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REVIEW

Molecular aspects of flower development in grasses

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Abstract The grass family (Poaceae) of the monocotyledons includes about 10,000 species and represents one of the most important taxa among angiosperms. Their flower morphology is remarkably different from those of other monocotyledons and higher eudicots. The peculiar floral structure of grasses is the floret, which contains carpels and stamens, like eudicots, but lacks petals and sepals. The reproductive organs are surrounded by two lodicules, which correspond to eudicot petals, and by a palea and lemma, whose correspondence to eudicot organs remains controversial. The molecular and genetic analysis of floral morphogenesis and organ specification, primarily performed in eudicot model species, led to the ABCDE model of flower development. Several genes required for floral development in grasses correspond to class A, B, C, D, and E genes of eudicots, but others appear to have unique and diversified functions. In this paper, we outline the present knowledge on the evolution and diversification of grass genes encoding MIKC-type MADS-box transcription factors, based on information derived from studies in rice, maize, and wheat. Moreover, we review recent advances in studying the genes involved in the control of flower development and the extent of structural and functional conservation of these genes between grasses and eudicots.

Keywords Floral organogenesis · Floral transcription factors · MADS-box genes · Phylogenesis · Spikelet

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Introduction

Grasses belong to the Poaceae family, which includes about 10,000 species and represents one of the most interesting angiosperm taxa in terms of morphological diversity, systematics, ecology, and economic importance (Grass phylogeny working group 2001). Grasses are widespread throughout the world and include staple crops such as wheat, rice, maize, barley, oat, and sugar cane.

The Poaceae are monocotyledons, flowering plants that evolved from an ancestral monocotyledon lineage during the late Cretaceous, 60–80 million years ago (mya) (Bremer 2002; Magallon and Sanderson 2005). The grass inflorescence is composed of spikelets, each containing 1–40 florets (Schmidt and Ambrose 1998), wherein sepals are replaced by the leaf-like floral organs palea and lemma, and petals by lodicules that consist of fleshly or scale-like organs (Bommert et al. 2005; Zanis 2007). Genetic and molecular studies have allowed the identification of several genes that play key roles in regulating inflorescence architecture and floral organ identity and development (Bommert et al. 2005; Doust 2007; Kellogg 2007; Zanis 2007; Thompson and Hake 2009). These results provide a framework for comparing flower morphogenetic and developmental aspects among grass species and in relation to well-characterized nongrass model species.

The elaboration of the ABC model of floral development based on homeotic floral mutants of *Arabidopsis* and *Antirrhinum* has been one of the most significant advances in plant biology (Coen and Meyerowitz 1991). It explains how the combined functions of three classes of homeotic genes (A, B, and C) determine the organ identities (sepals, petals, stamens, and carpels) of the four concentric floral

whorls. The class A action specifies the sepal identity in the first whorl; the class A and B joint activities control the petal identity in the second whorl; the class B and C activities control the stamen identity in the third whorl; the class C activity specifies the carpels in the fourth whorl. In *Arabidopsis,* the class A genes include *APETALA1* (*AP1*) and *APETALA2* (*AP2*), the class B genes *APETALA3* (*AP3*) and *PISTILLATA* (*PI*), whereas the C function is provided by the single gene *AGAMOUS* (*AG*) (recently reviewed in Causier et al. 2010). Except for the class A gene *AP2*, all ABC genes encode MADS-box transcription factors of the MIKC type. The ABC model was later extended to include two more functions, yielding the "ABCDE model" (Theissen 2001). In *Arabidopsis,* the D function controls the ovule development and is contributed, redundantly with *AG*, by three further *AG*-like genes: *SEEDSTICK* (*STK*), *SHATTERPROOF1,* and *2* (*SHP1* and *SHP2*). The E function is required for sepal, petal, stamen, and carpel development and is provided by the *SEPALLATA* genes (*SEP1*, *SEP2, SEP3, and SEP4*), a set of four similar and redundant MIKC-type MADS-box genes, previously identified as *AGL2*-like. Analysis of the E-function proteins has shown that they act as "bridging molecules" and mediate the physical interactions between A- and B-, B- and C-, and C- and the ovule-specific D-class proteins. Based on these findings, a quaternary model has been proposed for the ABCDE functions of the MADS-domain proteins (reviewed in Immink et al. 2010).

The extent to which models based on eudicot flower development can be applied to grasses is an important issue from both evolutionary and applied points of view. Several genes required for floral development in grasses have been identified, cloned, and characterized by forward and reverse genetic approaches. Not surprisingly, most of them are homologous to class A, B, C, D, and E genes previously cloned from eudicots. However, experiments based on forward genetic strategies have also identified some floral regulators that do not have a functional eudicot counterpart and appear to have unique functions in grass floral development.

This paper provides an update of the present knowledge on the evolution and diversification of grass genes encoding MIKC-type MADS-box transcription factors, incorporating information based on comprehensive studies concerning their cloning and characterization in rice, maize, and wheat. Moreover, we review recent progress in elucidating the genetic and molecular mechanisms involved in the control of grass flower development. An understandable presentation of these results requires a summing up of the evolutionary history of the MIKC-type MADS-box gene family in flowering plants, of the phylogeny of grasses, and of the structural features of the grass inflorescence.

Evolution of floral MADS-box gene family in angiosperms

The prominent role of MIKC-type MADS-box genes in flower and ovule development has been established in model eudicot species. Phylogenetic studies have shown that MIKC-type MADS-box genes can be clustered into several major clades, or subfamilies, each enclosing genes produced by duplication events. On the basis of sequence similarity, 13 MIKC-type MADS-box gene subfamilies have been identified among eudicotyledonous angiosperms; each subfamily has been named after the first identified clade member (Becker and Theissen 2003). The class A, B, C, D, and E genes of different plant species can be assigned to separate subfamilies, named after the enclosed genes of *Arabidopsis* and *Antirrhinum*: *AP1/SQUAMOSA*-like (class A), *AP3/DEFICIENS*-like or *PI/GLOBOSA*-like (Class B), *AG*-like (class C and D), and *AGL2*-like (*SEP*) (class E). The establishment of different gene clades of the MIKC-type MADS-box gene family has been shown to correlate with the occurrence of major plant groups. Thus, it has been proposed that the duplication and diversification of these genes may have played an important role in the innovation of the regulatory network that determines floral morphology (Zahn et al. 2005; Veron et al. 2007; Litt and Kramer 2010). For example, gymnosperms (the closest living relatives of flowering plants) have a single B-function gene, whereas all angiosperms have at least two of them (*AP3*-like and *PI*-like genes). Evidently, the two B-function gene lineages with homologs of *AP3* and *PI* originated from the duplication of a single ancestral B-function gene, preceding the origin of the angiosperms, estimated approximately 260–290 mya (Kim et al. 2004). A similarly ancient duplication event in the C-function lineage has led to two lineages in angiosperms, one including *AG* homologs with roles in stamen and carpel identity, the other one with ovule-specific D function (Kramer et al. 2004). Likewise, gene duplications formed the *SEP* groups *SEP1/2/4* (*AGL2/3/4*) and *SEP3* (*AGL9*) in angiosperms (Zahn et al. 2005). The corresponding duplications of these key floral organ identity genes before the angiosperms' origin may have somehow promoted diversification and innovation in the plant reproductive program, ultimately resulting in the origin of the flower itself. Similarly, within each of the *AP1*, *AP3*, *AG,* and *SEP1/2/4* (*AGL2/3/4*) lineages, additional gene duplications have occurred before the diversification of extant core eudicots to create the gene lineages *euFUL*, *euAP1* and *AGL79* (*AP1*), *euAP3* and *TM6* (*AP3*), *euAG* and *PLE* (*AG*), *AGL2*, *AGL3,* and *FBP9* (*SEP1/2/4*) (Litt and Irish 2003; Kramer et al. 2004; Kim et al. 2004; Stellari et al. 2004; Zahn et al. 2005; Shan et al. 2007). Phylogenetically, close paralogs have similar but distinct expression patterns, suggesting that

they perform related but distinct functions. Moreover, several floral organ identity genes, such as *AP1* and *AP3* of *Arabidopsis*, appear as novel genes generated in pre-core eudicot duplication events (see below). In fact, due to frameshift mutations, the C-terminal regions of their encoded proteins are no longer homologous to those of the paleoAP1 and paleoAP3 proteins (Vandenbussche et al. 2003; Litt and Irish 2003). Considering that the core eudicots are a successful group with very elaborate and highly derived floral structures, many authors have suggested that the origin of the core eudicot-specific floral structures may have been caused by the inclusion of more regulatory genes and their complex interactions with the already well-organized regulatory network for floral development in basal eudicots (Irish 2006; Kramer and Zimmer 2006). Understanding the role of gene duplication in floral diversification represents the key to comprehending the evolution of the angiosperms and, among them, of the grasses.

Many gene duplications have arisen simultaneously via polyploidy, an important force throughout angiosperm evolution (Soltis et al. 2009). Genomic analyses showed the occurrence of polyploidization before or coincident with the origin of angiosperms and indicated that many lineages have since undergone additional whole-genome duplication (WGD) events (reviewed in Soltis et al. 2009 and Van de Peer et al. 2009). Sequence analysis of the whole *Arabidopsis* genome detected three ancient polyploidy events: the α duplication, which occurred within the Brassicales, and the more ancient β and γ duplications. The γ event has been dated before the origin of angiosperms and the β event just before or coincident with the divergence of the major core eudicot lineages (Bowers et al. 2003). The whole-genome sequences of *Populus trichocarpa* (poplar), *Vitis vinifera* (grapevine), and *Carica papaya* (papaya) have supplied new evidence on number and timing of early WGDs in angiosperm evolution and confuted previous interpretations (Tuskan et al. 2006; Jaillon et al. 2007; Ming et al. 2008). On the basis of several large-scale phylogenetic studies, *Vitis* is considered an early diverging rosid, sister to both *Arabidopsis* and poplar (Jansen et al. 2006, 2007). Jaillon et al. (2007) suggested that the common ancestor of grapevine, poplar, and *Arabidopsis* was an ancient hexaploid species (this is now considered the γ event), which possibly arose after the split between monocots and eudicots. Further independent genome duplications took place later in Brassicales (α and β) and in *Populus* lineages. Although papaya is not closely related to grapevine, its genome shows a triplicate structure similar to that of grapevine (Ming et al. 2008). Papaya belongs to the order Brassicales and has been estimated to have diverged from *Arabidopsis* only about 70 mya (Ming et al. 2008). The most parsimonious explanation would be that the hexaploid origin (leading to a triplicate genome structure) is ancestral and shared between grapevine and papaya. Additional duplications in *Arabidopsis* do not appear shared with papaya, meaning that the *Arabidopsis* lineage underwent two genome duplications (α and β) after its divergence from the papaya lineage. The recent completion of the soybean genome sequence (Schmutz et al. 2010) has brought new evidence to the hexaploid nature of the common ancestor of most eudicot species; soybean underwent two additional rounds of WGD: an allotetraploidization specific of the soybean lineage would have occurred approximately 13 mya, after a previous duplication that affected the legumes' progenitor (see below) about 60 mya (Bertioli et al. 2009).

The exact timing and nature of the events giving rise to the ancestral triplicate genome structure is still debated (Soltis et al. 2009; Van de Peer et al. 2009). For instance, although there is some evidence for an older duplication in the rice genome (Paterson et al. 2005), which might be shared by all monocots (Velasco et al. 2007), conclusive evidence of ancestral hexaploidy common to eudicots and monocots is still lacking (Jaillon et al. 2007; Tang et al. 2008). On the basis of extensive collections of expressed sequence tag (EST) data, a genome duplication has also been proposed to have occurred early in the evolution of magnoliids, at least 100 mya (Bell et al. 2005), in particular in the common ancestor of tulip poplar (*Liriodendron tulipifera*) and avocado (*Persea americana*) (Cui et al. 2006). However, since there is no genomic assembly for any magnoliid species yet, it has not been possible to investigate whether this large-scale gene duplication corresponds to the hexaploidy event or represents an independent WGD in the magnoliid lineage. Thus, although some monocot and magnoliid species do show traces of duplications early in their evolution, it has yet to be established whether they share the same hexaploidy event with other monocots and magnoliids and, if not so, which and how many lineages share the old duplications proposed in these groups. What is clear, however, based on a recent analysis considering collinearity between tomato (*Solanum lycopersicum*) and the triplicate regions in grapevine, is that the hexaploidy occurred before the split between asterids and rosids and therefore pre-dates the divergence of most eudicot lineages (Tang et al. 2008).

Besides the more ancient duplication events shared by most angiosperms, many plant lineages show traces of additional, more recent WGD events (Soltis et al. 2009; Van de Peer et al. 2009). As previously described for the Brassicaceae, some of the most diverse and species-rich clades, such as the families Poaceae (see below), Fabaceae, Solanaceae, and Asteraceae, have been suggested to have undergone a WGD before their diversification, although the exact timing has yet to be determined (Soltis et al. 2009; Van de Peer et al. 2009). Interestingly, many independent

WGDs, such as those in cereals (e.g., rice and sorghum), legumes (e.g., soybean and *Medicago truncatula),* tomato, lettuce (*Lactuca sativa*), cotton (*Gossypium hirsutum*), poplar, and banana (*Musa spp*.), appear to have occurred about 50–70 mya (Van de Peer et al. 2009). Recently, it has been suggested that these duplication events were related to the Cretaceous–Tertiary extinction event, which was the most recent large-scale mass extinction of plant and animal species, including the dinosaurs (Fawcett et al. 2009). However, one should be cautious when linking polyploidization events with adaptation or species diversification, as these are often difficult to test and require further investigation (Van de Peer et al. 2009).

As described, genome evolution in angiosperms has been complex, with a number of rounds of genome duplications followed, in some cases, by gene deletions, making difficult the identification of strictly homologous (same function) genes. Thus, while there is considerable interest in identifying homologous genes controlling floral development between grasses and higher eudicots such as *Arabidopsis*, the identification of strictly homologous genes is not always straightforward. In an effort to distinguish homology relationships in multigene families, Fitch (1970) used the terms "orthology" and "paralogy". Orthology refers to genes that diverged through speciation, whose homology can be traced through speciation events, such that the history of the orthologous genes would reflect the species history. Paralogy, in contrast, refers to the homology between genes deriving from duplication events. These two concepts are often confused, but their correct usage is critical for providing insight into the conservation of gene structure and function between species. Since the definition of paralogy does not refer to speciation events, genes in different organisms that arose from gene duplication in an ancestral genome should be considered as paralogous (Sonnhammer and Koonin 2002). However, when multiple gene copies are compared between different species, often they include both orthologous and paralogous genes, due to gene duplications following speciation events. In these cases, there is orthology between clades of paralogous genes, which have been termed co-orthologs by Sonnhammer and Koonin (2002). The orthology assignments of genes can be made even more complex by gene loss following gene duplication. After duplication, in fact, a copy of a particular gene may have been retained at a locus in a species, but lost in the second one, whereas the second copy may have been retained in both species at orthologous locations. Therefore, loss of orthologous copies may result in paralogues rather than orthologs being compared in genomic analyses. Understanding both the evolutionary history of the gene phylogeny as well as species phylogeny is essential to differentiate orthologous (speciation) and paralogous (gene duplications) evolutionary events. Several additional terms that have been proposed to distinguish various subtypes of orthology and paralogy include the already cited co-orthologs, outparalogues, and inparalogues (Sonnhammer and Koonin 2002). The term co-orthologs is referred to paralogous genes produced by duplications of orthologous genes subsequent to a given speciation event (also called lineage-specific expansions of paralogous families), which is commonly observed between distantly related species (e.g., rice and *Arabidopsis*). The terms outparalogues and inparalogues are introduced to distinguish paralogues that originated before a given speciation event from those that originated afterward.

Evolutionary history of the grasses

The evolutionary and phylogenetic relationships among the approximately 10,000 extant species of grasses have long been debated. The most comprehensive overview of grass phylogenetic relationships stemmed from the successful collaboration of the Grass Phylogeny Working Group (2001) (GPWG), which, using both morphological and molecular data and 62 exemplar species, recognized 12 monophyletic subfamilies within the Poaceae family, with Sanchez-Ken et al. (2007) later adding the subfamily Micrairoideae. Further analyses confirmed the major relationships found by the GPWG, showing additionally that the outgroup families Ecdeiocoleaceae and Joinvilleaceae form a clade sister to the Poaceae, whereas Flagellariaceae clusters into a clade, named graminid, which includes Poaceae, Ecdeiocoleaceae, and Joinvilleaceae and is sister to the Restionaceae (Marchant and Briggs 2007) (Fig. 1). The three earliest-divergent lineages of basal grasses—Anomochlooideae (*Anomochloa* and *Streptochaeta*), Pharoideae (*Pharus*), and Puelioideae (*Puelia* and *Guaduella*), previously included in the subfamily Bambusoideae—comprise few species and are generally herbaceous plants of tropical forest understoreys. The great radiation of species occurred in the "crown group" of grasses, which diverged approximately 50–70 mya (GPWG 2001; Bremer 2002). A wide clade (the BEP clade) comprises the basal sister subfamilies Bambusoideae (bamboos) and Ehrhartoideae (including rice and wild rice) and the Pooideae (including wheat, oats, barley, etc.) (Fig. 1). This large group of approximately 4,200 species is sister to the PACCMAD clade, which includes the subfamilies Panicoideae, Arundinoideae, Chloridoideae, Centothecoideae, Micrairoideae, Aristidoideae, and Danthonioideae (Sanchez-Ken et al. 2007). The large subfamily Panicoideae includes two tribes, the Paniceae, containing the millets, and the Andropogoneae, containing sorghum, maize, and sugar cane.

Studies on the phylogeny of grasses based on morphological and paleontological information have been integrated

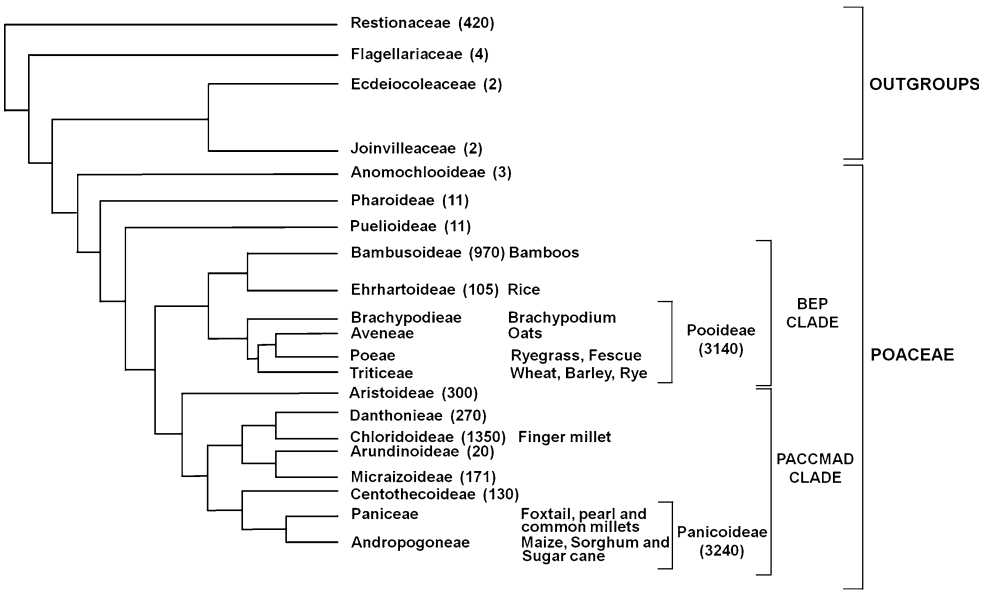

Fig. 1 Summary of grass (Poaceae) phylogeny including outgroup families based on: GPWG (2001), Doust (2007), Sanchez-Ken et al. (2007) and Marchant and Briggs (2007) Phylogenetic analyses recognized 13 monophyletic subfamilies within the Poaceae family. The "crown group" of grasses includes two major clades: BEP and PACCMAD. For the Pooideae and Panicoideae subfamilies, representative tribes are shown. The most economically important grass species are provided following the subfamily or tribe name. The approximate number of species is indicated beside the subfamilial labels

by genomic data of some major cereal grasses (see Doust 2007 and Bolot et al. 2009 for reviews). Molecular analyses have revealed that a WGD pre-dates the divergence of the wild relatives of modern cereal grasses. During the first third of their subsequent evolution, there was little molecular divergence (Paterson et al. 2004), whereas marked genomic divergence occurred in the last two-thirds of the 50–70 million years since the main cereal grass lineages separated (Paterson et al. 2004), resulting in genome size differences ranging from the 420 Mb genome of rice to the approx. 17,000 Mb genome of wheat (Goff et al. 2002). Integration of data from independent analyses of gene duplications within the genomes and of chromosomal synteny between the four major cereals (wheat, rice, maize and sorghum) led to the identification of seven shared duplications in the four compared species and the definition of five ancestral chromosomal groups (Salse et al. 2008). The characterization of the seven paleo-duplications and the relationships between conserved regions allowed the identification of the evolutionary events that shaped the grass genomes since their divergence from a putative ancestor species with an haploid number of five chromosomes ($n = 5$) (Bolot et al. 2009). After a WGD event ($n = 5 + 5 = 10$ chromosomes) about 50–70 mya, the ancestral genome underwent two inter-chromosomal translocations and fusions that resulted in an $n = 12$ intermediate ancestor, ($5 + 5 + 2 = 12$ chromosomes, A1–A12). Rice retained the 12 original haploid chromosome number, whereas the other grass genomes underwent further rearrangements of chromosome number and structure. In rice, additional segmental duplications, including the recent duplications (7–8 mya) over approximately 3 Mb at the terminal ends of chromosomes 11 and 12, occurred without modifying the basic structure of 12 chromosomes. The maize and sorghum karyotypes evolved from the ancestor with 12 chromosomes through two chromosomal fusions (between A3 and A10, A7 and A9) that resulted in a Panicoideae ancestor with $n = 10$ ($5 + 5 + 2 - 2$) chromosomes (Salse et al. 2008). Whereas the sorghum karyotype remained similar to that of the ancestral species, with $n = 10$ chromosomes, maize underwent a WGD event about 11 mya, resulting in an intermediate with $n = 20$ chromosomes (Gaut and Doebley 1997; Gaut 2001; Swigonova et al. 2004). After this tetraploidization, a number of chromosomal fusions led to the maize karyotype including 10 chromosomes, $n = 10$ ($(5 + 5 + 2 - 2) \times 2 - 10$). At least 17 chromosomal fusions must have occurred to explain the paralogous relationships that can be detected today among the maize chromosomes (Bolot et al. 2009). The intermediate ancestral genome with 12 chromosomes of the Triticeae underwent five chromosomal fusions (A5 + A10, A6 + A8, A9 + A12, A3 + A11, and A4 + A7) that resulted in a basic number of $n = 7$ ($5 + 5 + 2 - 5$) chromosomes for the wheat and barley karyotypes. The analysis of the recently released genome of *Brachypodium distachyon*, which is the first member of the Pooidae subfamily to be sequenced, is consistent with an evolutionary model that shaped the five *Brachypodium* chromosomes from a five-chromosome ancestral genome through a 12-chromosome intermediate involving seven major chromosome fusions (The International *Brachypodium* Initiative 2010).

Spikelets and florets, the structural units of the grass inflorescence

One of the most striking features of grasses is their complex floral and inflorescence structure, which includes

multiple meristem types, as revealed by mutants affecting discrete stages of inflorescence development in maize, rice, and wheat (for reviews see Bommert et al. 2005; Kellogg 2007; Thompson and Hake 2009).

After flag leaf initiation, the shoot apical meristem (SAM) of wheat shifts from the vegetative to reproductive phase, elongates to form a collar (the first reproductive primordium), and becomes an inflorescence meristem (IM) (Hay and Ellis 1998). This then forms a double-ridge structure, which represents the beginning of spikelet initiation. The upper part of the double-ridge, which consists of two upheavals, then acquires spikelet meristem (SM) identity and forms a spikelet; the lower part, originally a leaf meristem, disappears as growth proceeds. Spikelets are arranged to form two opposite rows along the rachis (Fig. 2a); their number is determined by the timing of terminal spikelet initiation, which depends upon the genotype and environmental conditions. The spikelet is composed of multiple florets (usually six to eight) joined alternately on opposite sides of an axis (rachilla); some of those in apical positions can be sterile due to hypoplasia.

In rice, the inflorescence meristem (IM) produces several primary branch meristems (PBMs) before terminating; each of them initiates secondary branches (SBM) as axillary meristems. Thus, the IM degenerates after making PBMs, and the internodes of both primary and secondary branches elongate to form the panicle architecture. The spikelet meristem (SM) initiates from SBM or directly from PBM, which then forms floret meristems (FMs) (Fig. 2b). Each rice spikelet contains a single fertile floret subtended by two pairs of small bracts called empty glumes (or sterile lemma) and rudimentary glumes, as described in more detail below.

Maize is a monoecious plant with two inflorescence types: the ear, which arises in the axil of a vegetative leaf and produces the female flowers, and the tassel, which develops the male flowers and is located at the apex of the plant. The tassel IM first initiates several lateral meristems (BMs) that become long branches and then switch to form transient spikelet pair meristems (SPMs) and produce a pair of SMs; these are transient as well and form the bracts, which are sterile leaves, and two floral meristems (FMs) (Fig. 2c). The unbranched ear does not produce BMs, and only one of the two FMs develops into a fertile flower (Fig. 2d).

The spikelet meristem is unique to all grasses except the subfamily Anomochlooideae (see below), which is sister to all of the Poaceae (Fig. 1), but the spikelet determinacy differs by species: in wheat, for example, the SMs are indeterminate (Murai et al. 2002), whereas in maize, spikelet and spikelet pair meristems are considered

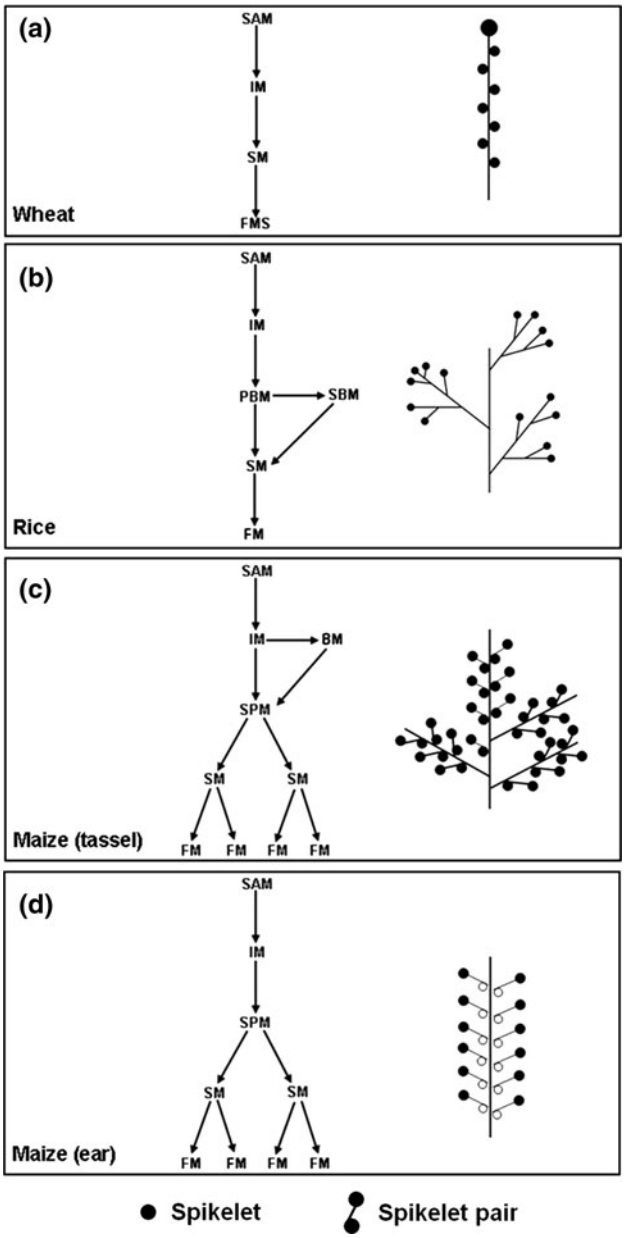

Fig. 2 Inflorescence development in wheat (**a**), rice (**b**) and maize tassel (**c**) and ear (**d**). *Left, diagram* illustrating the different meristems formed during the inflorescence development in each species; *right*, schematic drawing of the inflorescence of the three species. **a** Wheat inflorescence meristem (IM) transits from the shoot apical meristem (SAM) directly to form indeterminate spikelet meristem (SM) as lateral branch, which will form several floral meristems (FMs). **b** In rice, the IM initiates primary branch meristems (PBMs) and this initiates the secondary branch meristems (SBMs). The SMs initiate from SBMs or directly from PBMs and then form floret meristems (FMs). **c** The maize tassel IM first initiates several lateral meristems (BMs) that become long branches; the IM later switches to producing spikelet pair meristems (SPMs). SPMs produce a pair of SMs, which in turn form two floral meristems (FMs). **d** The maize ear is unbranched and does not produce BMs; in addition only one of the two produced FMs develops into a fertile flower

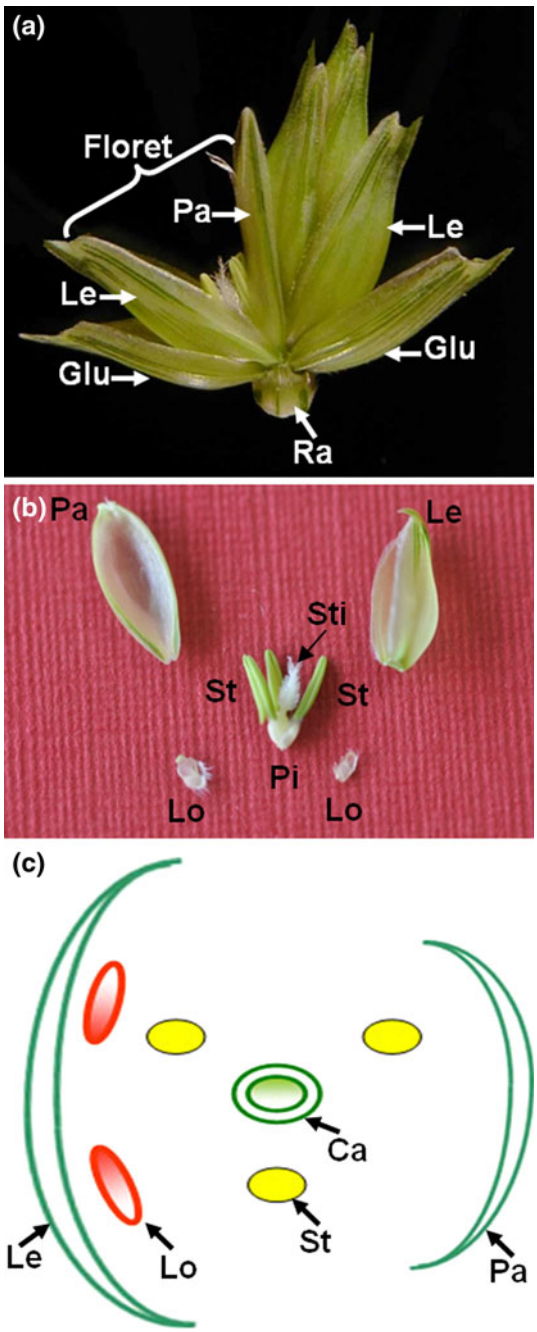

Fig. 3 Spikelet (**a**), single floret organs (**b**) and diagrammatic representation of a transverse section through a wheat floret (**c**). The wheat inflorescence (spike or head) develops at the tip of a stem and is composed of spikelets, which include florets. The florets join the axis (rachilla) alternately on opposite sides and are encompassed by two small bracts (glumes). Each spikelet encloses multiple florets (usually six to eight); some of those in apical position can be sterile, due to hypoplasia. The reproductive organs of each floret are enveloped by two leaf-like structures, lemma and palea, and at the base of the ovary, there are two small glandular organs, the lodicules, that, swelling at anthesis, spread the palea and lemma apart to open the floret for wind pollination. The reproductive organs of the floret comprise three stamens and a pistil, which is a unilocular carpel and consists of the ovary containing the ovule and two filamentous styles, each terminating with a feathery stigma. In **b**, the single floret organs have been detached from the rachilla. *Glu* glumes, *Pa* palea, *Le* lemma, *Lo* lodicules, *St* stamens, *Pi* pistil, *Sti* stigma, *Ra* rachilla, *Ca* carpel

determinate, because they produce a defined number of organs (Vollbrecht et al. 2005).

The spikelet represents the basic unit of the grass inflorescence architecture (Fig. 3); it is a compact axillary branch composed of one to many florets, subtended by glumes, which can be large and encompass the entire set of florets, as in maize and sorghum, or severely reduced structures at the base of the floret, as in rice. The two glumes encompass a characteristic and species-specific number of florets, e.g., two florets per spikelet in maize, just one in barley. Some species (e.g., wheat, Fig. 3) have multiple florets enclosed by the glume pair, some of them being sterile. Although rice is occasionally and erroneously described as having single-flowered spikelets, different developmental genetic studies (Prasad et al. 2001; Komatsu et al. 2003) have indicated that the rice spikelet contains three florets subtended by two tiny rudimentary glumes. The uppermost floret is bisexual and fertile, whereas the lower two florets are strongly reduced and sterile. The typical grass florets (Fig. 3) consist of: (1) the lemma, often considered a bract in whose axil the flower arises; (2) the palea, a bract-like organ, occasionally interpreted as prophyll of the floral branch subtended by the lemma; (3) two (rarely three or more) lodicules, small scale-like or fleshy organs that swell up at anthesis to open the floret and exert the anthers; in some grasses, for instance bamboos, there are three lodicules that alternate with the anthers, whereas in most grasses (e.g., wheat, Fig. 3), the medial (adaxial) lodicule aborts, leaving only the two abaxial lodicules adjacent to the lemma; (4) the stamens, the male reproductive organs, generally occur in one (e.g., wheat, Fig. 3) or two (e.g., rice) whorls of three; (5) the female reproductive unit (gynoecium) is composed of three fused carpels, generally with two stigmas and a single ovary. In rice, the lower sterile florets develop only a reduced lemma, known as empty glume or sterile lemma.

Grasses exhibit a variety of sexual systems, including bisexual, unisexual (staminate and pistillate), and sterile florets. Unisexual florets have evolved independently on multiple occasions within grasses, with as many as six estimated transitions in the species belonging to the Chloridoideae subfamily alone (Columbus 1999). Plants that make unisexual florets are most commonly monoecious, with male and female florets on separate inflorescences of the same plant (e.g., maize, subfamily Panicoideae; *Lithachne*, sub. Bambusoideae; *Pharus*, sub. Pharoideae); dioecious, with male and female florets on separate plants (*Bouteloua dioica*, *Distichlis spicata*, sub. Chloridoideae); or andromonoecious, with male and perfect flowers on the

same plant (e.g., *Sorghum*, sub. Panicoideae). Maize is the only grass wherein significant genetic and molecular data on sex determination are available (see Malcomber and Kellogg 2006; Thompson and Hake 2009 for reviews); like other grasses with unisexual florets, its florets are initially bisexual, but then the development of carpel and stamen primordia arrests in male and female florets, respectively.

Phylogenetic and morphological studies of grasses and other monocots have improved our understanding on the origin of the grass spikelet and its organs. The grass spikelet was probably derived from an ancestral monocot flower consisting of a perianth containing either two whorls of tepals or an outer whorl of sepals and an inner whorl of petals, perhaps similar to the perianth of *Joinvillea* and *Ecdeiocolea* (Rudall et al. 2005; Zanis 2007), whose families (Joinvilleaceae and Ecdeiocoleaceae) are closely related to Poaceae (Fig. 1). The androecium likely contained two whorls with three stamens each, the gynoecium contained an ovary with three stigmas. The earliest grasses had three stigmas, a relict of the three fused carpels inherited from their ancestors; this number was reduced to two after the speciation event that led to the genus *Pharus* (Pharoideae subfamily in Fig. 1) (Kellogg 2001). Like their nongrass ancestors, the earliest species also had six stamens; it is not clear from the grass phylogeny when the shift from six to three occurred, probably after the divergence of the *Puelia/Guaduella* group (Puelioideae subfamily in Fig. 1) (Kellogg 2001). Before or soon after the origin of the grasses, the perianth was highly modified, resulting in floret organs such as palea, lemma, and lodicules. Lodicules are present in the male floret of *Pharus* but not in the genera *Streptochaeta* and *Anomochloa*, the earliest lineage of the grasses (Anomoclooideae subfamily in Fig. 1). Moreover, neither *Streptochaeta* nor *Anomochloa* have structures that are clearly homologous with glumes, lemma or palea, and thus, neither can be described as possessing typical grass spikelets. The inflorescence structures of these two species are difficult to interpret when compared to the ancestral monocot condition or to the morphology of their sister taxa in the spikelet-bearing grasses. The inflorescence branches of both *Streptochaeta* and *Anomochloa* have been interpreted either as (1) partial inflorescences and termed pseudospikelets (Soderstrom 1981) or spikelet equivalents (Judziewicz et al. 1999) or as (2) highly modified spikelets (Sajo et al. 2008).

The inflorescence of *Streptochaeta* bears multiple, spirally arranged, primary branches, each terminating in a flower with six stamens and a three carpellary gynoecium (Whipple et al. 2007; Preston et al. 2009). The fertile floral organs of *Streptochaeta* are thus similar in position and number to those of many other monocots, including the sister groups of the grasses. Outside the stamens, there is a set of three bracts, which are comparable in position and number, but not phyllotaxis, to the inner perianth whorl of other monocots (Whipple et al. 2007). In addition, these extrastaminal bracts express the *AP3/DEF*-like and *PI/GLO*-like genes (B-class genes), which are markers of the extrastaminal domain in many angiosperms (Whipple et al. 2007) (see below). Thus, the extrastaminal bracts in *Streptochaeta* and the lodicules in the spikelet-bearing grasses are comparable to inner tepals. Below the three extrastaminal bracts, inflorescence branches of *Streptochaeta* bear eight or nine additional bracts. The bracts are conventionally numbered from the base of the branch, so those comprising the inner perianth (three extrastaminal bracts) are numbered 10–12 or 9–11. The homologies of the bracts, in particular eight or nine of the more proximal, are unclear and have been the subject of much discussion (Soderstrom 1981; Sajo et al. 2008). The most proximal bracts (numbered 1 and 2) are more or less opposite each other, whereas bracts 3, 4, and 5 (if present) are arranged as a spiral. Opposite and above bract 5, there is a large bract that develops a long awn (bract 6). The awn extends during development to become filamentous and twisted (hence the name *Streptochaeta*) and ultimately becomes entangled with other awns of the inflorescence; it is presumed to be an adaptation for dispersal. Bract 6 is adaxial to the main inflorescence axis, above it there are five to six more bracts (bracts 7–11/12) in two whorls or spirals. At maturity, the floral branch disarticulates below the basal bracts along a distinct abscission zone (Sajo et al. 2008; Preston et al. 2009).

Soderstrom (1981) postulated that all the bracts in *Streptochaeta* would be homologous to floral bracts of conventional monocots, and that none of them constitutes a perianth. His interpretation thus suggested that the primary inflorescence branch would be actually a complex of branches. While Soderstrom (1981) interpreted the inflorescence branch of *Streptochaeta* as a largely vegetative shoot, Sajo et al. (2008), investigating floral development of *Streptochaeta spicata* Schrad. ex Nees, proposed that the inflorescence branch is actually a highly modified spikelet. In their interpretation, bracts 1–5 would correspond to empty glumes, bracts 6–8 to lemma/palea and outer tepals, and bracts 9–11 to lodicules and inner tepals of grass spikelets and nongrass monocots, respectively. These conclusions were consistent with the gene expression analyses of the *AP3*-like, *PI*-like, *AP1/FRUITFULL*-like and *LEAFY HULL STERILE1*-like MADS-box genes in *Streptochaeta angustifolia* and a nongrass outgroup (*Joinvillea ascendens*) reported by Whipple et al. (2007) and Preston et al. (2009) (see below) and indicate that the highly modified spikelet of *Streptochaeta* could be considered as morphologically intermediate between the true spikelets of grasses and reproductive units of their close relatives.

MIKC-type genes of the MADS-box family in grasses

Although different MIKC-type genes have been isolated from several grass species (Bommert et al. 2005; Whipple and Schmidt 2006; Dwivedi et al. 2008), the most comprehensive cloning and characterization of genes encoding MADS-box transcription factors have been carried out in rice, maize, and sorghum (Munster et al. 2002; Lee et al. 2003a; Arora et al. 2007; Zhao et al. 2010), thanks to the completion of their whole-genome sequencing, and in wheat, wherein Paolacci et al. (2007) carried out an extensive screening for MIKC-type cDNAs.

The evolutionary relationships between MIKC-type genes of grasses and *Arabidopsis* were assessed through phylogenetic reconstruction based on the alignment of 134 amino acid sequences deduced from the nucleotide sequences of 34 genes of *Arabidopsis* (Parenicova et al. 2003), 31 of rice (Lee et al. 2003a; Arora et al. 2007), 40 of maize (Munster et al. 2002; Zhao et al. 2010), and 29 of wheat (Paolacci et al. 2007) (Fig. 4). This analysis identified putative co-orthologs of MIKC-type genes in *Arabidopsis* and grasses, thus providing a minimal estimate for the number of MIKC-type genes in the most recent ancestor of monocots and eudicots which, according to molecular evolution inference, existed about 125–150 mya (Soltis et al. 2008; Bell et al. 2010). Minimal clades containing only genes from grasses (maize, rice, and wheat) were also recognized within the phylogenetic tree; they allowed for the assessment of the number of MIKC-type genes that would have been present in the common ancestor of these grass species (orthologous or outparalogous genes). Moreover, phylogenetic analysis identified within each grass species the putative paralogous genes (inparalogues), which most likely originated by duplication events occurring after the separation of their lineages. All the rice, wheat, and maize genes were assigned to 11 of the 13 known plant subclasses of MIKC-type genes, except *OsMADS32*, *TaWM16,* and *ZmMADS2*/*ZmMADS14*, which formed a separate clade, referred to as *OsMADS32* in Fig. 4. More extensive analyses would be needed to verify whether the *Arabidopsis* co-orthologs of these genes have been lost or are present only in monocot or grass genomes. So far, *FLC*- and *AGL15*-like genes have been found only in *Arabidopsis* and other Brassicaceae species (Becker and Theissen 2003). Co-orthologs of these genes have not been found in any grass species, even in the sequenced genomes of rice, maize, and sorghum (Becker and Theissen 2003; Arora et al. 2007; Zhao et al. 2010). These results demonstrate that the complexity of the MIKC branch of the MADS-box family of wheat, maize, and rice is similar to that of eudicots and that at least 11 different MIKC-type genes were already present in the last common ancestor of monocots and eudicots about 125–150 mya. Moreover, it is evident that several gene duplications occurred in the original grass lineage before maize, rice, and wheat diverged. Phylogenetic analysis identified 24 minimal clades containing putative orthologs of all the three grass species, whereas two clades included only genes of maize and rice (*GGM13* subfamily in Fig. 4) and three clades only genes of rice and wheat (*AGL17* and *SOC1* subfamilies in Fig. 4). The lack of wheat orthologs in the two minimal clades belonging to the subfamily *GGM13* might be ascribed either to incomplete MIKC gene sampling in that species or to their deletion after wheat speciation. Due to the comprehensive analysis of the MADS-box gene family in the maize genome (Zhao et al. 2010), the absence of the maize orthologs in the two minimal clades of the *AGL17* subfamily and in one clade of the *SOC1* subfamily can only be explained by their loss during maize evolution. On the basis of these lines of evidence, at least 24 (probably 29) MIKC genes would have been present in the common ancestor of maize, rice, and wheat, before their divergence about 50–70 mya (Bremer 2002). This represents a significant increase in comparison to the 11 MIKC-type genes that would have been present about 125–150 mya in the common ancestor of monocots and eudicots. Most likely, the increase of MIKC genes in the progenitor of grass species was caused by multiple duplications followed by diversification and could have been a key factor in the evolution of the complex inflorescences and floral structures common to the species of the Poaceae family. Several studies found evidence that most duplications would result from the whole-genome duplication event that occurred before the origin of the Poaceae 50–70 mya (Preston and Kellogg 2007; Xu and Kong 2007). Arora et al. (2007) located 30 MADS-box genes lying on segmental duplicated regions of rice chromosomes, whereas only 16 were found to have been retained, suggesting that relevant changes leading to the loss of some duplicated genes might have taken place following segmental duplications. All but one of the paralogous gene pairs are of MIKC-type; most of them exhibit divergent expression patterns, probably because they underwent neofunctionalization or subfunctionalization, although functional analyses are needed to test this hypothesis. Furthermore, comparative genomic studies have shown that after gene duplication, exonization of intron sequences and pseudoexonization of exon sequences would have contributed to the divergence of duplicated genes in sequence structure and possibly gene function (Xu and Kong 2007). These observations and the distinct Poaceae floral structures imply that the duplication of MIKC-type homeotic floral genes of the MADS-box family may have contributed to the formation of a more complex gene network controlling floral development and leading to the origin/diversification of more advanced regulatory and morphogenetic systems.

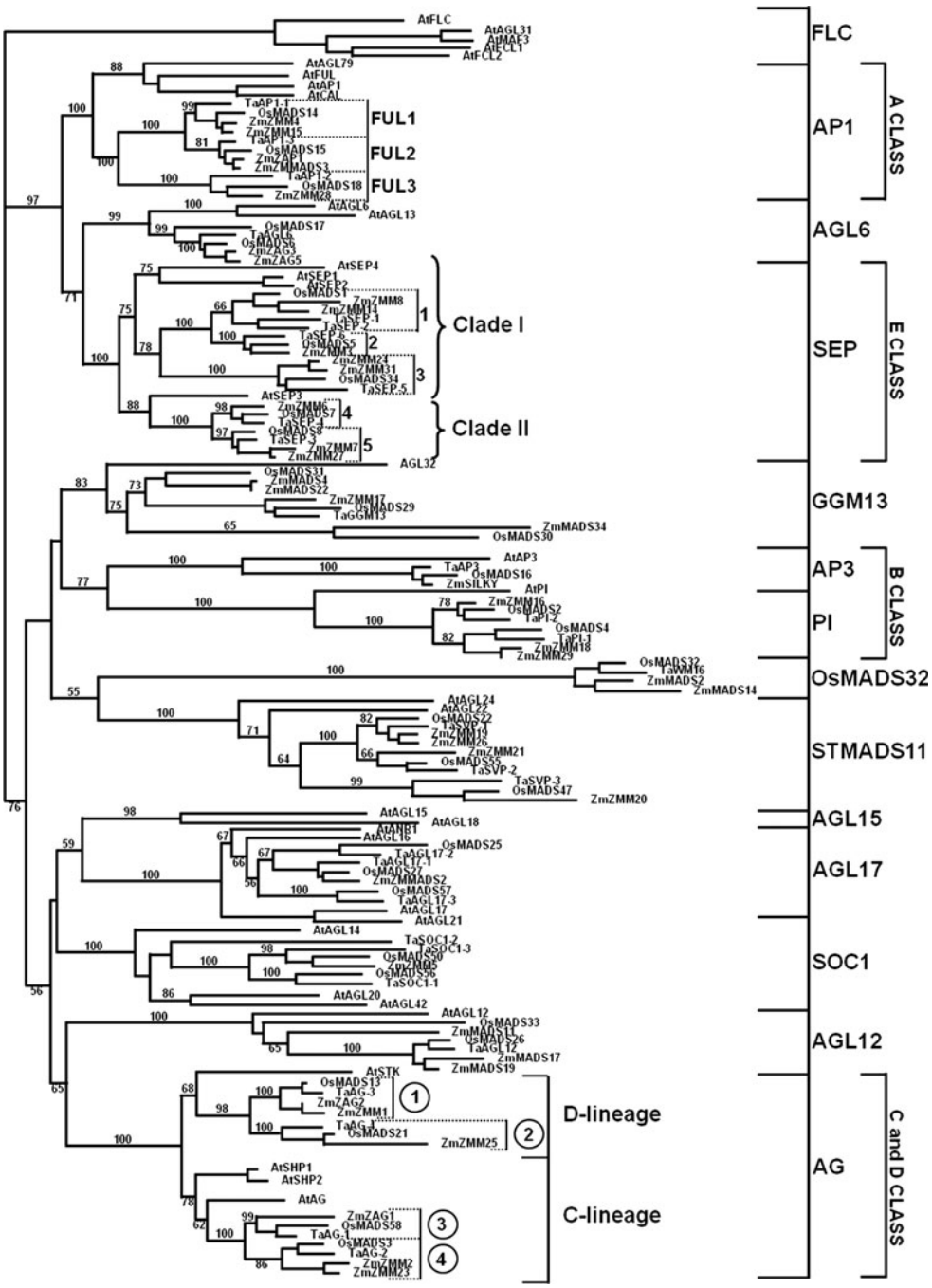

Fig. 4 Phylogenetic tree based on amino acid sequences of 134 MIKC-type MADS-box genes: 34 from *Arabidopsis* (At), 31 from rice (Os), 40 from maize (Zm), and 29 from wheat (Ta). Deduced amino acid sequences of the MIK domains were aligned using ClustalX1.83 software, and the phylogenetic tree was constructed by the neighbor-joining method and evaluated by bootstrap analysis (PHYLIP version 3.6). Five *Arabidopsis* sequences of the *FLC* subfamily were used as outgroups. Numbers on major branches indicate bootstrap percentage for 1,000 replicates. Subfamilies of the plant MIKC-type genes are indicated at the *right margin*. The three grass clades within the *AP1* subfamily *FUL1*, *FUL2,* and *FUL3* are enclosed by *square brackets,* and the two major clades of the *SEP* subfamily are enclosed by braces. The five grass clades within the *SEP* subfamily are also indicated by *numbers* showing their respective name according to Malcomber and Kellogg (2005). *1 = LHS1/OsMADS1*; *2 = OsMADS5*; *3 = OsMADS34*; *4 = OsMADS7/45*; *5 = OsMADS8/24*. Finally, the two grass clades putatively assigned to the D- (1 and 2) and C-lineages (3 and 4) within the *AG* subfamily are enclosed by *square brackets*

On the basis of these indications, it is possible to present the functional and evolutionary conservation of each class of ABCDE genes in grasses, focusing the attention on maize and rice, wherein the function of some floral MADS-box genes has been defined using mutant phenotypes. In contrast, the putative functions of the wheat homeotic floral genes will be deduced on the basis of both similar expression pattern and sequence clustering with characterized MADS-box sequences from *Arabidopsis*, maize and rice.

Class A genes

Of the two class A genes of *Arabidopsis* (*AP1* and *AP2*), only *AP1* encodes a MADS-box transcription factor; it has two distinct roles: the class A function for the identity of sepals and petals and the specification of the floral meristem identity (Mandel et al. 1992). Phylogenetic reconstructions of *Arabidopsis* genes encoding MADS-box factors have shown that *AP1* forms the clusters of *AP1/SQUA*-like genes with *CAULIFLOWER* (*CAL*), *FRUITFULL* (*FUL*), and

AGAMOUS-LIKE 79 (*AGL79*) (Fig. 4). *AP1* and *CAL* would derive from the polyploidization (probably the α event), that took place at the base of the Brassicaceae evolution (Litt and Irish 2003). *CAL* and *FUL* are involved redundantly with *AP1* in establishing the floral meristem (Kempin et al. 1995); besides this function, *FUL* is also involved in fruit development and specifies valve identity (Gu et al. 1998). The function of *AGL79* is unknown, although *DEFH28*, its orthologous gene of *Antirrhinum majus*, plays a dual role during the development of both inflorescences and carpels (Muller et al. 2001). Several studies have indicated that the *AP1/SQUA* subfamily experienced frequent gene duplications and the acquisition of novel sequence structures, which make difficult to elucidate the evolutionary history of this subfamily (see Litt 2007 for review). Recent phylogenetic analyses of *AP1/SQUA*-like genes have shown that within the core eudicots there are three major clades: *euAP1* (including *AP1* and *CAL*), *euFUL* (including *FUL*), and *AGL79* (comprising the *Arabidopsis* gene *AGL79*) (Shan et al. 2007). These three clades were probably generated through two very close duplication events preceding the diversification of core eudicots but following the split of Buxaceae and core eudicots. Moreover, phylogenetic analyses and sequence comparisons indicated that the angiosperm taxa that diverged before the two close duplications at the base of core eudicots carried only genes of *FUL*-like type. Notably, these genes showed much higher sequence similarity to the genes of the *euFUL* clade than to those of the *euAP1* and *AGL79* clades, suggesting a corresponding higher functional similarity of the *FUL*-like genes from early diverging eudicots, monocots, and basal angiosperms to the genes of the *euFUL* clade. The ancestral genes giving rise to the *euAP1* and *AGL79* clades may have been generated from an *euFUL* or *FUL*-like gene through two independent duplication events (Litt and Irish 2003; Litt 2007; Shan et al. 2007). Based on these observations and on the expression analyses of the *FUL*-like, *euFUL,* and *euAP1* genes, it has been proposed that the ancestral function of *AP1/SQUA*-like genes was the specification of floral meristem identity or phase change to reproduction, whereas their role in the specification of sepal, petal, and fruit valve identity was acquired later (Theissen et al. 2000; Litt 2007; Shan et al. 2007). So far, only in *Arabidopsis* has it been reported that the loss of an *AP1/SQUA*-like gene (*euAP1*, *euFUL,* or *FUL*-like) results in loss of identity of both the first- and second-whorl organs, suggesting that the A function performed by the *AP1/SQUA*-like genes may be confined to *Arabidopsis* or to the Brassicaceae family. The observation that *AP1* and *CAL* stem from a gene duplication that occurred within the Brassicaceae supports further the hypothesis that *AP1* may have acquired the class A function very recently. Therefore, one may expect that *AP1/SQUA*-like genes play a role in specifying floral meristem identity or in the transition from the vegetative to reproductive phase in grasses, but not in identity determination of the floral organs in the outermost whorls.

Previous phylogenetic analyses of *FUL*-like genes in grasses indicated the existence of three distinct clades: *FUL1*, *FUL2,* and *FUL3* (Preston and Kellogg 2006, 2007). Accordingly, the 11 *FUL*-like genes of wheat, maize, and rice form three subclades, each containing at least one gene for each of the three grass species (Fig. 4). This clustering has been explained by two duplication events that took place in their common ancestor (Preston and Kellogg 2006, 2007). Apparently, the first duplication occurred around the base of the monocots giving rise to the *FUL3* clade, the second occurred later, likely during a whole-genome duplication (polyploidization) at or near the base of grasses, giving rise to the *FUL1* and *FUL2* clades. The *FUL1*- and *FUL2*-like genes of rice (*OsMADS14* and *OsMADS15*) were located in regions of the chromosomes 3 and 7 identified as segmental duplications, providing genomic evidence that the gene duplication identified by phylogenetic analysis corresponds to a genome-wide duplication event (Preston and Kellogg 2007; Xu and Kong 2007). The presence of two maize genes in the *FUL1* and *FUL2* clades (Fig. 4), also observed in other subclasses of the grass MIKC-type MADS-box family, may reflect the duplication of the maize genome after its speciation, which occurred about 11 mya (Gaut 2001, Swigonova et al. 2004).

The rice *FUL3*-like gene *OsMADS18* (Fig. 4) was expressed in roots, leaves, inflorescences, and developing kernels, but not in young seedlings; its expression was detected 4 weeks after germination in leaves and surged when the plant reached the reproductive stage (Fornara et al. 2004). Functional analysis showed that RNAi-mediated silencing of *OsMADS18* did not result in detectable phenotypic alteration, indicating that this gene is probably redundant with one or more of the other *AP1/SQUA*-like genes found in rice. Over-expression of *OsMADS18* resulted in an early flowering phenotype and induced precocious initiation of axillary shoot meristems, suggesting that this gene is able to promote the differentiation program of the vegetative shoot. The expression pattern of *TaAP1-2*, the wheat *FUL3*-like gene, is similar to that of its rice ortholog *OsMADS18*, with high levels of transcripts in roots, stems, leaves, in spikes at different stage of development and in different spikelet organs at heading time (glumes, lemma, and palea). A low level of expression of *TaAP1-2* has also been detected in coleoptiles 20 days after germination, developing caryopses, reproductive spikelet organs (stamens and pistils), and lodicules (Paolacci et al. 2007).

Expression of *TaAP1-1*, the wheat *FUL1*-like gene (Fig. 2), was absent in roots, coleoptiles, and caryopses

collected 20 days after anthesis (DAA) but was high in leaves, stems, 5 DAA caryopses, and in all spikelet organs at heading time (Paolacci et al. 2007). Conversely, its rice ortholog *OsMADS14* was expressed only in inflorescence and developing caryopses (Pelucchi et al. 2002). The expression pattern of the two genes was different during flower development; in situ hybridization showed that in rice the expression started in the spikelet meristem, later was limited to the primordia of flower vegetative organs and finally switched to reproductive organs but was shut down in the vegetative organs (Pelucchi et al. 2002). Moreover, *TaAP1-1* is closely related to the wheat homoeologous genes *TaVRN-A1*, *TaVRN-B1,* and *TaVRN-D1* (Fu et al. 2005) and to *VRN1* of the diploid wheat *T. monococcum* (Yan et al. 2003), which are involved in the transition from the vegetative to the reproductive phase induced by the vernalization.

OsMADS15 (rice *FUL2*-like gene) expression was initially observed throughout the spikelet meristem, but after initiation of the spikelet organs, its transcripts were found only in the vegetative organs, i.e., lodicules, palea, lemma, and glumes (Kyozuka et al. 2000). The expression pattern of *TaAP1-3* was similar to that of its orthologous *OsMADS15*: it was expressed in all spike developmental stages and in the vegetative organs of the spikelets, but at lower level in the lodicules (Paolacci et al. 2007).

The *FUL2*-like genes of maize *ZAP1* and *ZmMADS3* (Fig. 4) are characterized by distinct expression patterns; in fact, *ZAP1* was expressed only in the nonreproductive organs of the spikelet (glumes, lemma, and palea) (Mena et al. 1995), whereas *ZmMADS3* was expressed in both vegetative and inflorescence tissues (Heuer et al. 2001). During flower development, *ZmMADS3* was expressed at intermediate stages in all the organ primordia of the ear spikelets, whereas at later stages, its expression was restricted to mature pistils. In the tassel, after organ differentiation, *ZmMADS3* was expressed only in the stamens. Among vegetative tissues, *ZmMADS3* was expressed in stem nodes and displayed a gradient, with highest expression in the uppermost node. Constitutive expression of *ZmMADS3* resulted in undifferentiated floral organs in the male spikelets and reduced male inflorescence branching (Heuer et al. 2001). This phenotype has been attributed to some interference with other MADS-box proteins, for example through inappropriate dimerization at particular stages of development. This interference hypothesis predicts that the maize *FUL2*-like genes' expression must be switched off during certain stages of floral meristem differentiation, or that *FUL2*-like genes and interacting proteins should be co-expressed only in specific floral organs.

As a whole, the function of *AP1/SQUA*-like genes in grasses is still unclear, as compared to other classes of MIKC-type MADS-box genes. Based on the available information, the grass *AP1/SQUA*-like genes would accomplish a general role in floral transition and/or in floral meristem identity. However, on the basis of *TaAP1-3*, *OsMADS15,* and *ZAP1* expression patterns, a specific role in the identity of the nonreproductive organs of spikelet (A function genes) cannot be excluded for the *FUL2*-like genes.

Class B genes

In *Arabidopsis,* the class B genes *AP3* and *PI*, required to specify petal and stamen identity, belong to the *AP3/DEF*- and *PI/GLO*-like gene groups, respectively (Fig. 4). All the single mutants of these genes display the same homeotic transformation of petals to sepals in the second whorl and of stamens to carpels in the third whorl (Jack et al. 1992; Goto and Meyerowitz 1994). This is consistent with the activity of their encoded proteins as obligate heterodimers (Goto and Meyerowitz 1994). The expression of either B-function gene is initiated independently in the second and third floral whorls, but the maintenance of high levels of *AP3* and *PI* transcripts by auto-regulation depends upon the presence of the heterodimeric protein complex (Honma and Goto 2000; Lamb et al. 2002). While the *AP3/DEF* and *PI/GLO* subfamilies originated from a gene duplication event after the separation of the lineages leading to the extant gymnosperms and angiosperms approximately 260–290 mya (Kim et al. 2004), it has become clear that in many species the B function has further been shaped and complicated by other rounds of gene duplications in both gene subfamilies (recently reviewed in Litt and Kramer 2010 and Rijpkema et al. 2010). One of the duplications in the *AP3/DEF* subfamily, which coincided with the radiation of the core eudicots and resulted in the *euAP3* lineage (to which *AP3* belongs) and *TM6* lineage (named after *tomato MADS-box gene 6*), is of special interest for the evolution of the core eudicot flower (Kramer et al. 1998). The euAP3 and TM6 proteins can easily be distinguished by their distinct C-terminal motifs, the so called euAP3 and paleoAP3 motifs. The TM6 lineage proteins share the paleoAP3 C-terminal motif with proteins found throughout the angiosperms (magnolids, monocots, and lower eudicots), whereas the proteins containing the euAP3 motif are found only in core eudicots. Remarkably, the euAP3 C-terminal motif seems to have originated from the paleoAP3 motif by a frameshift mutation (Kramer et al. 2006); thus, the euAP3 lineage, which is unique to higher eudicots, represents a divergent paralogous group. Many core eudicots have retained copies of both genes *euAP3* and *TM6*, whereas *Arabidopsis* and *Antirrhinum* have lost *TM6* (Kramer et al. 1998); as a consequence, function and regulation of *TM6*-like genes were not included in the

original ABC model. Recent studies have indicated that in species wherein both gene lineages are maintained, aspects of the B function appear to be partitioned between *TM6*- and *euAP3*-like genes. The *euAP3*-like genes appear mainly involved in the control of petal identity and development, whereas the *TM6*-like genes, redundantly with *euAP3*–like genes, participate in the control of stamen development (de Martino et al. 2006; Rijpkema et al. 2006).

Phylogenetic analysis indicated that the *AP3/DEF* subfamily includes a single grass clade clustering *TaAP3* of wheat and its putative orthologs *SILKY* of maize and *OsMADS16* of rice, whereas the *PI/GLO* subfamily is split into two grass clades, one comprising *TaPI-1* (wheat), *OsMADS4* (rice), and *ZMM18/ZMM29* (maize), the other including *TaPI-2*, *OsMADS2* (rice), and *ZMM16* (maize) (Fig. 4). The presence of at least a *PI/GLO*-like gene from wheat, rice, and maize in each of the two *PI/GLO* grass clades suggests that a duplication, likely ascribable to the WGD event that took place before the origin of the Poaceae 50–70 mya, occurred in the common ancestor of these three species. Accordingly, the *PI/GLO*-like genes of rice (*OsMADS2* and *OsMADS4*) were located in regions of chromosomes 1 and 5 identified as segmental duplications (Arora et al. 2007; Xu and Kong 2007). The two very similar genes of maize *ZMM18/ZMM2*, clustered in the clade including *TaPI-1* and *OsMADS4,* were mapped in close vicinity in the long arm of chromosome 8 (Munster et al. 2001), suggesting that they likely originated by a gene duplication event less than 11 mya, i.e., after the segmental allotetraploidization event that shaped the maize genome.

The putative B-function genes of wheat *TaAP3* (*AP3/DEF* subfamily), *TaPI-1,* and *TaPI-2* (*PI/GLO* subfamily) were expressed in spikes, 5 DAA (Days After Anthesis) developing caryopses, lodicules, stamens, and pistils from fully emerged spikes (heading stage) (Paolacci et al. 2007). The expression pattern of *TaAP3* was slightly different from that of its ortholog genes of maize (*SILKY*) and rice (*OsMADS16/SUPERWOMAN1),* which were expressed in lodicules and stamen primordia from their initiation to the later stages of their development, but not in developing carpels (Ambrose et al. 2000; Nagasawa et al. 2003). Thus, the expression of *TaAP3* in mature female organs is noteworthy and would need further investigations. *TaPI-1* and *TaPI-2* showed an expression pattern that was similar to that of their ortholog genes of maize (*ZMM18/ZMM29* for *TaPI-1* and *ZMM16* for *TaPI-2*) (Munster et al. 2001) and of rice (*OsMADS4* for *TaPI-1* and *OsMADS2* for *TaPI-2*) (Kyozuka et al. 2000); however, low levels of *ZMM16* transcripts were also detected in vegetative tissues of maize.

The analysis of floral homeotic mutants for *AP3/DEF*-like genes in maize and rice and of *PI/GLO-like* genes in rice showed that the function of class B genes is conserved in grass species. In *silky* mutants of maize and *osmads16/superwoman1* (*spw1*) of rice, stamens were replaced by carpels and lodicules by bracts that resemble palea/lemma (Ambrose et al. 2000; Nagasawa et al. 2003). These homeotic mutations were similar to those caused by mutations in class B genes of *Arabidopsis*, where sepals replaced petals in the second whorl and carpels replaced stamens in the third whorl. These observations first suggested the hypothesis of a homologous relationship between lodicules and petals and of the evolutionary conservation of the B-function genes in grass flowers. Although the *AP3/DEF-like* genes from rice and maize appeared to have conserved functions in organ identity specification in the second and third whorls, they seemed to accomplish additional functions in flower development, at least in rice (Nagasawa et al. 2003). In *spw1* mutants, the number of organs in the second whorl was higher than in the wild type, and they were completely sterile because no functional carpel was formed in the fourth whorl, whereas *apetala3* mutants of *Arabidopsis* were female fertile (Jack et al. 1992). The sterility of *spw1* mutants seemed to be related to overproduction of undifferentiated nucellar tissue (Nagasawa et al. 2003). It is noteworthy to mention that cell proliferation was also affected in *Arabidopsis* class B mutants *apetala3* and *pistillata*; however, in these mutants, there was a reduced organ number in the third whorl, whereas the organ number in the second whorl was not affected (Jack et al. 1992; Sakai et al. 2000). To this extent, the *OsMADS16/SUPERWOMAN1* gene appears to have a specific function, distinct from that of *AP3* and *PI*, in the regulation of whorl-specific proliferation.

As in *Arabidopsis* and *Antirrhinum*, rice, maize, and wheat possess a single copy of *AP3/DEF*-like genes, whereas the *PI/GLO*-like genes are duplicated (Fig. 4), though it is not clear whether this duplication has any functional relevance. In transgenic rice plants, whose *OsMADS4* expression had been reduced by antisense suppression, lodicules were changed to palea/lemma-like structures and stamens were changed to carpel-like structures, suggesting functional conservation of the *PI* co-ortholog of rice (Kang et al. 1998). However, it was not clear whether the observed phenotype of these antisense lines originated from reduced levels of *OsMADS4* alone or from the additional nonspecific suppression of *OsMADS2*. Later, Prasad and Vijayraghavan (2003) reported the down-regulation of *OsMADS2* by an RNAi approach. In wild-type rice plants, *OsMADS2* was specifically expressed in lodicules, stamens, and pistil. In these knock-down plants, only mRNA levels of *OsMADS2* were reduced, while *OsMADS4* was normally expressed. Interestingly, in these RNAi lines, only the lodicule identity was affected, whereas stamens were normal, indicating that *OsMADS2*

was only necessary to specify the identity of the lodicules, which were significantly enlarged in these transgenic rice plants and had characteristics of the marginal region of palea (mrp), similar but not identical to the homeotic transformation of whorl 2 organs that was observed in the *spw1* mutant. On the basis of these results and of the observation that *OsMADS2* RNAi plants are fertile, Prasad and Vijayraghavan (2003) suggested that *OsMADS2* would not be redundant with *OsMADS4*, the first being required for lodicules specification, the second for stamen identity. From these results, however, it is unclear whether *OsMADS2* plays any role in stamen development and what roles *OsMADS4* plays in lodicule and stamen development. More recently, Yoshida et al. (2007) generated RNAi transgenic plants whose *OsMADS4* and *OsMADS2* were suppressed independently in order to produce a detailed comparison of their phenotypes with that of the *spw1* mutant. They observed that, because of anther abnormalities, *OsMADS2* RNAi plants had much lower seed fertility (<10%) than wild-type plants, whereas *OsMADS4* RNAi plants showed normal lodicules or stamens and were completely fertile, implying the involvement of *OsMADS2* in stamen development. Moreover, although the lodicules in the *OsMADS2* RNAi plants were largely modified with a substantial apical growth, they differed from the organs derived from the homeotic transformation of whorl 2 in the *spw1* mutant, because they retained a wild-type-like morphology, with a wide base and rough surface, indicating that the lodicule identity was not completely lost. Furthermore, yeast two-hybrid experiments using the two PI/GLO-like proteins of rice indicated that SPW1, the rice AP3-like protein, interacted with both OsMADS4 and OsMADS2. In contrast with the hypothesis of Prasad and Vijayraghavan (2003), these results clearly indicated the unequal redundancy of the class B genes of rice. *OsMADS2* plays an important role in lodicule development, but *OsMADS4* also supports the specification of the lodicule identity, while both genes are roughly equally important in stamen development. Consistent with their redundant functions, the double-knockdown plants (*OsMADS2* RNAi + *OsMADS4* RNAi) exhibited a complete *spw1* phenotype, with homeotic conversion of the lodicules into palea-like organs (mrp-like organs) and stamens into carpel-like organs (Yao et al. 2008). Since duplication of *PI/GLO* subfamily genes is common within grasses, it would be interesting to verify whether the unequal contribution of the rice *PI/GLO*-like genes in the identity and development of the floral organs of the second and third whorls occurred also in other grass species, including wheat and maize.

Pistillody, consisting in the homeotic transformation of stamens into pistil-like structures, was observed in alloplasmic lines of the hexaploid *T. aestivum* carrying the cytoplasm of the wild relative *Aegilops crassa* (Murai et al. 2002). The gene responsible for this phenotype has yet to be identified; however, the three wheat class B genes *TaAP3* (*AP3/DEF* subfamily), *TaPI-1,* and *TaPI-2* (*PI/GLO* subfamily), which were normally expressed in stamen primordia, were down-regulated in the organs of the third whorl of the pistillody line (Hama et al. 2004). Then, the pistillody phenotype in alloplasmic wheat would be caused by alterations in the expression patterns of *AP3/DEF*- and *PI/GLO*-like genes, suggesting that in wheat, like in eudicot species, the class B genes are responsible for stamen development.

As described previously, in *Arabidopsis,* the proteins encoded by *AP3* and *PI* form heterodimers that would act as transcriptional regulators. Whipple et al. (2004) investigated the relationship between *SILKY1* (*SI1*), the *AP3/DEF*-like gene of maize, and *ZMM16*, the maize co-ortholog of *PI*; in vitro the proteins encoded by these genes formed a heterodimer that bound to DNA. Remarkably, the heterodimer between the proteins encoded by *ZMM16* and by *AP3* of *Arabidopsis* (the *SI1* co-ortholog) was also able to bind DNA, as was the heterodimer between SI1 and PI. Finally, the expression of *SI1* driven by the *AP3* promoter in an *ap3* mutant line of *Arabidopsis* resulted in partial rescue of the mutant phenotype, with both petal and stamen development being restored (although neither organ was completely normal). Similarly, *ZMM16* was able to rescue partially a *pi* mutant. The reported results reveal the remarkable degree of conservation and of functional homology between monocots and eudicots of the B-function genes, despite 150–200 mya of divergent evolution and the dissimilarity of the second-whorl structures that they control (petals, inner tepals, and lodicules).

Further evidence that the apparently very different organs of the second whorl are actually homologous was provided by the expression analysis of class B genes in three species representing the morphological transition from the typical monocot flower to the grass floret: *Elegia elephas* (Restionaceae), *Joinvillea ascendens* (Joinvilleaceae), and *Streptochaeta angustifolia* (Anomochlooideae subfamily of the Poaceae) (Whipple et al. 2007). Specifically, the flowers of *E. elephas* and *J. ascendens* are actinomorphic, possessing two trimerous whorls of tepals and three or six stamens, respectively (Wipple et al. 2007), whereas in the early diverging grass *S. angustifolia,* there are 11 or 12 bracts subtending the androecium. The arrangement of the six or seven inner bracts (6–11/12) has been interpreted as the first and second perianth whorls (Whipple et al. 2007; Sajo et al. 2008). In situ RNA hybridization analysis of *AP3/DEF*- and *PI/GLO*-like genes in these three species showed that the B-class genes were consistently expressed in stamens and in the organ whorl just outside the stamens, represented by the inner tepals in *E. elephas* and *J. ascendens* and by the inner three

bracts 9–11 (or 10–12) in *S. angustifolia* (Whipple et al. 2007). These data suggest that the inner three bracts of *Streptochaeta* are second-whorl organs, possibly a transitional form preceding the evolution of actual lodicules in grasses, and that both these different second-whorl organs evolved by modification of the inner tepals of a typical monocot flower. Although this evidence suggests a conserved role of the class B genes in establishing the second-whorl identity in both grass and nongrass monocots, the evolutionary pathway leading to the distinct morphology of the lodicules has yet to be explained. The identification of the gene targets of the class B MADS-box transcription factors could represent a significant contribution to the understanding of the evolutionary modifications affecting second-whorl organs.

In order to evaluate homologies between the outer sterile organs of grass spikelets and inflorescence structure of nongrass monocot flowers, Preston et al. (2009) analyzed the expression patterns of *AP1/FUL*-like and *LHS1*-like genes in the early diverging grass *S. angustifolia*, and in the nongrass outgroup *J. ascendens*. The *AP1/FUL*-like genes were expressed in all floral organs except bracts in most monocots and in all bract-like structures in the spikelet-bearing grasses (Preston and Kellogg 2006, 2007), whereas the *LHS1*-like genes were known to be expressed always in lemma and palea, but never in the glumes of the analyzed grass species (see below) (Malcomber and Kellogg 2004). On the basis of these observations, Preston et al. (2009) proposed these two groups of MADS-box genes as useful markers for interpreting the *Streptochaeta* inflorescence. *J. ascendens* has a single *AP1/FUL*-like gene (*JaFUL*), co-orthologous to the grass *FUL1-/FUL2*-like genes (Preston et al. 2009). These genes stem from a duplication event preceding the origin of extant grasses, but following the divergence of Joinvilleaceae (and presumably Ecdeicoleaceae) from the ancestor of grasses (Preston and Kellogg 2006). Moreover, this species has a single *LHS1*-like gene (*JaLHS1*) co-orthologous to the grass members of the *LHS1* and *OsMADS5* clades (see below) (Preston et al. 2009). As for the *AP1/FUL*-like genes of grasses, the gene duplication event that produced the *LHS1* and *OsMADS5* clades is estimated to have occurred prior to the origin of extant grasses and after the divergence of immediate grass relatives Joinvilleaceae and Ecdeicoleaceae (see below) (Preston et al. 2009). *Streptochaeta* has retained only one (*SaFUL2*) of the two *AP1-FUL*-like genes carried by most grass species following the *AP1/FUL* duplication event that produced the *FUL1* and *FUL2* clades prior to the divergence of grasses (Preston and Kellogg 2006). The single copy of *AP1/FUL* in *Streptochaeta* could be ascribed either to the duplication occurring after the split of Anomochlooideae from the rest of grasses or, as supported by the phylogenetic analyses of Preston and Kellogg (2006), to the loss or rapid divergence of *FUL1* following the duplication event. In situ RNA hybridization analyses showed that *JaFUL* was expressed only in floral organs of *J. ascendens*, supporting the hypothesis that *AP1/FUL*-like genes mark the floral boundary in nongrass monocots, and that *JaLHS1* was expressed in the inner and outer tepals, stamens and pistil (Preston et al. 2009). In *S. angustifolia, SaFUL2* was expressed in all the 11 (or 12) bracts of the primary inflorescence branch, but not in the suppressed floral bract below the abscission zone. In contrast, *SaLHS1* was only expressed in the inner bracts 6–11 (or 12). Together, these data are consistent with the hypothesis that bracts 1–5 of *S. angustifolia* primary inflorescence branches are homologous to the glumes of grass spikelets and that bracts 6–8 of *S. angustifolia* correspond to lemma/palea of grass spikelets and to the outer tepals of close grass relatives.

Since petals are replaced by sepals in class B mutants of eudicots, the loss-of-function phenotypes of rice and maize class B genes have been considered as evidence that palea and lemma are homologous to sepals (Kang et al. 1998; Ambrose et al. 2000). However, further investigations would be necessary, as comparative morphology studies have suggested that palea is a prophyll-like structure, whereas lemma is a bract-like structure. On the other hand, genetic analysis showed that in mutants, *osmad16* (*spw1*) of rice and *si1* of maize, lodicules were transformed into palea/lemma-like structures. On the basis of the analogy with the class B mutants of *Arabidopsis*, Ambrose et al. (2000) argued that this homeotic transformation implied that palea and lemma correspond to sepals in grasses. Nagasawa et al. (2003) put forward the alternative possibility that the organs formed in the second whorl of *spw1* mutants might not correspond to palea, but to novel bract-like structures that do not develop into normal spikelets but arise only in mutants with removed class B activity. Unlike the ectopic expression of *AG*, which affects the identity of both sepals and petals, the ectopic expression of *OsMADS3*, the rice co-orthologous gene of *AG*, did not cause any morphological alteration in palea and lemma but did cause the lodicules to be transformed into stamen-like structures (Kyozuka and Shimamoto 2002). This finding supports the idea that palea and lemma of grasses are different from the sepals of eudicots. The identification of genes whose silencing is able to disrupt completely the structures of palea and lemma will be needed to understand the nature of these structures.

Class C and class D genes

Phylogenetic analysis of MIKC-type MADS-box genes belonging to the *AGAMOUS* (*AG*) clade showed that gene

duplications were critical for shaping this subfamily (Kramer et al. 2004; Zahn et al. 2006). An ancient duplication after the divergence of the angiosperm and gymnosperm lineages resulted in the C-lineage, including the C-function genes involved in stamen and carpel development, and the D lineage, comprising the D-function genes involved in ovule identity determination (Kramer et al. 2004; Yamaguchi et al. 2006; Dreni et al. 2007). As this classification has been used in many phylogenetic studies, we decided to adopt the same nomenclature; however, since gene orthology does not always coincide with functional equivalence, as suggested by Zahn et al. (2006), this type of classification might be somewhat misleading. *Arabidopsis* has three genes of the C-lineage: *AG*, *SHP1* (*SHATTERPROOF1*) and *SHP2* (Fig. 4). The typical class C gene of *Arabidopsis AG* is involved in: specification of stamen and carpel identity, control of floral determinacy and negative regulation of A function gene activity in whorls 3 and 4 (Yanofsky et al. 1990; Coen and Meyerowitz 1991). The additional C-lineage genes of *Arabidopsis SHP1* and *SHP2* share largely redundant functions in specifying the fruit dehiscence zone and are involved with *AG* in carpel development (Liljegren et al. 2000; Pinyopich et al. 2003). *SEEDSTIK* (*STK*) is the only *Arabidopsis* gene belonging to the D lineage (Fig. 4). It is involved in ovule development and is required for seed dispersal of mature fruits (Pinyopich et al. 2003). Moreover, *STK* acts redundantly with *SHP1*, *SHP2,* and *AG* in promoting ovule identity (Pinyopich et al. 2003). Mutant analysis has shown that all the four *Arabidopsis* genes of the *AG* subfamily, which includes both the C- and D-lineages, are involved in ovule identity determination; consequently, a clear-cut distinction between the functional roles of the C- and D-lineages is not universally feasible. Since some of the C-function genes contribute to ovule development, the D-function genes may be considered as a more specialized version of the C-function genes (Zahn et al. 2006).

In grasses, phylogenetic analysis identified four *AG*-like subclades, each containing at least one gene of maize, rice, and wheat. Two subclades (1 and 2 in Fig. 4), being related to *STK* of *Arabidopsis*, were assigned to the D lineage, while the other two subclades (3 and 4 in Fig. 4) were assigned to the C-lineage because they were related to the C-function genes *AG, SHP1,* and *SHP2* of *Arabidopsis*. Accordingly, the putative D-lineage gene *TaAG-3* of wheat appeared orthologous to the genes *ZAG2/ZMM1* of maize and *OsMADS13* of rice (subclade 1 in Fig. 4), whereas *TaAG-4* would be orthologous to *OsMADS21* of rice and *ZMM25* of maize (subclade 2 in Fig. 4). Both the other grass subclades contain putative C-lineage genes from the three grass species: one subclade comprising *TaAG-1* of wheat, *OSMADS58* of rice and *ZAG1* of maize, the other including *TaAG-2* of wheat, *OSMADS3* of rice and *ZMM2/ZMM23* of maize (Fig. 4). These observations are consistent with the results obtained by Kramer et al. (2004) and Dreni et al. (2007) and can be explained by a major gene duplication event that occurred in both grass C and D clades before the separation of the maize, rice, and wheat lineages. Comparative genomic studies in rice indicate that the duplication that gave rise to the two C clades of grasses would have been produced by the WGD event that occurred 50–70 mya before the origin of the Poaceae (Xu and Kong 2007). Accordingly, the C-lineage genes of rice *OsMADS3* and *OsMADS58* were located in regions of the chromosomes 1 and 5, which have been identified as segmental duplications (Arora et al. 2007; Xu and Kong 2007). Analogously, the C-lineage genes *ZAG1* and *ZMM2* of maize were located in regions of the chromosomes 6 and 8, which were duplicated before the segmental tetraploidization event that generated the maize genome (Theissen et al. 1995; Munster et al. 2002). On the other hand, the presence of two closely related maize genes in both the first D subclade (*ZAG2/ZMM1*) and the fourth C subclade (*ZMM2/ZMMM23*) of grasses may be explained by the allotetraploidization event that took place 11 mya in the maize lineage (Munster et al. 2002). Previous phylogenetic analyses showed some conflicting results in the placement of the genes *ZAG2/ZMM1* of maize and *OsMADS13* of rice in both the C-lineage (or *AG* clade *sensu* Zahn et al. 2006) and D lineage (or *AGL11* clade *sensu* Zahn et al. 2006). However, phylogenetic analyses performed by us (Fig. 4) and by Kramer et al. (2004), Yamaguchi et al. (2006) and Dreni et al. (2007) showed that the orthologous genes *OsMADS13* and *ZAG2/ZMM1* cluster together with the orthologous genes *OsMADS21* and *ZMM25* within the D lineages. In contrast, Zahn et al. (2006) placed the orthologous genes *OsMADS13* and *ZAG2/ZMM1* into the C-lineage (*AG* clade). Since the correct placement of these genes within the C- or D-lineages would be relevant to infer their evolutionary relationships, more extensive analyses would be needed to assess whether, according to the first hypothesis, the members of the grass *AG* subclades 1 and 2 (Fig. 4) are the result of a more recent duplication preceding the divergence of the Poaceae or, according to the second hypothesis by Zahn et al. (2006), the genes of subclade 1 were produced by a more ancient duplication within the C-lineage.

According to their putative function as class C genes, the expression of *OsMADS3* and *OsMADS58* was restricted to stamens, carpels, and ovule primordia, although their temporal expression was quite different (Yamaguchi et al. 2006). *OsMADS3* was repressed in differentiated organs, whereas *OsMADS58* remained expressed during differentiation and development (Yamaguchi et al. 2006). A knockout line of *OsMADS3* showed the homeotic transformation of stamens into lodicules, whereas carpels developed

almost normally (Yamaguchi et al. 2006). On the contrary, *OsMADS58* RNAi knock-down lines showed indeterminate development of floral organs, producing a reiterated set of floral organs consisting of lodicules, stamens/ectopic lodicules, and carpel-like organs. The morphology of the carpels was severely affected; they developed into thick cup-like structures without fusing along their margins and no differentiation of stigmatic tissue was observed (Yamaguchi et al. 2006). Unexpectedly, the analysis of the *osmads3* and *osmads58* mutants showed that these genes were also involved in controlling the number of lodicules, since additional ectopic lodicules developed in whorl two of the mutant flowers. However, *osmads3* mutant lines developed a number of ectopic lodicules higher than *osmads58*, indicating that *OsMADS3* played a more relevant role than *OsMADS58* in controlling lodicule formation. The introduction of the *OsMADS58* RNAi construct into the milder *osmads3–2* mutant showed that the observed defect in indeterminacy was very similar to that of the *osmads58* knock-down line, indicating the weak contribution of *OsMADS3* to meristem determinacy. The study of these mutants showed that both *OsMADS3* and *OsMADS58* performed some functions typical of class C gene activity, similar to those observed for *AG*, although they were functionally diversified, with predominant functions in different whorls. *OsMADS3* had a stronger role in repressing the ectopic development of additional lodicules and in specifying stamen identity, whereas *OsMADS58* was more relevant in conferring floral meristem determinacy and in regulating carpel morphogenesis. The mechanism underlying this functional diversification of the class C genes in rice may be partially explained by the variation in their temporal expression, as reported above. However, the predominant function of *OsMADS58* in floral meristem determinacy cannot be explained by its temporal expression profile alone because it was expressed at the same rate in both whorls 3 and 4. Another hypothesis is that differences in the proteins interacting with the two MADS-domain proteins encoded by these genes may have led to their functional diversification. In other terms, OsMADS3 and OsMADS58 proteins may interact specifically and independently with different factors that are preferentially expressed in whorl 3 and whorl 4, respectively. The exact molecular mechanism underlying the functional diversification of *OsMADS3* and *OsMADS58* during rice evolution remains an interesting research topic.

As previously described, phylogenetic analysis suggests that multiple class C genes arose by gene duplication before the species of the grass family diverged. The subfunctionalization of class C genes may have been closely associated with this gene duplication event. In maize, a loss-of-function mutant of *ZAG1* showed a phenotype similar to that of the *OsMADS58* RNAi knock-down lines of rice (Mena et al. 1996). In the *zag1* mutant line, floral meristem determinacy was partially lost and carpels were not fused in the female flowers, whereas stamens were almost normal in male flowers, suggesting that other class C genes, such as *ZMM2* and *ZMM23*, orthologs to *OsMADS3*, may be responsible for stamen specification. The expression pattern of *ZAG1* and *ZMM2* was consistent with this hypothesis (Schmidt et al. 1993; Mena et al. 1996); both floral-specific genes were expressed in the tassel as well as in the ear; however, the relative abundance of their transcripts in the developing male and female inflorescences was different. *ZAG1* expression was higher in developing ears than in tassels, whereas *ZMM2* transcripts were more abundant in the tassel. Moreover, both genes were expressed in carpels and stamens, but *ZMM2* showed a higher expression in the stamens (Mena et al. 1996). The preferential expression of *ZAG1* in carpels and *ZMM2* in stamens could explain the phenotype of *zag1* mutant plants, in which only carpel development was disturbed, and could suggest the potential involvement of *ZMM2* in stamen identity determination. In this context, it would be interesting to determine the phenotypes produced by single or double loss-of-function mutations of *ZMM2* and *ZMM23*. Thus, the subfunctionalization of class C genes may have begun before the divergence of rice and maize, but its differential fine tuning may be responsible for the slight divergences observed between *osmads58* and *zag1* mutants (Yamaguchi et al. 2006).

The expression of the two putative class C genes of wheat *TaAG-1* and *TaAG-2* increased during spike development, and their transcripts were detected only in stamens and pistils of the spikelet organs at heading time (Paolacci et al. 2007). However, the expression of *TaAG-2* was ten times higher in stamens than in pistils, whereas the expression of *TaAG-1* was similar in both organs.

Interestingly, on the basis of the results reported previously, mutants for class C genes of rice and maize were affected for carpel development but did not show any clear homeotic conversion of this organ, whereas stamens were transformed into lodicules. Thus, the genetic control for carpel specification in grasses seems different from that of *Arabidopsis* and other eudicots although more recent analysis of *osmads3 osmads58* double mutants reported by Dreni et al. (2011) indicate that the two rice class C genes might redundantly regulate inner floral organ identity, including the carpel (see below).

OsMADS13 and *OsMADS21* of rice are the only grass genes putatively assigned to the D lineage whose functional analysis has been reported (Dreni et al. 2007). *OsMADS13* was specifically expressed in the ovule primordia and along their development, with a pattern very similar to that of *STK*, the class D gene of *Arabidopsis* (Lopez-Dee et al. 1999). Inside the ovule, *OsMADS13* was

expressed in both integuments and nucellus tissues and, after anthesis, in developing seeds (Lopez-Dee et al. 1999). Interestingly, the expression pattern of *OsMADS13* was very similar to its orthologous maize gene *ZAG2* (Schmidt et al. 1993), whose expression was detected in the carpel primordium and persisted during all stages of ovule development. Like *OsMADS13*, *ZAG2* was also expressed in the epidermal cells on the inner side of the carpel wall (Schmidt et al. 1993). The *osmads13* knock-out mutant was completely female sterile, and its ovules were converted into a reiteration of ectopic carpels or into more amorphous structures with carpel identity (Dreni et al. 2007). *OsMADS21* accumulated at very low levels in developing anthers, carpels, styles/stigmas, and ovules (Dreni et al. 2007); this low, diffuse expression throughout the two inner whorls of the flower was different from that of other genes of the D lineage, such as *STK*, *OsMADS13,* and *ZAG2*, which were all specifically expressed in the ovule. During later stages of flower development, *OsMADS21* expression was particularly evident in the inner cell layers of the ovary and in the ovule integuments, where it overlapped with that of *OsMADS13* (Dreni et al. 2007), although steady-state mRNA levels of *OsMADS21* transcripts seemed lower than those of *OsMADS13*. Interestingly, knock-out analysis showed that the *osmads21* mutant had a normal phenotype; moreover, analysis of the *osmads13*/*osmads21* double mutant revealed that loss of the *OsMADS21* function did not modify the *osmads13* phenotype (Dreni et al. 2007). These data suggest that only *OsMADS13* plays a role in ovule identity determination and that *OsMADS21* has probably lost this function during evolution. Moreover, the presence of carpels inside carpels showed the indeterminate development of the *osmads13* flower and demonstrated that *OsMADS13* was also involved in floral meristem determinacy.

The putative D-lineage wheat genes *TaAG-3* and *TaAG-4* showed very divergent expression patterns (Paolacci et al. 2007). The temporal expression of *TaAG-4* was similar to that of *OsMADS21*, its orthologous rice gene: it was higher during late stages of spike development, very weak in stamens, very high in pistils and 5 DAA caryopses, but fading in 20 DAA caryopses (Paolacci et al. 2007). By contrast, *TaAG-3* was expressed in vegetative tissues such as coleoptiles of seedlings 20 days after germination, developing spikes, caryopses 5 DAA and in all the spikelet organs, suggesting a functional divergence from its orthologs in other grass species. In fact, as described above, the *TaAG-3* orthologous genes *OsMADS13* of rice (Lopez-Dee et al. 1999) and *ZAG2* of maize (Schmidt et al. 1993) were specifically expressed in ovules. Since the duplication that produced *TaAG-4* and *TaAG-3* probably occurred before the divergence of wheat from rice and maize, as indicated by phylogenetic analysis, their functions may have diversified during the independent evolution of these species.

Class E genes

In *Arabidopsis,* the *SEPALLATA* (*SEP*) genes of class E are involved in the specification of sepal, petal, stamen, carpel, and ovule identity (Pelaz et al. 2000; Ditta et al. 2004). Their encoded proteins interact 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), which regulate the formation of floral organ identity (floral quartet model, Theissen and Saedler 2001). In these complexes the B-, C-, and D-function proteins are considered important for organ-specific gene regulation, whereas the E-function proteins would act as mediators for the formation of the protein complexes (Melzer et al. 2009; Immink et al. 2009).

Phylogenetic analysis clustered the class E genes into the *SEP* subfamily (previously *AGL2* subfamily), which has two major clades, one of them (clade I) including the *SEP1/2/4*-like genes (*AGL2/3/4* clade *sensu* Zahn et al. 2005 or *LOFSEP* clade *sensu* Malcomber and Kellogg 2005), the other one (clade II) comprising the *SEP3*-like genes (*AGL9* clade *sensu* Zahn et al. 2005 or *SEP3* clade *sensu* Malcomber and Kellogg 2005) (Fig. 4). The two clades were generated by a gene duplication probably predating the origin of the most recent common ancestor of the extant angiosperms (Zahn et al. 2005). Five distinct subclades, named on the basis of the included rice gene, have been identified in grasses (Malcomber and Kellogg 2005; Paolacci et al. 2007). Each subclade includes the orthologous genes of wheat, rice and maize (Fig. 4), most likely because the common ancestor of these three species contained at least five *SEP*-like genes. The major clade I (*SEP1/2/4*-like genes) clusters three grass lineages: the subclade 1, including *OsMADS1* (also known as *LHS1*) of rice, two closely related genes of wheat (*TaSEP-1* and *TaSEP-2*) and two of maize (*ZMM8* and *ZMM14*); the subclade 2, grouping *OsMADS5* of rice, *TaSEP-6* of wheat and *ZMM3* of maize; the subclade 3, containing *OsMADS34* of rice, *TaSEP-5* of wheat and *ZMM24*/*ZMM31* of maize (Fig. 4). This clustering could be explained by two duplication events that took place in the common ancestor of the three grass species (Zahn et al. 2005; Malcomber and Kellogg 2005; Xu and Kong 2007). Apparently, the first duplication occurred around the base of the monocots, probably after the split of Arecales from other commelinids (Xu and Kong 2007), giving rise to the grass subclade 3 (*OsMADS34* subclade in Malcomber and Kellogg 2005). The second occurred later, just before the diversification of the Poaceae (Xu and Kong 2007), and

gave rise to the subclades 1 and 2 (*LHS1* and *OsMADS5* subclades, respectively, in Malcomber and Kellogg 2005). The second major clade (clade II, *SEP3*-like genes) includes the remaining two grass subclades: the subclade 4 contains *OsMADS7* (also known as *OsMADS45*) of rice, *TaSEP-4* of wheat and *ZMM6* of maize, whereas the subclade 5 includes *OsMADS8* (also known as *OsMADS24*) of rice, *TaSEP-3* of wheat and two closely related maize genes *ZMM7*/*ZMM27* (Fig. 4). These two grass lineages of *SEP*-like genes originated from a duplication event at or near the base of grasses, likely as the result of the WGD event that occurred 50–70 mya before the origin of the Poaceae (Xu and Kong 2007). *OsMADS7*/*45* (grass subclade 4) and *OsMADS8*/*24* (grass subclade 5) were located in regions of chromosomes 8 and 9 identified as segmental duplications, providing genomic evidence that the gene duplication identified by phylogenetic analyses corresponded to a genome-wide duplication event (Xu and Kong 2007; Arora et al. 2007). Likewise, the presence of two maize genes in the subclades 1, 3, and 5, also observed in other subclasses of the grass MIKC-type MADS-box family, may reflect the duplication of the maize genome after its divergence from other grass species. As for *ZMM8* and *ZMM14* (subclade 1 in Fig. 4), this observation is supported by the mapping of the two genes into syntenic duplicated regions of maize chromosomes 1 and 9 (Cacharon et al. 1999). The presence of two wheat genes in subclade 1 (*TaSEP-1* and *TaSEP-2*) also suggests that they would be paralogous genes produced by a duplication that occurred after wheat speciation.

Rice *OsMADS1* was the first *SEP*-like gene whose function was partially characterized in grasses. *OsMADS1* was first expressed in the spikelet meristem before the glume primordia emerge and then restricted to lemma and palea, with weak expression in the carpel (Chung et al. 1994; Prasad et al. 2001). In rice mutations at amino acid positions 24 and 27 in the MADS domain of *OsMADS1* caused the *leafy hull sterile 1* (*lhs1*) phenotype, which had flowers with lemma, palea and lodicules transformed into leaf-like organs, a decreased number of stamens and, occasionally, an extra pistil or floret (Jeon et al. 2000). Since the *lhs1* mutation was caused by a semi-dominant allele, the observed phenotype might not reflect the precise function of *OsMADS1*. More recently, *osmads1 Tos17* knock-out and RNAi knock-down lines were reported (Agrawal et al. 2005; Prasad et al. 2005). Both *osmads1* mutants showed phenotypes similar to *lhs1*, with elongated and leaf-like lemma and palea. The lemma was more affected than the palea and mimicked glumes, whereas the palea was never glume like. These phenotypic differences indicate that *OsMADS1* seems to function mostly as a lemma identity gene, since its inhibition results in loss of lemma identity, whereas ectopic expression of *OsMADS1* causes homeotic transformation of glumes into lemma-like organs (Prasad et al. 2001). However, severe loss-of-function *OsMADS1* mutants had underdeveloped palea and lemma and displayed complete homeotic transformation of the organs of the three inner whorls (lodicules, stamens, and carpels) into lemma- and palea-like structures, and a loss of determinacy of the flower meristem (Agrawal et al. 2005). If palea/lemma are considered equivalent to sepals, this phenotype strikingly resembles that of the *sep1/2/3* triple mutant of *Arabidopsis*, where petals, stamens, and carpels were all converted into sepal-like organs, and flower development became indeterminate (Pelaz et al. 2000), which led to the definition of the class E floral homeotic function (Theissen 2001). However, there is a contradiction between the E function role of *OsMADS1* and the observation that it is not expressed in lodicules and stamens; this might be explained by a very early action of *OsMADS1* in the spikelet primordium, before the development of organ primordia. The effect of *OsMADS1* at this stage may be required for the specification of floral organs at later stages of ontogeny (Prasad et al. 2005). Because OsMADS1 protein physically interacts with the AP1-like proteins OsMADS14 and OsMADS15 (Lim et al. 2000), it may be involved in the formation of a complex with proteins encoded by class A genes. An alternative explanation for the homeotic transformations of the inner floral whorls might be that at early stages of floret development *OsMADS1* activates accessory factors that control organogenesis in these whorls. One such gene might be *OsMGH3*, which encodes a flower-specific GH3-type factor of rice (Prasad et al. 2005) and is first expressed in young floret meristems, then in all the organs of the four whorls. Down-regulation of *OsMADS1* resulted in a drastic decrease of *OsMGH3* expression, whereas ectopic expression of *OsMADS1* induced *OsMGH3* expression in leaves, indicating that *OsMADS1* (possibly indirectly) regulates this gene. The phenotypic effects observed in *osmads1* mutants indicate 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.

On the basis of phylogenetic analyses, the paralogous genes *TaSEP-1*/*TaSEP-2* of wheat and *ZMM8*/*ZMM14* of maize are orthologs to *OsMADS1* (Fig. 4). The expression patterns of *TaSEP-1* and *TaSEP-2* were similar; their transcripts were detected at low levels in such vegetative tissues as coleoptile, leaf, and stem, at moderate levels in developing caryopses and at very high levels in spikes at heading time, but only *TaSEP-1* was expressed in roots. In spikelets at heading time, the transcription levels of two wheat paralogous genes were very high in lemma and palea, moderate in stamens and pistils, and very low in

lodicules and glumes (Paolacci et al. 2007). Their expression patterns differed from that of the rice ortholog *OsMADS1* (*LHS1*), whose transcripts, as previously reported, were found only in spikes. In particular, it was strongly expressed in palea and lemma, weakly in pistils, and was not detectable in glumes, lodicules, and stamens (Chung et al. 1994; Prasad et al. 2001). However, the higher transcription of *TaSEP-1* and *TaSEP-2* in lemma and palea suggests their involvement in controlling the identity and development of these organs, as shown for their rice orthologous *OsMADS1/LHS1*.

Interestingly, the three homoeologous wheat sequences of *WLHS1*, a gene corresponding to *TaSEP-2*, showed genetic and epigenetic alterations (Shitsukawa et al. 2007). The gene located in chromosome 4A (*WLHS1-A*) had a structural alteration containing a large novel sequence in place of the K domain, which in rice is essential for protein–protein interaction between *OsMADS1* and other MADS-box proteins (Lim et al. 2000). Yeast two-hybrid and three-hybrid analyses showed that the protein encoded by the other two homoeologous genes located in chromosomes 4B (*WLHS1-B*) and 4D (*WLHS1-D*), which had an intact MIKC modular structure, interacted with class B gene products forming a heterodimer; by contrast, WLHS1-A did not interact with any MADS-box gene product. Furthermore, over-expression of *WLHS1-A* did not induce any morphological change in transgenic *Arabidopsis* plants, whereas transgenic plants over-expressing *WLHS1-B* or *WLH1-D* showed early flowering. These observations suggest that the protein encoded by *WLSH1-A*, lacking the K domain, has lost the normal MADS function (Shitsukawa et al. 2007). Moreover, real time PCR analysis indicated that the expression of *WLHS1-B* was down-regulated, compared with its homoeologous gene *WLHS1-D*. Transient promoter assay demonstrated that the promoter regions of *WLHS1-B* possessed transcriptional activation functions, indicating that alteration of the cis-element was not the cause of *WLHS1-B* silencing. Using a bisulfite genome sequencing analysis, Shitsukawa et al. (2007) examined the methylation levels of the 5′ CpG and CpNpG islands of the *WLHS1* homoeologs. This analysis indicated that gene-specific hypermethylation was present in exon 1 of *WLHS1-B*, which seemed to be associated with the down-regulation of this gene. Consequently, of the three *WLSH1* homoeologous genes present in hexaploid wheat, *WLHS1-D* provides the largest contribution to their function.

Also the expression pattern of *ZMM8* and *ZMM14* was different from that of *OsMADS1*. Similar to rice, *ZMM8* and *ZMM14* expression was restricted to inflorescence tissues. They were first expressed throughout the upper floret meristem when the primordium of the lower floret became visible (Cacharrón et al. 1999; Malcomber and Kellogg 2004). Unlike *OsMADS1*, *ZMM8* was expressed at the same level in all floral organs of the upper floret in mature spikelets (Cacharrón et al. 1999). *ZMM14* was also expressed in all floral organs of the upper floret, but its expression in the pistil was more pronounced than that of *ZMM8* (Cacharrón et al. 1999). *ZMM8* and *ZMM14* were never detected in the lower floret. *ZMM14* was closely linked to *INDETERMINATE FLORAL APEX1* (*IFA1*) on maize chromosome 1 (Cacharrón et al. 1999); *ifa1* mutants made extra spikelets and extra flowers in the spikelets and often had proliferating gynoecial tissue (Laudencia-Chingcuanco and Hake 2002). This phenotype was consistent with *lhs1*, but whether a mutated *ZMM14* caused *ifa1* has yet to be demonstrated. *ZMM8* and *ZMM14* have been hypothesized to act as selector genes involved in distinguishing the upper and lower floret in maize (Cacharrón et al. 1999).

The different patterns detected in rice, wheat, and maize indicate that *LHS1* expression has diversified during the evolution of the grass family, acquiring diversified functions in different grass species. Notably, Malcomber and Kellogg (2004) examined *LHS1* expression in several distantly related grass species; their data support the *LHS1* role in specifying the determinacy of the spikelet meristem and in determining the lemma and palea identity. Furthermore, they noticed that the expression of *LHS1* orthologs varied from species to species with the developmental pattern of the florets. In spikelets of several species such as maize, whose florets develop basipetally, *LHS1* orthologs were expressed only in the terminal floret, whereas in the species with spikelets whose florets develop acropetally, including most likely also wheat, the same genes were expressed in multiple florets. *LHS1* expression limited to the upper flowers appears as the ancestral state, whereas its expression in all flowers would be derived later. Thus, differences in the expression of the *LHS1*-like genes may be associated with the developmental patterns of the spikelet, and these genes may have been involved in morphological diversification of the inflorescence during the evolution of grass species.

As described previously, the phylogenetic tree shown in Fig. 4 reveals a close relationship between the *Arabidopsis SEP3* gene and the monocot branch that includes *OsMADS7/45* (grass subclade 4) and *OsMADS8/24* (grass subclade 4). There is evidence that in *Arabidopsis SEP3* is the most important among the class E *SEP* genes (reviewed by Melzer et al. 2009), accordingly *OsMADS7/45* and *OsMADS8/24* appear as candidates, more likely than *OsMADS1*, in providing the class E gene function in rice, at least in terms of phylogenetic relationships. As shown by northern blot analysis, the expression of *OsMADS7/45* and *OsMADS8/24* was restricted to reproductive organs such as inflorescences and developing seeds (Pelucchi et al. 2002).

In situ RNA hybridization analyses showed that during flower development the expression of *OsMADS7/45* and *OsMADS8/24* was first detected in spikelet meristems; in later stages, it was not detected in lemma and palea primordia, but transcripts of both genes were found to accumulate in developing lodicules, stamens, and carpels during spikelet development (Pelucchi et al. 2002; Cui et al. 2010). The expression patterns of these two genes were thus similar to those of *SEP1*, *SEP2,* and *SEP3* in *Arabidopsis*, suggesting that *OsMADS7* and *OsMADS8* play a corresponding role in rice. In agreement with their expression pattern, the morphogenetic alterations caused by knockdown of both *OsMADS7* and *OsMADS8* were restricted to the organs of the innermost three whorls (Cui et al. 2010). Specifically, lodicules, stamens, and carpels were transformed into palea/lemma-like structures, whereas glumes, lemma, and palea were not affected. Flower apical meristems in the center of the transgenic carpels were reverted into reproductive meristems, which developed into stamen-like structures or carpel-like structures. As none of the single gene knockdowns causes any morphological change (Cui et al. 2010), the phenotypes of double mutants suggest strongly that *OsMADS7* and *OsMADS8* would be functionally redundant, with either of them required for the proper development of organ identities in the inner three floral whorls and for the maintenance of flower meristem determinacy. Furthermore, the knockdown *OsMADS7/8* transgenic plants were delayed in flowering time by approximately 2 weeks, but not in the transition from shoot meristem to inflorescence meristem, thus resembling *sep1/2/3* loss-of-function mutants in *Arabidopsis* (Pelaz et al. 2000).

As summarized in the "floral quartet model", the SEP proteins form higher order complexes together with class A, B, or C proteins to control various transcriptional programs required and sufficient for specifying floral organ identity in eudicots (Theissen 2001; Theissen and Saedler 2001). The SEP-like proteins OsMADS7 and OsMADS8 had an interaction profile similar to the SEP proteins of *Arabidopsis*; they interacted with the SQUA-like protein OsMADS18 (similar to AP1), with OsMADS16 (similar to AP3) and with the AG-like protein OsMADS13 (similar to STK) (Kater et al. 2006; Favaro et al. 2002). In *Arabidopsis,* all the AG-like proteins interacted with SEP3. Furthermore, exchange experiments showed that the rice SEP-like proteins OsMADS7 and OsMADS8 also interacted with STK from *Arabidopsis* and with FBP7 from *Petunia*, indicating that the interactions between SEP and class D proteins are evolutionarily conserved (Favaro et al. 2002, 2003). It is therefore highly probable that the two *SEP3* co-orthologs of rice also interact with the other members of the rice *AG*-like gene clade. Protein interaction experiments in vitro and in vivo showed that OsMADS7, OsMADS8, and OsMADS1 share similar interaction patterns, with all three proteins able to form homodimers and heterodimers with each other (Cui et al. 2010). Moreover, the results of Cui et al. (2010) showed that some phenotypes caused by silencing of the *SEP3* co-orthologs (*OsMADS7* and *OsMADS8*) resembled those of the class B (*OsMADS16*) or class C (*OsMADS3* and *OsMADS58*) gene mutants (Nagasawa et al. 2003; Yamaguchi et al. 2006). These data suggest that "floral quartet" complexes, similar to those deduced in eudicot species (Honma and Goto 2001; Favaro et al. 2003), may also be formed to control flower development in rice. More data on protein–protein and protein–DNA interactions are required to clarify this issue.

The expression of *TaSEP-3* and *TaSEP-4* of wheat was very similar to that of their respective rice orthologous genes *OsMADS8* and *OsMADS7*. Their transcription was restricted to spikes and developing caryopses, and in spikelets at heading time, their expression was restricted to lodicules, stamens, and pistils. A lower amount of *TaSEP-4* transcripts was also detected in the palea (Paolacci et al. 2007). Yeast two- and three-hybrid experiments showed that the protein encoded by the gene *WSEP*, corresponding to *TaSEP-4* (orthologous to *OsMADS7*), formed a complex with class A, B, and C genes of wheat and that overexpression of the same gene in *Arabidopsis* caused early flowering and terminal flower formation (Shitsukawa et al. 2007). These characteristics were similar to the phenotypes caused by ectopic expression of *Arabidopsis SEP3*, or its counterparts of *Petunia* and lily (Pelaz et al. 2001; Ferrario et al. 2003; Tzeng et al. 2003). Furthermore, transgenic *Arabidopsis* plants over-expressing *TaMADS1*, which corresponds to *TaSEP3* (orthologous to *OsMADS8*), showed a phenotype displaying early flowering and formation of a terminal flower, similar to that obtained with the overexpression of *WSEP* (Zhao et al. 2006).

The expression patterns of the *SEP*-like genes belonging to the grass subclades 2 and 3 (Fig. 4) were quite heterogeneous. Both *TaSEP-5* and *TaSEP-6* were expressed in coleoptiles, leaves, stems, spikes, and developing caryopses, but *TaSEP-5* expression was much higher at all developmental stages of spikes (Paolacci et al. 2007). At heading time *TaSEP-5* and *TaSEP-6* were expressed in all floral organs of the spikelets, but at very high level in glumes, lemma, and palea, indicating that they could be involved mainly in the development of the outer sterile organs of the spikelet (Paolacci et al. 2007). The expression pattern of *TaSEP-5* was similar to that of its rice ortholog *OsMADS34*, which was initially expressed throughout the floral meristem and subsequently in palea, lemma, and in the sporogenic tissue of the anthers in the mature flower

(Pelucchi et al. 2002). In contrast, the expression pattern of *TaSEP-6* differed from its rice ortholog *OsMADS5*, whose transcripts were not detected in vegetative tissues and developing caryopses but were restricted only to stamens and pistils (Malcomber and Kellogg 2005). In case of the loss-of-function of *OsMADS5*, the only deviation from the wild-type phenotype found in the whole plant was that lodicules were attached to the lemma and palea, suggesting that the gene was not required for class E function (Agrawal et al. 2005), even though it may contribute to it in a redundant way. In this context, it may be worthwhile to observe that simultaneous knockdown of the four rice *SEP*-like genes *OsMADS1*, *OsMADS5*, *OsMADS7,* and *OsMADS8* led to homeotic transformation of all floral organs except the lemma into leaf-like organs (Cui et al. 2010). This mimicked the phenotype observed with the *sep1 sep2 sep3 sep4* quadruple mutant of *Arabidopsis* and suggests that the four rice genes cover the full class E floral homeotic function, even though we cannot completely exclude the possibility that a quintuple mutant also comprising *OsMADS34* would not reveal an even more severe class E loss-of-function phenotype. More recently, Gao et al. (2010) showed that *OsMADS34* was a key regulator of rice inflorescence and spikelet architecture. Compared with the wild type, *osmads34* mutants developed altered inflorescence morphology, with an increased number of primary branches and a decreased number of secondary branches. In addition, they displayed a decreased spikelet number and altered spikelet morphology, with lemma/leaf-like elongated sterile lemmas. This phenotypic analysis suggests that *OsMADS34* is able to regulate the inflorescence architecture by restricting the primary branch number and preventing the early differentiation of primary branch meristems into secondary branches/spikelets. Although *OsMADS34* expression was detected in the floral meristem, no defects of floral organs were observed in *osmads34* mutants. However, compared with the *osmads1* single mutant, the *osmads1 osmads34* double mutant displayed increased defects of the inner floral organs, suggesting that *OsMADS34* was also involved, redundantly with *OsMADS1*, in the identity determination of the inner flower organs (Gao et al. 2010).

In conclusion, based on mutant analyses in rice and phylogenetic, expression, and protein interaction studies, the putative co-orthologs of the *Arabidopsis* class E genes may be more likely the grass genes of the subclades 4 and 5, even though the other grass *SEP*-like genes may act redundantly or in an interdependent way to provide the same floral homeotic function.

The characterization of the rice *mosaic floral organs1* (*mfo1*) and the maize *bearded-ear* (*bde*) mutants indicated that the *AGL6*-like genes might be involved in the regulation of floral organ identity and floral meristem determinacy in grasses, acting as class E genes like the *SEP* genes (Ohmori et al. 2009; Li et al. 2009; Thompson et al. 2009). Phylogenetic analysis showed that *AGL6*-like genes are sister to the *SEP*-like genes (Zahn et al. 2005), but, whereas the *AGL6*-like genes were identified in both angiosperms and gymnosperms, *SEP*-like genes were identified only in angiosperms. Consequently, either an ancestor of *SEP*-like genes existed in the common ancestor of angiosperms and gymnosperms but was lost in extant gymnosperms or *AGL6*-like genes are basal members compared with the clade of angiosperm *SEP*-like genes (Becker and Theissen 2003; Zahn et al. 2005). Although *AGL6*-like genes are ancient and widely distributed in seed plants, their role in development has long remained elusive due to a lack of phenotypes in single loss-of-function mutants. The *Arabidopsis* genome contains two closely related *AGL6*-like genes, *AGL6* and *AGL13* (Fig. 4), probably produced by a recent duplication event (Becker and Theissen 2003) and whose function has yet to be ascertained. The main grass clade included in the *AGL6* subfamily (Fig. 4) contains *OsMADS6* of rice, *TaAGL6* of wheat and *ZAG3* and *ZAG5* of maize, two closely paralogous maize genes duplicated in the tetraploidization event that produced the maize genome after its divergence from the other grass species (Mena et al. 1995; Reinheimer and Kellogg 2009). The clustering of *OsMADS17*, a second rice gene divergent from the other *AGL6*-like grass genes (Fig. 4), could be explained either by a gene duplication that occurred after rice speciation, which was followed by a high rate of molecular evolution, or by a more ancient gene duplication that occurred before the diversification of the grass species. This second hypothesis assumes that *OsMADS17* has been retained only in the rice genome, whereas its orthologous genes of wheat and maize got lost during their evolution (Reinheimer and Kellogg 2009). This is supported by the finding that the *Sorghum* genome contains two *AGL6*-like genes, *SbMADS6* and *SbMADS17*, which are orthologs to *OsMADS6* and *OsMADS17*, respectively (Li et al. 2009).

The expression of grass *AGL6*-like genes seems largely restricted to inflorescence (Reinheimer and Kellogg 2009). In situ RNA hybridizations showed that *ZAG3* was expressed in upper and lower floral meristems of both tassel and ear. In the tassel, *ZAG3* was expressed in developing lodicules and palea, but not in lemma and stamens, whereas it was detected in carpel primordia and later is strongly expressed in the inner integument and in the region underlying the ovule primordium (Thompson et al. 2009). The characterization of the *ZAG3* mutant named *bearded-ear (bde)* showed that this gene plays pleiotropic roles in controlling floral meristem determinacy, organ specification, and sex determination

(Thompson et al. 2009). The *bde* mutation affected floral development differently in the upper and lower meristem. The upper floral meristem initiated extra floral organs that were often mosaic or fused, whereas the lower floral meristem initiated additional floral meristems, indicating that in the upper floret *ZAG3* was required to specify floral organ number and fate, and in the lower floret, it was required to regulate floral meristem determinacy. Unfortunately, no data on functional and expression studies are available for the other *AGL6*-like maize gene *ZAG5*. In rice, the two *AGL6*-like genes *OsMADS6* and *OsMADS17* had similar expression patterns (Ohmori et al. 2009). Both genes were first expressed in the floral meristem and later in palea, lodicules, and pistil and at lower levels in stamens (Ohmori et al. 2009). Only *OsMADS17* was expressed in the lemma (Ohmori et al. 2009). The characterization of an *OsMADS6* mutant named *mosaic floral organs1* (*mfo1*) indicated this gene as a key regulator of floral organ identity and floral meristem determinacy in rice (Ohmori et al. 2009). The *mfo1* mutants showed altered flower morphology with disturbed palea identity, homeotic transformation of lodicules into glume-like or mosaic organs and occasionally abnormal carpel development (Ohmori et al. 2009). Furthermore, the determinacy of the floral meristem was lost and extra carpels or spikelets developed in *mfo1* florets (Ohmori et al. 2009). Suppression of the other *AGL6*-like gene *OsMADS17* did not cause any morphological abnormality in the wild-type background, but it increased the phenotypic effect in the *mfo1* background, indicating that *OsMADS17* has a minor but redundant function with *OsMADS6* (Ohmori et al. 2009). Strikingly, the mutation of the *SEP*-like gene *OsMADS1*/*LHS1* increased the defect in *osmads6*/*mfo1* flowers (Ohmori et al. 2009; Li et al. 2009). In the severe phenotypes of the *osmads1*/*osmads6* double mutants, the floral organs of whorls 2 and 3 were transformed into glume-like organs or completely repressed during flower development. In addition, *osmads1*/*osmads6* flowers displayed more severe defects caused by loss of meristem determinacy. Taking into account the similarities and differences between single mutants for *OsMADS1* and *OsMADS6*, these genes likely play both cooperative and independent roles in the floral organ identity control and in the establishment and determinacy of the floral meristem.

Recently, Li et al. (2011a) investigated the genetic interaction between *OsMADS6* and the floral homeotic genes *OsMADS16/SPW1* (class B), *OsMADS3* and *OsMADS58* (class C), *OsMADS13* (class D) and *DROOPING LEAF (DL),* a non-MADS-box gene involved in carpel specification (see below). They demonstrated that the interactions of *OsMADS6* with these floral homeotic genes play key roles in specifying flower development and determining floral meristem fate in rice. The analysis of double mutants indicated that *OsMADS6* specifies the palea identity, most likely through repressing the expression of *DL* in the palea, regulates the lodicule development by interacting with *SPW1*, defines the stamen, carpel, and meristem identities with *OsMADS3* and *OsMADS58*, specifies the carpel/ovule development and the floral meristem determinacy together with *OsMADS13*, and acts synergistically with *DL* in terminating the floral meristem. Moreover, expression analyses revealed that in the *osmads6* mutant, the transcript levels of class B (*OsMADS4* and *SPW1*), class C (*OsMADS3* and *OsMADS58*), and class E (*OsMADS7* and *OsMADS8*) genes were reduced at early flower development stages. This suggests that *OsMADS6* may be an upstream regulator that activates the expression of these six floral homeotic genes during early flower development. Consequently, in addition to its interaction with class A (OsMADS14 and OsMADS15), class B (OsMADS4 and SPW1), class D (OsMADS13), and class E (OsMADS7 and OsMADS8) proteins (Moon et al. 1999; Favaro et al. 2002; Lee et al. 2003b; Seok et al. 2010), OsMADS6 regulates also the expression of some of their encoding genes, thus providing novel insights into the molecular mechanism by which *AGL6*-like genes regulate flower development.

The expression of *TaAGL6* of wheat was very similar to that of its orthologous genes of maize (*ZAG3*) and rice (*OsMADS6*); its transcription was restricted to spikes and developing caryopses, and in spikelets at heading time, its expression was restricted to palea, lodicules, and pistils (unpublished results), indicating that *TaAGL6*, like its orthologous genes of maize and rice, may be important for the identity specification and/or development of these floral organs.

Considering the importance of *AGL6*-like genes during flower development in grasses, as determined by the characterization of loss-of-function mutants in maize and rice, Reinheimer and Kellogg (2009) reconstructed the evolutionary history of *AGL6*-like genes in grasses and analyzed gene expression patterns during spikelet and floret development. They found that the *AGL6*-like genes of grasses had discrete expression domains, acquired sequentially in evolutionary time. Expression of *AGL6*-like genes in the carpel, and particularly in the inner integument of the ovule, seems to be rather ancient and closely related to the expression of the *AGL6*-like genes in the mega sporangium and integument of gymnosperms (Mouradov et al. 1998; Shindo et al. 1999; Winter et al. 1999). On the other hand, angiosperms have acquired the new expression pattern in floral meristems and in second-whorl organs in monocots. Expression in the palea (presumptive first whorl) is a novel trait that correlates with the origin of the grass spikelet.

Non-MADS-box genes involved in grass floral development

While research results evidence that MIKC-type MADS-box genes play a very important role in floral organ identity and patterning, it is not equally clear to what extent the different aspects of the eudicot ABCDE model are conserved and can be transferred to grasses. It would be incorrect to assume a priori that only MADS-box genes control flower development in grasses.

As described previously, none of the mutants for class C genes of rice exhibited evident homeotic conversions of the carpel, and only carpel development was affected, whereas the stamens were clearly transformed into lodicules (Yamaguchi et al. 2006); thus other genes appear to regulate carpel identity. *DROOPING LEAF* (*DL*), belonging to the YABBY transcription factor family, has been suggested as one such candidate gene in determining carpel identity in rice (Nagasawa et al. 2003; Yamaguchi et al. 2004, 2006). The same could be true for other grasses including maize and wheat, wherein some similarities were observed (Mena et al. 1996; Ishikawa et al. 2009).

The leaves of the rice *dl* mutant drooped, instead of standing erect, as in the wild type. The drooping leaf phenotype was caused by the absence of the midrib, a strong structure enabling the leaves to stand erect. *DL* was expressed in the central region of the leaf primordia and seemed to promote cell proliferation along the adaxial-abaxial axis, which may be required to yield the number of cells necessary for the construction of the midrib structure (Yamaguchi et al. 2004). Floral homeotic phenotypes wherein carpels were replaced by stamens were identified subsequently; this abnormality has always resulted in association with the drooping leaf phenotype. Flowers of severe rice homeotic *dl* mutants showed the complete conversion of the carpels into stamens, with defects in the flower structure restricted to whorl 4 (Nagasawa et al. 2003; Yamaguchi et al. 2004). This homeotic transformation in rice *dl* mutants contrasts strongly with the standard floral homeotic mutants for class C genes of *Arabidopsis*, in which defects always affected both whorls 3 and 4. *DL* encodes a YABBY protein, a putative transcription factor specific to plants, and was expressed in the presumptive region (the carpel anlagen) where the carpel primordia initiate (Yamaguchi et al. 2004). Shortly after *DL* induction, the carpel primordia began to form and *DL* expression continued in the carpel primordia. Thus, both phenotype and expression analyses suggest that *DL* function would be necessary for carpel specification. This was the first indication that a *YABBY* gene controlled flower organ specification, like MADS-box genes. The *DL* gene would have two additional functions in the control of rice flower development: floral meristem determinacy and antagonistic regulation with class B genes. As already described, *dl* mutants had carpels replaced by stamens, suggesting that class B genes were ectopically expressed in whorl 4. Conversely, stamens were replaced by ectopic carpels in mutants for the *spw1* gene, suggesting that in these mutants *DL* is also expressed in whorl 3. These predictions were confirmed by verifying the spatial expression pattern of both genes in the *spw1* and *dl* mutants; in other words, *DL* was ectopically expressed in whorl 3 of the *spw1* mutant, and *SPW1* was ectopically expressed in whorl 4 of the *dl* mutant (Nagasawa et al. 2003; Yamaguchi et al. 2004), indicating the mutual antagonistic regulation of *DL* and *SPW1*. The ectopic expression of *OsMADS16* (*SPW1*) caused the transformation of carpels into stamens in whorl 4 (Lee et al. 2003b), most likely due to *DL* repression in whorl 4 by the ectopic expression of *OsMADS16* in the transgenic lines. *DL* expression was maintained in carpels of rice plants wherein *OsMADS3* and *OsMADS58* had both been inactivated and abnormal carpel-like organs still developed in these double mutant lines (Yamaguchi et al. 2006), demonstrating that *DL* may function independently of class C genes. It is not yet clear whether carpel development depends on *DL* expression *per se*, or *DL* is mainly responsible for preventing B-function gene expression in whorl 4. Recent experiments combining mutations of class C and D and *dl* genes in rice have provided new insights into the molecular mechanisms that regulate reproductive floral organ development and floral meristem determinacy, and in particular, have shed some new light on the relative contribution of MADS-box genes and *DL* in the specification of carpel identity (Li et al. 2011b; Dreni et al. 2011) (see below).

Mutants showing phenotypes similar to those of rice *dl* were observed in several grass species, such as *Pennisetum americanum* and *Panicum aestivum* (Rao et al. 1988; Fladung et al. 1991). Moreover, *DL* orthologs of maize and wheat were expressed in carpel primordia with a similar pattern (Bommert et al. 2005; Ishikawa et al. 2009). These observations suggest that the *DL* functions, which consist in controlling carpel specification and midrib development, have been conserved throughout the grass family evolution. *CRABS CLAW* (*CRC*) of *Arabidopsis*, the co-ortholog of *DL*, shares its same functions in meristem determinacy and regulation of class B genes. However, unlike *dl* mutants, the *crc* mutation affected carpel development but did not result in homeotic transformations of carpels and showed normal leaves (Bowman and Smyth 1999). Consequently, during grass evolution *DL* may have been recruited for accomplishing new critical functions, such as the control of carpel identity and midrib formation.

The rice mutant *aberrant panicle organization 1* (*apo1*) resembles class C mutants, suggesting that *APO1* regulates class C genes or that it has some class C function. The *apo1*

mutant formed small inflorescences with reduced numbers of branches and spikelets, indicating that *APO1* increases the spikelet number by suppressing the precocious conversion of inflorescence meristems into spikelet meristems (Ikeda et al. 2005, 2007). In addition, *apo1* mutants exhibited abnormal floral organ identity and a loss of floral determinacy. Stamens were frequently replaced by lodicules, and carpels were produced indeterminately. The *DL* gene was expressed in ectopic carpels, and ectopic glumes with partial carpelloid characters were occasionally produced outside the fourth whorl (Ikeda et al. 2005, 2007). These mutant phenotypes, together with the reduced expression of the class C gene *OsMADS3* (Ikeda et al. 2005), suggest that *APO1* regulates positively the class C genes. Considering the precocious formation of spikelet meristems and prolonged formation of lodicules and carpels, *APO1* is likely involved in the temporal regulation of meristem identity. Interestingly, *apo1*/*spw1* (*superwoman1*) double mutants generated novel phenotypes in comparison to those observed in each single mutant (Ikeda et al. 2007). The *apo1*/*spw1* flowers formed a few narrow bracts resembling palea/lemma in whorls 2, identical to those observed in the same whorl in *spw1* flowers. However, unlike *spw1* flowers wherein carpels replaced stamens due to ectopic expression of *DL*, *apo1*/*spw1* formed the same narrow organs of whorl 2 in whorl 3 (Ikeda et al. 2007). The phenotype observed for the double mutant *apo1*/*spw1* indicated that *DL* was not expressed in whorl 3, even when *SPW1* activity was lost, and thus *APO1* activity was required for the expression of *DL* in whorl 3. These results suggest that *APO1* activates MADS-box class C genes and *DL* but does not affect the class B gene *SPW1*.

APO1 encodes an F-box protein closely related to *Arabidopsis UNUSUAL FLORAL ORGANS* (*UFO*). The F-box motif provides substrate specificity for the large protein complexes SCFs (SKP1-cullin-F-box), which have E3 ubiquitin ligase activity and target proteins for degradation. Although phylogenetic analysis indicated that *APO1* is co-ortholog of *UFO* (Ikeda et al. 2007), the loss-of-function phenotypes differed largely between *ufo* and *apo1* mutants. Both *UFO* and *APO1* were expressed in inflorescence meristems (Ikeda et al. 2007; Lee et al. 1997), but *apo1* formed small panicles with reduced numbers of branches and spikelets (Ikeda et al. 2007), whereas *ufo* produced more co-florescences than the wild type (Wilkinson and Haughn 1995). This different behavior indicated that *APO1* and *UFO* had opposite effects on the fate of the inflorescence meristem. As for floral organ identity, *APO1* interacts with class C (*OsMADS3*) genes and *DL*, while *UFO* and its orthologous genes in eudicot species are required to activate class B genes (Weigel and Meyerowitz 1993). In contrast to *apo1* mutants, which resembled class C mutants, the *ufo* mutants were very similar to the class B mutants, showing a reduced number of petals and stamens and a phenotype consistent with a lower level of *AP3* expression during early stages of flower development (Wilkinson and Haughn 1995; Levin and Meyerowitz 1995). Moreover, ectopic expression of *UFO* (35S:*UFO*) resulted in the partial conversion of sepals of the first whorl to petals and of carpels of the fourth whorl to stamens; these organ identity conversions resembled those in 35S:*AP3* and 35S:*PI* (Lee et al. 1997). Thus, both *UFO* and *APO1* play key roles in inflorescence architecture and floral development and likely have similar biochemical functions, whereas their roles in the inflorescence and floral regulatory network diverged considerably during evolution. Seemingly, the *UFO* co-ortholog of rice *APO1* has acquired novel functions by recruiting different target proteins.

OPEN BEAK (*OPB*) is the only other identified gene involved in the regulation of the rice floral organ identity genes (Horigome et al. 2009). The *opb* mutants exhibited pleiotropic defects that affected leaf morphogenesis, inflorescence architecture, and floral organ identity. Abnormal cell proliferation was observed in their epidermal and mesophyll tissues of the second and third leaves and in the parenchyma cells of the glumes. In addition, the determinacy of rachis and floral meristems was reduced because of prolonged meristematic activity. Ectopic and increased expression of the class 1 *knox* genes (*OSH1, OSH6*, and *OSH15*) was observed in the flower primordia of the *opb* mutants, and over-expression of *OSH1* was detected in the second and third leaf. Thus, the abnormal cell proliferation observed in the *opb* mutants was probably caused by ectopic expression of class1 *knox* genes, which are regulated negatively by *OPB*. The *opb* mutants also showed defects in floral organ identity, resulting in the development of mosaic organs such as gluminous lodicules, staminoid lodicules, and pistiloid stamens. Concomitantly, the expression of the class B gene *SUPERWOMAN1* (*SPW1*) decreased; since the expression domains of *OPB* and *SPW1* overlapped, *OPB* would be required for the proper expression of *SPW1*. This function of *OPB* is probably independent of its function in cellular proliferation. The appearance of mosaic organs was restricted to whorls 2 and 3. In particular, the formation of a gluminous lodicule suggests that the balance between the expression of the genes potentially involved in the development of the outer sterile organs of the spikelet (putative class A genes) and the expression of the class B genes is disturbed in whorl 2, and a pistiloid stamen would result from the unbalanced expression of class B and C genes in whorl 3. Then, the mosaic organs of the *opb* mutants may be explained by the down-regulation of *SPW1*, which entails relatively abundant expression of class A and C genes in whorls 2 and 3, respectively.

The map-based cloning of *OPB* showed that it encodes a transcription factor containing a zinc-finger motif; in line with its presumed function, it was expressed in leaf primordia, inflorescence meristems, rachis branch meristems, floral meristems, and floral organ primordia (Horigome et al. 2009). The protein encoded by *OPB* showed 78.4% and 69.1% sequence similarity, respectively, with the proteins encoded by the genes *JAGGED* (*JAG*) and *NUBBIN* (*NUB*) of *Arabidopsis*, which are highly similar and functionally redundant (Dinneny et al. 2006). Plants with the *jag* mutation showed abnormal lateral organs, such as serrated leaves, narrow floral organs, and petals containing fewer cells larger than normal (Dinneny et al. 2004; Ohno et al. 2004). *JAG* would thus suppress the precocious maturation of tissues and promote cell proliferation in lateral organs (Dinneny et al. 2004). Furthermore, ectopic expression of *JAG* resulted in leaf fusion and ectopic leaf-like outgrowths (Dinneny et al. 2004; Ohno et al. 2004). These *jag* phenotypes differed from those observed in the *opb* mutants, wherein cell proliferation was not suppressed, whereas the pattern of cell proliferation was modified. Moreover, *jag* affected the morphology of floral organs but not their identity, whereas the identity of the organs in whorls 2 and 3 was modified in the *opb* mutants. Also the expression domains of *JAG* and *NUB* differed from those of *OPB*. During the vegetative phase, both *JAG* and *OPB* were expressed in leaf primordia, but not in the SAM or in mature leaves, and during the reproductive phase, *JAG* was neither expressed in inflorescence meristems nor in floral meristems at early stages, whereas *OPB* expression was evident. Like *JAG*, *NUB* expression took place in organ primordia but not in the SAM and later was restricted to the adaxial side of lateral organs. Expression domain and function of *NUB* were fully included in those of *JAG*. In fact, the *nub* single mutant did not display any abnormal phenotype. However, the phenotype exhibited by the *jag nub* double mutant represented a more severe version of the *jag* single mutant, indicating the functional redundancy of the two genes, although with a stronger action of *JAG* (Dinneny et al. 2006). The phenotypic differences between the *jag*/*nub* and the *opb* mutants suggest that *OPB* has acquired novel functions during evolution by recruiting new target genes, such as the class 1 *knox* genes and the floral homeotic gene *SPW1*.

As previously discussed, the evolutionary relationship between lemma and palea of grasses and their relationships to eudicot floral organs have been interpreted variously and widely debated. In the *palealess* (*pal*) mutant of rice, the palea was replaced by two leaf-like structures, whereas the other floral organs (lemma, lodicules, stamens, and carpels) remained normal (Luo et al. 2005). The *pal* mutation was mapped within a 35-kb region located in chromosome 6, wherein none of the predicted genes belongs to the MADS-box gene family or to any other gene families of transcription factors, such as *AP2* or *YABBY*, which have been associated with floral organ identity in both grasses and higher eudicots. In a survey of all the predicted genes within the 35-kb region, Luo et al. (2005) found a candidate gene for *PAL* encoding a putative unknown transcription factor. They suggested that the gene responsible for the *pal* phenotype could be a palea identity gene and thus may represent a class A function gene in rice. If true, this would suggest that grasses either have evolved a distinct mechanism to specify a peculiar organ or that the outer first whorl is specified differently than in eudicots. Identification and functional studies of the *PAL* gene will provide further molecular evidence for these hypotheses. Considering the *pal* phenotype, in which only the palea was replaced by two leaf-like structures, while lemma and other floral organs were unaffected, and on the basis of the observation that in the *spw1* mutant, the lodicules were transformed into organs resembling palea more than lemma, Luo et al. (2005) have suggested that lemma and palea are not homologous organs and that palea could be the evolutionary equivalent of the eudicot sepal. On the other hand, the replacement of the palea by two leaf-like organs in the *pal* mutant would suggest that the palea might be composed of two fused perianth parts which, along with the lemma, would represent a modified trimerous calyx (Zanis 2007). The isolation and characterization of the *PAL* gene in other grasses and outgroups may shed light on the origin and evolution of the outermost whorls of the grass floret.

The cloning and characterization of the rice gene *RETARDED PALEA1* (*REP1*), which has a specific role in the regulation of palea development (Yuan et al. 2009), showed that in the *rep1* mutant, palea growth was delayed and reduced and cell differentiation was strongly affected. Furthermore, the reduced palea had five vascular bundles, similar to the vascular pattern of the wild-type lemma, indicating partial loss of palea identity in the *rep1* mutant. *REP1* was expressed only in the palea primordium during early flower development, but it was radially dispersed in stamens and vascular bundles of lemma and palea during later floral stages. *REP1* encodes a putative protein belonging to a plant-specific TCP transcription factor family (Yuan et al. 2009), whose members were previously identified only in angiosperms and are essential in specifying plant morphology by regulating cell proliferation and differentiation (Navaud et al. 2007). The detection of reduced cell size in the palea of *rep1* mutants implies a role of REP1 similar to other TCP proteins in regulating cell expansion and differentiation. Further experiments with cell proliferation markers will be necessary to verify whether this observation excludes a role for *REP1* in regulating cell division, as for other reported TCP proteins.

The reported examples of non-MADS-box gene mutants affecting grass floral identity and development have elucidated the importance and need of a forward genetic strategy—in addition to the more common reverse genetic approach—to study grass flowering. Unfortunately, grass floral organs are tightly enclosed in the developing spikelet, making large-scale mutant screenings difficult; consequently, many interesting grass mutants carrying florets with altered structures may have been missed. Future forward genetic studies will be important for understanding which of the pathways regulating flower development are novel and specific to grasses and which of them have been conserved during evolution and are similar to those of model eudicot species.

Conservation and diversification of the ABCDE eudicot model of floral development in grasses

The ABCDE model, which explains the specification of floral organ identity in *Arabidopsis* in terms of gene interactions, represents a useful framework for interpreting the genetic control of flower development in a wide variety of species including rice and maize (Ferrario et al. 2004; Theissen and Melzer 2007; Soltis et al. 2007; Thompson and Hake 2009; Litt and Kramer 2010). In the present review, the current knowledge on the flower identity genes discovered and studied in rice, maize, and wheat has been integrated into a comprehensive modified ABCDE model, in the attempt to adapt it to the peculiarities of flower development in each of these grass species (Fig. 5). When loss-of-function mutants were not available, gene functions have been postulated on the basis of sequence similarity and comparison of expression patterns with characterized genes and of protein interaction analyses. Considering the grass ABCDE MADS-box genes included in the models, it is evident that further research efforts are needed to understand their functions, particularly in maize and wheat. In rice, suitable loss-of-function mutants are available for the genes of class B *OsMADS16* (*SPW1*), *OsMADS2,* and *OsMADS4*, of class C *OsMADS3* and *OsMADS58*, of class D *OsMADS13,* and of class E *OsMADS1* (*LHS1*), *OsMADS7/8* (*SEP*-like genes), and *OsMADS6* (*AGL6*-like gene). Currently, only the functions of the genes *SILKY1* (class B), *ZAG1* (class C), and *ZAG3* (class E) have been characterized in maize, whereas no loss-of-function mutants are available in wheat.

The comparison between the ABCDE model based on *Arabidopsis* and the adapted models of rice, maize, and wheat indicate that the regulation of flower development in grasses is somewhat different from higher eudicots. Patterns of gene expression and phenotypes of the available mutants appear consistent with the predictions on their functions based on studies in eudicot model species. This appears mostly true for the genes responsible of the class B-, C-, and D-organ identity functions. In fact, since the reproductive organs are considered homologous throughout all angiosperms, a single ancestral genetic mechanism controlling their specification may have been conserved even in divergent angiosperm groups. However, the function of the class C genes in grasses puts forward some questions. For instance, mutations of these genes in rice and maize affect carpel development but do not cause its clear homeotic conversion, whereas stamens are clearly transformed into lodicules. Thus, in grasses, the genetic control for carpel specification seems different from *Arabidopsis* and other eudicots. According to Yamaguchi et al. (2006), *DROOPING LEAF (DL)*, which in rice encodes a member of the plant-specific YABBY family of transcription factors, specifies carpel identity and meristem determinacy and its loss-of-function mutations cause complete homeotic transformation of carpels into stamens. The *DL* orthologous genes of grasses would have acquired critical functions for carpel specification during their evolution (Yamaguchi et al. 2004; Fourquin et al. 2005; Ishikawa et al. 2009). In addition, the grass co-orthologs of the genes of class C *AGAMOUS* (*AG*) and of class B *PISTILLATA* (*PI*) of *Arabidopsis* are duplicated, and analysis of their mutants in rice and maize showed that in grasses, both B and C functions may be partially subfunctionalized to distinct genes. For instance, rice has two *AG* homologs, *OsMADS3* and *OsMADS58* (Fig. 5); Yamaguchi et al. (2006) observed that in *OsMADS3* mutants, almost all stamens were homeotically transformed into lodicules, but they had only minor defects in floral meristem determinacy. Contrastingly, *OsMADS58* RNAi knockdown mutants had only minor defects in floral organ identity, but were greatly affected in floral meristem determinacy. Thus, *OsMADS3* appears to have a central role in specifying stamen identity, whereas *OsMADS58* would have a central role in floral meristem determinacy. Moreover, mutant analysis of the *PI*-like genes of rice *OsMADS2* and *OsMADS4* (Fig. 5) indicated unequal redundancy of their class B function. Besides its major role in specifying lodicule identity, *OsMADS2* also shows a contribution to stamen development equivalent to that of *OsMADS4*. As in *Arabidopsis*, some grass species such as wheat, rice, and maize possess a single copy of the *APETALA3*-like gene, whose function seems conserved across eudicots and grasses. Mutations of the *AP3* co-orthologs *SILKY1* of maize and *SUPERWOMAN1* (*SPW1*) of rice result in homeotic transformations of stamens into carpels and lodicules into palea-like organs. Thus, as in eudicots, *AP3*-like genes are required to promote whorl 2 and 3 identities in grasses.

Two recently published papers have investigated the genetic interactions between the rice genes of class C and

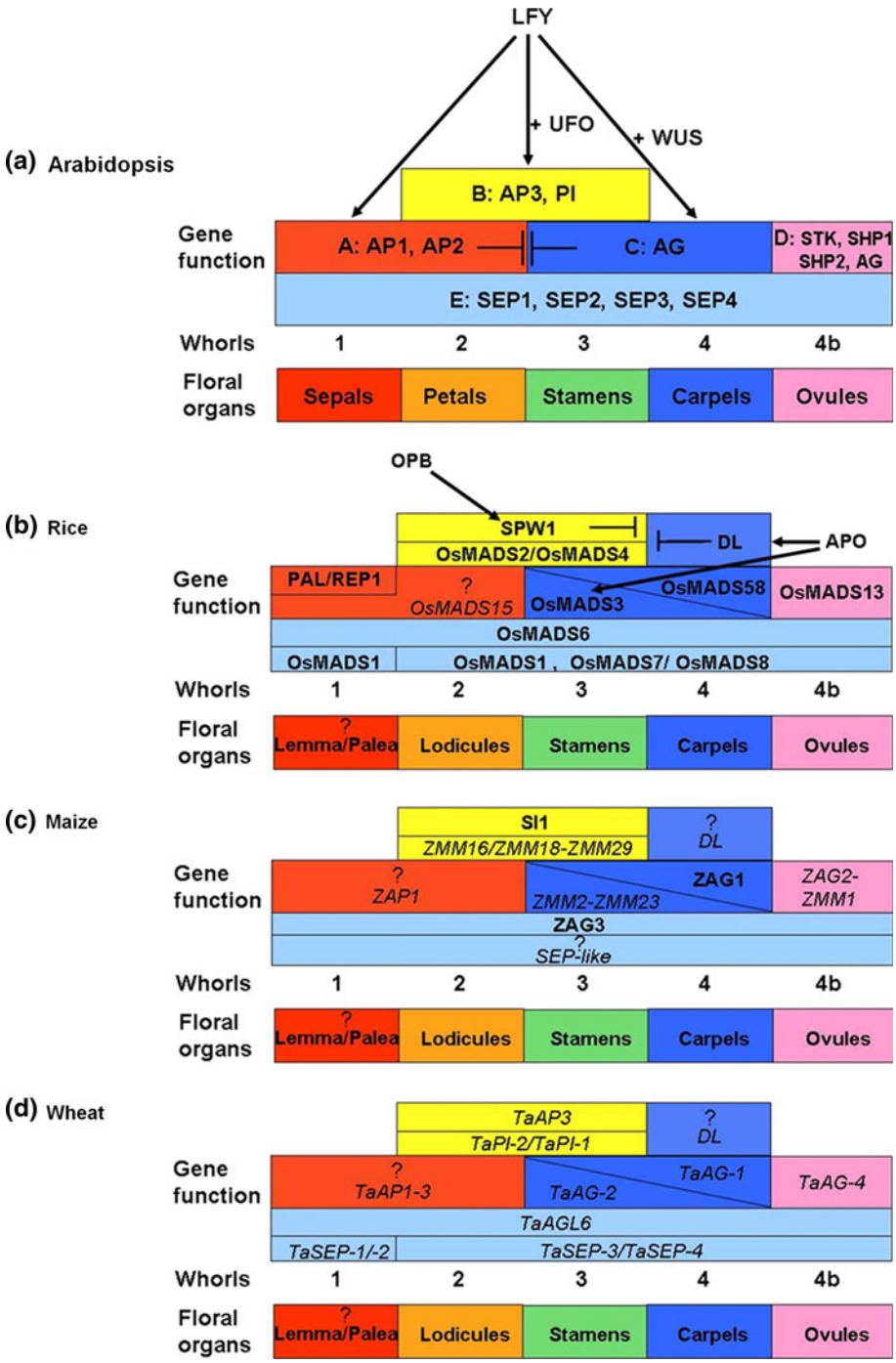

Fig. 5 Comparison of the ABCDE models for floral organ identity of *Arabidopsis* (**a**) and of some grass species such as rice (**b**), maize (**c**), and wheat (**d**). According to the basic ABC model, the class A genes specify the organs of whorl 1, class A and B of whorl 2, class B and C of whorl 3, and class C of whorl 4. The extended ABCDE model includes class D genes promoting ovule identity and class E genes acting as cofactors for the class A, B, C, and D genes and required for the development of all the categories of floral organs. The function domains are indicated by colors: A = *red*, B = *yellow*, C = *blue*, D = *pink*, and E = *light-blue*. The ABCDE schemes include the names of the genes assigned in each species to one of the five functional classes. Genes whose function has been determined by mutant analysis are reported in *bold*, whereas those whose putative function has been inferred by sequence similarity, expression pattern and protein interaction analyses are typed in italics. In the ABCDE models of *Arabidopsis* (**a**) and rice (**b**) the genes involved in the regulation of floral organ identity genes are also indicated. Symbols "?" indicate uncertainty about the A and E functions in grasses, the DL function in maize and wheat and the homology between floral organs in the first whorl in grasses (lemma/palea) and eudicots (sepals). See text for details

D and *DL* and have provided new insights into the conservation/diversification of the molecular mechanisms that regulate flower development in rice and *Arabidopsis* (Li et al. 2011b; Dreni et al. 2011).

Through double mutant analysis, Li et al. (2011b) investigated the genetic interactions among *OsMADS3* (class C gene), *OsMADS13* (class D gene), and *DL* in specifying floral organ identities and floral meristem determinacy. The *osmads13/osmads3* double mutant displayed loss of floral meristem determinacy and generated abundant carpelloid structures containing severe defective ovules in the flower center, which were not detectable in each of the single mutants. In addition, in situ hybridization and yeast two-hybrid analyses revealed that *OsMADS13* and *OsMADS3* neither regulated each other's transcription nor interacted at the protein level. Consequently, *OsMADS3*

would play a synergistic role with *OsMADS13* in both ovule development and floral meristem termination. These results confirm that the C- and D-class genes retained their functions in grasses, even though, after duplication within the *AG* clade, they underwent multiple subfunctionalization and several neofunctionalization events. Strikingly, the *osmads3/dl* double mutant displayed a severe loss of floral meristem determinacy and produced supernumerary whorls of lodicule-like organs at the fourth whorl. In addition, since the *osmads3* single mutant showed normal expression pattern of the *DL* gene and *OsMADS3* expression was detected in ectopic stamens of *dl* flowers, *OsMADS3* and *DL* would not affect their mutual transcription. These results indicate that *OsMADS3* and *DL* play a redundant role in terminating floral meristem, although through distinct pathways. On the contrary, the comparison between the characteristics of the *osmads13/dl* double mutant and those of the two single gene mutants suggest that *DL* and *OsMADS13* may be involved in the same pathway specifying the identity of carpel/ovule and floral meristem. The defective phenotype of *osmads13/dl* flowers appeared identical to that of the single mutant *dl* and no obvious *OsMADS13* expression was detectable in *dl* flowers; on the contrary, *DL* transcripts were ectopically detected in *osmads13*, thus suggesting that *DL* may directly or indirectly regulate *OsMADS13* expression.

Even more recently, Dreni et al. (2011) carried out a more detailed and extensive analysis of the four rice genes of the *AG* subfamily (*OsMADS3*, *OsMADS58*, *OsMADS13,* and *OsMADS21*) and of their interaction using mutant plants for single and multiple genes. They found that the *osmads3* single mutant showed a milder phenotype than that reported by Yamaguchi et al. (2006) and *osmads58* mutant flowers do not have significant developmental defects. On the contrary, the phenotypic effect was very dramatic in the *osmads3 osmads58* double mutants, which showed complete loss of sexual organ identity and massive accumulation of lodicules in the third and fourth floral whorls. Interestingly, most *osmads3 osmads58* double mutant flowers developed one to three palea-like structures replacing the carpel, and again a large number of ectopic lodicules developed in the center of the flower. These *osmads3 osmads58* double mutant phenotypes revealed that a double knockout of *OsMADS3* and *OsMADS58* produced a rice mutant flower in which reproductive organ identity was completely lost and the floral meristem became indeterminate, a phenotype very similar to that of the *ag* mutant in *Arabidopsis*. Therefore, in contrast with previous reports, these findings clearly indicate that *OsMADS58* and *OsMADS3* redundantly determine stamen and carpel identity, even though partial subfunctionalization was confirmed between the two genes; thus, the C function may also be highly conserved in monocotyledonous plants. Furthermore, the *osmads13 osmads3* and *osmads13 osmads58* double mutants displayed a more severe loss of floral meristem determinacy than that observed in each of three single mutants, confirming that *OsMADS13*, together with *OsMADS3* and *OsMADS58*, plays an important role in floral meristem determinacy. On the contrary, analysis of single and multiple mutants carrying the mutated *osmads21* gene showed that this gene has marginal, if any, role in the formation of reproductive organs and floral meristem determinacy and does not seem to contribute to the C and D functions.

Moreover, on the basis of expression analysis of *DL* in *osmads3 osmads58* double mutants and *osmads3 osmads13 osmads58* triple mutants, Dreni et al. (2011) proposed that *DL* cannot specify carpel identity without *OsMADS3* and *OsMADS58*; thus, it is possible that *DL* does not possess any C function and its function would be limited to a negative regulator of class B gene expression in the fourth whorl. This specific *DL* function can explain the *dl* mutant phenotype, since the simultaneous expression of B- and C-function genes in the fourth whorl leads to stamen formation. On the other hand, a full C function of the *DL* gene would have caused a different phenotype in the *dl* mutant, because the lack of C function would have determined the conversion of the stamens (third whorl) and carpels (fourth whorl) into sterile perianth organs, such as palea and lodicules.

Although the results of Dreni et al. (2011) provided important new insights into the function of the four rice genes of the *AG* subfamily in reproductive organ development, more extensive experiments combining mutations of class B, C, and D and *dl* genes in rice will be necessary to clarify the true function of MADS-box genes and *DL* in the specification of reproductive floral organs.

Although in grasses sound functional data for class E genes are available only in rice, it is tempting to draw some deductions from the models of wheat and maize shown in Fig. 5, which are based on phylogenetic and expression data. In particular, the genes responsible for the E function appear conserved among grasses and eudicots, at least for some *SEP*-like genes such as *OsMADS1* (*LHS1*), *OsMADS7,* and *OsMADS8* of rice and *TaSEP-1*/*TaSEP-2*, *TaSEP-3,* and *TaSEP-4* of wheat. Mutant analysis and detailed studies of the spatial and temporal mRNA expression and protein interaction patterns, corresponding to the different rice *SEP*-like genes (Cui et al. 2010; Gao et al. 2010), have indicated the conservation of *SEP*-like gene function in specifying floral determinacy and organ identities, since the separation of eudicots and monocots about 125–150 mya (Soltis et al. 2008; Bell et al. 2010), but have also revealed grass-specific neo- and sub-functionalization events, thus indicating an unexpected evolutionary dynamic of *SEP*-like genes. The situation in *Arabidopsis* is largely dominated by functional redundancy

among the four *SEP* genes, whereas in rice, the mutant phenotypes of *OsMADS1* and *OsMADS7/8* are strikingly similar, indicating that these genes do not act redundantly, like *SEP1/2/3/4*, but in an interdependent way. According to Cui et al. (2010), the functional interdependence of *OsMADS1* and *OsMADS7/8* may reflect a dosage effect during spikelet meristem development. Assuming that the genes are functionally almost equivalent, but dosage-dependent partial knock-down of either *OsMADS7* or *OsMADS8* may still leave enough protein, whereas knockdown of both genes or of *OsMADS1* may reduce *OsMADS1/7/8* products in the spikelet meristem cells below a critical threshold for proper SEP-like protein function. Both *osmads1* and *osmads7/8* mutants reveal some aspects of the severe and complete *sep* phenotype when the four *SEP*-like genes (*OsMADS1/5/7/8*) are down-regulated, suggesting that these class E rice genes have undergone subfunctionalization but retain partially overlapping functions.

The characterization of the rice *mfo1/osmads6* and maize *bde/zag3* mutants has provided key insights into the role of *AGL6*-like genes. Several lines of evidence have indicated that these genes play a very similar functional role to that of class E (*SEP*) genes. Both the *AGL6*-like genes of rice (*OsMADS6*) and maize (*ZAG3*) regulate the development of all four whorls of floral organs and the floral meristem determinacy (Ohmori et al. 2009; Li et al. 2009, 2011a; Thompson et al. 2009). As previously described, in rice, *osmads6 osmads1* double mutants display more glume-like organs inside the flower, a phenotype that is similar to that of the *sep1/2/3* triple mutant of *Arabidopsis*, which exhibits sepal-like floral organs, and to that of the *sep1/2/3/4* quadruple mutant that exhibits leaf-like floral organs. Furthermore, *AGL6*-like genes of grasses have an expression pattern similar to that of the *SEP* genes (Reinheimer and Kellogg 2009; Ohmori et al. 2009; Li et al. 2009; Thompson et al. 2009), indicating that their products may function in multiple complexes interacting with B-, C-, and D-class proteins to promote floral meristem determinacy and to regulate floral organ development. For instance, OsMADS6 is able to form protein complexes with rice B and D proteins in yeast cells (Favaro et al. 2002; Lee et al. 2003b), which resembles the complex formation of SEP proteins with class A, B, C, and D proteins in *Arabidopsis* and other eudicot species. Moreover, physical interaction both in yeast and plant between ZAG3 and ZAG1 (class C protein) in maize has also been reported (Thompson et al. 2009). The conclusion that *AGL6*-like genes from rice and maize resemble class E genes is consistent with the finding that the *Petunia AGL6*-like gene *PhAGL6* functions redundantly with the *SEP*-like genes *FBP2* and *FBP5* in petal and anther development (Rijpkema et al. 2009). Conservation of a *SEP*-like function in *Petunia*, maize, and rice *AGL6*-like genes indicates that comparative functional analyses for the class E genes should also include members of the *AGL6* subfamily.

Little is known on the genes controlling the specification of the nonreproductive organs of the grass floret, in particular whether and how MADS-box genes are involved. A major exception is the role of the class B genes in controlling lodicule identity, although even the interpretation of their action remains controversial. Consequently, it would be interesting to know how genes predicted to have a role in the control of first- and second-whorl organ identities (specifically A- and E-class genes) actually affect flower development in grasses. The A function has been well studied and documented in *Arabidopsis*, although it seems confined to this species or to the Brassicaceae family (Litt 2007). Moreover, some lines of evidence suggest that the *AP1/SQUA*-like genes may have changed their function during angiosperm evolution and, in that case, their function inferred from *Arabidopsis* should not be extended to all angiosperms (Litt and Irish 2003; Litt 2007). In addition, the correspondence of the eudicot sepals to the grass lemma/palea has long been controversial and remains unclear. As mentioned previously, the interpretation of the nonreproductive structures unique to grasses is difficult because homeotic mutations of class B and C genes of maize generate leaf-like organs with intermediate characteristics (Ambrose et al. 2000). In rice, however, mutations of the class B gene *SPW1* transform lodicules into palea-like organs (Nagasawa et al. 2003). A recent hypothesis suggests that lemma arose from reduction and fusion of bracts that formed outside the flower in the common ancestor of the grasses and of their sister lineages, meaning palea would be the only floral organ homologous to sepals (Whipple and Schmidt 2006). Moreover, homeotic conversion of the carpel into a palea-like organ, as recently observed in the *osmads3 osmads58* double mutants by Dreni et al. (2011), confirmed that only the palea, and not the lemma, can be considered as a first-whorl organ. So far, no class A gene with functional equivalence (i.e., specifying the identity of lemma and palea) to the corresponding genes of higher eudicots has been identified in grasses. Considering the complex rounds of gene duplications of the *AP1* and *FUL* lineages in both grasses and higher eudicots and their structural and functional diversification, the class A function as observed in *Arabidopsis* cannot be simply extended to the grasses. Based on available information, the grass *AP1/SQUA*-like genes appear more involved in floral transition and/or in floral meristem identity than in floral organ identity. Other MADS-box genes, such as the class E genes *OsMADS1* and *OsMADS6*, or genes encoding other transcription factors, such as *PALEALESS* and *RETARDED PALEA1,* may be involved in specifying the organ identity of the outermost whorl

(Fig. 5). Although the existence of A function genes (*sensu strictu*) in grass species has not been demonstrated, on the basis of the expression analyses of the *AP1/SQUA*-like genes belonging to the *FUL2* grass clade (*TaAP1-3* in wheat, *OsMADS15* in rice and *ZAP1* in maize), a specific role in the identity and/or development of the nonreproductive organs of spikelets cannot be excluded for the grass *FUL2*-like genes. As previously described, investigations on the molecular evolution and expression of the grass *AGL6*-like genes have suggested that expression in the inner integument of the ovule is likely an ancient expression pattern in seed plants, but expression in the palea might reflect a new expression domain in grasses (Reinheimer and Kellogg 2009). Therefore, further investigation on the role of the rice *AGL6*-like gene *OsMADS6* in controlling palea identity and its relationship with other rice genes involved in the development of the nonreproductive spikelet organs, such as *PAL1*, *REP1,* and *OsMADS1*, may help to gain more insights into the mechanism of grass flower formation.

The diversification between *Arabidopsis* and grass species for floral identity genes suggests that the regulation of homeotic gene expression would not be tightly conserved between eudicots and monocots. In *Arabidopsis*, the gene network leading to proper flower patterning is being discovered, and several genes regulating the expression of floral homeotic genes have been found, including *LEAFY* (*LFY*) and *UNUSUAL FLORAL ORGAN* (*UFO*) (reviewed in Liu and Mara 2010). *LFY* activates directly the class A gene *APETALA1* (*AP1*), but also the class B gene *AP3* and the class C gene *AG* together with *UFO* and *WUSCHEL* (Parcy et al. 1998; Wagner et al. 1999; Lohmann et al. 2001). The expression of *SUPERMAN* (*SUP*) is required for the proper patterning of the boundary between whorls 3 and 4; in fact, it restricts the expression of class B genes to whorls 2 and 3 by down-regulating them in whorl 4 (Bowman et al. 1992; Sakai et al. 1995). *RABBIT EARS* (*RBE*), which encodes a SUP-like zinc-finger protein, acts downstream of *UFO* to promote the development of whorl 2 organs by repressing *AG* expression in this whorl (Takeda et al. 2004; Krizek et al. 2006). In grasses, mechanisms of organ identity gene regulation are almost unknown and the only available data come from the characterization of the rice genes *OPEN BEAK* (*OPB*) and *ABERRANT PANICLE ORGANIZATION 1* (*APO1*), which would be involved in the regulation of class B and C genes, respectively. As previously described, the phenotype of *apo1* mutants resembles that of class C mutants, showing extra lodicules at the expense of stamens; in addition, they make extra carpels, implicating *apo1* in flower meristem determinacy, another class C function. Consistent with this phenotype, the expression of the class C gene *OsMADS3* is reduced in *apo1* mutants, indicating that *APO1* regulates positively class C gene expression. *APO1* encodes an F-box protein, similar to *UFO* of *Arabidopsis*, which instead is required to activate class B genes. Thus, whereas *UFO* and *APO1* both play key roles in floral development and likely have similar biochemical functions, their roles in the floral regulatory network appear to have diverged during the evolution of monocots and eudicots. In contrast, the *opb* mutants showed defects in floral organ identity mainly in the second and third whorls, wherein different types of mosaic organs develop, including gluminous and staminoid lodicules, and pistiloid stamens. These mutant phenotypes, together with the reduced expression of the class B gene *SPW1*, indicate that *OPB* increases the expression of the class B genes. The gene *OPB* encodes a transcription factor with a zinc-finger motif co-orthologous to the *Arabidopsis* pair of paralogous genes *JAGGED* and *NUBBIN*. As previously discussed, the functions of *OPB* divergent from those of its co-orthologous genes of *Arabidopsis* would suggest a significant role in determining the peculiar morphology and flower morphogenesis of grasses.

Conclusions

The genetic and molecular mechanisms involved in the control of flower development have mainly been studied in eudicot model species, such as *Arabidopsis thaliana*, *Antirrhinum majus,* and *Petunia hybrida*. Consequently, it is not surprising that current knowledge on flower development and morphogenesis in grasses lags behind. However, as shown in this review, the knowledge on the genes and their interactions controlling grass flower development is moving forward rapidly, aided by the genomic tools that are becoming increasingly available. In rice and maize, new exciting discoveries elucidating the developmental mechanisms in grasses can be expected in the very near future, thanks to the progress in functional genomics. These advances may contribute significantly to the understanding of the evolution of development patterns in higher plants. Last but not least, the grass family includes the agronomically and economically most important crops, and inflorescences and flowers are closely related to grain productivity. Consequently, in the long run, these studies may contribute to their substantial yield increase.

References

Agrawal GK, Abe K, Yamazaki M, Miyao A, Hirochika H (2005) Conservation of 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 Mol Biol 59:125–135

Ambrose BA, Lerner DR, Ciceri P, Padilla CM, Yanofsky MF, Schmidt RJ (2000) Molecular and genetic analysis of the *Silky1* gene reveals conservation in floral organ specification between eudicots and monocots. Mol Cell 5:569–579

Arora R, Agarwal P, Ray S, Singh AK, Singh VP, Tyagi AK, Kapoor S (2007) MADS-box gene family in rice: genome-wide identification, organization and expression profiling during reproductive development and stress. BMC Genomics 8:24

Becker A, Theissen G (2003) The major clades of MADS-box genes and their role in the development and evolution of flowering plants. Mol Phylogenet Evol 29:464–489

Bell CD, Soltis DE, Soltis PS (2005) The age of the angiosperms: a molecular timescale without a clock. Evolution 59:1245–1258

Bell CD, Soltis DE, Soltis PS (2010) The age and diversification of the angiosperms re-revisited. Am J Bot 97:1296–1303

Bertioli DJ, Moretzsohn MC, Madsen LH, Sandal N, Leal-Bertioli SCM, Guimaraes PM, Hougaard BK, Fredslund J, Schauser L, Nielsen AM, Sato S, Tabata S, Cannon SB, Stougaard J (2009) An analysis of *Arachis* with *Lotus* and *Medicago* sheds new light on the structure, stability and evolution of legume genomes. BMC Genomics 10:45

Bolot S, Abrouk M, Masood-Quraishi U, Stein N, Messing J, Feuillet C, Salse J (2009) The "inner circle" of the cereal genomes. Curr Opin Plant Biol 12:119–125

Bommert P, Satoh-Nagasawa N, Jackson D, Hirano HY (2005) Genetics and evolution of inflorescence and flower development in grasses. Plant Cell Physiol 46:69–78

Bowers JE, Chapman BA, Rong J, Paterson AH (2003) Unraveling angiosperm genome evolution by phylogenetic analysis of chromosomal duplication events. Nature 422:433–438

Bowman JL, Smyth DR (1999) *CRABS CLAW*, a gene that regulates carpel and nectary development in *Arabidopsis*, encodes a novel protein with zinc finger and helix-loop-helix domains. Development 126:2387–2396

Bowman JL, Sakai H, Jack T, Weigel D, Mayer U, Meyerowitz EM (1992) *SUPERMAN,* a regulator of floral homeotic genes in *Arabidopsis*. Development 114:599–615

Bremer K (2002) Gondwanan evolution of the grass alliance of families (Poales). Evolution 56:1374–1387

Cacharrón J, Saedler H, Theissen G (1999) Expression of MADS box genes *ZMM8* and *ZMM14* during inflorescence development of *Zea mays* discriminates between the upper and the lower floret of each spikelet. Dev Genes Evol 209:411–420

Causier B, Schwarz-Sommer Z, Davies B (2010) Floral organ identity: 20 years of ABCs. Semin Cell Dev Biol 21:73–79

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 Mol Biol 26:657–665

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

Columbus JT (1999) An expanded circumscription of *Bouteloma* (Gramineae: Chloridoideae): new combinations and names. Aliso 18:61–65

Cui L, Wall PK, Leebens-Mack JH, Lindsay BG, Soltis DE, Doyle JJ, Soltis PS, Carlson JE, Arumuganathan K, Barakat A, Albert VA, Ma H, dePamphilis CW (2006) Widespread genome duplications throughout the history of flowering plants. Genome Res 16:738–749

Cui R, Han J, Zhao S, Su K, Wu F, Du X, Xu Q, Chong K, Theissen G, Meng Z (2010) Functional conservation and diversification of class E floral homeotic genes in rice (*Oryza sativa*). Plant J 61:767–781

de Martino G, Pan I, Emmanuel E, Levy A, Irish VF (2006) Functional analyses of two tomato *APETALA3* genes demonstrate diversification in their roles in regulating floral development. Plant Cell 18:1833–1845

Dinneny JR, Yadegari R, Fischer RL, Yanofsky MF, Weigel D (2004) The role of *JAGGED* in shaping lateral organs. Development 131:1101–1110

Dinneny JR, Weigel D, Yanofsky MF (2006) *NUBBIN* and *JAGGED* define stamen and carpel shape in *Arabidopsis*. Development 133:1645–1655

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

Doust A (2007) Architectural evolution and its implications for domestication in grasses. Ann Bot 100:941–950

Dreni L, Jacchia S, Fornara F, Fornari M, Ouwerkerk PB, An G, Colombo L, Kater MM (2007) The D-lineage MADS-box gene *OsMADS13* controls ovule identity in rice. Plant J 52:690–699

Dreni L, Pilatone A, Yun D, Erreni S, Pajoro A, Caporali E, Zhang D, Kater MM (2011) Functional analysis of all AGAMOUS subfamily members in rice reveals their roles in reproductive organ identity determination and meristem determinacy. Plant Cell. doi:10.1105/tpc.111.087007

Dwivedi S, Perotti E, Ortiz R (2008) Towards molecular breeding of reproductive traits in cereal crops. Plant Biotech J 6:529–559

Favaro R, Immink R, Ferioli V, Bernasconi B, Byzova M, Angenent G, Kater M, Colombo L (2002) Ovule-specific MADS-box proteins have conserved protein–protein interactions in monocot and dicot plants. Mol Genet 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*. Plant Cell 15:2603–2611

Fawcett JA, Maere S, Van de Peer Y (2009) Plants with double genomes might have had a better chance to survive the cretaceous- tertiary extinction event. Proc Natl Acad Sci USA 106:5737–5742

Ferrario S, Immink RG, Shchennikova A, Busscher-Lange J, Angenent GC (2003) The MADS box gene *FBP2* is required for *SEPALLATA* function in *Petunia*. Plant Cell 15:914–925

Ferrario S, Immink RG, Angenent GC (2004) Conservation and diversity in flower land. Curr Opin Plant Biol 7:84–91

Fitch WM (1970) Distinguishing homologous from analogous proteins. Syst Zool 19:99–113

Fladung M, Bossinger G, Roeb GW, Salamini F (1991) Correlated alterations in leaf and flower morphology and rate of leaf photosynthesis in a *midribless* (*mbl*) mutant of *Panicum maximum Jacq*. Planta 184:356–361

Fornara F, Parenicova 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 *AP1/SQUA* subfamily of MADS box genes. Plant Physiol 135:2207–2219

Fourquin C, Vinauger-Douard M, Fogliani B, Dumas C, Scutt CP (2005) Evidence that *CRABS CLAW* and *TOUSLED* have conserved their role in carpel development since the ancestor of the extant angiosperms. Proc Natl Acad Sci USA 102:4649–4654

Fu D, Szucs P, Yan L, Helguera M, Skinner JS, von Zitzewitz J, Hayes PM, Dubcovsky J (2005) Large deletion within the first intron *VRN-1* are associated with spring growth habit in barley and wheat. Mol Genet Genomics 273:54–65

Gao X, Liang W, Yin C, Ji S, Wang H, Su X, Guo C, Kong H, Xue H, Zhang D (2010) The *SEPALLATA*-like gene *OsMADS34* is required for rice inflorescence and spikelet development. Plant Physiol 153:728–740

Gaut BS (2001) Patterns of chromosomal duplication in maize and their implications for comparative maps of the grasses. Genome Res 11:55–66

Gaut BS, Doebley JF (1997) DNA sequence evidence for the segmental allotetraploid origin of maize. Proc Natl Acad Sci USA 94:6809–6814

Goff S, Ricke D, Lan T, Presting G, Wang R, Dunn M et al (2002) A draft sequene 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 Dev 8:1548–1556

GrassPhylogenyWorking Group (2001) Phylogeny and subfamilial classification of the grasses (Poaceae). Ann Missouri Bot Gard 88:373–457

Gu Q, Ferrandiz C, Yanofsky MF, Matienssen R (1998) The *FRUITFULL* MADS-box gene mediates cell differenziation during *Arabidopsis* fruit development. Development 125:1509–1517

Hama E, Takumi S, Ogihara Y, Murai K (2004) Pistillody is caused by alterations to the class-B MADS-box gene expression pattern in alloplasmic wheats. Planta 218:712–720

Hay RKM, Ellis RP (1998) The control of flowering in wheat and barley: what recent advances in molecular genetics can reveal. Ann Bot 82:541–554

Heuer S, Hansen S, Bantin J, Brettschneider R, Kranz E, Lorz H, Dresselhaus T (2001) The maize MADS box gene ZmMADS3 affects node number and spikelet development and is co-expressed with ZmMADS1 during flower development in egg cells, and early embryogenesis. Plant Physiol 127:33–45

Honma T, Goto K (2000) The Arabidopsis floral homeotic gene PISTILLATA is regulated by discrete cis-elements responsive to induction and maintenance signals. Development 127:2021–2030

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

Horigome A, Nagasawa N, Ikeda K, Ito M, Ito JI, Nagato Y (2009) Rice OPEN BEAK is a negative regulator of class 1 *knox* genes and a posite regulator of class B floral homeotic gene. Plant J 58:724–736

Ikeda K, Nagasawa N, Nagato Y (2005) ABERRANT PANICLE ORGANIZATION 1 temporally regulates meristem identity in rice. Dev Biol 282:349–360

Ikeda K, Ito M, Nagasawa N, Kyozuka J, Nagato Y (2007) Rice ABERRANT PANICLE ORGANIZATION 1, encoding an F-box protein, regulates meristem fate. Plant J 51:1030–1040

Immink RGH, Tonaco I, de Folter S, Shchennikova A, van Dijk A, Busscher-Lange J, Borst JW, Angenent GC (2009) *SEPALLATA3*: the glue for MADS box transcription factor complex formation. Genome Biol 10:24

Immink RGH, Kaufmann K, Angement GC (2010) The "ABC" of MADS domain protein behaviour and interactions. Semin Cell Dev Biol 21:87–93

Irish V (2006) Duplication, diversification, and comparative genetics of angiosperm MADS-box genes. In: Soltis DE, Soltis PS, Leebens-Mack J (eds) Developmental genetics of the flower, advances in botanical research 44. Academic Press, San Diego, London, pp 130–151

Ishikawa M, Ohmori Y, Tanaka W, Hirabayashi C, Murai K, Ogihara Y, Yamaguchi T, Hirano HY (2009) The spatial expression patterns of *DROOPING LEAF* orthologs suggest a conserved function in grasses. Genes Genet Syst 84:137–146

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

Jaillon O, Aury JM, Noel B, Policriti A, Clepet C, Casagrande A, Choisne N, Aubourg S, Vitulo N, Jubin C et al (2007) The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature 449:463–467

Jansen RK, Kaittanis C, Saski C, Lee SB, Tomkins J, Alverson AJ, Daniell H (2006) Phylogenetic analyses of *Vitis* (Vitaceae) based on complete chloroplast genome sequences: effects of taxon sampling and phylogenetic methods on resolving relationships among rosids. BMC Evol Biol 6:32

Jansen RK, Cai Z, Raubeson LA, Daniell H, Depamphilis CW, Leebens-Mack J, Muller KF et al (2007) Analysis of 81 genes from 64 plastid genomes resolves relationships in angiosperms and identifies genome-scale evolutionary patterns. Proc Natl Acad Sci USA 104:19369–19374

Jeon JS, Jang S, Lee S, Nam J, Kim C, Lee SH, Chung YY, Kim SR, Lee YH, Cho YG, An G (2000) *Leafy hull sterile 1* is a homeotic mutation in a rice MADS-box gene affecting rice flower development. Plant Cell 12:871–884

Judziewicz EJL, Clark G, Londono X, Stern MJ (1999) American bamboos. Smithsonian Institution Press, Washington

Kang HG, Jeon JS, Lee S, An G (1998) Identification of class B and class C floral organ identity genes from rice plants. Plant Mol Biol 38:1021–1029

Kater MM, Dreni L, Colombo L (2006) Functional conservation of MADS-box factors controlling floral organ identity in rice and *Arabidopsis*. J Exp Bot 57:3433–3444

Kellogg EA (2001) Evolutionary history of the grasses. Plant Physiol 125:1198–1205

Kellogg EA (2007) Floral displays: genetic control of grass inflorescences. Curr Opin Plant Biol 10:26–31

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

Kim S, Yoo MJ, Albert VA, Farris JS, Soltis PS, Soltis DE (2004) Phylogeny and diversification of B-function MADS-box genes in angiosperms: evolutionary and functional implication of a 260-million-year-old duplication. Am J Bot 91:2102–2118

Komatsu M, Chujo A, Nagato Y, Shimamoto K, Kyozuka J (2003) FRIZZY PANICLE is required to prevent the formation of axillary meristems and to establish floral meristem identity in rice spikelets. Development 130:3841–3850

Kramer EM, Zimmer EA (2006) Gene duplication and floral developmental genetics of basal eudicots. In: Soltis DE, Soltis PS, Leebens-Mack J (eds) Developmental genetics of the flower, advances in botanical research 44. Academic Press, San Diego, pp 354–376

Kramer EM, Dorit RL, Irish VF (1998) Molecular evolution of genes controlling petal and stamen development: duplication and divergence within the *APETALA3* and *PISTILLATA* MADS-box gene lineages. Genetics 149:765–783

Kramer EM, Jaramillo MA, Di Stilio VS (2004) Patterns of gene duplication and functional evolution during the diversification of the *AGAMOUS* subfamily of MADS box genes in angiosperms. Genetics 166:1011–1023

Kramer EM, Su H, Wu C, Hu J (2006) A simplified explanation for the frameshift mutation that created a novel C-terminal motif in the APETALA3 lineage. BMC Evol Biol 6:30

Krizek BA, Lewis MW, Fletcher JC (2006) *RABBIT EARS* is a second-whorl repressor of *AGAMOUS* that maintains spatial boundaries in *Arabidopsis* flowers. Plant J 45:369–383

Kyozuka J, Shimamoto K (2002) Ectopic expression of *OsMADS3*, a rice ortholog of *AGAMOUS*, caused a homeotic transformation of lodicules to stamens in transgenic rice plants. Plant Cell Physiol 43:130–135

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 Physiol 41:710–718

Lamb RS, Hill TA, Tan QKG, Irish VF (2002) Regulation of *APETALA3* floral homeotic gene expression by meristem identity genes. Development 129:2079–2086

Laudencia-Chingcuanco D, Hake S (2002) The *indeterminate floral apex1* gene regulates meristem determinacy and identity in the maize inflorescence. Development 129:2629–2638

Lee I, Wolfe DS, Nilsson O, Weigel D (1997) A *LEAFY* co-regulator encoded by *UNUSUAL FLORAL ORGANS*. Curr Biol 7:95–104

Lee S, Kim J, Son JS, Nam J, Jeong DH, Lee K, Jang S, Yoo J, Lee J, Lee D-Y, Kang HG, An G (2003a) Systematic reverse genetic screening of T-DNA tagged genes in rice for functional genomic analyses: MADS-box genes as a test case. Plant Cell Physiol 44:1403–1411

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

Levin JZ, Meyerowitz EM (1995) *UFO*: An *Arabidopsis* gene involved in both floral meristem and floral organ development. Plant Cell 7:529–548

Li H, Liang W, Jia R, Yin C, Zong J, Kong H, Zhang D (2009) The *AGL6*-like gene *OsMADS6* regulates floral organ and meristem identities in rice. Cell Res 20:299–313

Li H, Liang W, Hu Y, Zhu L, Yin C, Xu J, Dreni L, Kater MM, Zhang D (2011a) Rice *MADS6* interacts with the floral homeotic genes *SUPERWOMAN1*, *MADS3*, *MADS58*, *MADS13* and *DROOPING LEAF* in specifying floral organ identities and meristem fate. Plant Cell. doi:10.1105/tpc.111.087262

Li H, Liang W, Yin C, Zhu L, Zhang D (2011b) Genetic interaction of *OsMADS3*, *DROOPING LEAF* and *OsMADS13* in specifying rice floral organ identities and meristem determinacy. Plant Physiol 156:263–274

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

Lim J, Moon YH, An G, Jang SK (2000) Two rice MADS domain proteins interact with *OsMADS1*. Plant Mol Biol 44:513–527

Litt A (2007) An evaluation of A-function: evidence from the *APETALA1* and *APETALA2* gene lineages. Int J Plant Sci 168:73–91

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

Litt A, Kramer MK (2010) The ABC model and the diversification of floral organ identity. Semin Cell Dev Biol 21:129–137

Liu Z, Mara C (2010) Regulatory mechanisms for floral homeotic gene expression. Semin Cell Dev Biol 21:80–86

Lohmann JU, Hong RL, Hobe M, Busch MA, Parcy F, Simon R, Weigel D (2001) A molecular link between stem cell regulation and floral patterning in *Arabidopsis*. Cell 105:793–803

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. Dev Genet 25:237–244

Luo Q, Zhou K, Zhao X, Zeng Q, Xia H, Zhai W, Xu J, Wu X, Yang H, Zhu L (2005) Identification and fine mapping of a mutant gene for palealess spikelet in rice. Planta 221:222–230

Magallon SA, Sanderson MJ (2005) Angiosperm divergence times: the effect of genes, codon positions, and time constraints. Evolution 59:1653–1670

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

Malcomber ST, Kellogg EA (2005) *SEPALLATA* gene diversification: brave new whorls. Trends Plant Sci 10:427–435

Malcomber ST, Kellogg EA (2006) Evolution of unisexual flowers in grasses (Poaceae) and the putative sex determination gene, *TASSEL-SEED2* (*TS2*). New Phytol 170:885–899

Mandel MA, Gustafson-Brown C, Savidge B, Yanofsky MF (1992) Molecular characterization of the *Arabidopsis* floral homeotic gene *APETALA1*. Nature 360:273–277

Marchant AD, Briggs BG (2007) Ecdeiocoleaceae and Joinvilleaceae, sisters of Poaceae (Poales): evidence from rbcL and matK data. Telopea 11(4):437–450

Melzer R, Verelst W, Theissen G (2009) The class E floral homeotic protein SEPALLATA3 is sufficient to loop DNA in "floral quartet"-like complexes in vitro. Nucl Acids Res 37:144–157

Mena M, Mandel MA, Lerner DR, Yanofsky MF, Schimidt RJ (1995) A Characterization of the MADS-box gene family in maize. Plant J 8:845–854

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

Ming R, Hou S, Feng Y, Yu Q, Dionne-Laporte A, Saw JH, Senin P, Wang W, Ly BV, Lewis KLT et al (2008) The draft genome of the transgenic tropical fruit tree papaya (*Carica papaya* Linnaeus). Nature 452:991–996

Moon YH, Kang HG, Jung JY, Jeo JS, Sung SK, An G (1999) Determination of the motif responsible for interaction between the rice *APETALA1/AGAMOUS-LIKE9* family proteins using a yeast two-hybrid system. Plant Physiol 120:1193–1204

Mouradov A, Glassick TV, Hamdorf BA, Murphy LC, Marla SS, Yang Y, Teasdale RD (1998) Family of MADS-box genes expressed early in male and female reproductive structures of Monterey Pine. Plant Physiol 117:55–61

Muller BM, Saedler H, Zachgo S (2001) The MADS-box gene DEFH28 from *Antirrhinum* is involved in the regulation of floral meristem identity and fruit development. Plant J 28:169–179

Munster T, Wingen LU, Faigl W, Werth S, Saedler H, Theissen G (2001) Characterization of three *Globosa*-like MADS-box genes from maize: evidence for ancient paralogy in one class of floral homeotic B-function gene of grasses. Gene 262:1–13

Munster T, Deleu W, Wingen LU, Ouzunova M, Cacharron J, Faigl W, Werth S, Kim JTT, Saedler H, Theissen G (2002) Maize MADS-box genes galore. Maydica 47:287–301

Murai K, Takumi S, Koga H, Ogihara Y (2002) Pistillody, homeotic transformation of stamens into pistil-like structures, caused by nuclear-cytoplasm interaction in wheat. Plant J 29:169–181

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

Navaud O, Dabos P, Carnus E, Tremousaygue D, Herve C (2007) TCP transcription factors predate the emergence of land plants. J Mol Evol 65:23–33

Ohmori S, Kimizu M, Sugita M, Miyao A, Hirochita H, Uchida E, Nagato Y, Yoshida H (2009) *MOSAIC FLORAL ORGANS1*, an *AGL6*-like MADS box gene regulates floral organ identity and meristem fate in rice. Plant Cell 21:3008–3025

Ohno CK, Reddy GV, Heisler MG, Meyerowitz EM (2004) The *Arabidopsis JAGGED* gene encodes a zinc finger protein that promotes leaf tissue development. Development 131:1111–1122

Paolacci AR, Tanazarella OA, Porceddu E, Varotto S, Ciaffi M (2007) Molecular and phylogenetic analysis of MADS-box genes of MIKC type and chromosome location of SEP-like genes in wheat (*Triticum aestivum* L.). Mol Genet Genomics 278:689–708

Parcy F, Nilsson O, Busch MA, Lee I, Weigel D (1998) A genetic framework for floral patterning. Nature 395:561–566

Parenicova L, de Folter S, Kieffer M, Horner DS, Favalli C, Busscher J, Cook HE, Ingram RM, Kater MM, Davies B, Angenent GC, Colombo L (2003) Molecular and phylogenetic analysis of the complete MADS-box transcription factor family in *Arabidopsis*: new openings to the MADS world. Plant Cell 15:1538–1551

Paterson AH, Bowers JE, Chapman BA (2004) Ancient polyploidization predating divergence of the cereals, and its consequences for comparative genomics. Proc Natl Acad Sci USA 101:9903–9908

Paterson AH, Bowers JE, Van de Peer Y, Vandepoele K (2005) Ancient duplication of cereal genomes. New Phytol 165:658–661

Pelaz S, Ditta GS, Bauman 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-Lopez R, Alvarez-Buylla ER, Yanofsky MF (2001) Conversion of leaves into petals in *Arabidopsis*. Curr Biol 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. Sex Plant Reprod 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, Vijayraghavan U (2003) Double-stranded RNA interference of a rice *PI/GLO* paralog, *OsMADS2*, uncover its second-whorl-specific function in floral organ patterning. Genetics 165:2301–2305

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

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. Plant J 43:915–928

Preston JC, Kellogg EA (2006) Reconstructing the evolutionary history of paralogous *APETALA1/FRUITFULL*-like genes in Grasses (Poaceae). Genetics 174:421–437

Preston JC, Kellogg EA (2007) Conservation and divergence of *APETALA1/FRUITFULL*-like gene function in grasses: evidence from gene expression analyses. Plant J 52:69–81

Preston JC, Christensen A, Malcomber ST, Kellogg EA (2009) MADS-box gene expression and implications for developmental origins of the grass spikelet. Am J Bot 96:1419–1429

Rao SA, Mengesha MH, Reddy CR (1988) Characteristics, inheritance, and allelic relationships of midribless mutants in pearl millet. J Hered 79:18–20

Reinheimer R, Kellogg EA (2009) Evolution of *AGL6*-like MADS box genes in grasses (Poaceae): ovule expression is ancient and palea expression is new. Plant Cell 21:2591–2605

Rijpkema AS, Royaert S, Zethof J, van der Weerden G, Gerats T, Vandenbussche M (2006) Analysis of the Petunia TM6 MADS-box gene reveals functional divergence within the *DEF/AP3* lineage. Plant Cell 18:1819–1832

Rijpkema AS, Zethof J, Gerats T, Vandenbussche M (2009) The *Petunia AGL6* gene has a SEPALLATA-like function in floral patterning. Plant J 60:1–9

Rijpkema AS, Vandenbussche M, Koes R, Heijmans K, Gerats T (2010) Variation on a theme: changes in floral ABCs in angiosperms. Semin Cell Dev Biol 21:100–107

Rudall PJ, Stuppy W, Cunnif J, Kellogg EA, Briggs BG (2005) Evolution of reproductive structures in grasses (Poaceae) inferred by sister-group comparison with their putative closest relatives, Ecdeiocoleaceae. Am J Bot 92:1432–1443

Sajo MG, Longhi-Wagner HM, Rudall PJ (2008) Reproductive morphology of the early-divergent grass *Streptochaeta* and its bearing on the homologies of the grass spikelet. Plant System Evol 275:245–255

Sakai H, Medrano LJ, Meyerowitz EM (1995) Role of *SUPERMAN* in maintaining *Arabidopsis* floral whorl boundaries. Nature 378:199–203

Sakai H, Krizek BA, Jacobsen SE, Meyerowitz EM (2000) Regulation of *SUP* expression identifies multiple regulators involved in Arabidopsis floral meristem development. Plant Cell 12:1607–1618

Salse J, Bolot S, Throude M, Jouffe V, Piegu B, Masood B, Calcagno T, Cooke R, Delseny M, Feuillet C (2008) Identification and characterization of conserved duplications between rice and wheat provide new insight into grass genome evolution. Plant Cell 20:11–24

Sanchez-Ken JG, Clark LG, Kellogg EA, Kay EE (2007) Reinstatement and emendation of subfamily Micrairoideae (Poaceae). System Bot 32:71–80

Schmidt RJ, Ambrose BA (1998) The blooming of grass flower development. Curr Opin Plant Biol 1:60–67

Schmidt RJ, Veit B, Mandel MA, Mena M, Hake S, Yanofsky MF (1993) Identification and molecular characterization of *ZAG1*, the maize homolog of the *Arabidopsis* floral homeotic gene *AGAMOUS*. Plant Cell 5:729–737

Schmutz J, Cannon SB, Schluter J, Ma J, Mitros T, Nelson W, Hyten DL, Song Q, Thelen JJ, Cheng J et al (2010) Genome sequence of the palaeopolyploid soybean. Nature 463:178–183

Seok HY, Park HY, Park JI, Lee YM, Lee SY, An G, Moon YH (2010) Rice ternary MADS protein complexes containing class B MADS heterodimer. Biochem Biophys Res Commun 401:598–604

Shan H, Zhang N, Liu C, Xu G, Zhang J, Chen Z, Kong H (2007) Patterns of gene duplication and functional diversification during the evolution of the AP1/SQUA subfamily of plant MADS-box genes. Mol Phylogenet Evol 44:26–41

Shindo S, Ito M, Ueda K, Kato M, Hasebe M (1999) Characterization of MADS genes in the gymnosperm *Gnetum parvifolium* and its implication on the evolution of reproductive organs in seed plants. Evol Dev 1:180–190

Shitsukawa N, Tahira C, Kassai KI, Hirabayashi C, Shimizu T, Takumi S, Mochida K, Kawaura K, Ogihara Y, Murai K (2007) Genetic and epigenetic alteration among three homoeologous genes of a class E MADS box gene in hexaploid wheat. Plant Cell 19:1723–1737

Soderstrom TR (1981) Some evolutionary trends in the Bambusoideae (Poaceae). Ann Missouri Bot Gard 68:15–47

Soltis DE, Chanderbali AS, Kim S, Buzgo M, Soltis PS (2007) The ABC model and its applicability to basal angiosperms. Ann Bot 100:155–163

Soltis DE, Bell CD, Kim S, Soltis PS (2008) Origin and early evolution of angiosperms. Ann NY Acad Sci 1133:3–25

Soltis DE, Albert VA, Leebens-Mack J, Bell CD, Paterson AH, Zheng C, Sankoff D, DePamphilis CW, Wall PK, Soltis PS (2009) Polyploidy and angiosperm diversification. Am J Bot 96:336–348

Sonnhammer ELL, Koonin EV (2002) Orthology, paralogy and proposed classification for paralog subtypes. Trends Genet 18:619–620

Stellari GM, Jaramillo MA, Kramer EM (2004) Evolution of the *APETALA3* and *PISTILLATA* lineages of MADS-box-containing genes in the basal angiosperms. Mol Biol Evol 21:506–519

Swigonova Z, Lai J, Ma J, Ramakrishna W, Llaca V, Bennetzen JL, Messing J (2004) Close split of sorghum and maize genome progenitors. Genome Res 14:1916–1923

Takeda S, Matsumoto N, Okada K (2004) *RABBIT EARS*, encoding a *SUPERMAN*-like zinc finger protein, regulates petal development in *Arabidopsis thaliana*. Development 131:425–434

Tang H, Wang X, Bowers JE, Ming R, Alam M, Paterson AH (2008) Unraveling ancient hexaploidy through multiply-aligned angiosperm gene maps. Genome Res 18:1944–1954

The International Brachypodium Initiative (2010) Genome sequencing and analysis of the model grass *Brachypodium distachyon*. Nature 463:763–768

Theissen G (2001) Development of floral organ identity: stories from the MADS house. Curr Opin Plant Biol 4:75–85

Theissen G, Melzer R (2007) Molecular mechanisms underliyng origin and diversification of the angiosperm flower. Ann Bot 100:603–619

Theissen G, Saedler H (2001) Plant biology: floral quartets. Nature 409:469–471

Theissen G, Strater T, Fischer A, Saedler H (1995) Structural characterization, chromosomal localization and phylogenetic evaluation of two pairs of *AGAMOUS*-like MADS-box genes from maize. Gene 156:155–166

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 Mol Biol 42:115–149

Thompson BE, Hake S (2009) Translational biology: from *Arabidopsis* flowers to grass inflorescence architecture. Plant Physiol 149:38–45

Thompson BE, Bartling L, Whipple C, Hall DH, Sakai H, Schmidt R, Hake S (2009) *Bearded-ear* encodes a MADS box transcription factor critical for maize floral development. Plant Cell 21:2578–2590

Tuskan GA, Difazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, Putnam N, Ralph S, Rombauts S, Salamov A et al (2006) The genome of black cottonwood, *Populus trichocarpa* (Torr. and Gray). Science 313:1596–1604

Tzeng TY, Hsiao CC, Chi PJ, Yang CH (2003) Two lily *SEPALLATA*-like genes cause different effects on floral formation and floral transition in *Arabidopsis*. Plant Physiol 133:1091–1101

Van de Peer Y, Fawcett JA, Proost S, Sterck L, Vadepoele K (2009) The flowering world: a tale of duplications. Trends Plant Sci 14:680–688

Vandenbussche M, Theissen G, Van de Peer Y, Gerats T (2003) Structural diversification and neo-functionalization during floral MADS-box gene evolution by C-terminal frameshift mutations. Nucl Acids Res 31:4401–4409

Velasco R, Zharkikh A, Troggio M, Cartwright DA, Cestaro A, Pruss D, Pindo M et al (2007) A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. PLoS ONE 2:e1326

Veron AS, Kuafmannn K, Bornberg-Bauer E (2007) Evidence of interaction network evolution by whole-genome duplications: a case study in MADS-box proteins. Mol Biol Evol 24:670–678

Vollbrecht E, Springer PS, Goh L, Buckler ES, Martienssen R (2005) Architecture of floral branch systems in maize and related grasses. Nature 436:1119–1126

Wagner D, Sablowski RWM, Meyerowitz EM (1999) Transcriptional activation of *APETALA1* by *LEAFY*. Science 285:582–584

Weigel D, Meyerowitz EM (1993) Activation of floral homeotic genes in *Arabidopsis*. Science 261:1723–1726

Whipple C, Schmidt RJ (2006) Genetics of grass flower development. In: Soltis DE, Soltis PS, Leebens-Mack J (eds) Developmental genetics of the flower, advances in botanical research 44. Academic Press, San Diego, pp 353–384

Whipple CJ, Ciceri P, Padilla CM, Ambrose BA, Bandong SL, Schmidt RJ (2004) Conservation of B-class floral homeotic gene function between maize and *Arabidopsis*. Development 131:6083–6091

Whipple CJ, Zanis MJ, Kellogg EA, Scmidt RJ (2007) Conservation of B class gene expression in the second whorl of a basal grass and outgroups links the origin of lodicules and petals. Proc Natl Acad Sci USA 104:1081–1086

Wilkinson MD, Haughn GW (1995) *UNUSUAL FLORAL ORGANS* Controls Meristem Identity and Organ Primordia Fate in *Arabidopsis*. Plant Cell 7:1485–1499

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. Proc Natl Acad Sci USA 96:7342–7347

Xu G, Kong H (2007) Duplication and divergence of floral MADS-box genes in grasses: evidence for generation and modification of novel regulators. Int J Plant Biol 49:927–939

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

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*. Plant Cell 18:15–28

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of the wheat vernalization gene *VRN1*. Proc Natl Acad Sci USA 100:6263–6268

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

Yao SG, Ohmori S, Kimizu M, Yoshida H (2008) Unequal genetic redundancy of rice *PISTILLATA* orthologs, *OsMADS2* and *OsMADS4*, in lodicules and stamen development. Plant Cell Physiol 49:853–857

Yoshida H, Itoh J, Ohmori S, Miyoshi K, Origome A, Uchida E, Kimizu M, Matsumura Y, Kusaba M, Satoh H, Nagato Y (2007) *Superwoman1-cleistogamy*, a hopeful allele for gene containment in GM rice. Plant Biotech J 5:835–846

Yuan Z, Gao S, Xue DW, Luo D, Li LT, Ding SY, Yao X, Wilson ZA, Qian Q, Zhang DB (2009) *RETARDED PALEA1* controls palea development and floral zygomorphy in rice. Plant Physiol 149:235–244

Zahn LM, Kong H, Leebens-Mack JH, Kim S, Soltis PS et al (2005) The evolution of the *SEPALLATA* subfamily of MADS-box genes: a preangiosperm origin with multiple duplications throughout angiosperm history. Genetics 169:2209–2223

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. Evol Dev 8:30–45

Zanis MJ (2007) Grass spikelet genetics and duplicate gene comparisons. Int J Plant Sci 168:93–110

Zhao XY, Cheng ZJ, Zhang XS (2006) Overexpression of *TaMADS1*, a *SEPALLATA*-like gene in wheat, causes early flowering and the abnormal development of floral organs in *Arabidopsis*. Planta 223:698–707

Zhao Y, Li X, Chen W, Peng X, Cheng X, Zhu S, Cheng B (2010) Whole-genome survey and characterization of MADS-box gene family in maize and sorghum. Plant Cell Tissue Organ Cult 105:159–173