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## Decisive Factors Controlling Flowering.

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### ABSTRACT

Biologists have studied flowering plants from ancient times. Physiological development of flowers is categorized into four stages viz., floral initiation, floral organization, floral maturation and anthesis. Initiation of flowering is dependent on hormones and environmental conditions. This activates the network of regulatory genes which sequentially signal differentiation of floral primordia into sepals, petals, stamens and carpels. Homeotic mutations have helped to understand the functions of genes involved in flowering. Regulatory genes have been classified into three classes as A, B and C. These genes belong to MADS box transcription family. Though ABC model was the first to be proposed new and modified models have also been presented as ABCE and ABCDE models.

**Keywords:** flowering, physiology, homeotic, models

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## INTRODUCTION

The origin of flowers has been a fascinating issue from the period of Linnaeus to Goethe and from Darwin to the present. Several theories have been proposed on the basis of evolutionary, morphological and physiological developments. On the basis of phylogeny and morphological data two theories were majorly accepted; Anthophyte theory and Neo-Pseudanthial theory. Goethe in 1790 had proposed that floral organs are modified forms of leaves. This suggested that vegetative leaf is the basis of development of flower. Darwin referred origin of flowering plants as “abominable mystery” [1]. It was found that initiation of flowering is also regulated by various physical and hormonal factors for example length of day and night, exposure to low temperature and also due to hormones like gibberellins. Models based on gene regulatory networks have been proposed. These models provide a framework for understanding floral development at genetic levels. Classical ABC model divides homeotic genes into Class A, B and C. Modified forms of ABC model includes Class D and Class E. However ABC and ABCE model are the two widely accepted models. Certain plants are used to understand the genetic complexity involved in flowering. These plants are called as model plants. Commonly used model plants are *Arabidopsis thaliana* and *Antirrhinum majus*.

### Physiology of flowering plants

The process of flowering can be divided into major stages viz., floral initiation, floral organization, floral maturation and anthesis.

#### Floral Initiation

Floral Initiation is the stage where differentiation of primordia occurs. It is the stage of transition from vegetative phase to reproductive phase of development in plants. Rate of vegetative growth and the various conditions affecting it determines the time of floral formation [2].

#### Photoperiodism

Plants are sessile in nature. Hence they have to adapt to the environmental changes caused due to the rotation of earth. Circadian clock enables plants to anticipate the changes. Therefore plants have evolved mechanisms to include these changes into their developmental processes. The theory of day length controlling flowering response was proposed individually by Tournois and Klebs in early 1900. In the year 1920, Wightman Garner and Henry Allard showed that shortening of the length of day induced flowering in Maryland Mammoth Tobacco plant [3]. On observing day length dependent flowering in many other plants, they concluded that the length of the day is a crucial factor that determines flowering. They coined the term photoperiod which means regular pattern of light and dark periods. Also response of the plant to photoperiod is termed as photoperiodism [4]. They categorized plants, on the basis of response to photoperiod, as short day (SDP) plants, long day (LDP) plants and day neutral plants [3]. LDPs are plants in which flowering occurs only when day length is more than the critical day length. SDPs are plants that require longer dark period for flowering. Day neutral plants are those that do not depend on day length for flowering [2].

Following the discovery of photoperiodism, it was identified that photoperiod is measured in leaves. Hence it was concluded that photoperiod induces flowering stimulus. This stimulus was perceived to be a floral hormone being transmitted from the leaves through the phloem to the shoot apex to initiate flowering. Mikhail Chailakhyan named this floral stimulus as florigen [5, 6]. Upon reaching a threshold value, flowering is not dependent of photoinduction. Since florigen could not be isolated and identified in any plant, Chailakhyan proposed that there are two groups of substance that are involved in the composition of flowering hormone that are involved in flowering process. First group is gibberellins that are involved in initiation and growth of flower system and the second group is anthesins which is essential for flowering initiation [7]. It was found that the levels of these hormones control flowering in short and long day plant.

#### Vernalization

In certain plants induction of flowering occurs at low temperature. Vernalization is the process of floral initiation by exposure to cold treatment. Papers describing the effect of vernalization have been published in the 19<sup>th</sup> century. The first report of plants requiring exposure to cold for flowering was given by

Gassner in 1918 [8, 9]. Purvis and Gregory worked on winter rye and observed certain biochemical changes that occur due to low temperature. Their work stated that low temperature treatment is effective from the beginning of the treatment. It does not require any external materials except oxygen to initiate the effect of low temperature. They also observed that vernalization is only effective in the presence of oxygen. The effect of cold treatment is lost if it is interrupted by a period of room temperature. Thus inference was made that the process depends on the synthesis of a particular substance which only exists at low temperature but is degraded at higher temperature [2].

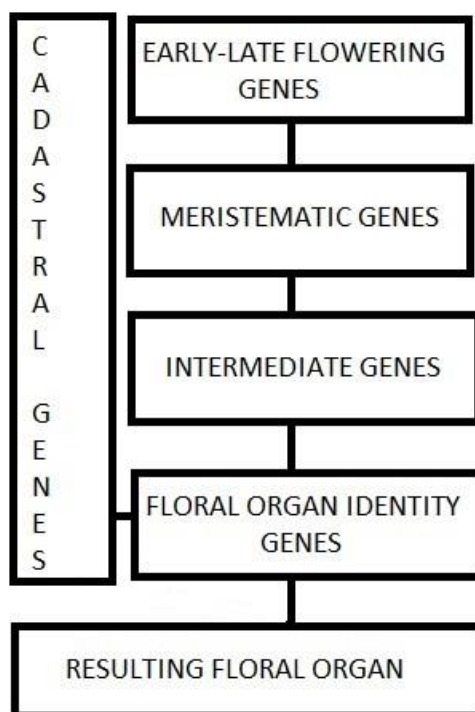
**Floral organization, maturation and anthesis**

While floral initiation involves floral primordia differentiation, floral organization includes differentiation of the floral parts. Flower begins to differentiate into sepals, petals, stamens and finally the carpels. Floral maturation involves growth of floral parts. This also involves meiosis, and development of reproductive structures like pollen and embryo sac. Floral maturation is regulated by hormones like auxins and gibberellins. Anthesis is the final stage of floral development. In this stage flower blooms completely. It becomes fully functional. Anthesis in certain plants is dependent on environmental factors [2].

**Mechanism Of flowering**

Over the years several studies have been done on networks of regulatory genes related to flowering. Environmental signals activate the early and late flowering genes. These genes in turn activate the meristematic genes that arbitrate to the conversion from vegetative to reproductive phase. Cadastral genes regulate expression of floral genes. They provide boundaries for their expression. Intermediate genes connect the floral meristematic genes and floral organ identity genes. These floral organ identity genes are responsible for the formation and function of floral organs. The genes involved in flowering are depicted in a hierarchical order through Fig 1.

**Figure 1: Regulation of flowering**



**MADS box**

The term “MADS” originated from the initials of Minichromosomal maintenance 1 (M), Agamous (A), Deficiens (D) and Serum Response Factor (S) [10]. Transcription factors with MIKC domain are coded by MADS

BOX genes. MIKC is a domain structure of protein. It comprises of MADS domain (M), Intervening domain (I), Keratin like domain (K) and C terminal domain(C) [11, 12, 13]. Plant MADS protein domain have modular structure and is a highly conserved domain [14]. The major function of MADS domain is to bind DNA. It is also associated with dimerization and accessory factor binding [15].

**Homeotic genes**

The genes involved in floral developmental pathways are called homeotic genes. Various homeotic genes determine the floral organ identity. The functions of these genes were discovered through homeotic mutations. These homeotic genes control the expression of the other genes involved in flowering. Many of them share a related type of sequence and belong to the MADS box gene sequence [16]. Three types of genes are involved in flowering viz., cadastral genes, meristematic genes and organ identity genes. Table 1. depicts the major genes involved in flowering in *Arabidopsis thaliana*.

**Table1: Genes involved in flowering in *Arabidopsis thaliana***

GENES	FUNCTIONS	REFERENCES
<b>Cadastral</b>		
SUP	Prevents class B genes to function in the fourth whorl	Bowman et. al (1992) [17]
LUG	Negatively regulates AG expression in sepal and petal formation	Liu and Meyerowitz (1995) [18]
AP2 and AG	Forms boundaries between A and C	Bowman et. al (1991) [19]
<b>Meristematic</b>		
LFY and AP1	Master regulator of flowering and initiates the floral program	Weigel et. al (1995) [20] Mandel et. al (1995) [21]
CAL	Affects LFY expression and therefore controls inflorescence architecture	Ferrándiz et. al. (2000) [22]
<b>Organ identity</b>		
AP1	Sepal formation, forms petals along with Class B genes	Mandel et. al. (1992) [23]
PI and AP3	Forms petals and stamen along with Class A and C genes respectively	Jack et. al. (1992) [24], Goto et. al. (1994) [25]
AG	Carpel formation, forms stamen along with Class B genes	Yanofsky et. al. (1990) [26]
STK, SHP1 and SHP2	Works with class E genes to form ovule	Favaro et. al. (2003) [27], Pinyopich et. al. (2003) [28]
SEP1, SEP2, SEP3 and SEP4	Partially works to define identity of the whorls	Pelaz et. al. (2000) [29], Ditta et. al. (2004) [30]
AGL6 and AGL13	Associated with ovule formation	Schauer et. al. (2009) [31]

**ABC model**

Initial steps in the genetic analysis of flowering led to the proposal of ABC model and were published in 1991. A model of organ specification in developing flower served as a basis for further studies. It was developed by using a series of homeotic mutants, double mutants and triple mutants. In ABC model the boundaries, A, B, C represent various genes involved in determining floral identity. The functions of A, B, C governing genes were determined by various mutation studies and its effect on flowering pattern of the plant [16]. Table 2. describes the ABC model for *Arabidopsis thaliana* and *Antirrhinum majus* which includes genes responsible for flowering and their specific functions. Various factors lead to mutation of these genes which affects the flowering pattern. On mutation of gene A there is a formation of outer whorl of carpel followed by two inner whorls of stamen and finally the innermost whorl of carpel. Similarly on mutation of gene B there is formation of two outer whorls of sepals and two inner whorls of carpels. On mutation of gene C there is formation of outer whorl of sepal followed by two inner whorls of petals and finally the innermost whorl of sepal.

**Table 2: Functions of genes involved in defining whorls**

BOUNDARIES	GENES	FUNCTIONS	REFERENCES
<b><i>Arabidopsis thaliana</i></b>			
A	AP1 and AP2	Controls first and the second whorl of flower i.e., sepals and petals	Bowman et. al. (1989) [32], Weigel et. al. (1994) [33]
B	AP3 and PI	Controls the second and third whorl of the flower i.e., petals and stamen	
C	AGL	Controls the third and fourth whorl of flower i.e., stamens and carpels	
<b><i>Antirrhinum majus</i></b>			
A	SQUA	Encodes a protein which is orthologous to AP1	Huijser et. al. (1992) [34], Mandel et. al. (1992) [23]
B	DEF	Controls second and third whorl of flower	Schwarz-Sommer et. al. (1990) [10], Tröbner et. al (1992) [35]
	GLO	Promote cell proliferation, involved in determining floral "Bauplan"	
C	PLE	Controls cell proliferation in formation of flower bud	Bradley et. al. (1993) [36]

**ABCE model**

ABCE model is the modified form of ABC model. This model includes an additional class of gene called as the Class E. In case of *Arabidopsis thaliana* class E genes constitute the SEP genes [29, 30, 37, and 38]. These genes coordinate with each other to form floral organs. Class A, B, E genes determine petals; Class A and E genes determine sepals; Class B, C, E genes determines stamens; Class C and E genes together determine carpels [29, 39, 40]. ABC class genes belong to MADS box transcriptor family. However AP2 gene is unique. It does not encode MADS box. It rather encodes a novel nuclear protein [41]. MADS domain proteins not only control the floral organ identity but is also involved in regulation of specific target gene sets.

**ABCDE model**

ABCDE model is an advanced and more recent model in floral development. According to this model floral organ identity is determined by five classes of genes viz., A, B, C, D, E [42]. Class A and E together form sepals in the first whorl. Class A, B and E determine the petals. Class B, C and E determine stamens in the third whorl while C and E together determine carpels [43].

**ABCDE model studied in *Arabidopsis***

Class A gene for *Arabidopsis* has been reported as AP (apetala) 1 [23]. AP3 and PI (pistilata) are classified under Class B [24, 25]. AG (agamous) gene is included in Class C [26]. Class D genes include SEEDSTICK (STK), SHATTERPROOF1 (SHP1) and SHP2 [27, 28]. Class E of homeotic genes have been divided into two clades: SEP and AGL6. Under clade SEP are SEPALLATA1 (SEP1), SEP2, SEP3 and SEP4 [29, 30]. Under Clade, AGL (agamouslike) 6 is AGL6 and AGL13 gene. They play an important role in floral organ formation and in ovule formation [31]. Class D and E genes interact to specify ovule identity. Table 3. depicts the classification and function of genes of *Oryza sativa* into the most recent ABCDE model.

**Table 3: Role of A, B, C, D and E class of genes in rice**

BOUNDARIES	CLADES	GENES	FUNCTIONS	REFERENCES
A		OsMADS14/RAP1B	Derived from FRUITFULL lineage, promotes flowering and determines the identity of floral meristem	Litt et. al. (2003) [44] Jeon et. al. (2000) [45]
		OsMADS15/RAP1A	Derived from FRUITFULL lineage and forms palea	Litt et. al. (2003) [44] Wang et. al. (2010) [46]
		OsMADS18	Derived from FRUITFULL lineage. Overexpression accelerates auxillary shoot meristem development and induces early flowering	Litt et. al. (2003) [44] Fornara et. al. (2004) [47]
		SNB and OsIDS1	Develops lodicule	Lee et. al. (2012) [48]
		MFS1	Regulates SNB and OsIDS1	Lee et. al. (2012) [48] Ren et. al. (2013) [49]
B		OsMADS16/SPW1	Works with class C genes to stop indeterminate growth in floral meristem	Dreni et. al. (2011) [50]
		OsMADS2 and OsMADS4	Lodicule and stamen formation. Orthologous to PI	Yao et. al. (2008) [51] Chung et. al. (1995) [52]
C		OsMADS3	Dominates stamen identity and prevents lodicule formation	Yamaguchi et. al. (2006) [53]
		OsMADS58	Regulates carpel morphogenesis and determines floral meristem	Yamaguchi et. al. (2006) [53], Dreni et. al. (2011) [50],
			Interacts with OsMADS16/SPW1 to suppress indeterminate growth in floral meristem	Yun et. al. (2013) [54]
D		OsMADS13	Associated with ovule identity and determines floral meristem along with Class C genes	Dreni et. al. (2011) [50], Lopez-Dee et. al. (1999) [55], Favaro et. al. (2002) [56]
		OsMADS21	Paralogous to OsMADS13	Dreni et. al. (2007) [57]
E	SEP	OsMADS7/OsMADS45 and OsMADS8/OsMADS24	Sequence is similar to Arabidopsis SEP genes and affect inner three whorls of the flower	Malcomber et. al. (2005) [58]
	LOFSEP	OsMADS1/LHS1	Associated with lemma and palea formation	Christensen et. al. (2012) [59] Prasad et. al. (2001) [60]
		OsMADS5/OSM5	Function not fully established	Agarwal et. al. (2005) [61]
		OsMADS34/PAP2	Specifies inflorescence and spikelet along with OsMADS1	Kobayashi et. al. (2012) [62]
	AGL-6	OsMADS6/MFO1	Regulates floral organ identity	Ohmori et. al. (2009) [63] , Duan et. al. (2012) [64]
		OsMADS17	Functions with MFO1	Li et. al. (2010) [65]

**CONCLUSION**

Multiple studies have sought to understand how different models correlate to floral physiology and morphology. Our knowledge about molecular mechanisms involved in flowering has increased over the years. Manipulations of these genes have enabled us to elucidate the functions of genes involved in regulation of flowering.

However functions of few genes like OsMADS7, OsMADS8 have not been completely discovered. Future prospects will involve discovery of the functions of these genes and application of these models to various range of plants. Also complete network of genes have to be found out. Over the years several flowering mysteries have been unravelled and many are yet to be revealed.

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