1*), have been isolated from *D. nobile* (Li et al., 2012; Liang et al., 2012). *FT* predominantly promotes flowering, while *MFT* regulates seed germination in *Arabidopsis*. Other *FT* orthologs have also been identified from *Cymbidium* orchids, such as *Cymbidium sinense* 'Qi Jian Bai Mo', *Cymbidium goeringii* and *Cymbidium ensifolium* 'Jin Si Ma Wei' (Huang et al., 2012). However, the function of orchid orthologs of *FT* and *MFT* is still largely unknown. Thus far, the genetics of flowering time control and flower development in *Dendrobium* and other orchids is still at a nascent phase of discovery. Consequently, it is important to develop a model orchid to advance research. With many homeotic mutants, *Phalaenopsis* is the best choice for floral organ development research, and great progress has been made in recent years (Pan et al., 2014) while for flowering time and flower senescence, a better alternative is needed. *D. crumenatum*, with its unique flowering process (Yap et al., 2008) and a few *Dendrobium* hybrids, which have been successfully induced to flower *in vitro* (Sim et al., 2007; Ding et al., 2013), make *Dendrobium* a potentially powerful candidate model orchid genus. However, while it is fascinating to suggest an international effort to develop a model orchid for studying the underlying molecular genetic mechanisms, it is difficult at this stage to make a concrete plan because of the diversity of orchid species and different interests in various orchid species in multiple countries.

Besides, it is important to further clarify the molecular mechanism of the floral transition and flower development using the following approaches.

(1) Genes that are homologous to those putative key regulators in the control of flowering and flower development in *Arabidopsis* or cereals could be isolated in orchids.
(2) Change in gene expression profiles could be examined during the floral transition and flower development using RNA-seq.
(3) The function of genes identified through the above approaches will be further examined by transgenic studies in model plants such as *Arabidopsis*.
(4) In the long run, transgenic orchids with altered expression of these genes should be obtained to evaluate their endogenous function in orchids. Considering the long time needed to generate transgenic plants, virus-induced gene silencing (VIGS) is an alternative for validating gene functions in a short period of time. VIGS has already been successfully applied to *Phalaenopsis* (Hsiao et al., 2011; Hsieh et al., 2013; Pan et al., 2014).
(5) Transcriptome analysis of *C. sinensis* indicated the presence of 120 flowering-associated unigenes: 73 MADS-box and 28 *CONSTANS-LIKE* (*COL*) (Zhang et al., 2013), highlighting the great impact of the next-generation sequencing techniques in the study of flower development in non-model plant species.

Conflicts of interest

The authors declare no conflicts of interest.

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