MADS-box Genes in Plant Evolution and Development

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ABSTRACT
Evolutionary developmental studies have shown that large transcription factor families underlie key morphological features. The duplication and diversification of these transcription factor families has been important to provide material for the generation of new interacting partners where duplicates could take on a new role or a sub-function of the original protein. Arguably one of the most important of these transcription factor families in plants is the MADS-box family of transcription factors. MADS-box genes are an ancient group found in animals, plants and fungi but have duplicated and diversified much more in plants than in animals and fungi. The plant MADS-box genes can be further sub-divided into Type I, Type II MIKCc and Type II MIKC* MADS-box genes. The Type II MIKCc MADS-box genes are best known for their role in the ABC model of floral organ identity. MIKCc MADS-box genes are also important for flowering time, fruit, endosperm and seed development. There have been many Type II MIKCc studies performed across the land plants however, comparatively little is known about the Type II MIKC* and Type I MADS-box genes. Therefore, recent functional analyses of the previously uncharacterized MIKC* and Type I MADS-box genes are particularly exciting. Recent genome analyses have shown that MIKC* and Type I MADS-box genes can also be found in Physcomitrella patens and Selaginella moellendorffii. Phylogenetic, functional and expression analyses of MADS-box genes across the land plants will provide insight into the role of all types of MADS-box genes in the evolution and development of land plant body plans.

Keywords: body plan, duplication, diversification, regulatory networks
Abbreviations: aa, amino acid; AGL, AGAMOUS-LIKE; Arabidopsis, Arabidopsis thaliana; CARG box, CC(A/T)6GG motif bound by MADS-box proteins; evo-devo, evolution and development; MEF2, myocyte enhancer factor-2; SRF, serum response factor

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INTRODUCTION
Large families of transcription factors have been the focus of evolutionary developmental (evo-devo) studies. In animals, the duplication and loss of members of the large homeobox (HOX) transcription factor family have been implicated in the evolution and development of diverse animal body plans (Swalla 2006). The animal regulatory protein paired box gene 6 (PAX6) has been co-opted multiple times during eye evolution to specify a light gathering cell that has then been elaborated into multiple different types of eyes (Gehring 2005). These studies in eye evolution and development also illustrate that complex organs such as eyes do not arise de novo but are built using existing building blocks that are co-opted for new or modified functions. In addition, transcription factors are usually part of a large regulatory network with each protein having several interacting partners (Davidson et al. 2002). These proteins are usually composed of multiple domains. The domains within the protein determine the interactions of these proteins within regulatory networks and the addition or loss of domains can modify the interactions within the regulatory network (Galant and Carroll 2002; Veron et al. 2007). The animal MADS-box protein myocyte enhancer factor-2 (MEF2) is part of a core regulatory network with other transcription factors and signaling proteins that directs heart development (Olson 2006). The duplication and diversification of the MEF2 regulatory network has been important for the evolution and development of the heart from a simple pump to a complex 4-chambered muscle. The duplication and div-
erisification of transcription factor families has been important to provide material for the generation of new interacting partners where duplicates could take on a new role or a sub-function of the original protein.

One of the best-known regulatory families underlying plant development is the MADS-box family of transcription factors. This family has been particularly well studied for its role in the ABC model of floral organ specification (Klein et al. 2000). However, the MADS-box gene family in the flowering plants (Parenicova et al. 2003). The complete MADS-box gene family is now known from the genomes of the flowering plants Arabidopsis thaliana (Arabidopsis), Oryza sativa (rice) and Populus trichocarpa (poplar) (AGI 2000; Goff et al. 2002; Yu et al. 2002; Parenicova et al. 2003; Leseger et al. 2006; Tuskan et al. 2006; Arora et al. 2007). Functional studies in Arabidopsis have shown that MADS-box genes have important roles in development besides floral organ specification (Theissen et al. 2000). In addition, the recent completion of the land plant genomes from the bryophyte, Physcomitrella patens and the lycophyte, Selaginella moellendorffii, has provided insights into the evolution of the MADS-box family of transcription factors and will be integral for future studies on the role of MADS-box genes in land plant evolution and development (Rensing et al. 2008).

There have been extensive studies on MADS-box genes in the land plants but these have focused on the MIKCC type of MADS-box gene, particularly the floral organ identity genes in angiosperms. These Type II MIKCC MADS-box genes have been identified across the land plants although little is known of their function outside of the angiosperms (Munster et al. 1997; Hasebe et al. 1998; Winter et al. 1999; Krogan and Ashton 2000; Carlsecker et al. 2003; Jager et al. 2003; Tanabe et al. 2003). I will briefly review the background of these important Type II MIKCC MADS-box genes with particular emphasis on functional characterization and protein-protein-interactions. These analyses illustrate how duplications and diversifications within the MIKCC group may provide starting material for the elaboration of key morphological features. Recent functional analyses of the previously uncharacterized Type II MIKCC* and Type I MADS-box genes are particularly exciting. Recent genome analyses have shown that MIKCC* and Type I MADS-box genes can also be found in mosses and lycophytes. Comparative expression analyses, phylogenetic analyses, functional analyses and protein-protein interaction studies will elucidate the function of the MIKCC* and Type I MADS-box genes and provide insight into their contribution to land plant evolution and development.

**MADS-BOX TRANSCRIPTION FACTORS**

**Antiquity of the MADS domain**

The MADS-box family of transcription factors is an ancient family of proteins found in animals, plants and fungi (Alvarez-Buylla et al. 2000; Carlsbecker et al. 2000). MADS domain proteins were first identified in Arabidopsis from the first 4 founding members of this family: Mini Chromosome Maintenance from yeast, AGAMOUS from Arabidopsis, DEFICIENS from Antirrhinum majus and Serum Response Factor from humans (Norman et al. 1988; Passmore et al. 1988; Schwarz-Sommer et al. 1990; Sommer et al. 1990; Yanofsky et al. 1990). The MADS domain is typically found at the N terminus of the protein. An ancient duplication in the last common ancestor of these 3 eukaryotic kingdoms is proposed to have given rise to 2 lineages of MADS-box proteins: Type I and Type II (Fig. 1) (Alvarez-Buylla et al. 2000). Animal, plant and fungal Type I and Type II MADS-box proteins were first distinguished by amino acid synapomorphies in the MADS DNA binding domain. Type I MADS-box proteins have an SRF-like MADS domain while Type II MADS-box proteins have a MEF2-like MADS domain (Shore and Starrcks 1995). There are no other conserved domains outside of the MADS domain shared between animals, plants and fungi (Alvarez-Buylla et al. 2000).

In plants, Type I and Type II MADS-box genes can be further distinguished by the protein domains C-terminal to the MADS domain and the intron-exon structure of Type I and Type II genes (Fig. 1) (Alvarez-Buylla et al. 2000; Parenicova et al. 2003). The plant Type I MADS-box proteins, or MIK proteins, have a well-defined structure (Alvarez-Buylla et al. 2000; Kaufmann et al. 2005). In addition to the MADS domain, there is an approximately 70 amino acid K domain recognized by its regularly spaced hydrophobic residues that is proposed to form a coiled-coil structure (Ma et al. 1991; Davies and Schwarz-Sommer 1994; Riechmann and Meyerowitz 1997). The MADS and K domains are divided by a less well-conserved intervening region or I region of variable length. At the C terminus of the protein is the aplyl named C domain. The K domain is involved in protein-protein interactions while the I region may confer some specificity to the protein-protein interactions. The function of the C region is less well defined and has been suggested that this region is important as a trans-activation domain and has been shown to be important for ternary complex formation (Riechmann and Meyerowitz 1997; Egea-Cortines et al. 1999). The protein domains C-terminal to the MADS-domain in Type I are less well defined however, shared motifs of unknown function have been recognized in some of these proteins (Parenicova et al. 2003). Type I MADS-box genes are composed of one or two exons while Type II MADS-box genes are composed of 5-11 exons (Kaufmann et al. 2005).

Phylogenetic and sequence analyses in Arabidopsis and the moss, Physcomitrella patens have revealed that the Type I and Type II MADS-box proteins can be further subdivided based on sequence and/or shared motifs (Henschel et al. 2002; Parenicova et al. 2003). The Type II MADS-box genes can be further subdivided into MIKCC classic (MIKCC) and MIKCC based on the intron-exon structure of their coding sequences (Henschel et al. 2002; Kofufi et al. 2003). The MIKCC MADS-box genes have a longer I domain encoded by multiple exons instead of 1 exon (Henschel et al. 2002). MIKCC and MIKC* (also known as M6) MADS-box genes can also be distinguished by phylogenetic analyses using the MADS domain alone (Parenicova et al. 2003). The Type I MADS domain proteins were divided into 3 groups: MI, MII and MII, based on phylogenetic analyses of the MADS domain and some conserved motifs in the C-terminal region (Parenicova et al. 2003).

**MIKC CLASSIC MADS-BOX GENES**

**The role of MIKCC MADS-box genes in flower development**

MIKCC MADS-box genes are the best-characterized MADS-box genes in any land plant and are well known for their role in flower development (Krizek and Fletcher 2005). The ABC model of floral organ identity describes how dif-
different combinations of MADS-box proteins are necessary for specifying the 4 floral organ identities sepals, petals, stamens and carpels (Bowman et al. 1989; Meyerowitz et al. 1991). A loss of A, B or C class gene function results in the homeotic conversion of floral organs and a loss of all ABC functions in Arabidopsis results in a flower composed entirely of leaves. Although there is a large amount of floral diversity in the angiosperms, all flowers have the same basic ground plan of sepals, petals, stamens and carpels. Comparative studies across the angiosperms have shown that the ABC model of floral organ identity is largely conserved across the 2 major groups of angiosperms: monocots and eudicots (Bowman 1997; Ambrose et al. 2000). Network analyses have also shown that the ABC model of floral organ identity is a modular gene regulatory network providing theoretical support for the conservation of the ABC model of floral organ identity (Mendoza et al. 1999; Espinosa-Soto et al. 2004). Morphological, functional and theoretical analyses on floral organ development suggest that the basic ground plan of the flower is relatively conserved due to developmental drive.

Fig. 1 The evolution of MADS-box proteins in the land plants. The Type I (M) and Type II (MIKC) protein structures are mapped onto a generalized land plant phylogeny. The Type I and Type II MADS-box proteins have similar DNA binding MADS (M) domain but differ in the domains 3’ to the M domain. The C-terminal domain of the Type I proteins is not well defined. The region 3’ to the MADS domain of Type II proteins have 3 recognizable domains: I, K and C. There are 2 kinds of Type II MADS-box proteins: MIKCc and MIKC* and 3 kinds of Type I MADS-box proteins: Mα, Mγ and Mβ. An ancient duplication gave rise to the Type I and Type II lineages so both types of proteins should be found in the green plants although only MIKCc have been isolated from the charophycean green algae. MIKCc MADS-box proteins have been identified throughout the land plants. Genomic and phylogenetic analyses have identified MIKC* in the bryophyte Physcomitrella patens, the lycophyte Lycopodium annotinum and the angiosperms Arabidopsis thaliana, Oryza sativa and Populus trichocarpa suggesting that MIKC* MADS-box proteins were present in the last common ancestor of bryophytes and vascular plants. Mα MADS-box genes have been identified in the bryophyte P. patens, the lycophyte Selaginella moellendorffii and the angiosperms A. thaliana, O. sativa and P. trichocarpa also suggesting that Mα MADS-box proteins were present in the last common ancestor of bryophytes and vascular plants. My MADS-box proteins have been identified in the lycophyte S. moellendorffii and the angiosperms A. thaliana, O. sativa and P. trichocarpa suggesting that Mγ MADS-box proteins were present in the last common ancestor of vascular plants. Mβ proteins have only been identified in the angiosperms A. thaliana, O. sativa and P. trichocarpa.
Duplication and diversification of MIKCc clades

MIKCc MADS-box genes have been isolated from charophycean green algae that are sister to all land plants, and ancient duplications and subsequent diversifications have given rise to distinct clades (Karol et al. 2001; Tanabe et al. 2005; Riano-Pachon et al. 2008). Phylogenetic analyses of MIKCc MADS-box genes indicates that there are 12 clades: A, B, C, D, E, F, G, H, I, J, K, and L, containing at least 7 out of these 12 clades existed in the last common ancestor of land plants (Veron et al. 2007). Duplication and diversification within the A clade in flowering plants has given rise to CAULIFLOWER (CAL) and FRUITFULL (FUL) in Arabidopsis (Purugganan et al. 1995). CAL is functionally redundant with the A class gene APETALA1 (API) in specifying floral meristem identity (Kempin et al. 1995). While FUL has a role in floral meristem identity it also has diversified into a new role in fruit differentiation (Gu et al. 1998; Ferrandiz et al. 2000). Within the C clade, duplications have given rise to two functionally redundant proteins SHATTERPROOF 1 and 2 (SHP1 and SHP2) that are important for patterning the fruit (Purugganan et al. 1995; Liljegren et al. 2000). These functional studies in Arabidopsis have illustrated the importance of duplication and diversification of MADS-box proteins in plant evolution and their roles in flower development. Across the angiosperms, duplications within the A, B and C floral organ identity clades have also occurred and have important functional implications (Litt and Irish 2003; Kramer et al. 2004; Irish and Litt 2005). Not only the presence of particular clades but their regulatory networks have been important for morphological evolution at least within the angiosperms.

MIKC* MADS-BOX GENES

Antiquity of MIKC* MADS-box genes

MIKC* MADS-box genes were first identified in the moss Physcomitrella patens (Henschel et al. 2002). Phylogenetic analyses at the time indicated that there was only one other MIKC* MADS-box protein, LAMB1, identified from the lycophyte, Lycopodium annotinum (Svensson et al. 2000; Henschel et al. 2002). Phylogenetic analyses of MADS-box genes from Arabidopsis revealed the presence of 6 MIKC* MADS-box genes (AGL30, AGL65, AGL66, AGL67, AGL94 and AGL104) present in the genome (AGI 2000; Parenicova et al. 2003). The identification of MIKC* MADS-box genes in bryophytes, lycophytes and angiosperms indicates that an MIKC* MADS-box gene was present in the last common ancestor of bryophytes and flowering plants (Fig. 1).

Role of MIKC* MADS-box genes in plant development

The role of MIKC* MADS-box genes have recently been studied in Arabidopsis. Transcriptome analyses and RT-PCR have shown that MIKC* MADS-box genes are preferentially expressed in Arabidopsis pollen (Kofuji et al. 2003; Honys and Twell 2004). MIKC* MADS-box genes bind a particular CArG box motif that is predominantly found in late stage pollen specific genes (Verelst et al. 2007). MIKC* mutants have reduced pollen germination that results in reduced fertility (Verelst et al. 2007; Adamczyk and Fernandez 2009). AGL104 is expressed is detected throughout pollen development from the single nuclear stage to the deposition of the sperm cells in the female gametophyte (Reddy and Ambrose, unpublished data). MIKC* MADS-box genes form 2 monophyletic subgroups P (AGL30, AGL65 and AGL94) and S (AGL66, AGL67 and AGL104) (Nam et al. 2004; Adamczyk and Fernandez 2009). Yeast-2-hybrid and bimolecular fluorescence complementation protein interaction studies have shown that MIKC* MADS-box genes form heterodimers and
heterodimers are only formed between members of the S and P MIKC* groups in Arabidopsis (de Folter et al. 2003; Verelst et al. 2007; Adamczyk and Fernandez 2009). Functional analyses have shown that there is functional redundancy within the S and P MIKC* subgroups (Verelst et al. 2007; Adamczyk and Fernandez 2009). Recent studies have also shown that these MIKC* MADS-box genes are part of a large pollen regulatory network, that is composed of additional MIKCc genes, particularly Type I MADS-box genes (Verelst et al. 2007; Adamczyk and Fernandez 2009). Therefore similar to MIKCc MADS-box gene duplications, MIKC* duplicates maintain their interaction partners. It is not known whether maintenance of S and P MIKC* interactions has facilitated the neo- or sub-functionalization of Arabidopsis MIKC* paralogs.

There are 23 MADS-box genes in the Physcomitrella patens genome and astonishingly 12 of these are MIKC* (Rensing et al. 2008). RT-PCR demonstrated that 2 MADS-box genes, PPM6 and PPM7, are expressed throughout Physcomitrella patens development but are more highly expressed in protonemata compared to gametophores and sporophytes (Riese et al. 2005). Phylogenetic analyses of Arabidopsis and P. patens MADS-box genes are necessary to determine if the P. patens MIKC* MADS-box genes cluster with the Arabidopsis S and P MIKC* subgroups and whether there are novel subgroups in P. patens. Although there is little information on MIKC* MADS-box genes in P. patens, it is enticing to hypothesize on an ancestral function of MIKC* MADS-box genes in land plants. In Arabidopsis, MIKC* MADS-box genes are important for pollen tube germination and growth. In Arabidopsis, the pollen grain is the male gametophyte that germinates and grows by tip growth. Bryophytes, as represented by P. patens, have a dominant gametophyte life cycle and some cells of the gametophyte such as the protonemata grow by tip growth (Menand et al. 2007). Functional analyses of MIKC* orthologs in P. patens will allow us to determine whether MIKC* MADS-box genes had an ancestral function in tip growth, gametophyte development or specifically in male reproductive development.

**TYPE I MADS-BOX GENES**

Until recently, the only MADS-box genes that had been functionally characterized in any land plant species were the Type II MIKCc MADS-box genes and expression and phylogenetic analyses of Type I MADS-box genes suggested that these were non-functional (De Bodt 2003; Kofuji 2003). Genomic analyses in Arabidopsis indicated a higher birth and death rate of Type I MADS-box genes which means there was rapid duplication of these genes but these duplicates were not maintained (Nam et al. 2004). Phylogenetic analyses also suggested that Type I and Type II MADS-box genes have evolved differently (De Bodt et al. 2003; Nam et al. 2004). Closely related Type I MADS-box genes are clustered on the Arabidopsis chromosomes indicating that these were generated by recent segmental duplications as opposed to whole genome duplications (De Bodt et al. 2003; Nam et al. 2004; Veron et al. 2007).

**Type I functional analyses in Arabidopsis**

Recently, 5 Type I MADS-box proteins have been functionally characterized: AGL23, AGL28, AGL61 DIANA (DIA), AGL62 and AGL80 (FEM111) (Portereiko et al. 2006; Yoo et al. 2008; Colombo et al. 2008; Kang et al. 2008; Steffen et al. 2008). These characterized Type I MADS-box proteins belong to 2 of the 3 sub-groups of Type I MADS-box proteins. AGL23, AGL28, DIA and AGL62 belong to the Ma subgroup while FEM111 belongs to the My subgroup (Parenicova 2003). Over-expression analyses of AGL28 suggest that it promotes flowering while functional analyses of AGL23, DIA, AGL62 and FEM111 show that they all have a role in female gametophyte and/or endosperm development (Portereiko et al. 2006; Yoo et al. 2006; Bemer et al. 2008; Colombo et al. 2008; Kang et al. 2008; Steffen et al. 2008).

AGL23::GUS is expressed in the functional megasporo- genome and maintains until female gametophyte development is complete (Colombo et al. 2008). After fertilization, AGL23:: GUS expression is detected in the embryo and endosperm until the torpedo stage of embryogenesis (Colombo et al. 2008). Loss-of-function analyses indicate that AGL23 is necessary for the proper progression of megagametogenesis (Colombo et al. 2008). FEM111-GFP expression is detected in the central cell and the endosperm before cellularization and in fem111 the central cell does not develop properly and subsequent development of the endo- sperm does not occur (Portereiko et al. 2006). DIA is also expressed in the central cell and endosperm (Bemer et al. 2008; Steffen et al. 2008). The dia central cell does not develop properly and subsequent endosperm development does not occur (Bemer et al. 2008; Steffen et al. 2008). Ectopic expression of female gametophyte synergid and antipodal marker genes in dia central cells suggest that the dia central cell identity is mis-specified (Steffen et al. 2008). Protein-protein interaction studies demonstrated that DIA and FEM111 interact (Bemer et al. 2008; Steffen et al. 2008). AGL62 expression is detected in the developing endosperm until the cellularization stage (Kang et al. 2008). In agl62, the endosperm cellularizes prematurely suggesting that AGL62 is important for the timing of endosperm cellularization (Kang et al. 2008).

**The role of Type I MADS-box genes in land plant development**

It appears that no Type I mutants were identified previously by forward genetic screens as they are lethal or affect discrete developmental processes (Portereiko et al. 2006; Bemer et al. 2008; Colombo et al. 2008; Kang et al. 2008; Steffen et al. 2008). Expression analyses suggest that additional Type I MADS-box genes are involved in gametophyte and/or endosperm development. Transcriptome analyses show that of the MADS-box genes preferentially expressed in the endosperm, a majority are Type I MADS-box genes (Day et al. 2008). Expression analyses also indicate a role for the My MADS-box gene PHERESI (AGL37) in endosperm development (Kohler et al. 2003). Expression studies have also shown that Type I Ma MADS-box genes, AGL29 and AGL97, are expressed in pollen (male gameto- phyte) (Adamczyk and Fernandez 2009). More recent expression analyses show that the Type I genes PHERESI, PHERES2, AGL35, AGL36, AGL40, AGL62 and AGL90 are upregulated in incompatible interspecific crosses (Walia et al. 2009). These results indicate that the down regulation of these genes is necessary for proper seed development.

Although there are no defined domains C-terminal to the MADS domain of Type I proteins, there are conserved motifs that suggest that these regions could act as transcription activation domains or form coiled-coil structures (De Bodt et al. 2003; Parenicova et al. 2003). Protein-protein interaction and genetic studies show that Type I Ma and My proteins preferentially form heterodimers (de Folter et al. 2005; Bemer et al. 2008; Steffen et al. 2008). Functional analyses of the domain C terminal to the MADS domain in Type I proteins and additional protein interaction studies will help to determine if Type I proteins are part of large regulatory networks. Comparative functional analyses of Type I MADS-box proteins across the land plants may help to explain why Type I MADS-box proteins appear to have distinct evolutionary trajectories.

The recent completion of the bryophyte genome, P. patens, and the lycopod, S. moellendorffii, has provided some insight into the evolution of Type I MADS-box genes in land plants (Fig. 1) (Rensing et al. 2008). Preliminary analyses indicate that only the Ma subgroup is present in P. patens while the Ma and My subgroups are found in S. moellendorffii genome (Ambrose, unpublished data). These preliminary analyses indicate that the Ma Type I MADS-
box genes can be found in the most recent common ancestor of bryophytes and vascular plants while the Mγ can be found in the common ancestor of the vascular plants. Additional genome sequences and comprehensive phylogenetic analyses are needed to assess the evolution of the Type I MADS-box genes across the land plants. Functional analyses in *P. patens* will help to determine the ancestral function of Mα MADS-box genes and interaction and functional studies need to be performed to determine if Mα and Mγ also preferentially form heterodimers in *S. moellendorffii*. In Arabidopsis, Mα and Mγ proteins preferentially interact and are important for female gametophyte and/or endosperm development. Functional analyses will help to elucidate if a Mα - Mγ regulatory module is present in lycophytes and whether this regulatory module was important for subsequent seed evolution.

MADS-box proteins are conserved genes across the land plants, and their role in the regulation of organ growth and development, Functional analyses will help to elucidate if a Mα - Mγ regulatory module was important for subsequent seed evolution. Functional analyses will help to elucidate if a Mα - Mγ regulatory module was important for subsequent seed evolution. Functional analyses will help to elucidate if a Mα - Mγ regulatory module was important for subsequent seed evolution. Functional analyses will help to elucidate if a Mα - Mγ regulatory module was important for subsequent seed evolution.

**CONCLUDING REMARKS**

Large regulatory networks underlie morphological features and regulatory networks evolve by the loss and gain of protein domains (Veron et al. 2007). MADS-box proteins are modular in organization although Type I and Type II MADS-box genes differ in the domains C-terminal to the MADS domain. Type II MADS-box genes are necessary for the specification of modular structures such as flowers in angiosperms and fruits in Arabidopsis (Krizek and Fletcher 2005). The duplication and subsequent diversification of Type II MADS-box genes is facilitated by the higher order complexes that it forms (Veron et al. 2007; Lesebeg 2008). Although, Type I MADS-box genes, by definition, do not have a recognizable I or K domain, regions necessary for protein-protein interactions in Type II MADS, there are conserved domains that could form coiled-coil structures (Parenicova et al. 2003). Protein-protein interaction studies and genetic studies show that Type I proteins do form heterodimers and these heterodimers are important for their function (de Folter et al. 2005; Bemer et al. 2008; Struebig et al. 2008). Functional analyses indicate that Type I MADS-box genes are also important for specifying another modular structure, the female gametophyte (Friedman and Williams 2003). Phylogenetic studies indicate that Type I and Type II proteins have evolved differently (De Bodt et al. 2003; Nam et al. 2004; Veron et al. 2007). Type II regulatory networks have facilitated the diversification of this group of MADS-box genes. Functional and interaction analyses of Type I proteins should reveal the role of regulatory networks in the evolution of MADS-box proteins and the morphological innovations they underlie. A majority of the MADS-box genes that have been functionally characterized are Type II MIKC. Surprisingly not all of the Type II MIKC genes have been functionally characterized even in Arabidopsis. Recent functional analyses of an Arabidopsis B-sister MADS-box gene, *GORIDTA*, has shown a novel role for a MADS-box gene in the regulation of organ growth (Pressel et al. 2010). There have been many Type III MADS-box genes studied across the land plants however, little is known about the Type II MIKC and Type I MADS-box genes. To understand the role of MADS-box genes in the land plants, genetic functional systems need to be developed not only in additional angiosperm species but also in lycophytes, fern and gymnosperm species. Broad comparative functional analyses of Type II MIKC and Type I MADS-box proteins across the land plants will help to elucidate their role in the evolution and development of land plants.

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