Journal of Experimental Botany, Vol. 57, No. 13, pp. 3433–3444, 2006
Major Themes in Flowering Research Special Issue
doi:10.1093/jxb/erl097 Advance Access publication 12 September, 2006

Journal of Experimental Botany

Functional conservation of MADS-box factors controlling floral organ identity in rice and *Arabidopsis*

Martin M. Kater[1,*], Ludovico Dreni[1] and Lucia Colombo[2]

[1] *Dipartimento di Scienze Biomolecolari e Biotecnologie, Università degli Studi di Milano, Via Celoria 26, I-20133 Milano, Italy*

[2] *Dipartimento di Biologia, Università degli Studi di Milano, Via Celoria 26, I-20133 Milano, Italy*

Received 14 March 2006; Accepted 26 June 2006

Abstract

Studies on MADS-box genes in *Arabidopsis* and other higher eudicotyledonous flowering plants have shown that they are key regulators of flower development. Since *Arabidopsis* and monocotyledonous rice are distantly related plant species it is interesting to investigate whether the floral organ identity factors have been conserved in their functions, and if not, to understand the differences. *Arabidopsis* and rice are very suitable for these studies since they are both regarded as models for plant functional genomics. Both their genomes are sequenced and tools are available for the analysis of gene function. These developments have accelerated experiments and increased our knowledge on rice gene function. Therefore it is the right moment to perform a comparative analysis on MADS-box factors controlling floral organ identity as reported in this review.

Key words: ABCDE model, *Arabidopsis thaliana*, evolution, flower development, MADS-box genes, *Oryza sativa*.

Introduction

The lineages that led to the eudicot *Arabidopsis* and the monocot rice separated, according to molecular data, about 150 million years ago, which is quite early during flowering plant evolution (Theissen *et al*., 2000). Therefore, the analysis of functional conservation of orthologous MADS-box genes that control flower development in these two species promises to give a good impression of conservation and divergence of these floral regulators throughout most of the flowering plants. *Arabidopsis* and rice are typically chosen for these kinds of analyses since they are both considered to be most important model plants. The tiny weed *Arabidopsis thaliana* has served as a model plant for quite some time now. This is due to the fact that *Arabidopsis* is diploid, has a short life-cycle (from seed to seed under optimal conditions in 6–8 weeks), does not need much greenhouse space, has a large offspring (hundreds of seeds per plant), a small genome (130 Mb), and is easy transformable. These advantages have made *Arabidopsis* a favourite species and many tools such as T-DNA-, transposon insertion- and EMS mutagenized-populations have been developed to study gene function. Furthermore, since the start of this millennium the complete annotated genome sequence is available which has further boosted reverse genetic approaches (*Arabidopsis* Genome Iniative, 2000).

Rice (*Oryza sativa*) is a model 'crop' rather than just a model 'plant' since it is the major staple food source for half of the world's population. Moreover, rice is relatively closely related to all the other agronomically important cereal grasses including wheat, maize, barley, rye, oat, and sorghum which makes it a reference plant for the world's most important crop plants. The rice genome, which has been almost completely sequenced as well (Goff *et al*., 2002; Yu *et al*., 2002), is about four times larger than that of *Arabidopsis* (430 Mb), but is much smaller than those of other cereals such as maize (2500 Mb), barley (5500 Mb), oat (11 300 Mb), and wheat (16 000 Mb). Besides a relatively small genome size, some rice varieties also have a rather short life-cycle of about 4 months when grown under specific greenhouse conditions (high density and small pots). Furthermore, very efficient transformation protocols are available for both subspecies *Oryza sativa* ssp. *indica* and *Oryza sativa* ssp. *japonica*. These features have resulted in the adoption of rice as a model system

* To whom correspondence should be addressed. E-mail: martin.kater@unimi.it

and, in the last few years, powerful tools for functional genomics have been developed.

Plants initially form only vegetative organs. When the plant reaches a certain developmental stage and environmental conditions like temperature and day-length are favourable, they start to flower. This process is called the floral transition. *Arabidopsis* plants flower under long-day conditions whereas rice is typically a short-day plant. Despite these differences in day length requirements the genetic mechanisms controlling photoperiodic flowering in rice and *Arabidopsis* appear to be closely related (recently reviewed by Hayama and Coupland, 2004). After the floral transition, the shoot apical meristem initiates the production of inflorescence meristems from which floral meristems develop. From these floral meristems the floral organs develop. The structure of *Arabidopsis* and rice flowers is markedly different, although for the reproductive organs, i.e. stamens and carpels, homology between these two species is evident (Fig. 1). For the sterile perianth organs the common origin is still quite controversial and under discussion. The *Arabidopsis* flower consists of four concentric whorls comprising (from the outer to the inner whorl) four green sepals, four white petals, six stamens, and, in the centre, two fused carpels in which the ovules develop. An individual rice flower consists of one single carpel at the centre of the flower, six stamens, and two lodicules which are enclosed by two leaf-like structures the lemma and palea. These organs together form a floret. Palea and lemma might be homologues of eudicot sepals, alternatively, the palea may represent a prophyll and the lemma a true bract. The lodicules are small glandular organs that swell at anthesis to spread the palea and lemma apart to open the flower for wind-pollination. Two extra bracts called upper and lower sterile glumes envelop a floret, thus constituting a structure called a spikelet.

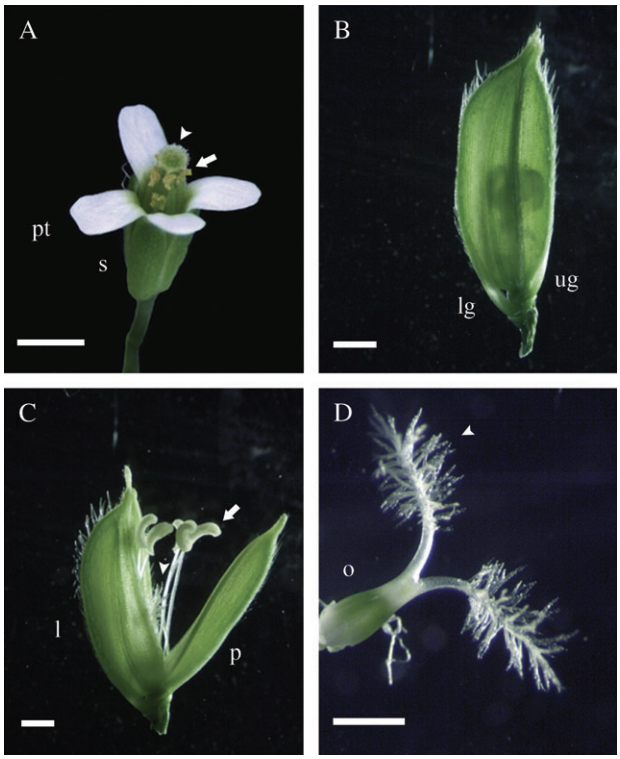

Fig. 1. *Arabidopsis* and rice wild-type flowers. (A) *Arabidopsis* flower showing all four floral whorls; (B) mature rice flower; (C) open rice flower showing the inner whorl organs; (D) rice pistil. s, Sepals; pt, petals; lg, lower glume; ug, upper glume; l, lemma; p, palea; o, ovary. Arrows and arrowheads indicate anthers and stigmas, respectively. Bar length represents 1 mm.

The analysis of floral homeotic mutants in *Arabidopsis* and *Antirrhinum majus* resulted in the beginning of the nineties in the formulation of the genetic ABC model which predicts that the combinatorial action of three classes of homeotic floral organ identity genes determine the identity of the four floral organ types: sepals, petals, stamens, and carpels (Coen and Meyerowitz, 1991) (Fig. 2). The model predicts that class A genes specify sepals in the first floral whorl, class A and B genes specify petals in the second whorl, class B and C genes specify stamens in the third whorl, and class C genes specify carpels in the inner fourth whorl. Furthermore, the model maintains that class A and C genes are mutually antagonistic, this means that, in the absence of A, C activity is present throughout the flower and likewise, in the absence of C, A activity is present throughout the flower. Class C genes also have a function in meristem determination since class C mutants are indeterminate in whorl 4 forming a new class C mutant flower instead of carpels. Cloning of these class A, B, and C organ identity genes (in the beginning mainly from *Arabidopsis* and *Antirrhinum*) showed that these mainly encode MADS-box transcription factors. In *Arabidopsis*, class A genes comprise *APETALA1* (*AP1*) and *APETALA2* (*AP2*), of which the latter is not encoding a MADS-box but an AP2 DNA binding domain. Class B genes are represented by *APETALA3* (*AP3*) and *PISTILLATA* (*PI*) and the class C gene is *AGAMOUS* (*AG*).

In recent years the ABC model has been extended with two classes of genes, named class D and E genes (Fig. 2). Class D genes control ovule identity and were first identified in *Petunia* were they have been termed *FLORAL*

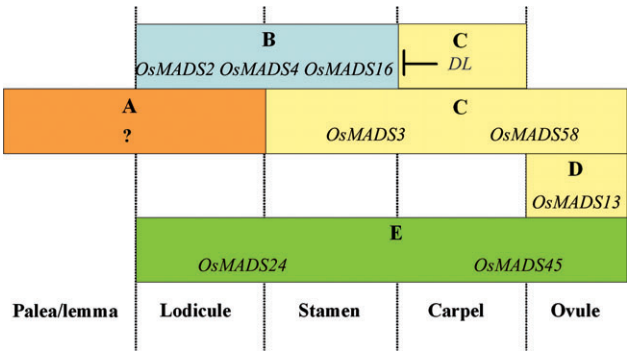

Fig. 2. ABCDE model for rice.

BINDING PROTEIN 7 (*FBP7*) and *FBP11* (Angenent and Colombo, 1996). These two genes are both necessary and sufficient to determine ovule identity in *Petunia* flowers, since cosuppression of both genes caused loss of ovule identity whereas ectopic expression resulted in ectopic ovule formation on sepals and petals. In *Arabidopsis* the class D gene is *SEEDSTICK* (*STK*), which is like *FBP7* and *FBP11* specifically expressed in ovules (Pinyopich *et al*., 2003; Favaro *et al*., 2003).

In *Arabidopsis* class E genes or *SEPALLATA* (*SEP*) genes consist of four members, *SEP1*, *SEP2*, *SEP3*, and *SEP4*, encoding MADS-box factors that show partial redundant functions in floral organ identity determination. The triple knock-out *sep1 sep2 sep3* has indeterminate flowers with petals, stamens, and carpels homeotically transformed into sepals. Class B and C expression was not altered in the *sep* triple mutant which shows that *SEP* genes do not act down-stream of B and C genes and that they are not required for the activation of these genes (Pelaz *et al*., 2000).

Recently, a *sep1 sep2 sep3 sep4* quadruple mutant was described in which all floral organs were transformed into organs similar to leaves (Ditta *et al*., 2004). These results show that the *SEP* genes are necessary for the function of class A, B, and C genes since the quadruple *sep1 sep2 sep3 sep4* mutant phenocopies the *abc* triple mutant.

The molecular basis for the genetic relation among floral organ identity genes has been explained by experiments of Honma and Goto (2001) and Pelaz *et al*. (2001). They addressed the question whether the expression of combinations of class A, B, C, and *SEP* genes would be sufficient to convert leaves into floral organs. They generated transgenic *Arabidopsis* lines that ectopically expressed a combination of A, B, and *SEP3* genes or B, C, and *SEP3* genes. These experiments showed that these factors were indeed sufficient to affect leaf identity. Transgenic plants expressing A, B, and *SEP3* genes had vegetative leaves converted into petal-like organs and those expressing B, C, and *SEP3* genes had cauline leaves and all floral organs converted into stamen-like structures. Interaction studies in yeast showed that SEP3 proteins interact with A, B, and C MADS-box factors forming multimeric complexes. These interaction data explained the molecular nature of the genetic interactions among A, B, C, and E factors.

In the following paragraphs the function and evolutionary conservation of these class ABCDE genes in rice will be discussed. Similar reviews have been reported previously (Fornara *et al*., 2003; Theißen and Becker, 2004), however, since then significant new data have been published which makes it timely to update these overviews.

Class A genes

Arabidopsis has two class A genes: *AP1* and *AP2,* of which only *AP1* encodes a MADS-box transcription factor. *AP1* has two genetically separable functions, it has a class A function for the determination of the identity of sepals and petals and furthermore it specifies floral meristem identity. These functions are already reflected in the single *ap1* mutant which has leaf-like bracts instead of sepals and the petals are mostly absent (Bowman *et al*., 1993). Furthermore, within the primary *ap1* flower a new ectopic inflorescence arises from the axil of the first whorl organs. Phylogenetic reconstructions of *Arabidopsis* MADS-box factors show that the AP1 MADS-box factor groups together in the so-called group of *SQUA*-like genes (named after the *Antirrhinum SQUAMOSA* (*SQUA*) gene, the founding member of this group) together with *CAULIFLOWER* (*CAL*) and *FRUITFULL* (*FUL*) (Fig. 3). Combining the *ap1* mutant with the *cal* mutant results in a 'cauliflower' phenotype, which is characterized by an extensive proliferation of inflorescence meristems at each position that in wild-type would give rise to a single flower (Kempin *et al*., 1995). This phenotype is further enhanced by combining the *ap1 cal* double mutant with *ful* in the *ap1 cal ful* triple mutant (Ferrándiz *et al*., 2000). These mutant phenotypes show that all these *SQUA*-like genes are floral meristem identity genes. Besides specifying floral meristem identity, *FUL* also has a function in valve

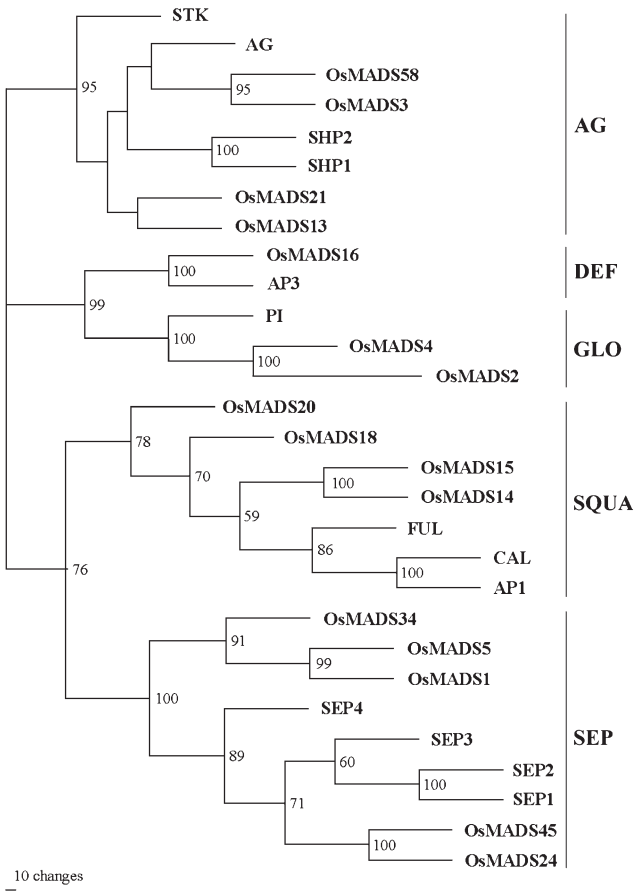

Fig. 3. Phylogenetic tree of MADS-box proteins cited in this review. Phylogenetic tree construction was performed as described previously (Pelucchi *et al*., 2002) using the M, I, and K domains of these proteins.

identity specification from which this gene derived its name. Phylogeny reconstructions suggest that the ancestral function of SQUA-like genes was in specifying floral meristem identity and that functions in specifying sepal and petal, or fruit valve identity are functions that have been derived later (Theissen, 2000; Theissen *et al*., 2000). Therefore, one may expect that *SQUA*-like genes in rice are playing a role in specifying floral meristem identity, but are not necessary in sepal and petal identity determination.

Litt and Irish (2003) carefully analysed the *SQUA* clade and distinguish, based on specific conserved amino acid motifs in the C-terminal domain, AP1-like and FUL-like proteins. They showed that, in contrast to the dicot lineage which contains both AP1- and FUL-like proteins, the monocot lineage seems to have evolved only FUL-like proteins. As shown in Fig. 3, in the rice genome there are four genes that encode such FUL-like proteins, *OsMADS14*, *OsMADS15*, *OsMADS18*, and *OsMADS20*. *OsMADS14* (Moon *et al*., 1999*b*) represents most likely the genes named *FDRMADS6* (Jia *et al*., 2000) and *RAP1B* (Kyozuka *et al*., 2000). Its expression is restricted to inflorescences and developing kernels. Expression is observed throughout the spikelet meristem. After organ primordia initiation, *OsMADS14* expression is restricted to sterile glumes, palea and lemma. Interestingly, in mature spikelets *OsMADS14* expression switches to the reproductive organs, stamens and carpels and its expression in the sterile organs is not observed anymore (Jia *et al*., 2000; Pelucchi *et al*., 2002). Jeon and colleagues (Jeon *et al*., 2000) found that ectopic expression of *OsMADS14* in rice results in extreme early flowering at the callus or young plantlet stage. From their results they suggest that *OsMADS14* might be involved in floral meristem identity determination.

OsMADS15 (Moon *et al*., 1999*b*) is very likely the same gene as *RAP1A* (Kyozuka *et al*., 2000). *OsMADS15* expression is initially observed throughout the spikelet meristem. After initiation of the different organs of the spikelet, transcripts accumulate only in the sterile organs (i.e. lodicules, palea, lemma, and empty glumes) (Kyozuka *et al*., 2000).

OsMADS18 is expressed in roots, leaves, inflorescences, and developing kernels; however, expression analysis using seedlings showed that *OsMADS18* is not expressed at the early stages of plant development (Masiero *et al*., 2002; Fornara *et al*., 2004). Expression is detectable at 4 weeks after germination in leaves and its expression increases when the plant reaches the reproductive stage. *In situ* hybridization analysis showed that *OsMADS18* is expressed in all parts of the plant with high expression levels in the root and flower meristems (Fornara *et al*., 2004). Functional analysis showed that RNAi-mediated silencing of *OsMADS18* did not result in a detectable phenotypic alteration which indicates that this gene is probably redundant with one or more of the other *SQUA*-like genes from rice. Overexpression of *OsMADS18* resulted in an early flowering phenotype. Furthermore it induced precocious initiation of axillary shoot meristems. This observation together with the early transition to flowering suggests that *OsMADS18* is able to promote the differentiation program of the vegetative shoot. Expression of *OsMADS18* in *Arabidopsis* surprisingly resulted in an *ap1* mutant phenotype (Fornara *et al*., 2004). Yeast two-hybrid interaction studies showed that OsMADS18 interacts with the *Arabidopsis* proteins SEP1 and SEP3 which are partners of AP1. These results suggest that OsMADS18 can form similar complexes as AP1 but cannot functionally complement AP1, which results in the dominant negative effect when expressing *OsMADS18* in *Arabidopsis*.

OsMADS20 is expressed in seedling shoots at 5 d after germination and in developing seeds, no transcripts were detected in developing panicles (Lee *et al*., 2003*b*). This expression profile is quite aberrant in relation to the evolutionary conserved function that *SQUA*-like genes are expected to have in flower development.

Based on the *OsMADS14* and *OsMADS18* overexpression experiments one could speculate that these genes have a role similar to *AP1* and *FUL*. Ectopic expression of *AP1* (Mandel and Yanofsky, 1995) and *FUL* (Ferrandiz *et al*., 2000) also induces precocious flowering. Furthermore, both these genes are linked to the flowering pathway. In rice, this situation seems to be the same since recent data from Lee *et al*. (2004) showed that *OsMADS14*, *OsMADS15*, and *OsMADS18* act down-stream of *OsMADS50*, a flowering time regulator that is a putative orthologue of *SUPPRESSOR OF OVER-EXPRESSION OF CO 1* (*SOC1*).

Unfortunately, besides the *OsMADS18* RNAi lines there are no other single or double knock-out or knock-down lines available for these *SQUA*-like genes from rice. Based on the information that is available so far, it is more likely that rice *SQUA*-like genes have a role in floral organ meristem identity than that they have an A-function.

Class B genes

Arabidopsis has two class B floral organ identity genes, *APETALA3* (*AP3*) and *PISTILLATA* (*PI*) which belong to the *DEF*-like and *GLO*-like gene groups, respectively (Jack *et al*., 1992; Goto and Meyerowitz, 1994) (Fig. 3). In *ap3* and *pi* loss-of-function mutants, petals are replaced by sepals and stamens by carpels clearly showing that these genes control petal and stamen identity. Analysis of extant gymnosperms revealed that class B genes may have an ancestral function in specifying male reproductive organ development: B gene expression results in stamen development, whereas the absence of B expression leads to carpel development. This mechanism of reproductive organ specification was probably already established in the last common ancestor of extant seed plants about 300 million years ago (Albert *et al*., 1998; Mouradov *et al*.,

1999; Sundström *et al*., 1999; Winter *et al*., 1999, 2002*a*; Fukui *et al*., 2001). During angiosperm evolution distinct *DEF*- and *GLO*-like genes were established by gene duplication and their gene products evolved from homodimerizing to obligatory heterodimerizing proteins. These *DEF*- and *GLO*-like genes were, besides specifying stamen development, also recruited for petal identity determination (Winter *et al*., 2002*b*).

As expected from these evolutionary data, *GLO*- (*OsMADS2* and *OsMADS4*) and *DEF*-like (*OsMADS16*) genes have been identified in rice as well (Chung *et al*., 1995; Moon *et al*., 1999*a*) (Fig. 3). *OsMADS16* is also named *SUPERWOMAN1* (*SPW1*) and is specifically expressed in lodicules and stamens (Moon *et al*., 1999*a*, *b*; Nagasawa *et al*., 2003). Recessive mutations in *SPW1* transform stamens into carpels and lodicules into palea- or outer bract-like organs. The cell-types found in these second whorl organs resemble cells that are found in the marginal tissue of the palea which are similar to cell types of the outer glume (Nagasawa *et al*., 2003; Prasad and Vijayraghavan, 2003). A similar phenotype as observed for the *spw1* mutant was obtained when *OsMADS16* was down-regulated by using an RNAi approach (Xiao *et al*., 2003). Ectopic expression of *OsMADS16* results in a homeotic conversion of carpels into stamens whereas no alterations of the outer whorl organs was observed (Lee *et al*., 2003*a*). The homeotic transformation of carpels is probably the result of active heterodimer formation with OsMADS4 which is, in wild-type plants, besides being in lodicules and stamens also expressed in carpels (Chung *et al*., 1995). Interestingly, the presence of two GLO-like class B genes was found in rice which might point to a diversification of function. Recently, Prasad and Vijayraghavan (2003) reported the down-regulation of OsMADS2 by a RNAi approach. In wild-type plants *OsMADS2* is specifically expressed in lodicules and stamens. In these knock-down plants only *OsMADS2* mRNA levels were reduced whereas *OsMADS4* was normally expressed. Interestingly, in these RNAi lines only the lodicule identity was affected whereas stamens were normal, indicating that *OsMADS2* is only necessary to specify the identity pf lodicules. The lodicules were significantly enlarged in these transgenic rice plants and had characteristics of the outer glume or palea marginal tissue similar to the homeotic transformation of whorl 2 organs that were observed in the *spw1* mutant. These results suggest that *OsMADS2* is not redundant with *OsMADS4* and that *OsMADS2* is necessary for lodicules specification, whereas *OsMADS4* is needed to determine the identity of stamens. However, Kang *et al*. (1998) reported that antisense plants in which *OsMADS4* was down-regulated were defective for both stamen and lodicule development. Unfortunately, it is not clear whether, in these antisense lines, the observed phenotype originates from reduced levels of *OsMADS4* alone or from the additional non-specific suppression of *OsMADS2*.

Since in eudicot class B gene mutants, petals are replaced by sepals, the loss-of-function phenotypes of rice class B genes have been used as evidence that lodicules are homologous to petals, and palea/lemma are homologous to sepals (Kang *et al*., 1998; Ambrose *et al*., 2000). Although this is possible it will require more investigation to clarify this point. Homologous organs can be expected to have common descent, but monocot and eudicot petals are thought to have arisen independently (Irish, 2000). This indicates the possibility of species-specific mechanisms for petal formation. In rice, this could originate from the duplication event of the *GLO*-like class B genes.

Class C genes

In *Arabidopsis* there is one typical class C gene which is *AGAMOUS* (*AG*) (Yanofsky *et al*., 1990). An *ag* flower develops petals instead of stamens and in the centre of the flower a new *ag* flower develops instead of the pistil which shows that the *AG* gene, as proposed by the ABC model, is necessary for specifying stamen and carpel identity and for floral determinacy. Interestingly, in the *ap2 ag* double mutant ectopic carpelloid organs develop instead of sepals indicating that a carpel pathway exists independent of *AG* (Bowman *et al*., 1991). Pinyopich *et al*. (2003) showed that the *SHATTERPROOF1* (*SHP1*) and *SHP2* genes are responsible for the *AG*-independent carpel development. These two MADS-box genes are, besides controlling carpel identity, also involved in carpel dehiscence zone formation, for which they obtained their name (Liljegren *et al*., 2000). The two *SHP* genes are closely related to *AG* and *SEEDSTICK* (*STK*) of *Arabidopsis* (Fig. 3). Within this *AG*-group two classes can be defined, the class C genes (expressed in stamens and/or carpels) and the class D genes (expressed specifically in ovules) (Zahn *et al*., 2006). In the rice genome four *AG*-like genes have been found, termed *OsMADS3* (Kang *et al*., 1995), *OsMADS58* (Yamaguchi *et al*., 2006), *OsMADS13* (Lopez-Dee *et al*., 1999), and *OsMADS21* (Lee *et al*., 2003*a*, *b*). The expression of *OsMADS3* and *OsMADS58* is restricted to stamens and carpels which suggest that they might have functions similar to class C genes (Kang *et al*., 1995; Kyozuka *et al*., 2000; Yamaguchi *et al*., 2006). However, the temporal expression of these two genes is quite different. *OsMADS3* is mainly expressed in stamen, carpel, and ovule primordia, but its expression is excluded when these organs differentiate. This is in contrast to *OsMADS58* which is expressed in stamen, carpel and ovule primordia, but its expression also remains during the differentiation and maturation of these organs (Yamaguchi *et al*., 2006). In rice plants in which *OsMADS3* was silenced by an antisense approach, partial transformations of stamens into lodicules were observed, while carpels were replaced by abnormal flowers with undifferentiated

stamens and carpels (Kang *et al*., 1998). Recently, Yamaguchi and colleagues (Yamaguchi *et al*., 2006) reported the phenotypes of a stable *osmads3* mutant and *OsMADS58* RNAi knock-down lines. This analysis shed more light on the functional specification of these two *AG*-like MADS-box factors. The *osmads3-3* T-DNA knock-out line has almost all stamens homeotically transformed into lodicule-like organs. Some of these whorl 3 lodicules were indistinguishable from wild-type. Furthermore, in whorl 4 an increased number of carpels were observed. Occasionally, new carpels developed within the carpels of the fourth whorl indicating a partial loss of meristem determinacy. *OsMADS58* RNAi knock-down lines show an indeterminate development of floral organs, producing a reiterated set of floral organs consisting of lodicules, stamens/ectopic lodicules, and carpel-like organs. Stamen identity was affected, but much less when compared with the *osmads3* mutant. Carpel morphology was severely affected, they did not fuse and no differentiation of stigmatic tissue was observed. Unexpectedly, the analysis of the *osmads3* and *osmads58* mutants showed that these genes are also involved in repressing lodicule development, since ectopic lodicules developed in whorl two of these mutant flowers.

Introduction of the *OsMADS58* RNAi construct in the milder *osmads3-2* mutant showed that the defect in indeterminacy was very similar to the *osmads58* knock-down line indicating that *OsMADS3* only weakly contributes to meristem determinacy.

The analysis of these mutants showed that both *OsMADS3* and *OsMADS58* have class C gene activity with functions similar to those observed for *AG*. However, there is clear functional diversification between these genes giving them predominant functions in different whorls. *OsMADS3* has a stronger role in repressing lodicule development in whorl two and in specifying stamen identity, whereas *OsMADS58* contributes more to conferring floral meristem determinacy and to regulate carpel morphogenesis.

Interestingly, none of these class C gene mutants caused clear homeotic conversions of the carpel, they only affect carpel development. This in contrast to the stamens that are clearly transformed into lodicules. It might be that, in rice, and this could be true for all grasses including maize where some similarities have been observed (Mena *et al*., 1996), the homeotic function for carpel specification is attributed by a YABBY domain protein named *DROOPING LEAF* (*DL*) (Nagasawa *et al*., 2003; Yamaguchi *et al*., 2004). *DL* is the orthologue of the *Arabidopsis* gene *CRABS CLAW* (*CRC*). The *DL* gene has three functions in rice flower development, specification of carpel identity, control of floral meristem determinacy, and the antagonistic regulation with class B genes. In *Arabidopsis CRC* shares the functions in meristem determinacy and regulation of class B genes. However, unlike loss-of-function mutations in *DL* the *crc* mutant does not result in homeotic transformations of carpels, although carpel development is affected. Since mutants with similar phenotypes have been observed in several grass species it might be that *DL* and its orthologues in other grasses have acquired critical functions in carpel identity during grass evolution (Yamaguchi *et al*., 2004; Fourquin *et al*., 2005). It will be interesting to establish whether rice class C MADS-box genes have lost this capacity of carpel specification or that there is a genetic interaction between *DL* and MADS-box genes that will explain these observations.

Class D genes

As discussed above, class D genes determine ovule identity and belong to the monophyletic *AG*-like clade (Fig. 3). The class D gene of *Arabidopsis* is *STK*, which is exclusively expressed in ovules. Pinyopich *et al*. (2003) showed that in *stk* single mutants ovule identity is not affected and only shows a defect in the development of the funiculus, an umbilical-cord-like structure that connects the ovule to the placenta. Furthermore, by combining different mutants they revealed that all four members of the *AG*-clade, *STK*, *SHP1*, *SHP2*, and *AG* are involved in specifying ovule identity.

In rice, based on phylogenetic reconstruction, two *AG*-like genes belonging to the class D gene lineage have been identified, namely *OsMADS13* (Lopez-Dee *et al*., 1999) and *OsMADS21* (Lee *et al*., 2003*a*, *b*). *OsMADS13* is specifically expressed in the ovule with an expression pattern very similar to *STK*. Its expression is first detected in the ovule primordium where it persists during further development of the ovule. Inside the ovule, *OsMADS13* is expressed in both integuments and nucellus tissues. After anthesis *OsMADS13* is also expressed in developing seeds.

RT-PCR analysis showed that *OsMADS21* is only expressed in developing seeds (Lee *et al*., 2003*b*). However, *in situ* analysis performed in this laboratory suggests that *OsMADS21* is also weakly expressed in the carpel wall and ovules (MM Kater, unpublished results).

For both genes a functional analysis has not yet been reported, therefore at present it is not possible to assign a D-function to *OsMADS13* and/or *OsMADS21*, although the expression pattern of *OsMADS13* makes it very likely that this gene has such a function. This hypothesis is further strengthened by the protein interaction studies performed by Favaro *et al*. (2002, 2003). They showed that OsMADS13 interacts with the partner proteins of FBP7 and FBP11 from *Petunia* and those of STK from *Arabidopsis*, indicating that the specificity for protein–protein interactions is also maintained in these proteins.

Class E genes

In *Arabidopsis* it has been shown that the *SEPALLATA* (*SEP*) MADS-box genes are involved in the specification

of sepal, petal, stamen, carpel, and ovule identity. Their activity is based on complex formation with the class A, B, C, and D gene products to form higher order MADS-box protein complexes (Honma and Goto, 2001; Pelaz *et al*., 2001; Favaro *et al*., 2003). In line with the ABC nomenclature this new class of floral-organ-identity genes were termed E-function genes (Theissen, 2001). Phylogenetic analysis clusters these class E genes together in the so-called *SEP* clade (previously called *AGL2* clade) (Fig. 3). In rice five *SEP*-like genes have been identified, which are *OsMADS1*, *OsMADS5*, *OsMADS24* (allelic to *OsMADS8*), *OsMADS34* (allelic to *OsMADS19*), and *OsMADS45* (allelic to *OsMADS7*) (Chung *et al*., 1994; Greco *et al*., 1997; Kang and An, 1997; Kang *et al*., 1997; Pelucchi *et al*., 2002; Malcomber and Kellogg, 2004). Of these five *SEP*-like genes *OsMADS1* is the one that has been studied in most detail (Jeon *et al*., 2000; Lim *et al*., 2000; Prasad *et al*., 2001, 2005; Malcomber and Kellogg, 2004; Agrawal *et al*., 2005; Chen *et al*., 2006). Mutations at amino acid positions 24 and 27 in the MADS domain of *OsMADS1* were found to cause the *leafy hull sterile 1* (*lhs1*) mutant phenotype in rice (Jeon *et al*., 2000). *Lhs1* has leafy lemma and palea, a decreased number of stamens and, occasionally, an extra pistil or floret (Khush and Librojo, 1985; Jeon *et al*., 2000). The *lhs1* mutation is a semi-dominant allele, therefore the observed phenotype might not reflect the precise function of *OsMADS1* (Theissen 2001; Malcomber and Kellogg, 2004). Recently *osmads1 Tos17* knock-out and RNAi knock-down lines have been reported (Agrawal *et al*., 2005; Prasad *et al*., 2005). Both types of *osmads1* mutants have similar phenotypic effects. Severe *Tos17* mutants have an underdeveloped palea and lemma. Lodicules and stamens were homeotically transformed into leafy lemma- and palea-like structures and in whorl four an additional abnormal floret develops composed of lemma- and palea-like structures. These phenotypic observations indicate that *OsMADS1* has a function in floral organ specification and in establishing floral determinacy (Agrawal *et al*., 2005). Prasad and co-workers (Prasad *et al*., 2005) analysed the homeotic transformations observed in the *OsMADS1* RNAi lines in more detail by histological analysis. They showed that, in severe RNAi lines, the lemma and palea were elongated and leaf-like. The lemma was more affected than the palea and mimics glumes, whereas the palea was never glume-like. Lodicules and stamens were also homeotically transformed into glume-like organs and, in place of the carpel, a new floret develops composed of glume-like organs. *OsMADS1* seems to function as a lemma identity gene since inhibition results in loss of lemma identity, whereas the ectopic expression of *OsMADS1* in glumes results in homeotic transformation of these organs into lemmas (Prasad *et al*., 2001).

The mutant phenotypes suggest that *OsMADS1* has a typical E-function, since they resemble those observed in the *Arabidopsis sep1 sep2 sep3* triple mutant (all floral organs get sepalloid) and the *sep1 sep2 sep3 sep4* quadruple mutant (all floral organs get leaf-like) (Pelaz *et al*., 2000; Ditta *et al*., 2004). Analysis of class B and C gene expression in the *osmads1* mutant showed that, as in the *sep* mutants, the expression of these genes has not been changed. This suggests that OsMADS1 functions, like the SEP proteins, in establishing protein complexes that determine floral organ identity. However, this is perhaps unlikely because *OsMADS1* expression in the floret meristem precedes that of the class B gene *OsMADS16*. The latter is activated after the emergence of lemma and palea primordia and, by this stage, *OsMADS1* is not expressed anymore (Nagasawa *et al*., 2003; Prasad *et al*., 2005). Another indication that OsMADS1 does not form complexes with class B and C proteins is that interactions between these proteins have never been observed in yeast two-hybrid screens (Fig. 4B).

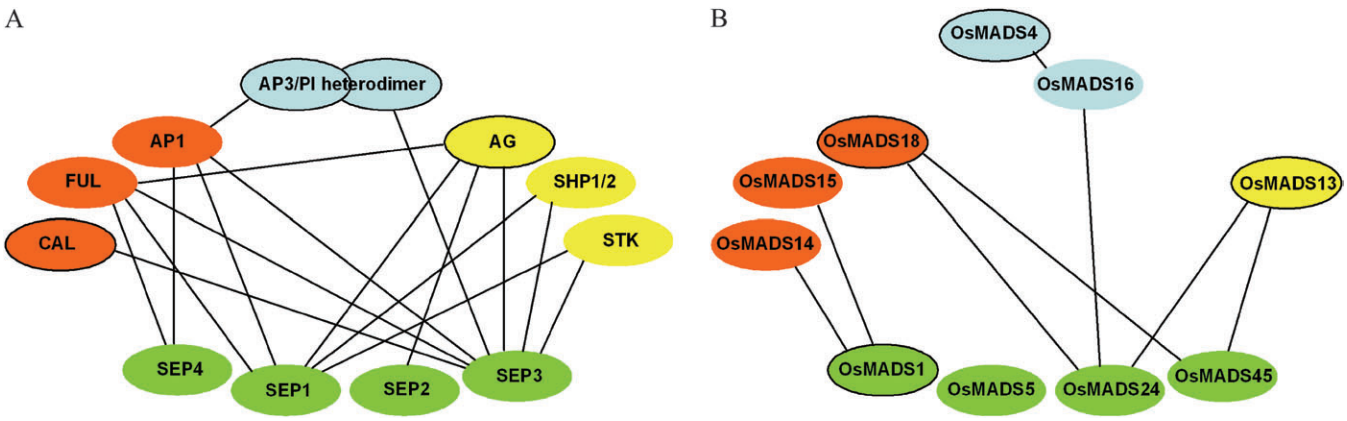

Fig. 4. MADS-box protein interactions in *Arabidopsis* and rice. Interactions between *Arabidopsis* (A) and rice (B) MADS-box proteins observed in yeast two-hybrid screens (de Folter *et al*., 2005, and references therein; Moon *et al*., 1999*a*; Fornara *et al*., 2002; Lee *et al*., 2003*a*; Favaro *et al*., 2004). Proteins that were used as a bait against cDNA libraries are surrounded by a black circle. Orange, SQUA-like proteins; blue, DEF- and GLO-like proteins; yellow, AG-like proteins; green, SEP-like proteins.

A possible explanation for the homeotic transformations in the inner floral whorls might be that *OsMADS1* activates at early stages of floret development accessory factors that control organogenesis in these whorls. One such gene might be *OsMGH3* which encodes a rice flower specific GH3-type factor (Prasad *et al*., 2005). This factor is expressed in young floret meristems and its mRNAs are subsequently localized predominantly in the second, third, and fourth whorl organs. Down-regulation of *OsMADS1* results in a drastic decrease of *OsMGH3* expression and ectopic expression of *OsMADS1* induces *OsMGH3* expression in leaves, indicating that *OsMADS1* (possibly indirectly) regulates this gene.

The phenotypic effects observed in *osmads1* mutants indicates its relatedness to the *Arabidopsis SEP* genes. However, regardless of whether *OsMADS1* has a function as cofactor or regulator of accessory factors, it is clear that early effects of *OsMADS1* are crucial for the specification of floral organs at later stages of development.

OsMADS5 is another rice *SEP*-like gene that has been functionally analysed (Agrawal *et al*., 2005). A *Tos17* insertions in the 4th exon resulted in a loss-of-function of the *OsMADS5* gene. Analysis showed that the *osmads5* mutation has almost no effect on flower development. Only lodicule development was slightly disturbed since they were found to be attached to the lemma and palea.

Based on phylogenetic, expression and interaction studies (see below) the more likely orthologues of the *Arabidopsis* class E genes are *OsMADS24* and *OsMADS45* (Favaro *et al*., 2002; Pelucchi *et al*., 2002; Malcomber and Kellogg, 2004; Prasad *et al*., 2005). Future functional studies will have to clarify this hypothesis.

The quartet-model for rice

Interactions between proteins are essential for their functioning and the biological processes they control. MADS-box transcription factors form specific homo- or hetero-dimers and these protein–protein interactions are essential for binding to a specific *cis*-element called a CArG box (Shore and Sharrocks, 1995). For specifying floral organ identity MADS-box proteins seem to form higher order complexes. Pioneer work in the laboratory of Hans Sommer showed that the class B proteins DEFICIENS (DEF) and GLOBOSA (GLO) form a ternary complex with the meristem identity protein SQUAMOSA to control floral architecture in *Antirrhinum majus* (Egea-Cortines *et al*., 1999). Later, similar complexes were observed between class A-B-E and B-C-E proteins (Honma and Goto, 2001) and between class C-D-E proteins (Favaro *et al*., 2003).

Protein studies in yeast allow the analysis of interactions at a larger scale and they provide a framework of proteins that possess the capacity and specificity to interact (Uetz *et al*., 2000). Recently, de Folter *et al*. (2005) determined the comprehensive interaction map of the *Arabidopsis* MADS-box transcription factors using a matrix-based yeast two-hybrid screen of more than 100 members of the *Arabidopsis* MADS-box family. This analysis provides a perfect starting point for functional analysis of this transcription factor family since clustering of proteins with similar interaction patterns pinpoints proteins that participate in a specific process and provides information about uncharacterized proteins that participate in this process. Unfortunately, such a large-scale analysis has not yet been done for rice MADS-box proteins. However, some information is available for MADS-box protein–protein interactions in rice.

Figure 4A shows that, in *Arabidopsis*, the PI-AP3 dimer interacts with AP1 and SEP3. Furthermore, AG interacts with SEP3, which mediates the interaction with the PI-AP3 dimer (Honma and Goto, 2001). These interactions were the basis for the so-called 'quartet-model' which proposes that complexes composed of AP1-PI-AP3-SEP3 and PI-AP3-SEP3-AG, respectively, specify petal and stamen identity (Honma and Goto, 2001; Theissen and Saedler, 2001). As Fig. 4B shows, in rice some similarities can be observed. The SEP-like proteins OsMADS24 and OsMADS45 have a similar interaction profile as the SEP proteins in *Arabidopsis*. They interact with the SQUA-like protein OsMADS18 (similar to AP1), with OsMADS16 (similar to AP3) and with the AG-like protein OsMADS13 (similar to STK).

In *Arabidopsis* all the AG-like proteins interact with SEP3. Furthermore, exchange experiments showed that the rice SEP-like proteins OsMADS24 and OsMADS45 also interact with STK from *Arabidopsis* and with FBP7 from *Petunia* showing that the interactions between SEP and class D proteins is evolutionarily conserved (Favaro *et al*., 2002, 2003). It is therefore highly possible that the two rice SEP orthologues also interact with the other members of the rice *AG*-like gene clade. This makes it tempting to speculate that a kind of quartet-model might also hold for rice lodicules, stamens, carpel and ovule specification.

The direct interaction between the AP3 homologue, OsMADS16, and OsMADS24 highlights a difference from *Arabidopsis*, where such interactions between individual class B proteins and a SEP protein have not been observed. In *Arabidopsis* it is the PI-AP3 heterodimer that interacts with SEP proteins. Although OsMADS16 has been shown not to homodimerize (Moon *et al*., 1999*a*), it remains conceivable that an OsMADS16 homodimer could become stabilized by interaction with OsMADS24.

SEP proteins in *Arabidopsis* are considered to establish ternary complex formation but they are also supposed to give transcriptional activity to these complexes. It was shown that SEP3 is able to activate transcription in yeast and in plant cells (Honma and Goto, 2001). Interestingly, in this respect is that OsMADS24 and OsMADS45 have also transcriptional activity in yeast (MM Kater, unpublished

results) which suggest that in analogy with SEP proteins of *Arabidopsis* these rice proteins can add transcription activity to a ternary complex as well.

Tool development for rice genomics

Arabidopsis is the premiere model species for functional genomics in plants. The availability of almost the entire genome sequence (*Arabidopsis* Genome Initiative, 2000) has boosted significantly the development of tools for functional genomics. Now that the genome sequence of rice is available it is moving up fast as a second model species (Goff *et al*., 2002; Yu *et al*., 2002). Two features make rice attractive as a model species: it represents the taxonomically distinct monocots and it is a crop species. With respect to tools for *Arabidopsis* functional genomics, deep sequence-tagged lines, inexpensive spotted oligonucleotide arrays, and a near-complete whole genome Affymetrix array are publicly available.

For rice, the development of similar functional genomics resources is in progress (Table 1) and their development is, to some extent, more streamlined since it is based on the lessons learned from *Arabidopsis* (Rensink and Buell, 2004). There are microarrays for rice available from Affymetrix, Agilent, and via a NSF supported rice oligo array project (Table 1). Tiling arrays covering the whole genome sequence, including intergenic regions, introns etc, to study genome-wide expression or to use for the analysis of chromatin immunoprecipitation analysis will soon be available for Arabidopsis. Since the genome of rice is almost four times larger than the genome of *Arabidopsis* this valuable tool, tiling the whole rice genome, is unlikely to be available very soon. Nevertheless, tiling analyses have been done for specific rice chromosomes (Jiao *et al*., 2005; Li *et al*., 2005).

For functional analysis of rice genes several insertion populations based on transposons (i.e. *Tos17* and Ac-Ds) and T-DNA insertions are available (Table 1; Hirochika *et al*., 2004). Furthermore, tilling using ethane methyl sulphonate-induced mutations is also available for reverse genetics-based gene function analysis (Wu *et al*., 2005).

It is interesting to notice that, within a few years, functional genomics tools for rice have been developed and that the international effort has been taken to establish databases that collect rice genomics information and resources (Table 1).

Concluding remarks

MADS-box gene function was mainly studied in *Arabidopsis, Antirrhinum*, and *Petunia*. It is therefore not surprising that our knowledge on flower development in rice lags behind these dicot species. However, this review makes it clear that our knowledge about the function of MADS-box genes that control rice flower development is moving forward rapidly and with the genomics tools that are becoming available for rice functional genomics it is to be expected that new exciting discoveries will be reported in the very near future.

Table 1. *Overview of resources for functional genomics in rice*

Microarrays		
Affimetrix	Oligos array	http://www.affymetrix.com
Agilent	Spotted oligos	http://www.home.agilent.com
NSF consortium	Spotted oligos	http://www.ricearray.org/index.shtml
RED (Rice Expression Database)	Database	http://red.dna.affrc.go.jp/RED
Mutant populations[a]		
CIRAD-INRA-IRD-CNRS, Genoplante	T-DNA	http://urgi.infobiogen.fr
Cereal Gene Tags, EU	Ds	
National Institute of Agrobiological Sciences	Tos17	http://tos.nias.affrc.go.jp/
University of California at Davis	Ds	
Gyeongsang National University	Ds	
Zhejiang University	T-DNA	
National University of Singapore	Ds	
Postech	T-DNA	http://141.223.132.44/pfg/index.php
CSIRO	T-DNA	http://www.pi.csiro.au/fgrttpub/
Taiwan Rice Insertional Mutants database (TRIM)	T-DNA	http://trim.sinica.edu.tw/
National Center of Plant Gene Research (Wuhan)	T-DNA	http://rmd.ncpgr.cn/
Rice genomics websites		
NCBI		http://www.ncbi.nlm.nih.gov/
TIGR		http://www.tigr.org/
Gramene		http://www.gramene.org/
NIAS		http://www.nias.affrc.go.jp/index_e.html
OrygenesDB		http://orygenesdb.cirad.fr

[a] Source: OrygenesDB.

From the data available so far (see the supplementary table at JXB online), emerges the picture that B- and C-function genes are relatively well conserved during evolution, which is in line with the conservation of these reproductive organs in flowering plants. Although, for C-function genes it is not clear whether they, as in the model dicots, are involved in specifying carpel identity. This function might, in rice and other grasses, be accomplished by none-MADS-box factors. Although no real functional data for class E genes are available it is tempting to speculate, based on phylogenetic expression and protein interaction data, that the E-function is also conserved, at least for some of the *SEP*-like genes. With respect to the A-function genes, apart from *Arabidopsis*, their existence in most flowering plants remains an enigma.

It is clear that much can be learned by comparing *Arabidopsis* with rice but, as the two floral structures already indicate, many significant differences can be observed. Moreover it makes clear that the evolution of MADS-box genes had a significant role in the biodiversity of floral structures that can be observed today in nature. The challenge is to sort out the molecular mechanisms that form the fundamentals for this biodiversity.

Supplementary data

Supplementary data are available at JXB online.

Acknowledgements

We thank Simona Masiero for critical reading of the manuscript, Stefano Berri for helping with the bioinformatics analysis, and Guenter Theissen for his helpful suggestions.

References

Agrawal KG, Abe K, Yamazaki M, Miyao A, Hirochika A. 2005. Conservation of the E-function for floral organ identity in rice revealed by the analysis of tissue culture-induced loss-of-function mutants of the *OsMADS1* gene. *Plant Molecular Biology* **59,** 125–135.

Albert VA, Gustafsson MHG, Di Laurenzio L. 1998. Ontogenetic systematics, molecular developmental genetics, and the angiosperm petal. In: Soltis DE, Soltis PS, Doyle JJ, ed. *Molecular systematics of plants*, Vol. II. Boston, USA: Kluwer Academic Publishers, 349–374.

Ambrose BA, Lerner DR, Ciceri P, Padilla CM, Yanofsky MF, Schmidt RF. 2000. Molecular and genetic analyses of the *Silky1* gene reveal conservation in floral organ specification between eudicots and monocots. *Molecular Cell* **5,** 569–579.

Angenent GC, Colombo L. 1996. Molecular control of ovule development. *Trends in Plant Science* **1,** 228–232.

***Arabidopsis* Genome Initiative.** 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana. Nature* **408,** 796–815.

Bowman JL, Alvarez J, Weigel D, Meyerowitz EM, Smyth DR. 1993. Control of flower development in *Arabidopsis thaliana* by *APETALA1* and interacting genes. *Development* **119,** 721–743.

Bowman JL, Smyth DR, Meyerowitz EM. 1991. Genetic interactions among floral homeotic genes of *Arabidopsis. Development* **112,** 1–20.

Chen ZX, Wu JG, Ding WN, Chen HM, Wu P, Shi CH. 2006. Morphogenesis and molecular basis on naked seed rice, a novel homeotic mutation of *OsMADS1* regulating transcript level of *AP3* homologue in rice. *Planta* **223,** 882–890.

Chung YY, Kim SR, Finkel D, Yanofsky MF, An G. 1994. Early flowering and reduced apical dominance result from ectopic expression of a rice MADS box gene. *Plant Molecular Biology* **26,** 657–665.

Chung YY, Kim SR, Kang HG, Noh YS, Park MC, Finkel D, An G. 1995. Characterization of two rice MADS-box genes homologous to *GLOBOSA*. *Plant Science* **109,** 45–56.

Coen ES, Meyerowitz EM. 1991. The war of the whorls: genetic interactions controlling flower development. *Nature* **353,** 31–37.

de Folter S, Immink RG, Kieffer M, *et al.* 2005. Comprehensive interaction map of the *Arabidopsis* MADS box transcription factors. *The Plant Cell* **17,** 1424–1433.

Ditta G, Pinyopich A, Robles P, Pelaz S, Yanofsky MF. 2004. The *SEP4* gene of *Arabidopsis thaliana* functions in floral organ and meristem identity. *Current Biology* **14,** 1935–1940.

Egea-Cortines M, Saedler H, Sommer H. 1999. Ternary complex formation between the MADS-box proteins SQUAMOSA, DEFICIENS, and GLOBOSA is involved in the control of floral architecture in *Antirrhinum majus. EMBO Journal* **18,** 5370–5379.

Favaro R, Immink RGH, Ferioli V, Bernasconi B, Byzova M, Angenent GC, Kater M, Colombo L. 2002. Ovule-specific MADS-box proteins have conserved protein–protein interactions in monocot and dicot plants. *Molecular Genetics and Genomics* **268,** 152–159.

Favaro R, Pinyopich A, Battaglia R, Kooiker M, Borghi L, Ditta G, Yanofsky MF, Kater MM, Colombo L. 2003. MADS-box protein complexes control carpel and ovule development in *Arabidopsis. The Plant Cell* **15,** 2603–2611.

Ferrandiz C, Gu Q, Martienssen R, Yanofsky MF. 2000. Redundant regulation of meristem identity and plant architecture by FRUITFULL, APETALA1, and CAULIFLOWER. *Development* **127,** 725–734.

Fornara F, Marziani G, Mizzi L, Kater MM, Colombo L. 2003. MADS-box genes controlling flower development in rice. *Plant Biology* **1,** 16–22.

Fornara F, Pařenicova L, Falasca G, Pelucchi N, Masiero S, Ciannamea S, Lopez-Dee Z, Altamura MM, Colombo L, Kater MM. 2004. Functional characterization of *OsMADS18*, a member of the *AP1/SQUA* subfamily of MADS box genes. *Plant Physiology* **135,** 2207–2219.

Fourquin C, Vinauger-Douard M, Fogliani B, Dumas C, Scutt CP. 2005. Evidence that *CRABS CLAW* and *TOUSLED* have conserved their roles in carpel development since the ancestor of the extant angiosperms. *Proceedings of the National Academy of Sciences, USA* **102,** 4649–4654.

Fukui M, Futamura N, Mukai Y, Wang Y, Nagao A, Shinohara K. 2001. Ancestral MADS box genes in Sugi, *Cryptomeria japonica*, D. Don (Taxodiaceae), homologous to the B function genes in angiosperms. *Plant and Cell Physiology* **42,** 566–575.

Goff SA, Ricke D, Lan T-H, *et al.* 2002. A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). *Science* **296,** 92–100.

Goto K, Meyerowitz EM. 1994. Function and regulation of the *Arabidopsis* floral homeotic gene *PISTILLATA*. *Genes and Development* **8,** 1548–1556.

Greco R, Stagi L, Colombo L, Angenent GC, Sari-Gorla M, Pè ME. 1997. MADS box genes expressed in developing inflorescences of rice and sorghum. *Molecular Genetics and Genomics* **253,** 615–623.

Hayama R, Coupland G. 2004. The molecular basis of diversity in the photoperiodic flowering responses of *Arabidopsis* and rice. *Plant Physiology* **135,** 677–684.

Hirochika H, Guiderdoni E, An G, *et al.* 2004. Rice mutant resources for gene discovery. *Plant Molecular Biology* **54,** 325–334.

Honma T, Goto K. 2001. Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. *Nature* **409,** 525–529.

Irish VF. 2000. Variations on a theme: flower development and evolution. *Genome Biology* **2,** 1015.1–1015.4.

Jack T, Brochman LL, Meyerowitz EM. 1992. The homeotic gene *APETALA3* of *Arabidopsis thaliana* encodes a MADS box and is expressed in petals and stamens. *Cell* **68,** 683–697.

Jeon JS, Lee S, Jung KH, Yang WS, Yi GH, Oh BG, An G. 2000. Production of transgenic rice plants showing reduced heading date and plant height by ectopic expression of rice MADS-box genes. *Molecular Breeding* **6,** 581–592.

Jia HW, Chen R, Cong B, Cao KM, Sun CR, Luo D. 2000. Characterization and transcriptional profile of two rice MADS-box genes. *Plant Science* **155,** 115–122.

Jiao Y, Jia P, Wang X, *et al.* 2005. A tiling microarray expression analysis of rice chromosome 4 suggests a chromosome-level regulation of transcription. *The Plant Cell* **17,** 1641–1657.

Kang HG, An G. 1997. Isolation and characterization of a rice MADS box gene belonging to the *AGL2* gene family. *Molecules and Cells* **7,** 45–51.

Kang HG, Jang S, Chung JE, Cho YG, An G. 1997. Characterization of two rice MADS box genes that control flowering time. *Molecules and Cells* **7,** 559–566.

Kang HG, Jeon JS, Lee S, An G. 1998. Identification of class B and class C floral organ identity genes from rice plants. *Plant Molecular Biology* **38,** 1021–1029.

Kang HG, Noh YS, Chung YY, Costa MA, An K, An G. 1995. Phenotypic alteration of petal and sepal by ectopic expression of a rice MADS box gene in tobacco. *Plant Molecular Biology* **29,** 1–10.

Kempin SA, Savidge B, Yanofsky MF. 1995. Molecular basis of the cauliflower phenotype in *Arabidopsis. Science* **267,** 522–525.

Khush GS, Librojo AL. 1985. Naked seed rice (NSR) is allelic to *op* and *lhs. Rice Genetics Newsletters* **2,** 71.

Kyozuka J, Kobayashi T, Morita M, Shimamoto K. 2000. Spatially and temporally regulated expression of rice MADS-box genes with similarity to *Arabidopsis* class A, B and C genes. *Plant Cell Physiology* **41,** 710–718.

Lee S, Jeon JS, An K, Moon YH, Lee S, Chung YY, An G. 2003*a.* Alteration of floral organ identity in rice through ectopic expression of *OsMADS16. Planta* **217,** 904–911.

Lee S, Kim J, Han JJ, Han MJ, An G. 2004. Functional analyses of the flowering time gene *OsMADS50*, the putative *SUPPRESSOR OF OVEREXPRESSION OF CO 1/AGAMOUS-LIKE 20 (SOC1/AGL20)* ortholog in rice. *The Plant Journal* **38,** 754–764.

Lee S, Kim J, Son JS, *et al.* 2003*b.* Systematic reverse genetic screening of T-DNA tagged genes in rice for functional genomic analyses: MADS-box genes as a test case. *Plant and Cell Physiology* **44,** 1403–1411.

Li L, Wang X, Xia M, *et al.* 2005. Tiling microarray analysis of rice chromosome 10 to identify the transcriptome and relate its expression to chromosomal architecture. *Genome Biology* **6,** R52.

Liljegren SJ, Ditta GS, Eshed Y, Savidge B, Bowman JL, Yanofsky MF. 2000. *SHATTERPROOF* MADS-box genes control seed dispersal in *Arabidopsis. Nature* **404,** 766–770.

Lim J, Moon Y-H, An G, Jang SK. 2000. Two rice MADS domain proteins interact with *OsMADS1*. *Plant Molecular Biology* **44,** 513–527.

Litt A, Irish VF. 2003. Duplication and diversification in the *APETALA1/FRUITFULL* floral homeotic gene lineage: implications for the evolution of floral development. *Genetics* **165,** 821–833.

Lopez-Dee ZP, Wittich P, Pè ME, Rigola D, Del Buono I, Sari Gorla M, Kater MM, Colombo L. 1999. *OsMADS13*, a novel rice MADS-box gene expressed during ovule development. *Developmental Genetics* **25,** 237–244.

Malcomber ST, Kellogg EA. 2004. Heterogeneous expression patterns and separate roles of the *SEPALLATA* gene *LEAFY HULL STERILE1* in grasses. *The Plant Cell* **16,** 1692–1706.

Mandel MA, Yanofsky MF. 1995. A gene triggering flower development in *Arabidopsis. Nature* **377,** 522–524.

Masiero S, Imbriano C, Ravasio F, Favaro R, Pelucchi N, Sari Gorla M, Mantovani R, Colombo L, Kater MM. 2002. Ternary complex formation between MADS-box transcription factors and the histone fold protein NF-YB. *The Journal of Biological Chemistry* **277,** 26429–26435.

Mena M, Ambrose BA, Meeley RB, Briggs SP, Yanofsky MF, Schmidt RJ. 1996. Diversification of C-function activity in maize flower development. *Science* **274,** 1537–1540.

Moon YH, Jung JY, Kang HG, An G. 1999*a.* Identification of a rice *APETALA3* homologue by yeast two-hybrid screening. *Plant Molecular Biology* **40,** 167–177.

Moon YH, Kang HG, Jung JY, Jeon JS, Sung SK, An G. 1999*b.* Determination of the motif responsible for interaction between the rice APETALA1/AGAMOUS-LIKE9 family proteins using a yeast two-hybrid system. *Plant Physiology* **120,** 1193–1203.

Mouradov A, Hamdorf B, Teasdale RD, Kim JT, Winter KU, Theissen G. 1999. A *DEF/GLO*-like MADS-box gene from a gymnosperm: *Pinus radiata* contains an ortholog of angiosperm B class floral homeotic genes. *Developmental Genetics* **25,** 245–252.

Nagasawa N, Miyoshi M, Sano Y, Satoh H, Hirano H, Sakai H, Nagato Y. 2003. *SUPERWOMAN1* and *DROOPING LEAF* genes control floral organ identity in rice. *Development* **130,** 705–718.

Pelaz S, Ditta GS, Baumann E, Wisman E, Yanofsky MF. 2000. B and C floral organ identity functions require *SEPALLATA* MADS-box genes. *Nature* **405,** 200–203.

Pelaz S, Tapia-López R, Alvarez-Buylla ER, Yanofsky MF. 2001. Conversion of leaves into petals in *Arabidopsis. Current Biology* **11,** 182–184.

Pelucchi N, Fornara F, Favalli C, Masiero S, Lago C, Pè ME, Colombo L, Kater MM. 2002. Comparative analysis of rice MADS-box genes expressed during flower development. *Sexual Plant Reproduction* **15,** 113–122.

Pinyopich A, Ditta GS, Savidge B, Liljegren SJ, Baumann E, Wisman E, Yanofsky MF. 2003. Assessing the redundancy of MADS-box genes during carpel and ovule development. *Nature* **424,** 85–88.

Prasad K, Parameswaran S, Vijayraghavan U. 2005. *OsMADS1*, a rice MADS-box factor, controls differentiation of specific cell types in the lemma and palea and is an early-acting regulator of inner floral organs. *The Plant Journal* **43,** 915–928.

Prasad K, Sriram P, Kumar CS, Kushalappa K, Vijayraghavan U. 2001. Ectopic expression of rice *OsMADS1* reveals a role in specifying the lemma and palea, grass organs analogous to sepals. *Development Genes and Evolution* **211,** 281–290.

Prasad K, Vijayraghavan U. 2003. Double-stranded RNA interference of a rice *PI/GLO* paralog, *OsMADS2*, uncovers its second-whorl-specific function in floral organ patterning. *Genetics* **165,** 2301–2305.

Rensink WA, Buell CR. 2004. *Arabidopsis* to rice. Applying knowledge from a weed to enhance our understanding of a crop species. *Plant Physiology* **135,** 622–629.

Shore P, Sharrocks AD. 1995. The MADS-box family of transcription factors. *European Journal of Biochemistry* **229,** 1–13.

Sundström J, Carlsbecker A, Svensson ME, Svenson M, Johanson U, Theissen G, Engström P. 1999. MADS-box genes active in developing pollen cones of Norway spruce (*Picea abies*) are homologous to the B-class floral homeotic genes in angiosperms. *Developmental Genetics* **25,** 253–266.

Theissen G. 2000. Shattering developments. *Nature* **404,** 711–713.

Theissen G. 2001. Development of floral organ identity: stories from the MADS house. *Current Opinion in Plant Biology* **4,** 75–85.

Theissen G, Becker A. 2004. The ABCs of flower development in *Arabidopsis* and rice. *Progress in botany*, Vol. 65. Berlin, Heidelberg: Springer-Verlag, 193–215.

Theissen G, Becker A, Di Rosa A, Kanno A, Kim JT, Münster T, Winter KU, Saedler H. 2000. A short history of MADS-box genes in plants. *Plant Molecular Biology* **42,** 115–149.

Theissen G, Saedler H. 2001. Floral quartets. *Nature* **409,** 469–471.

Uetz P, Giot L, Cagney G, *et al.* 2000. A comprehensive analysis of protein–protein interactions in *Saccharomyces cerevisiae. Nature* **403,** 623–631.

Winter KU, Becker A, Münster T, Kim JT, Saedler H, Theissen G. 1999. MADS-box genes reveal that gnetophytes are more closely related to conifers than to flowering plants. *Proceedings of the National Academy of Sciences, USA* **96,** 7342–7347.

Winter KU, Saedler H, Theissen G. 2002*a.* On the origin of class B floral homeotic genes: functional substitution and dominant inhibition in *Arabidopsis* by expression of an ortholog from the gymnosperm *Genetum. The Plant Journal* **31,** 457–475.

Winter KU, Weiser C, Kaufmann K, Bohne A, Kirchner C, Kanno A, Saedler H, Theissen G. 2002*b.* Evolution of class B floral homeotic proteins: obligate heterodimerization originated from homodimerization. *Molecular Biology and Evolution* **19,** 587–596.

Wu JL, Wu C, Lei C, *et al.* 2005. Chemical- and irradiation-induced mutants of indica rice IR64 for forward and reverse genetics. *Plant Molecular Biology* **59,** 85–97.

Xiao H, Wang Y, Liu D, Wang W, Li X, Zhao X, Xu J, Zhai W, Zhu L. 2003. Functional analysis of the rice *AP3* homologue *OsMADS16* by RNA interference. *Plant Molecular Biology* **52,** 957–966.

Yamaguchi T, Lee DY, Miyao A, Hirochika H, An G, Hirano HY. 2006. Functional diversification of the two C-class genes *OsMADS3* and *OsMADS58* in *Oryza sativa. The Plant Cell* **18,** 15–28.

Yamaguchi T, Nagasawa N, Kawasaki S, Matsuoka, Nagato Y, Hirano HY. 2004. The *YABBY* gene *DROOPING LEAF* regulates carpel specification and midrib development in *Oryza sativa. The Plant Cell* **16,** 500–509.

Yanofsky MF, Ma H, Bowman JL, Drews GN, Feldmann KA, Meyerowitz EM. 1990. The protein encoded by the *Arabidopsis* homeotic gene *AGAMOUS* resembles transcription factors. *Nature* **346,** 35–39.

Yu J, Hu S, Wang J, *et al.* 2002. A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). *Science* **296,** 79–92.

Zahn LM, Leebens-Mack JH, Arrington JM, Hu Y, Landherr LL, dePamphilis CW, Becker A, Theissen G, Ma H. 2006. Conservation and divergence in the AGAMOUS subfamily of MADS-box genes: evidence of independent sub- and neofunctionalization events. *Evolution and Development* **8,** 30–45.