Genetic Control of Floral Transition in Cereals

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The transition from vegetative to reproductive phase is of great fundamental and applied interest but is still poorly understood. Recently genetic-molecular approaches have been used to dissect this process in *Arabidopsis*. As the genes controlling floral induction in *Arabidopsis* become better defined, there is a need to address how they correspond to genes that regulate flowering in other agronomically important species. A focused effort on comparative mapping will be required to establish the potential correspondence of different genes in different species. Isolation of putative orthologs in different species and mutant's expression patterns reveal evolutionary conserved elements in flower development pathway. Here, overviewed with respect to the recent progress is molecular and genetic approaches for the elucidation of the mechanisms controlling reproductive development of rice and other grass species.

Key Words: ABC model, MADS-box, Flower development, *Arabidopsis*, Rice

Introduction

After seed germination, morphogenesis in higher plants originates from shoot and root apical meristem. After a certain phase of vegetative growth, the transition to flowering is brought about by the concerted action of endogenous and environmental factors that synchronize plants of a given species to ensure their reproductive development under optimal conditions. The most important environmental factors that control flower induction are low temperatures (vernalization) and the length of the day light period (photoperiodism). Whereas the vernalization temperature is recognized by the shoot apical meristem, flower induction by a favourable day length takes place in the leaves. In photoperiodic plants growing under the appropriate day length, a flowering stimulus is released from the leaves and transported to the apical meristem (Bernier *et al.*, 1993). If the apical meristem is competent to respond to this signal, the vegetative meristem becomes either an inflorescence or a floral meristem, from which floral development continues.

Although different plant species exhibit a wide range of flowering responses to environmental and developmental signals, physiological and genetic studies indicate that some of the basic mechanisms controlling flowering time may be conserved. Genetic analyses have revealed that single gene differences can alter the flowering response type; for example mutations that introduce or eliminate an effect of photoperiod on flowering have been described in tobacco (Allard, 1919), maize (Galinat and Naylor, 1951), pea (Murfet, 1989), sorghum (Quinby and Karper, 1945) and *Arabidopsis* (Zagotta *et al.*, 1992). A mutation in gibberellin biosynthesis has recently been shown to inhibit the ability of *Arabidopsis* to flower in noninductive photoperiods (Wilson *et al.*, 1992).

Unlike many developmental transitions in animals, the shoot apical meristem (SAM) of plants is not irreversibly "committed" to reproductive development once flowering commences. In some species and genotypes under certain environmental conditions, leafy shoots are formed after flowers in a phenomenon known as "inflorescence reversion" (Battey and Lyndon, 1990). This observation implies that the genes and processes involved in the transition to flowering are required to both initiate and maintain reproductive development.

Studies beginning in the early 1980s of mutations in *Arabidopsis* that alter either floral induction or floral meristem fate or floral organ fate were starting points for analysis of molecular genetics of flowering (Raghavan, 2001). In *Arabidopsis*, upon seed germination the shoot apical meristem (SAM) produces on its flanks primordia/ meristems for leaves. The leaves are generated in spiral fashion and are separated by short lengths of stem (internode). Upon floral induction, SAM reorganizes to form an inflorescence meristem that first produces a few spirally placed leaf primordia with axillary second order inflorescence meristems. These leaves are called cauline leaves and will be separated by long internodes. After this, the inflorescence meristem produces meristems for individual flowers, again in spiral fashion. The floral

meristem is determinate in its development. Each floral meristem specifies formation of concentric rings of floral organ primordia in an invariant order: sepals, petals, stamens and carpels from periphery to the centre of the flower. The result is a plant with a basal rosette of leaves and racemose inflorescence, where individual flowers bear organs in the pattern (sepals) 4, (petals) 4, (stamens) 6 and (carpels) 2.

From a genetic perspective, two phenotypic changes that control vegetative and floral growth are programmed in the plant. The first genetic change involves the switch from the vegetative to the floral state. If this genetic change is not functioning properly, then flowering will not occur. The second genetic event follows the commitment of the plant to form flowers. The observation that the organs of the plant develop in a sequential manner suggests that a genetic mechanism exists in which a series of genes are sequentially turned on and off. This switching is necessary for each whorl to obtain its final unique identity.

A series of *Arabidopsis* mutants have been identified in which normal flowers are replaced with structures that resemble inflorescence meristems and the shoots that normally develop from them. One such mutant is *LEAFY*. *LEAFY* mutants often do not develop floral meristems and late flowers lack petals and stamens. This gene must be involved in the development of these flower organs (Weigel *et al.,* 1992). The analogous gene in snapdragon to *LEAFY* is *floricaula* (*flo*). *flo* mutants also fail to undergo transition from inflorescence to floral meristem, and the flowers have the appearance of an inflorescence shoot. *flo* does differ from *LEAFY* with regards to organ development in that it does not appear to affect petal and stamen development (Coen *et al.,* 1990). This clearly shows a functional deviation during the evolution of the two species.

Flowers of *APETALA1* mutants express a partial inflorescence meristem phenotype where secondary floral meristems appear in the axis region of the sepal. But when the *APETALA1* and *LEAFY* mutants are combined, the flowers appear as an inflorescence shoot. *APETALA1* also affects the normal development of sepals and petals (Greg *et al.*,2001). The snapdragon analog to the *APETALA1* gene, *SQUAMOSA* (Mandel *et al.,* 1992) is much more severe, and the flowers appear as inflorescence shoots. Another gene, *CAULIFLOWER*, does not express its effects unless coupled with another mutant. *CAULIFLOWER* and *APETALA1*double mutants have inflorescence meristems developing in place of floral meristems. Phenotypic functions maintained by the *CAULIFLOWER* gene are duplicated by *APETALA1*. The *ap2* mutations also enhance flower meristem defects of *ap1* and *lfy* mutants, indicating that *ap2* also contributes floral meristem identity.

In *Arabidopsis*, *TERMINAL FLOWER1* (*TFL1*) and its *Antirrhinum* ortholog *CENTRORADIALIS* (*CEN*), respectively, are required for the inflorescence meristem to maintain its indeterminate growth fate. Mutations in *TFL1* and *CEN* result in the inflorescence meristem becoming a terminal flower. In *Arabidopsis*, *TFL1* inhibits the expression of *LFY* and *AP1* at the centre of the shoot apex to prevent the inflorescence meristem from becoming a floral meristem (Liljegren *et al.,* 1999); in turn *LFY* and *AP1* inhibit *TFL1* expression in the lateral meristems committed to a floral fate.

The ABC Model of Flower Development

The flowers of well studied dicot species like *Arabidopsis* and *Antirrhinum* consist of four whorls with, from outside to inside, the sepals, petals, stamens and carpels. The determination of the identity of these floral organs has been extensively studied. In these two species using homeotic flower mutants and these studies resulted in the formation of so called ABC model (Weigel and Meyerowitz, 1994). This model proposes that floral organ identity is regulated by three classes of master genes, A, B and C. These genes act in over lapping domains, each of which extends over two adjacent whorls. The model suggests that the A and C functions specify sepals and carpels, respectively, whereas the combined activities of A and B, and B and C specify petals and stamens, respectively. The model further suggests that the A and C activities are mutually antagonistic, such that in class A loss of function mutants, the C domain expands to include all whorls, the converse occurring in class C loss of function mutants. The proposed domains of A, B and C gene actions were supported by the observed expression patterns for the several of the cloned homeotic genes in wild type flowers as well as flowers mutant for one A, B or C function; this being the case for the *Arabidopsis AP1*, *AG*, *PI*, *AP3*, and as well the *Antirrhinum SQUA*, *DEFA*, *GLO* and *PLE* genes. Therefore, organ identity is controlled to great extent at the level of transcription of these regulators in specific domains of the floral meristem (Raghavan, 2001). The model was later extended to *Arabidopsis* and Petunia with the class D genes, necessary for ovule development (ABCD model) (Colombo *et al.,* 1995) and with class E

genes (*SEP* genes) indispensable for the determination of petal, stamen, carpel and ovule identity.

Examples of A, B, and C group genes involved in flowering, these have been identified in *Arabidopsis thaliana*.

A **group**	*APETALA1* **(***AP1***) and** *APETALA2* **(***AP2***)**
B **group**	*APETALA3* **(***AP3***) and** *PISTILLATA* **(***PI***)**
C **group**	*AGAMOUS* **(***AG***)**

Floral Organ Identity genes

The two A function genes are *APETALA2* and *APETALA1*. Alleles of these two genes have been isolated that show varying degrees of effect, but in general if an A function gene is mutated, the first whorl develops as a carpel and the second whorl develops as a stamen. *ovulata* is an A function gene of snapdragon similar to *APETALA2*. The B gene functions are defined by the genes *APETALA3* and *PISTILLATA*. The net effect of B gene mutations is that whorl 2 develops as a sepal rather than a petal, and whorl 3 develops as a carpel not a stamen. *deficiency* and *globosa* are snapdragon genes that have homologous functions to the *Arabidopsis* B function genes (Sommer *et al.,* 1990)

Finally, C gene functions are defined by the gene *AGAMOUS*. Mutants of this gene have the third whorl stamen replaced by a petal, and the fourth whorl develops into a new flower with the sepal-petal-petal pattern. Furthermore, flower development in *AGAMOUS* mutants is indeterminate, not determinate. A snapdragon gene similar to *AGAMOUS* is *pleniflora*.

	Phenotype			
Mutation	**Whorl 1**	**Whorl 2**	**Whorl 3**	**Whorl 4**
Wild Type	Sepal	Petal	Stamen	Carpel
A Function	Carpel	Stamen	Stamen	Carpel
B Function	Sepal	Sepal	Carpel	Carpel
C Function	Sepal	Petal	Petal	New Flower

The genetic model predicts that the A organ identity genes will be expressed in the tissues from which sepals and petals are derived. Although *APETALA2* is classified as an A function gene (Jofuku *et al.,* 1994), mutants of this genes also affect stamen and carpel development. This gene is shown to be expressed in all four whorls. The expression of the other A gene, *APETALA1* appears to be restricted to whorls 1 and 2, which is consistent with mutant patterns. The genetic model has also suggested that C organ identity genes are negatively regulated by the expression of A genes. This would lead to a hypothesis stating that the expression of C genes such as *AGAMOUS* would not appear in cells giving rise A function organs. *In situ* hybridizations with the *AGAMOUS* genes demonstrated that early expression of this gene is restricted to whorls 3 and 4.

B gene function genes have been suggested to control petal and stamen function (whorl 2 and 3, respectively) (Goto and Meyerowitz, 1994). Both *APETALA3* and *PISTILLATA* are found to be expressed in the appropriate whorls. *PISTILLATA* though is also found to be expressed in whorl 4 that gives rise to carpels.

Later in development, the expression of *AGAMOUS* is restricted to specific cell types. In stamens, the gene is not found in any cells that give rise to the pollen, nor it is expressed in the pollen grain itself. And in the carpel cells, *AGAMOUS* is only expressed in the outer cells of the ovule.

Arabidopsis	**Snapdragon (*Antirrhinum majus*)**
LEAFY	FLORICAULA
APETALA1	***SQUAMOSA***
APETALA 3	***DEFICIENS***
PISTILLATA	***GLOBOSA***
AGAMOUS	***PLENIFLORA/FARINELLI***

Several conclusions can be drawn regarding the functions of these genes by studying single and double mutants. Because a mutation of an A function gene results in the expression of organ phenotypes controlled by C function genes, it appears that A gene functions repress the expression of the C gene functions in the whorls giving rise to sepals and petals. Likewise, the appearance of the petal in the third whorl of C gene mutants, suggest that C genes repress the activities of A genes in the organs that they control. These conclusions are based on single mutants. For example, *APETALA2* A function mutants develop C function organs, carpels and stamens, in the first two whorls, respectively. A and C double mutant would not be expected to have any functions exclusively controlled by the A and C function genes. And indeed this is what was seen when the *APETALA2*/*AGAMOUS* double mutant was developed. The first whorl develops as a leaf and the second whorl has stamen-like petals. This second whorl phenotype of this mutant is the result of the activities of the B gene functions. A, B and C

function triple mutant would have no genes functioning that determine normal floral organ development. As expected, the triple mutants lack any floral organs, and the flower essentially consists of leaves developing form each of the whorls.

In *Arabidopsis*, A-function (specification of sepal and petal identity) is attributed to two unrelated genes, *APETALA1* (*AP1*) and *APETALA2* (*AP2*). An examination of the available information regarding orthologues and paralogues of these genes in other species shows that although some are required for sepal identity, none is required for both sepal and petal identity. Combined with phylogenetic analyses that show gene duplication and loss specific to Brassicaceae, this suggests that the two-whorl phenotype attributed to loss of A-function in *Arabidopsis* may be unique to Brassicaceae. Furthermore, all genes that are required for proper sepal identity, including *AP1* and *AP2*, are also implicated in floral meristem identity, suggesting that these two functions may not be separable. Available data are all consistent with a previous *Antirrhinum*-based model for floral organ identity that required only two gene functions. The loss of sepal identity seen in some *AP1*- and *AP2*-lineage mutants can be explained as loss of floral meristem identity; the available evidence suggests that a discrete perianth identity gene function is not required (Amy Litt, 2007).

The ABCDE Model

The ABC model (Coen and Meyerowitz, 1991) proposes that class A genes specify sepals and, together with class B genes, specify petals. Class B and C genes specify stamens, and C alone determines the identity of carpels. The model was later extended, both in *Arabidopsis* and Petunia, with the class D genes, necessary for ovule development and with class E genes (*SEP* genes), indispensable for the determination of petal, stamen, carpel, and ovule identity.

In *Arabidopsis* the class D gene is *SEEDSTICK (STK)*, which is like *FBP7* and *FBP11* specifically expressed in ovules (Pinyopich *et al*., 2003; Favaro *et al*., 2003). In *Arabidopsis* class E genes or *SEPALLATA (SEP)* genes consist of four members, *SEP1, SEP2, SEP3,* and *SEP4*, encoding MADS-box factors that show partial redundant functions in floral organ identity determination. The triple knock-out *SEP1, SEP2* and *SEP3* has indeterminate flowers with petals, stamens, and carpels homeotically transformed into sepals. Class B and C expression was not altered in the sep triple mutant which shows that SEP genes do not act down-stream of B and C genes and that they are not required for the activation of these genes (Pelaz *et al*., 2000). Recently, a *SEP1 SEP2 SEP3 SEP4* quadruple mutant was described in which all floral organs were transformed into organs similar to leaves (Ditta *et al*., 2004). These results show that the SEP genes are necessary for the function of class A, B, and C genes since the quadruple *SEP*1 *SEP*2, *SEP*3, *SEP*4 mutant phenocopies the abc triple mutant. The majority of the class A, B, C, D, and E homeotic genes belong to the MADS box transcription factor family and they are characterized by a typical modular structure

The MADS box (M), a highly conserved DNA-binding domain, is located at the N terminus. This domain is followed by a less conserved I region (I) and by the moderately conserved K box (K; keratin-like coiled-coil structure), both important for dimerization. The C terminus (C) is the most variable part and is involved in ternary complex formation and transcriptional activation (Egea-Cortines *et al*., 1999). The ABC model has been shown to be widely applicable in dicot species (Pnueli *et al*., 1994; Kater *et al*., 1998; Berbel *et al*., 2001; Kater *et al*., 2001; Immink *et al*., 2003).

Furthermore, MADS box genes that are homologous to the dicot (*Arabidopsis thaliana* and *Antirrhinum majus*) ABC genes have also been identified in monocot species including rice, maize, barley and orchids (Mena *et al*., 1995; Ambrose *et al*., 2000; Jeon *et al*., 2000a; Lim *et al*., 2000; Schmitz *et al*., 2000; Fornara *et al*., 2003; Xiao *et al*., 2003).

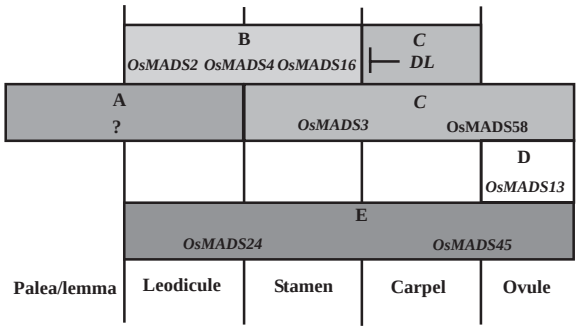
MADS-Box genes

The coding regions of most of the floral regulatory genes share nucleotide and amino acid sequence similarities with the DNA binding and dimerization domains of two previously identified transcription factors: *Mini Chromosome Maintenance* gene product of yeast (*MCM1*) and mammalian *Serum Response Factor* (*SRF*). This region of homology has been termed MADS box that stands for *MCM1*, *AG*, *DEFA*, *SRF* genes that are important developmental regulators found first in animals and yeast, and subsequently in plants. Each of these genes contains a 56 amino acid that is necessary for the protein to bind to DNA. This sequence is located near amino terminal end of the protein. The plant MADS domain containing proteins have additional with moderate sequence similarity. One such is the K domain, with predicted structural similarity to the intermediate filament protein Keratin. The predicted role for the K domain is to mediate protein-protein interactions. In addition, plant

MADS domain proteins have two divergent regions, the I region (for Inter domain) that lies between the MADS and K domain, and the C region that contributes the most C-terminal portion of the protein.

ABCDE Model for Rice

In recent years the ABC model has been extended with two classes of genes, named class D and E genes. Class D genes control ovule identity and were first identified in Petunia were they have been termed *FLORAL BINDING PROTEIN 7 (FBP7)* and *FBP11* (Angenent and Colombo, 1996). These two genes are both necessary and sufficient to determine ovule identity in Petunia flowers, since co suppression of both genes caused loss of ovule identity whereas ectopic expression resulted in ectopic ovule formation on sepals and petals.



ABCDE model for rice (Martin *et al.*, 2006)

Class A genes

In the rice genome there are four genes that encode such *FUL*-like proteins, *OsMADS14*, *OsMADS15*, *OsMADS18*, and *OsMADS20*. *OsMADS14* (Moon *et al*., 1999) represents most likely the genes named *FDRMADS6* (Jia *et al*., 2000) and *RAP1B* (Kyozuka *et al*.,2000). Its expression is restricted to inflorescences and developing kernels.

Class B genes

GLO- (*Os-MADS2* and *OsMADS4*) and *DEF*-like (*OsMADS16*) genes have been identified in rice (Chung *et al*., 1995; Moon *et al*., 1999). *OsMADS16* is also named *SUPERWOMAN1 (SPW1)* and is specifically expressed in lodicules and stamens (Moon *et al*., 1999; Nagasawa *et al*., 2003). Recessive mutations in SPW1 transform stamens into carpels and lodicules into palea- or outer bract like organs.

Class C genes

In *Arabidopsis* there is one typical class C gene which is *AGAMOUS (AG)*. An *AG* flower develops petals instead of stamens and in the centre of the flower a new *AG* flower develops instead of the pistil which shows that the *AG* gene, as proposed by the ABC model, is necessary for specifying stamen and carpel identity and for floral determinacy.

In the rice genome four *AG*-like genes have been found, termed *OsMADS3* (Kang *et al*., 1995), *OsMADS58* (Yamaguchi *et al*., 2006), *OsMADS13* (Lopez-Dee *et al*., 1999), and *OsMADS21* (Lee *et al*., 2003). The expression of *OsMADS3* and *OsMADS*58 is restricted to stamens and carpels which suggest that they might have functions similar to class C genes (Kang *et al*., 1995; Kyozuka *et al*., 2000; Yamaguchi *et al*., 2006). However, the temporal expression of these two genes is quite different. *OsMADS*3 is mainly expressed in stamen, carpel, and ovule primordia, but its expression is excluded when these organs differentiate. In rice plants in which *OsMADS*3 was silenced by an antisense approach, partial transformations of stamens into lodicules were observed, while carpels were replaced by abnormal flowers with undifferentiated stamens and carpels (Kang *et al*., 1998).

Class D genes

In rice, based on phylogenetic reconstruction, two AG like genes belonging to the class D gene lineage have been identified, namely *OsMADS*13 (Lopez-Dee *et al*., 1999) and *OsMADS*21 (Lee *et al*., 2003). *OsMADS*13 is specifically expressed in the ovule with an expression pattern very similar to *STK (Arabidopsis*). RT-PCR analysis showed that *OsMADS*21 is only expressed in developing seeds (Lee *et al*., 2003). However, *in situ* analysis suggests that *OsMADS*21 is also weakly expressed in the carpel wall and ovules.

Class E genes

In line with the ABC nomenclature this new class of floral-organ-identity genes were termed E-function genes (Theissen, 2001). Phylogenetic analysis clusters these class E genes together in the so-called *SEP* clade (previously called AGL2 clade). In rice five *SEP*-like genes have been identified, which are *OsMADS*1, *OsMADS*5, *OsMADS*24 (allelic to *OsMADS*8), *OsMADS*34 (allelic to *OsMADS*19), and *OsMADS*45 (allelic to *OsMADS*7) (Kang and An, 1997; Kang *et al*., 1997; Pelucchi *et al*., 2002; Malcomber and Kellogg, 2004).

Of these five *SEP*-like genes *OsMADS*1 is the one that has been studied in most detail (Jeon *et al*., 2000; Lim *et al*., 2000; Prasad *et al*., 2005; Malcomber and

Kellogg, 2004; Agrawal *et al*., 2005; Chen *et al*., 2006). Mutations at amino acid positions 24 and 27 in the MADS domain of *OsMADS*1 were found to cause the leafy hull sterile 1 (lhs1) mutant phenotype in rice (Jeon *et al*., 2000).

Rice, a model cereal

Rice (*Oryza sativa* L.) is the cereal that has been selected to be sequenced as a priority. It has the smallest genome of all the cereals: 430 million nucleotides. The corn genome is five times larger, and that of wheat, 40 times larger. However, preliminary comparisons between different cereal genomes revealed large blocks of homologous genes whose order is relatively conserved. This phenomenon, which is known as synteny, makes rice a good entry point for characterizing the genes of other cereals, and associating them with various agronomic traits. Furthermore, rice can serve as a model genome for one of the two main groups of flowering plants, the monocotyledons, in the same way as *Arabidopsis thaliana* is the model for the other group, the dicotyledons.

Rice reproductive development

Rice reproductive development exhibits several features that are not observed in *Arabidopsis.* One big difference between rice and *Arabidopsis* is found in their photoperiodic responses. Transition to the reproductive phase is the induced under short day (SD) conditions in rice, whereas it is enhanced under long day (LD) in *Arabidopsis*. Differences in their inflorescence morphologies are also of great interest.

Rice inflorescence development

The grass inflorescence, called either spike or panicle, according to its branching pattern, is formed from the original apical meristem at the top of the plant. Rice inflorescence is a panicle because it is highly branched. In grass species, flowers, called florets, are produced into a group that is enclosed by a pair of small leaf like structures called glumes. Only a single floret is formed in rice or a barley spikelet.

The genetics of inflorescence and flower development in maize and other grasses has been recently reviewed by other authors (McSteen *et al*., 2000; Bommert *et al*., 2005).The basic unit of grass inflorescence architecture is the spikelet, a compact axillary branch that consists of two bracts subtending one to several reduced flowers (Clifford, 1987). Maize is a monoecious plant that produces male flowers on a terminal tassel and female flowers on lateral ears, which arise in the axils of vegetative leaves. The tassel initiates several long, indeterminate branches at the base while the ear consists of a single spike with no long branches. The tassel's main spike and branches, and the entire ear, produce short branches (spikelet pairs) that bear two spikelets. The branches and spikelet pairs arise in the axils of small, undeveloped leaves referred to as bracts. In maize, spikelet and spikelet pair meristems are considered determinate because they produce a defined number of organs (Vollbrecht *et al*., 2005).

Maize and rice appear to have conserved mechanisms of meristem maintenance and organ identity. Other pathways, such as sex determination, are likely to be found only in maize with its separate male and female flowers. A rich genetic history has resulted in a large collection of maize mutants. The advent of genomic tools and synteny across the grasses now permits the isolation of the genes behind inflorescence architecture and the ability to compare function across the Angiosperms (Esteban Bortiri and Sarah Hake, 2007)

Genes involved in floral transition in rice

Recently, several genes involved in the determination of flowering time have been isolated from rice. *Hd1* and *Hd6* correspond to quantitative trait loci (QTLs) controlling the heading date of rice (Yano *et al.,* 2000). *Hd1* encodes a homolog of *CONSTANS (CO)*, which functions on the photoperiodic control of flowering in *Arabidopsis*. *Hd1* might function in the promotion of flowering under SD conditions and inhibition under LD conditions. *Hd6* is a weaker QTL than *Hd1*. It encodes an á sub unit of casein kinase 2 *(CK2)* which is important for photoperiodism. In the *photoperiodic sensitivity5* (*se5*) mutant of rice, photoperiod sensitivity is completely lost, which results in very early flowering under both SD and LD (Yokoo and Okuno, 1993). Photoperiod response is not greatly altered in the loss of function mutants of *HY1*, a putative *se5* ortholog in *Arabidopsis*.

Meristem identity genes in rice

Putative orthologs of dicot floral meristem identity genes have been isolated from several grass species. Two MADS box genes *RAP1A* and *RAP2B* were isolated (Kyozuka *et al.,* 2000). They are having extensive sequence similarities to *AP1*. The *RAP1A* was specifically expressed in very young floral meristems and outer whorls of the young rice floret, as *AP1* in *Arabidopsis*. On the other hand, the expression pattern of *RFL*, rice *LFY* was distinct from that of *LFY*. *RFL* expression started in the inflorescence meristem from a very early stage of rice panicle development, whereas *LFY* is expressed in floral meristems. Ectopic expression of *LFY* in *Arabidopsis* confers a striking change in the inflorescence form from

indeterminate to determinate with production of a terminal flower in addition to an extreme early flowering phenotype (Weigel and Nilsson, 1995). In contrast, ectopic expression of a same gene in rice did not cause a dramatic change in panicle morphology but only conferred weak early flowering phenotypes. *LFY* homology was isolated from rice and rye grass. *RFL* of rice is divergent from *Arabidopsis LFY* gene.

In rice *FZP2* (Himi *et al.*, 2001) mutant plants, spikelets are led to the indeterminate generation of meristems to inflorescence shoots, as shown in the *lfy* mutants as well as mutants of *LFY* orthologs in other dicot species.

Determinacy of meristems in rice

The determinacy of the meristem is an important feature for the establishment of the inflorescence structure. When the growth of the main axis ends in a flower, the inflorescence is classified as determinate, whereas in an indeterminate inflorescence, the main axis continues to produce lateral structures without turning into a terminal flower. The primary and secondary inflorescences of *Arabidopsis* do not produce a terminal flower. Thus, *Arabidopsis* inflorescences are indeterminate. This is also the case in maize inflorescence. Rice has determinate inflorescence.

There are several possibilities to explain the mechanism that controls the production of the terminal flower in the rice panicle.

1. *TFL1* functions may be lacking
2. *TFL1* functions are present in rice; however owing to the divergence in their expression pattern, they do not prevent the expression of floral meristem identity genes at the centre of the shoot apex, allowing the formation of a terminal flower.
3. Over expression of rice *TFL1* homologs in transgenic rice plants causes delay in the transition from the vegetative to the reproductive phase and from branch shoot to floral meristems (Ratcliffe *et al.*, 1998).

LFY homologs in various genera

LFY	-	*Arabidopsis*
RFL	-	Rice
LOLIUM LFY	-	Lolium
PRFLL	-	Pinus
NEEDY	-	Pinus
UNI	-	Pisum
NFL1, NFL2	-	Nicotiana
ALF	-	Petunia
FLO	-	*Antirrhinum*

The determinacy of spkelet meristem is controlled by *IDS* gene in maize. In the *indeterminate spikelet* (*IDS*) mutant, the spikelet meristem acquires a partial indeterminacy, leading to the production of additional florets in a single spikelet instead of the two florets observed in normal maize spikelets. The *IDS1* gene was cloned and shown to have a strong homology to *APETALA2* although it is still unclear whether it is the closest homology of *APETALA2* in maize (Jofuku *et al.*, 1994).

The determinacy of floral meristem of *Arabidopsis* is controlled by *AGAMOUS* (*AG*). Rice and maize orthologs have been isolated and their loss of function phenotypes reported. Reduction of floral meristem determinacy was observed in maize and rice plants, in which *AG* orthologs, *ZAG1* and *OsMADS3*, respectively was decreased (Mena *et al.*, 1996).

Genes Involved in Floral Organ Development

Grass species have flowers with highly derived structures. Basic mechanisms of flower development are probably conserved between grasses and dicots; therefore ABC Model can be extended to grass species.

The expression pattern of putative rice and maize class B and class C genes strongly suggest the applicability of the ABC model to grass species. In the mutant of maize *AP3* ortholog, *Silky1* (*sil1*), stamens and lodicules are homeotically transformed into carpels and palea/lemma like structures respectively (Ambrose *et al.*, 2000). Reduction of rice PI ortholog (*OsMADS4*) function by antisense methods also resulted in similar phenotype. These findings imply that there is a homologous relationship between lodicules and petals and that the B function is conserved in grass flowers.

In rice, homeotic conversion from lodicules to stamens is caused by the ectopic expression of *OsMADS3*, a rice *AG* ortholog, by a strong *Actin1* promoter. This added further strong evidence for the interpretation of the lodicule's identity. However, in contrast to the progress made toward understanding the identity of lodicules, the nature of lemma and palea is still unclear. The expression pattern of *RAP1A* and the phenotype of *sil1* mutant suggest that the palea/lemma are probably the equivalent of the sepals. To confirm this assumption, analysis of loss of function mutants of the grass class A genes is necessary.

MADS Box genes in rice

MADS box genes	Family
Os MADS1, Os MADS5	*AGL2*
Os MADS7 and *Os MADS8*	
Os MADS2 and *Os MADS4*	*GLO*
Os MADS3	*AGAMOUS*

OsMADS1

OsMADS1 is also involved in the determinacy of a floret. It is most similar to *AGL2*, *AGL4*, *AP1* and *SQUA*. The *OsMADS1* gene is actively expressed at the young inflorescence stage, and the expression continues into the early and vacuolated pollen stage (Chung *et al.,* 1994) and it is initially expressed in young flower primordia but becomes more localized in the in palea, lemma and ovary at later developmental stages. Vegetative tissues do not show any expression of the gene. Ectopic expression of the *OsMADS1* in homologous and heterologous plants results in early flowering. Therefore it is likely that the rice *OsMADS1* product regulates expression of genes involved in the induction of flowers.

Its expression pattern is most similar to *AP1* and *SQUA*. Southern blot analysis revealed that there are at least ten genes which share a significant homology with *OsMADS1*. Over expression of *OsMADS1* results in an extremely early flowering phenotype. Although *RAP1A* and *RAP1B* are closer to *AP1* than *OsMADS1* on the basis of their sequence, *OsMADS1* seems to have a closer function than *AP1* with respect to its function. The *OsMADS1* amino acid sequences shows 56.2% identity to *AGL2* and 44.4% identity to *AP1* (Chung *et al.*, 1994).

OsMADS3

OsMADS3 is an *AG* homologue in both *AG* gene from *Arabidopsis* and *PL* gene from *Antirrhinum* share similarities in amino acid sequences, expression patterns and effects of ectopic expression. The transgenic plants expressing the antisense *OsMADS3* transcript produced abnormal flowers and sterile seeds. Flowers of these plants showed homeotic alterations in their carpels and stamens. In the fourth whorl carpel is replaced by several abnormal flowers with undifferentiated stamens and carpels. The third whorl stamen was changed into lodicule like structure. Such alterations in the inner two whorls of the flower are similar to the phenotypes of *Arabidopsis AG* mutants and *Antirrhinum PLE* mutants (Kang *et al*., 1998).

OsMADS4

Antisense expression of the *OsMADS4* gene caused the lodicules to change so that they resembled the palea/lemma like organs and stamens changed to carpel like organs. In dicots, an alteration of petals towards sepal is a typical phenotype in the mutants that have lost the class B organ identity genes. Therefore, the observations are consistent with the hypothesis that the palea/lemma and sepals have a common ancestry (Krizek and Meyerowitz, 1996).

The *OsMADS4* protein is most homologous to *GLO* (54%) and *PI* (51%) and the homology was much lower with *AP3* (35%) and *DEF* (32%). However, the expression pattern of *OsMADS4* was more similar to *DEF*, since the *OsMADS4* transcript is present in the fourth whorl.

OsMADS14* and *-15

Two MADS box *OsMADS14* and *-15* were highly homologous to the maize MADS box gene *ZAP1* which is an orthologue of the floral homeotic gene *APETALA1* (*AP1*). These were identified by their identification with *OsMADS1* in the yeast two hybrid system. It was supposed that these two proteins were major *OsMADS1*-binding proteins expressed at the early stage of rice flower development, since only these genes were repeatedly found during the two hybrid screening process using a cDNA library generated from mRNAs of young rice flowers (Lim *et al.*, 2000). The *OsMADS15* protein shows high similarity to *OsMADS14* and *ZAP1* (Mena *et al.*, 1995). The amino acid sequence comparison of *OsMADS15* revealed 66% sequence identity and 67% sequence homology to *ZAP1*.

OsMADS16

OsMADS16 gene was isolated by yeast two-hybrid screening using *OsMADS4* as bait. The protein is most homologous to various MADS genes of *AP3* family. In mature floral organs, *OsMADS16* was expressed in lodicules and stamens, whereas *OsMADS4* was in lodicules, stamens, and carpels. These organ specific expression patterns of *OsMADS4* and *OsMADS16* are identical to those *PI* and *AP3*, respectively, indicating functional similarity between these MADS genes (Goto and Meyerowitz, 1994).

Heading date is an important agronomic trait of cereal crops such as rice and early heading varieties are required for certain regions in which rice is cultivated. Constitutive expression of *LEAFY* from the cauliflower mosaic virus 35S promoter caused early flowering in transgenic rice, with a heading date that was 26-34 days earlier than that of wild type plants. Early flowering was accompanied by a small yield penalty and some panicle abnormality (He *et al.,* 2000).

OsMADS18

OsMADS18 from rice *(Oryza sativa)* belongs to the phylogenetically defined *AP1/SQUA* group. The MADS box genes of this group have functions in plant

development, like controlling the transition from vegetative to reproductive growth, determination of floral organ identity, and regulation of fruit maturation. Fabio Fornara *et al.*, 2004 reported the functional analysis of *OsMADS18*. This rice MADS box gene is widely expressed with its transcripts accumulated to higher levels in meristems. Overexpression of *OsMADS18* in rice induced early flowering, and detailed histological analysis revealed that the formation of axillary shoot meristems was accelerated. Silencing of *OsMADS18* using an RNA interference approach did not result in any visible phenotypic alteration, indicating that *OsMADS18* is probably redundant with other MADS box transcription factors. Surprisingly, overexpression of *OsMADS18* in *Arabidopsis* caused a phenotype closely resembling the ap1 mutant. Yeast two-hybrid experiments showed that some of the natural partners of AP1 interact with *OsMADS18*, suggesting that the *OsMADS18* overexpression phenotype in *Arabidopsis* is likely to be due to the subtraction of AP1 partners from active transcription complexes. Thus, when compared to *AP1*, *OsMADS18* during evolution seems to have conserved the mechanistic properties of protein-protein interactions, although it cannot complement the *AP1* function.

Wheat

The genetic control of floral transition or heading time in wheat (*Triticum aestivum)*, is determined by three characters, vernalization requirement, photoperiodic sensitivity and narrow-sense earliness (earliness per se), that is the autonomous promoting pathway (reviewed in Worland and Snape, 2001). Vernalization requirement refers to the sensitivity of the plant to cold treatment for accelerating spike primordium formation, and vernalization insensitivity is controlled mainly by three major genes, *Vrn-A1*, *Vrn-B1* and *Vrn-D1*, earlier designated *Vrn1*, *Vrn2* and *Vrn3* (Mcintosh *et al*. 1998), each having two or more allelic forms (Puggsley 1971; Snape *et al.* 1976) located on chromosomes 5A, 5B and 5D, respectively (reviewed in Flood and Halloran, 1986). The photoperiodic (long-day) response is determined by the dominant genes, *Ppd-A1, Ppd-B1 and Ppd-D1* (formerly *Ppd3, Ppd2 and Ppd1)* that control sensitivity to photoperiod. These genes are located on chromosomes 2A, 2B and 2D, respectively (reviewed in Laurie, 1997).

Wheat *APETALA1* homolog *WAP1* identified on the group 5 homoeologous chromosomes is a promising candidate of the *Vrn* genes. The *vrn* and *ppd* genes controlling flowering response provide an example of genes that can be manipulated to improve adaptation (Halloran, 1975; Pirasteh & Welsh, 1980;). Narrow-sense earliness or earliness per se is the earliness of fully vernalized plants grown under long-day conditions, and involves polygenes with minor effects (G. Ortiz Ferrara, 1998; Koji Murai *et al*., 2005).

Wheat *APETALA1* (*WAP1*) is a key gene in the regulatory pathway that controls phase transition from vegetative to reproductive growth in common wheat. *WAP1* is an ortholog of the *VRN1* gene that is responsible for vernalization insensitivity in einkorn wheat (*Triticum monococcum*) mutant, *maintained vegetative phase (mvp)*. The *mvp* mutant resulted from deletion of the *VRN1* coding promoter regions, demonstrated that *WAP1/VRN1* is an indispensable gene for phase transition in wheat. Expression analysis of flowering related genes in *mvp* plants indicated that wheat *GIGANTIA (GI),CONSTANS (CO)* and *SUPRESSOR OF OVER EXPRESSION OF CONSTANS 1 (SOC1)* genes act in a different pathway to *WAP1/VRN1* (Naoki Shitsukawa *et al*, 2007)

The molecular and genetic bases of the interaction between environmental factors and the floral transition in winter cereals are still unknown. However, the recent identification of the wheat, TaVRT-1 gene provides an opportunity to decipher the molecular basis of the flowering-time regulation in cereals. Kane *et al*, 2005 described the characterization of another gene, named TaVRT-2, possibly involved in the flowering pathway in wheat. Molecular and phylogenetic analyses indicated that the gene encodes a member of the MADS-box transcription factor family that belongs to a clade responsible for flowering.

Maize

During maize ear and tassel development, male and female organs are initiated, but stamen in ear spikelets and the gynoeceum in tassel spikelet do not reach maturity. Some maize MADS box genes have been isolated and exclusive expression in developing ears has been shown for *ZAG2*, where the expression is largely restricted to developing carpels (Schmidt *et al.*, 1993). Other maize MADS box genes are expressed in developing male and female inflorescences.

Two novel maize MADS box cDNAs, *ZmMADS1*and *ZmMADS3*, were isolated after screening cDNA libraries of maize egg cells (EC) and mature pollen. Comparisons of *ZmMADS1*and *ZmMADS3* protein sequences with the other MADS box proteins revealed that *ZmMADS1* can

be classified as a member of the *TM3* subfamily of the MADS box proteins, whereas *ZmMADS3* belongs to the *SQUMOSA* subfamily. *ZmMADS3* exhibits 95% overall AA identity to the maize MADS box protein *ZAP1* (Mena *et al.*, 1995). Transcripts of both the genes *ZmMADS1*and *ZmMADS3* are detectable in egg cells and in *in vivo* zygotes of maize. *ZmMADS1* is additionally expressed in synergids and in central antipodal cells. During early somatic embryogenesis, *ZmMADS1*expression is restricted to cells with the capacity to form somatic embryos and to globular embryos at later stages. During flower development *ZmMADS1*and *ZmMADS3* are co-expressed in all ear spikelet organ primordia at intermediate stages. Among vegetative tissues, *ZmMADS3* is expressed in stem nodes and displays a gradient with highest expression in the uppermost node (Heuer *et al.*, 2001).

The *ZAP1* gene, an *AP1* homologue in maize, was isolated but its function has not been well characterized (Mena *et al.*, 1995). A transposan induced mutation *ZAG1*, the maize *AG* homologue, did not greatly affect the identity of reproductive organs. However, a loss of function experiment showed that the *ZAG1* mutation generated indeterminate floral meristems instead of a carpel in the center of the ear. Northern blot experiments from male and female inflorescences of maize revealed *ZAP1* expression only in non reproductive parts of the florets (Mena *et al.*, 1995).

To elucidate the molecular determinants involved in the process of floral transition, Olga N. Danilevskaya *et al.*, 2008 performed genome-wide RNA expression profiling on maize shoot apices at vegetative and early reproductive stages using massively parallel signature sequencing technology. Profiling revealed two closely related MADS-box genes, *ZMM4* and *ZMM15*, which were significantly up regulated in post transitional apices. *ZMM4* and *ZMM15* are linked to other MADS-box genes and form duplicate gene pairs *ZMM4-ZMM24* and *ZMM15-ZMM31* that are syntenic to the wheat *vernalization1* (*vrn1*) locus that controls the floral transition in winter wheat (*Triticum monococcum*) varieties and similar loci in other cereals in response to a cold treatment (Yan *et al.*, 2005, Messing and Dooner, 2006 and Petersen *et al.*, 2006).

Analyses of temporal and spatial expression patterns indicated that the duplicated pairs *ZMM4-ZMM24* and *ZMM15-ZMM31* are coordinately activated after the floral transition in early developing inflorescences. More detailed analyses revealed *ZMM4* expression initiates in leaf primordial of vegetative shoot apices and later increases within elongating meristems acquiring inflorescence identity. Expression analysis in late flowering mutants positioned all four genes downstream of the floral activators *indeterminate1* (*id1*) and *delayed flowering1* (*dlf1*). Over expression of *ZMM4* leads to early flowering in transgenic maize and suppresses the late flowering phenotype of both the *id1* and *dlf1* mutations. The results suggest that, *ZMM4* may play roles in both floral induction and inflorescence development.

Barley

Six barley MADS box cDNA clones, *BM1, BM3, BM5, BM7, BM8* and *BM9* were isolated and characterized. The derived protein sequences reflect the typical modular structure common to most plant MADS box proteins. Phylogenetic analysis on the basis of conserved MADS domains classified the barley MADS box genes into three subfamilies (Schmitz *et al*., 2000). *BM3*, *BM5* and *BM8* are members of the *SQUA* subgroup. Highest sequence homologies within this group were found with *BpMADS5* from *Betula pendula* which shares 70% homology to *BM3*. *TaMADS11* from *Triticum aestivum* (Murai *et al.*, 1998) is 96% homologus to *BM5*, and *ZAP1* from *Zea mays* shows 92% homology to *BM8*. The MADS box gene *LtMADS2* from *Lolium temulentum* shows 95% homology to *BM8*.

BM7 and *BM9* are members of *AGL2* subfamily. Database comparison of the predicted BM7 protein showed 84% homology to *OsMADS1* from *Oryza sativa*, while *BM9* shares 91% homology to the rice gene *OsMADS5*. *BM1* is homologous to genes that form the orphan gene group. Database comparison revealed that the deduced *BM1* protein is most similar to *StMADS16* from *Solanum tuberosum* (Carmona *et al.*, 1998) with 65% overall homology at the protein level.

Expression analysis of the barley MADS box genes revealed expression patterns that are not characteristic of the barley MADS box genes of the *SQUA* subgroup, while expression of *BM7* and *BM9* was largely as expected for *AGL2* subgroup. *BM1* is mainly expressed in vegetative tissues and its primary transcript undergoes alternative splicing such that the corresponding mRNAs differ by two codons. The genes *BM1*, *BM3* and *BM8* were mapped by analysis of single nucleotide polymorphism onto barley chromosomes 4, 2 and 7, respectively. Previously, *BM7* was mapped on chromosome 1 in the vicinity of *nudum*,

a locus 3 cM distant from the *multiovary* mutant (Tazhin, 1980)

BM1 expression resembles that of *StMADS11* with the exception that *BM1* is not exclusively expressed in vegetative tissues, but also in young inflorescences. *BM1* was detected in a single layer present in the first node, which could suggest a role in the regulation of vegetative stem growth. The barley genes *BM3*, *BM5* and *BM8* of the *SQUA* subgroup are abundantly expressed in all organ primordial and the vascular tissue of the barley floret throughout inflorescence development. In contrast to the *BM8*, the transcripts of *BM3* and *BM5* were additionally detected in vegetative tissues (nodes, leaves). In this respect, *BM3* and *BM5* resemble that of *TaMADS11* from wheat (Murai *et al.*, 1998). The observed expression pattern of the barley *AGL2* like genes *BM7* and *BM9* is identical to the homologous rice genes *OsMADS1* and *OsMADS45*, respectively, with the expression that *BM7* transcripts were found additionally in lodicules of barley florets (Greco *et al.*, 1997).

Short Vegetative Phase *(SVP)*-Like MADS-Box Genes Inhibit Floral Meristem Identity

In *Arabidopsis* (*Arabidopsis* thaliana), the Short Vegetative Phase (SVP) gene encodes a MADS-box transcription factor that delays the floral transition (Hartmann *et al*., 2000). Mutations that disrupt SVP cause early flowering (Hartmann *et al*., 2000), whereas ectopic expression of SVP results in late flowering. Ectopic expression of SVP also inhibits floral meristem identity, causing floral abnormalities such as the conversion of sepals and petals to leaf-like structures (Brill and Watson, 2004; Masiero *et al*., 2004) and causing inflorescence-like structures to develop within flowers (Brill and Watson, 2004). The development of inflorescences within flowers indicates that meristematic cells within the flower have lost floral identity and have formed an inflorescence instead of floral organs, a phenomenon known as floral reversion (Tooke *et al*., 2005). Presumably, ectopic expression of SVP causes floral reversion by interfering with a mechanism that maintains floral meristem identity.

The *Arabidopsis* gene, *AGAMOUS-LIKE 24 (AGL24)*, is closely related to *SVP* (Yu *et al*., 2002; Michaels *et al*., 2003). Unlike *SVP, AGL24* promotes the floral transition. Mutations that disrupt *AGL24* cause late flowering, whereas over expression of *AGL24* accelerates flowering (Yu *et al*., 2002; Michaels *et al*., 2003). *AGL24* is expressed during vegetative development and is induced by treatments that accelerate floral transition, such as vernalization (prolonged exposure to low temperatures), long days, or the application of gibberellins (Yu *et al*., 2002; Michaels *et al*., 2003). These data suggest that *AGL24* acts to promote floral transition in response to vernalization and long-day conditions (Yu *et al*., 2002; Michaels *et al*., 2003). Although *AGL24* has the opposite effect on flowering time compared to *SVP*, plants that ectopically express *AGL24* exhibit floral abnormalities similar to those caused by ectopic expression of *SVP*, and ectopic expression of *AGL24* also causes floral reversion. Thus, *AGL24* promotes the floral transition but inhibits floral meristem identity. It has been suggested that *AGL24* promotes inflorescence meristem identity (Yu *et al*., 2004).

Analysis of the functions of *Short Vegetative Phase (SVP)*-like MADS-box genes in barley (*Hordeum vulgare*) indicated a role in determining meristem identity. Three *SVP*-like genes are expressed in vegetative tissues of barley: Barley *MADS1 (BM1), BM10*, and Vegetative to Reproductive Transition gene 2. These genes are induced by cold but are repressed during floral development. Ectopic expression of *BM1* inhibited spike development and caused floral reversion in barley, with florets at the base of the spike replaced by tillers. Head emergence was delayed in plants that ectopically express *BM1*, primarily by delayed development after the floral transition, but expression levels of the barley *VRN1* gene (*HvVRN1*) were not affected. Ectopic expression of *BM10* inhibited spike development and caused partial floral reversion, where florets at the base of the spike were replaced by inflorescence-like structures, but did not affect heading date. Floral reversion occurred more frequently when *BM1* and *BM10* ectopic expression lines were grown in short-day conditions. *BM1* and *BM10* also inhibited floral development and caused floral reversion when expressed in *Arabidopsis* (*Arabidopsis* thaliana). SVP-like genes function to suppress floral meristem identity in winter cereals (Ben Trevaskis *et al*., 2007)

MADS box genes control vernalization-induced flowering in cereals

Many plants from temperate regions are induced to flower by an extended exposure to low temperature: vernalization. In winter cereal crops, such as wheat and barley, plant breeders have selected for variation in vernalization responsiveness to produce cultivars suited to plantings in different climatic zones. Winter cultivars are sown in autumn, vernalized by the low temperatures of winter, and subsequently flower and develop grain in

spring. Spring cultivars do not require vernalization and usually are planted in the late winter period. The genetics of the vernalization response have been studied in a number of cereals.

In hexaploid bread wheat, where the effects of recessive traits are masked by the redundancy resulting from the three genomes, the dominant *Vrn-1* gene has been found to be the major determinant of vernalization responsiveness. *Vrn-1* is located on chromosome 5 in each of the A, B, and D genomes of wheat. Winter wheats carry only winter *Vrn-1*alleles (*vrn-A1_vrn-B1_vrn-D1*), and without vernalization are late flowering. Spring alleles of *Vrn-1* are dominant and reduce the requirement for vernalization. Spring alleles of the *Vrn-1* gene on the A genome, *Vrn-A1*, have the strongest effect on flowering time, and plants with the *Vrn-A1* spring allele do not require any vernalization (Pugsley, 1971). The effects of spring alleles of the *Vrn-1* genes from the B and D genomes are weaker. Plants that carry *Vrn-B1* or *Vrn-D1* spring alleles (in the absence of a *Vrn-A1*spring allele) flower earlier than winter wheats but still show some acceleration in flowering time when vernalized. Such plants are classed as semispring wheats.

By comparing expression levels of MADS box transcription factor genes between near-isogenic winter and spring lines of bread wheat, *Triticum aestivum*, (Ben Trevaskis *et al.*, 2003) have identified 10 wheat MADS box genes that are expressed in vegetative tissues before the floral transition, including two vernalization-responsive MADS box genes. One of these is the hexaploid wheat orthologue of *TmAP1*, *WAP1*, suggest that this gene corresponds to the *Vrn-1* locus of hexaploid wheat and *WAP1* was identified as the probable candidate for the *Vrn-1* gene, the major locus controlling the vernalization flowering response in wheat. *WAP1* is strongly expressed in spring wheats and moderately expressed in semispring wheats, but is not expressed in winter wheat plants that have not been exposed to vernalization treatment.

Vernalization promotes flowering in winter wheats and strongly induces expression of *WAP1*. *WAP1* is located on chromosome 5 in wheat and, by synteny with other cereal genomes, is likely to be collocated with *Vrn-1*. These results in hexaploid bread wheat cultivars extend the conclusion made by Yan *et al*., 2003 in the diploid wheat progenitor *Triticum monococcum* that *WAP1* (*TmAP1*) corresponds to the *Vrn-1* gene.

The role of *WAP1*-like genes in controlling the vernalization response of cereals was further examined in the important (diploid) cereal crop barley. The barley MADS box gene *BM5* shares a high degree of predicted amino acid similarity (95%) with *WAP1* (Schmitz *et al.*, 2000). The barley homologue of *WAP1*, *BM5*, shows a similar pattern of expression to *WAP1* and *TmAP1*. *BM5* is not expressed in winter barleys that have not been vernalized, but as with *WAP1*, expression of *BM5* is strongly induced by vernalization treatment (Murai, K *et al.*, 2002 and Schmitz *et al*., 2000). In spring barleys, the level of *BM5* expression is determined by interactions between the *Vrn-H1* locus and a second locus for spring habit, *Vrn-H2*.

In cereals, two MADS box proteins with opposite effects appear to be involved in the regulation of the vernalization response. This pathway resembles the vernalization response pathway of *Arabidopsis*, where *SOC1*, a MADS box gene that promotes flowering, is repressed by *FLC*, also a MADS box gene, in plants that have not been vernalized (Lee *et al.*, 2000; Sheldon *et al.*,1999 and Michaels *et al.*,1999). No *FLC*-like genes have been identified in cereals, making it unlikely that *Vrn-2* is closely related to *FLC*. There is no evidence that *AP1*-like genes mediate the vernalization response in *Arabidopsis*, but over expression of *AP1* does result in early flowering (Mandel and Yanofsky, 1995). The vernalization response may have evolved separately in the ancestors of the cereals (monocots) and the *Brassicaceae* (dicots) through the recruitment of different MADS box transcription factor genes into a cold regulated switch that promotes flowering in vernalized plants. There is now evidence that *AP1*-like genes determine the time of flowering in a range of cereal and grass species.

Floral Genome Project

Recently a floral genome project was established to extend knowledge of developmental genes known from model species more broadly across a selection of angiosperms (Solties *et al.*, 2002). This project will generate large EST datasets, capturing thousands of sequences of genes expressed during early flower development in each species families. This will help test the generality of function of already known genes, although genes not yet identified, or those that do not function in the model species, are unapproachable by this strategy (Baum *et al.*, 2002). Baum *et al.*, (2002) argue that a more informative approach would be to develop a wider range of model species in which function is examined in depth. The floral genome project will examine the site and timing of gene

expression for the unique genes detected in each species using a combination of microarray analysis and new methods of high throughput *in situ* hybridization. Expression patterns will be evaluated for hundreds of genes in each species. Already, functional and genomic information is accumulating in other model species, with rice (Shimamoto and Kyozuka, 2002) and maize (Lawrence *et al.,* 2004) providing divergent monocot information that is intrinsically important as well as allowing comparisons with data from established core eudicots. This data base will provide annotated links to genomic and functional information in *Arabidopsis*, rice and maize, and to expressed gene studies in tomato, maize, and many other important crop species. The floral genome project will provide a key resource for generating hypotheses about common gene functions in plants and potential sources of variation among diverse species.

Advantages

- Harnessing the regulatory genes controlling timing and differentiation of floral organs has opened the potential for increased yield through the manipulation of plant growth and development (Gynheungan, 1994).
- *FPF* gene can be manipulated to alter flowering time; suppression of *FPF* delays flowering, over expression of *FPF* causes earlier floral induction
- Helpful in managing flowering time for optimal flower, fruit and seed production
- Creation of transgenics – time of flowering can be altered (*FLC* genes)
- Suppression of flowering – increase timber, forage, sugarcane production
- Off-season flowering can be induced
- Induction of early flowering – escapes stresses due to biotic and abiotic factors
- Synchronization of flowering – helps in hybrid seed production
- Induce male sterility for the production of hybrids
- Mono sexual plants can be made to bisexual which avoids the need for maintenance of either sex
- Genes determining rice floral morphology have been identified allowing rice spikelet development to be manipulated.
- In wheat, vernalization is controlled by the MADS-box gene *WAP1*. Unlocking vernalization should allow quality wheat to be produced in warm climates.
- Bolting is another aspect of cold temperature-induced flowering. An anti-bolting MADS-box gene has been identified in Chinese cabbage.
- The main commercial transgenic crops now available are modified for herbicide resistance or insect resistance, the MADS-box constructions are modified with flowering controls or flowering timing, even alterations in yield are contemplated
- The anther-specific transcription regulator can be manipulated to produce male-sterile varieties used to produce high value hybrid seeds.
- A root nodule-specific MADS-box gene was identified in alfalfa root nodules. Transferring nitrogen-fixing ability to non-legumes has been discussed for decades, and this discovery may spur developments in that area
- In rice, for example, MADS-box genes have been identified which control the timing of flowering. As flowering time determines regional adaptability of rice varieties, manipulating that timing will allow greater use of regional varieties.
- Recently a floral transcription factor was found to control the agronomic trait - seed yield.

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