Molecular Mechanism Regulating Floral Architecture in Monocotyledonous Ornamental Plants

Akira Kanno

Graduate School of Life Sciences, Tohoku University, Sendai 980-8577, Japan

Molecular and genetic analyses of flower development have been conducted primarily in dicot model plants such as Arabidopsis thaliana and Antirrhinum majus. The obtained data are the basis for the ABC model, which was extended to the ABCE model of floral development. This model has been validated in many dicot species using genetic transformation studies and mutant analyses. Many dicot flowers have two distinctive perianth whorls, which include greenish sepals and showy petals. By contrast, the monocot lily flower has two almost identical petaloid whorls, the inner and outer tepals. To explain this type of floral morphology, a modified ABC model, the further extended modified ABCE model, was proposed. According to this model, B-class genes are expressed in whorls 1–3, and whorls 1 and 2 form petaloid structures. This review describes the molecular mechanisms regulating flower development in monocots. Since the showy perianth is one of the most important traits in floricultural crops, we focused on the B- and C-class genes, which are related to the development and modification of the perianth. The review describes the expression patterns of floral organ identity genes, and presents functional studies using double-flowered and viridiflora cultivars in some monocot species. Besides lily-type flowers, there are several types of monocot flower, such as commelina type with two whorls of distinctive perianth, orchid and grass flowers. The review also describes the molecular analyses of these types of monocot flower.

Key Words: ABCE model, double flower, MADS-box genes, modified ABCE model, viridiflora.

Introduction

A flower is the reproductive structure in angiosperms. The male and female reproductive organs (stamens and carpels, respectively) are surrounded by the perianth, which is composed of tepals. Angiosperm flowers vary with respect to tepal color and structure and the number of perianth whorls. The showy perianth (or sometimes a colorful bract) is one of the most important traits in floricultural crops, and many varieties such as double-flower mutants have been produced. Therefore, it is important to understand the molecular mechanism regulating angiosperm floral development, especially in floricultural crops.

To acquire a detailed understanding of floral development, it is important to describe the floral structures in each angiosperm group. Figure 1 shows various ornamental flowers that are widely commercially available. Ipomoea (morning glory, Fig. 1A), Rhododendron (azalea, Fig. 1B), and Eustoma (lisianthus, Fig. 1C) belong to the core eudicots; these flowers contain two tepal whorls, including the showy inner tepals (petals) and the greenish outer tepals (sepals). The model plants Arabidopsis thaliana (Brassicaceae) and Antirrhinum majus (Plantaginaceae) belong to this group. Aquilegia (columbine, Fig. 1D) and Clematis (Fig. 1E) belong to the basal eudicots, and all tepals are colored and showy. The remaining five species in Figure 1 belong to the monocots (Fig. 1F–J). The perianth of Tulipa (tulip, Fig. 1F) and Crocus (Fig. 1G) contains two whorls of almost identical petaloid tepals. Narcissus has two whorls of petaloid tepals and an additional showy trumpet-like structure called the paracorolla (corona, Fig. 1H). Ornamental orchid flowers such as Bletilla (urn orchid, Fig. 1I) have three outer and three inner tepals (sepals and petals, respectively), and these tepals are often petaloid in orchids. One petal, called the lip or labellum, can be distinguished from the other two petals because of its large size and shape; the lip is a specific characteristic of flowers in the orchid family. In contrast to monocot flowers containing two whorls of petaloid tepals, some monocot species such as Trillium...
canshatcense (Fig. 1J), which belongs to the Trilliaceae, have two distinctive perianth whorls including greenish sepals in whorl 1 and showy white petals in whorl 2.

It is widely accepted that the perianth evolved independently many times during angiosperm evolution. Andropetals, which are petals derived from stamens, evolved many times in lower eudicots and at least once in higher eudicots and monocots. Bracteopetals, which are petals derived from bracts, evolved independently in magnolid dicots such as *Liriodendron*, *Peperomia*, and *Aristolochia*. Independent derivations of andropetals and bracteopetals during angiosperm evolution have been discussed previously (Kramer et al., 1998).

How can the distinct floral morphologies of these plants be explained? Floral morphology is one of the most important traits in floricultural crops that determine their commercial value. Therefore, it is important to clarify the molecular mechanism of floral development. Molecular and genetic analyses of floral development have been conducted primarily in higher eudicot model plants such as *Arabidopsis thaliana* and *A. majus*, and the classical ABC model was proposed (Coen and Meyerowitz, 1991; Weigel and Meyerowitz, 1994). According to this model, a combination of three gene functions specifies the four floral organs (Fig. 2A). Class A function leads to the formation of sepals in whorl 1. The combination of Class A and B functions specifies petals in whorl 2, which have been characterized in monocots (Kanno, 2006; Kanno et al., 2007), lower eudicots (Kramer, 2009; Kramer and Hodges, 2010), and basal angiosperms (Liu et al., 2013; Soltis et al., 2009). Among these, monocots are very important groups for horticulture because many important floricultural species, such as lilies, tulips, and orchids, are included in them. This review summarizes the molecular studies of floral organ identity genes in monocots, including molecular analyses using the double-flowered and viridiflora cultivars.

Molecular Mechanisms Determining Floral Architecture in Dicots and Monocots

Typical higher eudicot flowers contain four organ whorls (Fig. 2A). The outer two whorls are the perianth, and these two whorls are distinct: the outer most whorl (whorl 1) contains the sepals, which are often small and greenish, whereas the inner perianth whorl (whorl 2) contains the petals, which are generally showy and colorful. The inner two organ whorls contain the reproductive organs, the stamens and carpels (whorls 3 and 4, respectively). To explain this floral development, molecular and genetic studies were carried out using model plants of *Arabidopsis thaliana* and *A. majus*, and the classical ABC model was proposed (Coen and Meyerowitz, 1991; Weigel and Meyerowitz, 1994). According to this model, a combination of three gene functions specifies the four floral organs (Fig. 2A). Class A function leads to the formation of sepals in whorl 1. The combination of Class A and B functions specifies petals in whorl 2,
and the combination of Class B and C functions specifies stamens in whorl 3. Class C function specifies carpels in whorl 4. A-class genes include \textit{APETALA1} (\textit{AP1}) and \textit{APETALA2} (\textit{AP2}), B-class genes include \textit{APETALA3} (\textit{AP3}) and \textit{PISTILLATA} (\textit{PI}), and the C-class gene is \textit{AGAMOUS} (\textit{AG}) in \textit{A. thaliana}. These genes primarily encode MADS-box transcription factors, except for \textit{AP2} (reviewed by Theissen et al., 2000). The pair of B-class proteins [\textit{AP3} and \textit{PI} in \textit{A. thaliana}; \textit{DEFICIENS} (\textit{DEF}) and \textit{GLOBOSA} (\textit{GLO}) in \textit{A. majus}] interact to form a heterodimer, which performs Class B function (Goto and Meyerowitz, 1994; Jack et al., 1992; Krizek and Meyerowitz, 1996; Riechmann et al., 1996; Schwarz-Sommer et al., 1992; Tröbner et al., 1992). The classical ABC model was extended to the ABCE model after discovering the Class E function \textit{SEP ALLATA} genes in \textit{A. thaliana}; \textit{SEPALLATA} also encodes MADS-box transcription factors (Honma and Goto, 2001; Krizek and Fletcher, 2005; Pelaz et al., 2000, 2001). The E-class proteins form tetrameric complexes with ABC-class proteins to specify floral organs, which is described as the floral quartet model (Theissen and Saedler, 2001; Theissen et al., 2000; Wellmer et al., 2014).

In contrast to higher eudicot flowers, monocot flowers such as lily and tulip often have two whorls of petaloid tepals (Fig. 2B). There are floral homeotic mutants in tulip such as the viridiflora cultivars and the multi-
tepal mutants (van Tunen et al., 1993). In the viridiflora tulip mutant, the stamens are homeotically replaced with carpels and two perianth whorls change to sepaloid or leaf-like structures, which is similar to the Class B mutants in \textit{A. thaliana}. The multitepal tulip mutant has a flower with tepal-like structures in whorls 1–3 and a new flower arising from the center of the flower, which is similar to the Class C mutants in \textit{A. thaliana}. On the basis of the morphologies of wild-type flowers and these mutant varieties, a modified ABC model was proposed for tulip floral development (van Tunen et al., 1993). In this modified model, Class B function is expressed in whorls 1–3, which results in the development of petaloid structures in the outer and inner tepals (Fig. 2B). Although this model lacked molecular and genetic analyses of the mutant varieties, it was widely accepted because it is very simple and consistent with the classical ABC model. B-class genes were subsequently identified in tulip, including two DEF-like genes (\textit{TGDEFA} and \textit{TGDEFB}) and one GLO-like gene (\textit{TGGLO}) (Kanno et al., 2003). \textit{TGDEFA} and \textit{TGDEFB} were expressed in the outer two perianth whorls and the stamen whorl, whereas \textit{TGGLO} was expressed in all floral organs. Both types of B-class gene were expressed in whorls 1–3, and this expression pattern was consistent with the modified ABC model (Figs. 2B and 3A; Kanno et al., 2003). This model was extended further as the modified ABCE model after discovering
Morphological Variation and Molecular Mechanism of Floral Architecture in Monocots

1. B-class genes and tepal development in monocots

1-1) Duplication of B-class genes during angiosperm evolution

The number of MADS-box genes in plant genomes increased during evolution due to extensive gene and genome duplications, which has resulted in the evolution of diverse functions for MADS-box proteins (Airoldi and Davies, 2012; Martinez-Castilla and Alvarez-Buylla, 2003). Two main B-function lineages have been described, which produced paleoAP3 and PI lineages (Kramer et al., 1998; Purugganan et al., 1995). Phylogenetic analyses have demonstrated that this duplication event occurred before diversification of the angiosperms (Kim et al., 2004; Kramer et al., 1998). A later duplication of the paleoAP3 lineage, which produced euAP3 and TM6 lineages, occurred before diversification of the core eudicots (Causier et al., 2010; Kramer et al., 1998). The euAP3- and TM6-lineage genes were maintained and represent subfunctionalization in tomato and petunia (de Martino et al., 2006; Rijpkema et al., 2006). TM6-lineage genes have been
lost during the evolution of *A. thaliana* and *A. majus*; these species have only one type of *euAP3* gene (*AP3* and *DEF*, respectively). The *euAP3* lineage is unique to the core eudicots, whereas core eudicots, lower dicots, and monocots have *paleoAP3/TM6*-lineage genes (Kim et al., 2004; Kramer et al., 1998). Thus, monocots have two types of B-class gene, which belong to *paleoAP3/TM6* and *PI* lineages.

1-2) Lily-type flowers—a perianth with two petaloid whorls

The *paleoAP3/TM6* (DEF-like) and *PI* (GLO-like) lineage genes have been isolated and characterized from lily in addition to tulip. *LMADS1* and *LFDEF* genes belonging to the *paleoAP3/TM6* lineage were isolated from *Lilium longiflorum* and *L. × formolongi*, respectively (Akita et al., 2008; Tzeng and Yang, 2001). *LMADS1* mRNA accumulated in all four whorls and predominantly in the inner tepals and stamens (Tzeng and Yang, 2001), whereas *LFDEF* was expressed in outer and inner tepals and stamens (Akita et al., 2008). The *PI*-lineage genes *LFGLOA/B* and *LMADS8/9* were isolated from *L. × formolongi* and *L. longiflorum*, respectively (Akita et al., 2008; Chen et al., 2012a). *LFGLOA* and *LMADS8* are closely related to *LFGLOB* and *LMADS9*, although *LFGLOB* and *LMADS9* lack C-terminal ends. Phylogenetic analysis demonstrated that duplication of *LFGLOA/LMADS8* and *LFGLOB/LMADS9* occurred after lily and tulip diverged (Akita et al., 2008; Chen et al., 2012a). *LFGLOA/B* and *LMADS8/9* genes were found to be strongly expressed in outer and inner tepals and stamens, whereas only *LFGLOB* was weakly expressed in whorl 4 (Fig. 3B).

The *paleoAP3/TM6* and *PI*-lineage gene expression patterns in lily are in good agreement with the modified ABC model. Transgenic *A. thaliana* ectopically expressing *LMADS1* produces short petals and stamens, whereas ectopic expression of *LMADS1* without the MADS-box region generated an *ap3*-like flower, in which petals were transformed to sepaloid structures and stamens were transformed to carpeloid ones (Tzeng and Yang, 2001). These results provided supporting evidence that *LMADS1* is the functional counterpart in monocots of the eudicot *AP3* gene. By contrast, ectopic expression of *LMADS8* or *LMADS9* in *A. thaliana* resulted in partial conversion of sepals into petaloid structures in whorl 1 and rescued the *pi* mutant phenotype in *pi-1* mutants, indicating that *LMADS8/9* is the functional counterpart in monocots of the eudicot *PI* gene (Chen et al., 2012a). Protein-protein interactions among lily *paleoAP3/TM6* and *PI*-lineage genes were investigated. *LMADS8* and *LMADS9* formed heterodimers with *LMADS1*, although these three proteins also formed homodimers (Chen et al., 2012a). These gene expression and protein function studies indicated that the lily *paleoAP3/TM6* and *PI*-lineage genes have Class B function in outer and inner tepals and stamens, which is in agreement with the modified ABC model.

Further functional analysis is still needed to clarify the function of *LMADS1/8/9* homodimers in lily.

The *paleoAP3/TM6* and *PI*-lineage genes were also identified in and isolated from other monocots with lily-type flowers, including *ApDEF* and *ApGLO* from *Agapanthus praecox* (Nakamura et al., 2005), *AlsDEFa/b* and *AlsGLO* from *Astroemeria ligu* (Hirai et al., 2007), *TriDEF* and *TriaGLO* from *Tricyrtis affinis* (Kanno et al., 2007), and *TrihDEFa/b* and *TrihGLO* from *T. hirta* (Otani et al., in preparation), respectively. The expression of both types of B-class gene from these plants overlaps in whorls 1–3; these expression patterns are consistent with the modified ABC model (Fig. 3C). The expression of *paleoAP3/TM6* and *PI*-lineage genes was also identified in crocus (*Crocus sativus*) and grape hyacinth (*Muscari armeniacum*). Both types of *paleoAP3/TM6* and *PI*-lineage gene from *C. sativus* (*CsatAP3* and *CsatPla/b/c*) and *M. armeniacum* (*MaDEF1/2* and *MaGLOA1/A2/B*), respectively, were expressed in whorls 1–3 (Fig. 3D, E; Kalivas et al., 2007; Kanno et al., 2007; Nakada and Kanno, unpublished data; Nakada et al., 2006; Tsafarlis et al., 2006), similar to that in tulip and lily. However, *CsatAP3* and *CsatPla/b/c* and *MaDEF2* and *MaGLOA1/A2/B* were also expressed in whorl 4. The B-class genes from *C. sativus* and *M. armeniacum* may have some functions in carpel development, although it has not yet been confirmed that these proteins exist in whorl 4. The expression patterns of *paleoAP3/TM6* and *PI*-lineage genes in asparagus (*Asparagus officinalis*; *AODEF* and *AOGLOA/B*, respectively) are not in agreement with the modified ABC model because these genes are expressed only in whorls 2 and 3 and not in whorl 1 (Fig. 3F; Park et al., 2003, 2004). However, an asparagus floral homeotic mutant with two whorls of sepaloid tepals in whorls 1 and 2 and stamens transformed to carpels in whorl 3 has been identified (Fig. 7C; Asada et al., 2006). This floral homeotic mutant is similar to the putative B tulip mutant (van Tunen et al., 1993). This suggests that asparagus floral development is explained by and consistent with the modified ABC model, although B-class gene expression is absent in whorl 1. The molecular mechanism of asparagus floral development was discussed previously (Kanno et al., 2004, 2007). In conclusion, current molecular genetic studies indicate that the modified ABC model is generally applicable to explain the development and morphology of lily-type flowers.

1-3) Orchid flowers

Orchids have highly differentiated zygomorphic flowers (Fig. 4A). The perianth contains two whorls. Three outer tepals in whorl 1 are called sepals, although they are often petaloid and rarely sepaloid. The lateral tepals in whorl 2 are called petals, and the median tepal is called the lip (labellum), which is a highly modified structure. The reproductive organs in whorls 3 and 4 are fused into a gynostemium, which forms a column...
To understand the morphological diversification of orchid perianth, B-class genes specifying petals and stamens in *A. thaliana* and *A. majus* were investigated in many orchid species. Phylogenetic analyses showed that orchid GLO-like genes form a single clade, which is duplicated once in the subfamily Orchidaceae (Pan et al., 2011), and these genes are expressed in all floral organs (Kim et al., 2007; Mondragón-Palomino and Theißen, 2011; Pan et al., 2011; Tsai et al., 2005). By contrast, orchid has four well-supported lineages of DEF-like genes, which belong to Clade 1 to Clade 4 (Fig. 4B; Mondragón-Palomino and Theißen, 2008; Mondragón-Palomino et al., 2009). The DEF-like orchid genes in each clade displayed conserved combinatorial expression patterns associated with the development of sepals, petals, and lip; these findings led to the orchid code model of floral development (Mondragón-Palomino and Theißen, 2008, 2009). The general expression patterns of orchid DEF-like genes are shown in Figure 4B. Outer tepal identity is associated with the combinatorial expression of Clade 1 and 2 genes, and inner lateral tepal identity is defined by lower expression of Clade 3 and 4 genes. The lip identity depends on higher expression of Clade 3 and 4 genes (Mondragón-Palomino, 2013; Mondragón-Palomino and Theißen, 2011).

1-4) Sepal-petal differentiation—the perianth with two dissimilar whorls

Some monocot groups produce flowers with two dissimilar perianth whorls, including *Tradescantia reflexa* (Commelinaceae), *Trillium camingense* (Trilliaceae), and *Habenaria radiata* (Orchidaceae) (Fig. 5). These flowers have greenish outer tepal whorls (sepals) and showy inner tepal whorls (petals). Because species exhibiting this type of flower are distantly related, sepal-petal differentiation in these monocots appears to have evolved independently from that of the ancestral species, which produces flowers containing two petaloid perianth whorls.

To clarify the molecular mechanism of the evolution-
ary transition between sepals and petals within monocots, B-class genes were isolated from these plants. Commelinaceae DEF-like and GLO-like genes were isolated from *T. reflexa* and *Commelina communis* (Ochiai et al., 2004). Gene expression analysis using RT-PCR indicated that both DEF-like and GLO-like genes were expressed in petals and stamens (whorls 2 and 3). However, one of the DEF-like B-class genes was not expressed in sepals (whorl 1), although GLO-like genes were expressed in them. These results indicated that the expression of DEF-like genes in these two Commelinaceae species correlated with sepal-petal differentiation in the outer two whorls (Ochiai et al., 2004), and were consistent with the ABC model of dicot floral development (Fig. 5). One DEF-like and two GLO-like genes were isolated from *H. radiata* (*HrDEF*, *HrGLO1*, and *HrGLO2*, respectively). Gene expression analysis using northern blots and RT-PCR showed that both types of *H. radiata* B-class gene were expressed in inner tepals (white petals and lip), but only GLO-like genes were expressed in outer tepals (greenish sepals) (Kim et al., 2007). The expression pattern of *HrDEF* is similar to that of DEF-like genes in Comme-
1-5) Grass flowers

Important crop plants such as rice and maize belong to the grass family, Poaceae. The floral morphology of these species differs significantly from that of other monocots. Poaceae flowers have stamens and carpels in whorls 3 and 4, respectively, but they lack a well-developed perianth. They have palea and lemma, which are leaf-like organs, instead of sepals in whorl 1, and lodicules, which are scale-like organs, instead of petals in whorl 2. Genetic models for floral development in grass species have been discussed in numerous reviews (for example, Ciaffi et al., 2011; Yoshida and Nagato, 2011). Poaceae B-class genes specify lodicules and stamens (whorls 2 and 3, respectively) rather than palea and lemma (whorl 1). These gene expression patterns in grass species resemble those in Commelinaeae and *H. radiata*, and are consistent with the *A. thaliana* ABCE model of floral development.

2. AGAMOUS-like genes and reproductive organ development in monocots

2-1) Duplication of AGAMOUS-like genes during angiosperm evolution

C-class genes such as *AG* in *A. thaliana* and *PLENA* (*PLE*) in *A. majus* belong to the *AG* subfamily of MADS-box genes. Members of the *AG* subfamily have conserved function for controlling reproductive organ development in gymnosperms and angiosperms. Gene duplication and subfunctionalization events in the *AG* subfamily have been investigated previously (Kramer et al., 2004; Zahn et al., 2006). The angiosperm *AG* subfamily is divided into two clades, which are named C and D lineages (*AG* and *AGL11* lineages, respectively). In general, C-lineage genes have C function, which promotes stamen and carpel identity, whereas most of the D-lineage genes are specifically expressed in ovules (Kramer et al., 2004). A further duplication event occurred in core eudicots, and the C lineage generated the *euAG* and *PLE* lineages. Subfunctionalization and neo-functionalization can be observed in these lineages (Airoldi and Davies, 2012). *Arabidopsis thaliana* *AG* and *A. majus* *PLE* belong to the *euAG* and *PLE* lineages, respectively; therefore, *AG* and *PLE* genes are paralogs rather than orthologs (Kramer et al., 2004; Zahn et al., 2006). By contrast, monocot *AG*-like genes belong to C or D lineages, and further duplication events occurred in grass species as described subsequently in section 2-4.

2-2) Lily-type flowers—a perianth with two petaloid whorls

C- and D-lineage genes were isolated and characterized in *Lilium longiflorum*. Two distinct C-lineage genes, *LLAG1* and *LMADS10*, and one D-lineage gene, *LMADS2*, have been isolated (Benedito et al., 2004; Hsu et al., 2010; Tzeng et al., 2002). *LLAG1* and *LMADS10* were expressed in stamens and carpels, whereas *LMADS2* mRNA accumulated primarily in ovules and weakly in style tissue of the carpel (Benedito et al., 2004; Hsu et al., 2010; Tzeng et al., 2002). Ectopic expression of *LLAG1* and *LMADS10* in *A. thaliana* affected the outer two whorls; sepals were converted into carpel-like structures in whorl 1, and petals were sometimes converted into stamen-like structures in whorl 2 in transformants expressing 35S::*LLAG1* (Benedito et al., 2004), whereas these were occasionally absent in transformants expressing 35S::*LMADS10* (Hsu et al., 2010). The conversion of sepals into carpelloid structures and petals into staminoid structures occurred in *A. thaliana* transformants overexpressing *LMADS2* (Tzeng et al., 2002); therefore, lily D-lineage genes may have stronger effects than C-lineage genes in *A. thaliana* (Hsu et al., 2010). C- and D-lineage genes (*HAG1* and *HoMADS1*, respectively) were also isolated and characterized in *Hyacinthus orientalis* (Li et al., 2002; Xu et al., 2004). *HAG1* was expressed in stamen and carpel, whereas *HoMADS1* mRNA accumulated exclusively in ovules. Overexpression of *HAG1* and *HoMADS1* in *A. thaliana* resulted in carpel-like organs with ovules in whorl 1 and staminoid petals or a petal-less form in whorl 2 (Li et al., 2002; Xu et al., 2004).

C-lineage *AG*-like genes were also isolated from other monocots, including *Asparagus virgatus* (AVAG1), *Crocus sativus* (CsAG1), *Hosta plantaginea* (HpAG/HpSHP), and *Tricyrtis macranthopsis* (*TrimAG*). All of these genes were expressed in stamens and carpels (Rao et al., 2012; Sharifi et al., 2015; Tsafaris et al., 2005; Wang et al., 2012; Yun et al., 2004a), similar to the expression patterns of C-lineage genes from *L. longiflorum* and *H. orientalis*. D-lineage *AG*-like genes were isolated from *A. virgatus* (AVAG2) and *Agapanthus praecox* (*ApMADS2*). *AVAG2* was expressed in stamens and carpels during the early stage of floral development, and expressed in ovules at later stages of floral development (Yun et al., 2004b). *ApMADS2* has not been characterized yet.

The expression patterns of C- and D-lineage genes in lily-type flowers indicated that C-lineage *AG*-like genes function in stamen and carpel development, whereas D-lineage *AG*-like genes function in ovule development. Functional analysis of *Arabidopsis* transformants over-expressing these C- and D-lineage *AG*-like genes showed similar phenotypes. Thus, the functional divergence between these monocot C- and D-lineage genes is not clear. Further genetic and functional analyses using non-grass monocots are needed to clarify these gene functions in monocot flower development.

2-3) Orchid flowers

The male and female reproductive organs of orchids are fused into a single column; therefore, the function of *AG*-like genes was investigated in orchids. Orchid *AG*-like genes were divided primarily into two groups, the C- and D-lineages, similar to other monocot *AG*-like genes. Subsequent duplication occurred among the...
orchid C-lineage genes in Epidendroideae and Orchidoideae, and there are two types of C-lineage genes in Cymbidium ensifolium (CeMADS1 and CeMADS2) and the Phalaenopsis hybrid cultivar ‘Athens’ (PhaMADS8 and PhaMADS10). Gene expression analyses in some orchid species such as Phalaenopsis equestris, C. ensifolium, and Orchis italica showed that the C- and D-lineage genes were expressed primarily in the column and ovaries (Acri-Nunes-Miranda and Mondragón-Palomino, 2014; Chen et al., 2012b; Hsu et al., 2010; Salemme et al., 2013; Skipper et al., 2006; Song et al., 2006; Wang et al., 2011; Xu et al., 2006). The D-lineage gene expression pattern in orchids differs from that of LMADS2 (L. longiflorum) and HoMADS1 (H. orientalis), which were expressed exclusively in ovules (Tzeng et al., 2002; Xu et al., 2004). Although the orchid C- and D-lineage genes are expressed in column and ovaries, the expression level of D-lineage genes is higher in ovaries at late flower development stages in C. ensifolium, O. italica, and Phalaenopsis hybrid (Acri-Nunes-Miranda and Mondragón-Palomino, 2014; Salemme et al., 2013; Wang et al., 2011). These results indicate that orchid C- and D-lineage genes have important roles in column and ovule development. Transcriptional divergence analysis indicated that D-lineage genes rather than C-lineage genes have important functions for ovule development.

2.4 Grass flowers

Monocot AG subfamily genes can be divided into two groups designated as C and D lineages (Kramer et al., 2004; Zahn et al., 2006). Further duplication occurred in these two groups within the grass family (Poaceae). Thus, there are two subclades of OSMADS3 and OSMADS58 in the C lineage and two subclades of OSMADS13 and OSMADS21 in the D lineage (Dreni et al., 2013; Yamaguchi et al., 2006). C-lineage genes (OSMADS3/58) are expressed in the floral meristem just before reproductive organ differentiation, and the expression continues in these organs during development (Dreni et al., 2011; Yamaguchi et al., 2006). The results of gene function studies using mutant and transgenic lines indicate that OSMADS3 and OSMADS58 determine stamen and carpel identity (Dreni et al., 2011; Yamaguchi et al., 2006). The two D-lineage genes of rice have different expression patterns; OSMADS13 expression is restricted to ovules, whereas OSMADS21 is weakly expressed in stamens, carpels, and ovules (Dreni et al., 2007). Ovules of the omsads13 mutant were converted into carpeloid structures, whereas the omsads21 mutant did not show any aberrant phenotype (Dreni et al., 2007). Although the grass family has two types of AG-like genes in the D lineage, only OSMADS13 is likely to be an ovule-identity gene (Dreni et al., 2007; reviewed by Dreni and Kater, 2014).

Poaceae C-lineage genes determine stamen and carpel identity, similarly to those in other dicot and monocot species, but an additional gene [DROPPING LEAF (DL)] has a function for specifying carpel identity. Rice DL belongs to the CRABS CLAW (CRC)/DL subfamily of the YABBY transcription factors, is expressed in the entire carpel primordium, and loss-of-function mutations cause homeotic conversion of carpels to stamens (Yamaguchi et al., 2004). The expression pattern of DL orthologs was found to be conserved in four grass species (rice, maize, wheat, and sorghum); therefore, DL function is likely to be conserved within the grass family (Ishikawa et al., 2009). A DL ortholog was isolated from the monocot Asparagus asparagoides, designated AaDL, and this gene is expressed specifically in the abaxial side of the developing carpels (Nakayama et al., 2010). The abaxial expression pattern and functional role in the carpel were determined to be ancestral characters according to a study in Amborella trichopoda (a sister to other angiosperms) and Eschscholzia californica (California poppy, a basal eudicot); therefore, the acquisition of carpel specification observed in Poaceae might have occurred after the order Asparagoales diverged in monocots (Nakayama et al., 2010).

Molecular Mechanisms Regulating Monocot Floral Mutant Varieties

1. Double-flowered cultivars

The Class C mutant in A. thaliana produces double flowers, in which the stamens are converted to petals and carpels are converted to a new flower. In a double-flower mutant of Japanese morning glory, a transposon insertion was identified in the second intron of the C-class gene DUPLICATED, and most of the gene was deleted due to transposon excision (Nitasaka, 2003). This mutant does not express this C-class gene, and produces flowers in which the stamens and carpels are transformed into petals and a new flower bud, respectively. Petunia expresses two C-class genes, FBP6 and PMADS3. Reduction of the expression of these genes by PMADS3-RNAi in a mutant background (fbp6) results in a double-flowered phenotype (Heijmans et al., 2012). Thus, loss of Class C function causes a double-flowered phenotype in dicot plants.

Many double-flowered cultivars are known in monocots such as lily, tulip, amaryllis, and Trillium. However, molecular studies of double-flowered cultivars are limited in monocots. In wild-type lily (Lilium × formolongi), the C-class gene LFAG is expressed in stamens and carpels (Fig. 6A; Akita et al., 2008). In the double-flowered lily cultivar ‘Aphrodite’, six stamens are converted into six petaloid tepals, and the top of the pistil forms a separated style that appears slightly petaloid. The ‘Aphrodite’ cultivar lost LFAG expression in whorl 3, although LFAG expression remained in the carpel whorl (Fig. 6B; Akita et al., 2008). The double-flowered lily cultivar ‘Elodie’ produces three types of individual with weak, intermediate, and strong phenotypes. Stamens of the strong-phenotype individual are
converted into petaloid tepals, similar to those in the ‘Aphrodite’ cultivar, whereas stamens of the weak-phenotype individual become slightly petaloid in whorl 3. Gene expression analysis of ‘Elodie’ determined that the expression level of AG-like genes is high in weak-phenotype individuals and low in strong-phenotype ones (Akita et al., 2011). These results indicate that the AG-like gene expression level correlates with the phenotype strength, although the molecular mechanism causing reduction of AG-like gene expression in ‘Elodie’ is still unclear.

Tricyrtis species belong to the family Liliaceae and are native to East Asia. Some of these species are popular in Japan as ornamentals for pot and garden uses. Tricyrtis macranthopsis bears yellow flowers with two whorls of identical petaloid organs, similar to the floral architecture of many Liliaceae plants (Fig. 6C). One double-flowered cultivar of T. macranthopsis is known, in which six stamens and three carpels are homeotically converted to petaloid organs and many petaloid tepals arise at the center of the flower, which is similar to the floral architecture of the A. thaliana ag mutant (Fig. 6C). An AG-like gene was isolated recently from T. macranthopsis, designated TrimAG; gene expression analysis showed that TrimAG was expressed in wild-type stamen and carpel, but expression was reduced dramatically in the double-flowered cultivar (Fig. 6C; Sharifi et al., 2015). The TrimAG amino acid sequence in wild-type plants and double-flowered cultivar is identical and there is no significant difference in the promoter and intron sequences, indicating that another factor regulating TrimAG gene expression may be mutated in the double-flowered cultivar (Sharifi et al., 2015).

It is easy to predict that the expression of C-class genes is reduced in double-flowered cultivars. However, double-flowered lily and T. macranthopsis cultivars weakly express full-length C-class mRNA gene transcripts. The molecular mechanism regulating AG gene expression in model plants such as Arabidopsis and Antirrhinum is still unclear. These double-flowered monocot cultivars will be useful for further investigations of the molecular mechanism regulating AG gene expression.

2. Viridiflora cultivars

In general, double-flowered cultivars are more attractive than wild-type flowers because they have more petals or petaloid tepals. Therefore, many double-flowered cultivars have been bred and/or maintained in ornamen-
tal species. In contrast to double-flowered cultivars, viridiflora cultivars have weakly or completely greenish petal/petaloid tepals in their flowers. Ornamental viridiflora cultivars are also in great demand, and viridiflora tulip is one of the best-known cultivars.

Tulip has three B-class genes, two DEF-like genes and one GLO-like gene. Both types of B-class gene are expressed in outer and inner petaloid tepals and stamens in wild-type tulip (Figs. 2 and 7A; Kanno et al., 2003). Viridiflora tulip produces flowers with a greenish stripe in the tepals of whors 1 and 2. The stamen size correlates with phenotype strength; stamen size is equivalent to that of the wild type in the weak phenotype, whereas stamen size is reduced in the strong phenotype. Gene expression analysis showed that the DEF-like gene expression level is lower in viridiflora tulip than in wild-type plants, whereas the GLO-like gene expression level is approximately identical in the viridiflora cultivar and wild-type plants (Fig. 7B; Hirai et al., 2010). Comparative analysis of the B-class gene sequence in wild-type and viridiflora cultivars identified one amino acid change in the MADS-box region of TGDEFB, which may reduce DEF-like gene expression in viridiflora tulip (Hirai et al., 2010). Although the detailed molecular mechanism remains unclear, reduced DEF-like gene expression may cause the viridiflora phenotype. A previous report showed that the strong viridiflora phenotype changes petaloid tepals to greenish sepaloid structures and replaces stamens with carpels (van Tunen et al., 1993), but molecular genetic analyses have not yet been conducted in this viridiflora tulip cultivar. Because the tepal edge in this cultivar is slightly petaloid with a red color, the B-class genes may be expressed very weakly.

Molecular analysis of the strong viridiflora phenotype was conducted in asparagus. In these mutant flowers, six stamens are converted into six carpels surrounding the central pistil, and the outer two tepal whors are changed to greenish sepaloid or leaf-like structures (Fig. 7C; Asada et al., 2006). The phenotypes of this asparagus mutant and the strong viridiflora tulip mutants are very similar. Northern blot analysis was performed, and the transcripts of B-class genes were not or scarcely detected in the asparagus mutant. Genomic Southern blot analyses indicated that there was no significant difference in B-class genes in wild-type plants and viridiflora mutants; therefore, the B-class genes are likely to exist in the asparagus mutant (Asada et al., 2006).

Gene expression analyses of B-class genes in viridi-
flora cultivars indicated that a reduction in B-class gene expression is associated with the viridiflora phenotype. The molecular mechanism regulating the viridiflora mutant has not yet been clarified. Viridiflora mutants will be very useful to clarify the regulatory mechanism of B-class gene expression in monocots.

**Future Prospects**

Monocot floral morphology is generally explained by the modified ABCE model, and B- and C-class genes in monocots are likely to function in the floral organ identity of petals (petaloid tepals) and reproductive organs. Expression analyses of viridiflora and double-flowered cultivars in Liliaceae and asparagus indicated that floral morphologies are likely to be related to the expression levels of B- and C-class genes, respectively.

Nucleic acid sequence information is needed to modify gene expression levels using molecular genetic approaches. Because B- and C-class genes are highly expressed in flower buds, RACE methods can be used to obtain sequence information (Frohman et al., 1988). In addition, next-generation sequencing has been utilized to obtain MADS-box gene sequences (Singh et al., 2013; Su et al., 2013). The sequence information can be used to isolate cDNA clones, and these can be used for plant transformation studies to modify gene expression levels. Although *Agrobacterium*- and/or particle bombardment-mediated transformation techniques are technically challenging in monocot plants, they have been successfully applied in some monocots including *Phalaenopsis*, *Agapanthus*, *Muscaria*, *Alstroemeria*, and *Tricyrtis* (Adachi et al., 2005; Akutsu et al., 2004; Mishiba et al., 2005; Suzuki and Nakano, 2002; Suzuki et al., 2001). These monocot species can be used for studies to modify floral organ development by over-expression or reduction of B- and C-class floral organ identity genes. The chimeric repressor of SRDX has been used successfully to reduce C-class gene expression and produce double-flowered mutants in morning glory and cyclamen (Sage-Ono et al., 2011; Tanaka et al., 2013). Recently, virus-induced gene silencing (VIGS) has been employed to reduce B- or C-class gene expression in ornamental plants such as *Aquilegia*, *Phalaenopsis*, and *Petunia* (Gould and Kramer, 2007; Hsieh et al., 2013; Kramer et al., 2007; Noor et al., 2014). VIGS may be useful to create viridiflora and double-flowered cultivars by reducing B- and C-class gene expression, respectively.

New double-flowered and viridiflora cultivars can be created by modifying B- and C-class gene expression levels. Organ-specific reduction of B- and E-class genes can be used to induce sepal-petal differentiation. Monocot orchid species produce a very attractive tepal called a lip; the molecular mechanism regulating lip development is unclear. The key factor regulating lip development could become an attractive factor for modifying tepal morphology in monocots.

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