Molecular basis of flower initiation—A review

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Plants have many differences, like protandry, protogyny, etc. However, amidst these differences, all angiosperms have a common mechanism of flowering, i.e. concentric pattern of flowering (sepal, petal, stamen and carpel). The mechanism or the genes through which plants maintain boundaries between the four, sepal-petal-stamen-carpel, are also given due importance. Once a plant attains competence, flowers may be produced through the reorganization of SAM directly to floral meristem, or through the inflorescence or co-inflorescence meristem, in response of exogenous and endogenous signals. Initiation, determination and differentiation are classed into four stages and this is the region which is studied here in detail. Some organ specificity genes, like MALE STERILITY (MS) and BICAUDAL (BIC), move us towards a better understanding of the mechanism like male sterility and self-incompatibility in plants. Models like ABC, biophysical, MCDK, etc., help in explaining the mechanism of flowering on a molecular basis. However, in general, the path towards flowering is laid when the floral repressor genes are down regulated. Recently identified miRNAs in plants authenticate them and also give out the mechanism by which they down regulate. It has also been found out that MADS-box gene family and CArG-box genes (where those MADS domain protein binds) are highly conserved. Their role in flower development is also touched upon.

Keywords: cDNA, floral development, mutant, photoperiod, shoot apical meristem
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Introduction

Animals and plants significantly differ in their developmental pattern. In case of animals, organogenesis occurs at the stages of embryonic development; whereas, it occurs as post-embryonic development in case of plants. This implies that most organs are spatially regulated in animals, while they are temporally regulated in plants⁵. The main reason behind this is the two important processes, viz. cell migration, which is totally absent in plants, and programmed cell death, which does not contribute in a major way to the plant development⁶. However, the lack of cell migration in plants is overcome by means of the other two important processes, viz. the rate of cell division at various tissues and the plane of cell division (anticlinal and periclinal). Since embryos in plants are seated deep within the ovaries and ovules, the understanding of the plant development has been delayed. However, its study is more amazing and gives astonishing truth of the evolutionary aspects too. A plant mostly develops from the seed, which in turn develops from the floral organs. Invariably all angiosperms conserve the concentric pattern of flowering — sepals, petals, stamens and carpels — irrespective of colour or size⁷. A flower temporarily arises from the meristem, from where the leaves are given out till then. These two fascinating aspects lead to the unravelling of facts about the development of flowers and have been covered in brief in the present review.

Basics of Flowering

Flowers, in general, are derived from shoot apical meristem (SAM)—a collection of undifferentiated cells set aside during embryogenesis—from where leaves, flowers and other appendages are temporally segregated such that leaves occur in earlier part and the flowers in later part of the plants’ life cycle. Those plant species that are determinate in nature, SAM finally reorganizes itself directly into a floral meristem as in sunflower (Helianthus annus) or through the inflorescence meristem that finally gives rise to the floral meristem as in rice (Oryza sativa); whereas, in case of indeterminate plant species, flowers will be formed on the lateral branches or through lateral inflorescence called as ‘co-inflorescence’⁸. Hence, in indeterminate plant species, SAM persists and continues to produce floral meristem laterally. Thus, both vegetative and reproductive phases occur simultaneously.
Irrespective of any species of angiosperm, the floral meristem generates a fixed array of organ primordia, wherein each primordia adopts itself to a specific fate that never changes—sepal, petal, stamen and carpel—and according to their relative position in the flower bud which shows that the organ primordia of a floral meristem are spatially regulated in the concentric whorls in the order as mentioned above. This is explained later with the biophysical and the ABC models. An important point to be noted in the basics of flowering is competence. Thus, only those plants that have competence to produce inflorescence start the flowering process, i.e. they receive and respond to the signal for flowering. In other words, only those plants that possess competence can flower when the signal for flowering is perceived.

Process of Flowering

Though it is not clearly demarcated, the process of flowering is classed into four steps, namely floral induction/evocation, floral initiation, identification of organ primordia (determination) and identification of organ specificity (differentiation), for the ease of research. Of which, the former two stages are considered as the phase I and are called flower initiation. While, the latter two stages are considered as phase II of the process of flowering and are called floral development.

Floral induction or evocation is a very important process that deals with the transition from the vegetative to reproductive phase. It involves various changes at cellular and molecular level that causes the SAM to reorganize itself to the inflorescence/floral meristem or to produce the inflorescence/floral meristem laterally by maintaining the SAM. Once the transition from vegetative to reproductive phase is made, the second step—floral initiation starts up. In case of inflorescence meristem, they further develop and produce floral meristem, from where flowers develop. In cases where there is no inflorescence meristem, these two stages are blended such that they occur simultaneously and no clear demarcation is made. As the floral meristem forms, they determine themselves for the formation of four floral primordia—sepal, petal, stamen and carpel—that are regulated spatially and are best explained by the ABC and the biophysical models. It is at this stage where the organs of identity are determined and are maintained in concentric whorls due to the absence of the internodes. Here, though, we cannot identify the organs, the fate of the cells at the floral meristem is decided.

The last and final stage of the process of flowering is the differentiation of the organ identity or organ primordia that are determined by their fate during the third stage of flowering. They grow from here towards their phenotypic expression by the growth of the individual organ primordia into a complete organ that are clearly distinguishable from the others. This stage leads to the development of a mature flower that may range from incomplete to complete or imperfect to perfect flower. The present review is focused on the mechanisms involved in the development of these four stages; the models that are proposed for explanation and the genes involved in these mechanism of action. For such studies, homeotic mutants are highly helpful. Homeotic mutants are those mutants that produce normal organ in a place where an organ of another type is typically found. They are first recognized in plants. For example, the gene AP2 is responsible for the normal growth of sepals and petals in the first and second whorls of the organ primordial, respectively. Under the mutant condition, however, it causes the growth of carpel and stamen in the first and second whorl, respectively. In wild type, the growth is in the order of sepal, petal, stamen and carpel; whereas, in case of ap2 mutant, the growth happens to be in the order of carpel, stamen, stamen and carpel. Thus, these homeotic mutants are helpful to study the expression of various genes by their absence.

Proposed Models for Flowering Mechanism

(a) Positional Information Theory

In 1979, Holder proposed the first and foremost theory for the flower development, which is known as positional information theory. According to the theory, the floral hormone level is thought to specify the floral responses to general positional cues provided by a morphogen system. However, there are no answers for floral determination etc., from the view of developmental biology.

(b) Reaction-Diffusion Theory

Meinhardt, in 1982, proposed a 2-D pattern in the meristem for flowering in terms of the concentration profiles of a pair of morphogens, viz. activator and inhibitor, that decides the plant to flower or not to flower. These two theories though answered some questions but left many of them unanswered, like the uniqueness of stamens, etc.
(c) Biophysical Theory

These earlier two theories when couldn't answer many questions, Green postulated a theory in 1988, called Biophysical theory. It unravelled the natures' secret regarding the aspects of flowering and answered almost all the questions from the conservativeness of the floral arrangement to the uniqueness of the stamen. Biophysical theory is mainly based on the principle that various organs are formed based on the differences or changes which occur in the cellulose reinforcement pattern during cell wall formation of those cells that are at the surface layers of the organs to be formed. Another interesting feature in this model is that inflorescence meristem is termed as the generator and floral meristem as product and, thus, it gives clues for the inflorescence cycle and the floral cascade. The difference in cellulose reinforcement is by means of polarity in the cell at the time of division. The cellulose reinforcement takes place in the normal to the elongation direction of the cell and it persists through mitosis. But in case of cells on the surface layers, the direction of division is the direction of stretch. This repeated operation is made into cycle and which as a result produce a pattern. This type of cyclic patterning is seen in case of SAM and in inflorescence meristem (IM). After few cycles, the direction of division of cells on the surface layers changes due to deformation and as a result there is a change from SAM or IM to the floral meristem. According to this theory, the mechanism of flowering has been explained by three postulates. First postulate deals with the transitional phase (floral induction) that occurs due to the change in physical boundary conditions of the cell wall during cell division rather than the changes in cell rules. It is based on two aspects; one is the change in the cell configuration of the apical dome during the onset of flowering (becomes larger) and the other one is the arching up or swollen dome. These two differences are caused due to the change by a single factor named dynamic equilibrium, i.e. there is a continuous outward (basipetal) displacement of cells in the SAM region, caused by growth between a cell and the dome's center. The resultant stretching action of appendages causes the reinforcement pattern on the dome in an acropetal action (as the reinforcement is normal to the stretch). These two processes are maintained in dynamic equilibrium. So, during transition for flowering, this equilibrium is changed. As during floral induction, the appendage size is reduced, leading to the basipetal shift in the equilibrium and, hence, to counteract this shift, it causes a swelling in the dome towards the acropetal accession, which leads to new appendages (floral parts) and a new dome configuration.

The second postulate deals with the floral development, i.e. the difference in the inflorescence cycle and the floral cascade. So, if the bract is large, it produces inflorescence cycle as there are fewer shifts. But to form a floral meristem, the bract size should be in reduced form, as a result more shift causing it to swell. Once the bract is reduced and causes a significant shift in equilibrium, bulging of dome occurs that produces floral organs through the steps called floral cascade, which is known as determination of organ identity in the flower development processes.

The final postulate is that the structure of the primordium gives specification for its later biochemical differentiation that leads to the fourth stage of the flower development. It occurs in two steps namely the formation of a polar axis and its subsequent local differentiation.

(d) ABC Model of Flowering

In 1991, Coen and Meyerowitz postulated a model especially for organ identity, which is known as ABC model of flowering. To explain how the homeotic genes control the organ identity, this model gives a clear idea in a simple way for the organ identity in each of the four whorls. As already mentioned above, these whorls are concentric due to the fact that they lack internodes. In a unique way, this model proposes that organ identity in each whorl is determined by the activities of three homeotic organ identity genes, namely A, B and C. The activity of type A specifies sepals and the activities of type A and B petals, whereas the activities of type B and C are required for stamens and the activity of type C for carpels. In Arabidopsis thaliana (Thale cress), the homeotic gene activity of A involves the gene called APETALA1 (AP1) and AP2. So, the mutant of A causes the sepals and petals to convert into carpels and stamens, respectively. The homeotic gene activity of B involves the genes called AP3 and PISTILLATA (PI). The mutant of B causes the petals and stamens to convert into sepals and carpels, respectively. Further, the homeotic gene activity of C involves the gene called AGAMOUS (AG). So, the mutant of C activity causes the stamens and carpels to convert to petals and sepals, respectively. This shows that A and C genes are mutually repressive, that is if A genes are absent, then C class genes are expressed throughout
the flower and if C genes are absent, then A class genes are expressed throughout the flower. Thus, by combining all these, they act like a chain for the recognition of the surrounding whorls. Similar gene activities have also been observed in a distantly related dicot plant, Antirrhinum majus (Snapdragon). Here, the gene activity of A involves the genes OVULATA (Ovu) and SQUAMOSA (Squ) in snapdragon, which are similar to AP2 and AP1 genes respectively in Thale cress. Similarly, the gene activity of B involves genes called DEFICIENS (Def), GLOBOSA (Glo), and SEPALOIDEA (Sep) that have similar effect of PI and AP3 of Thale cress. While the gene activity of C involves PLENA (Ple) that shows similarity with AG. It is also observed that AP3 is homologous to Def; and AG is homologous to Ple. In case of double mutant AB, carpels are formed in all the four whorls and, in double mutant BC, sepals are formed in all the four whorls. In the triple mutant ABC, the whorls are very much like vegetative leaves with stipules. The double mutant AC is similar to the triple mutant AC, as B doesn’t have any activity by its own.

The recent discovery of the SEP genes (SEP 1, 2, 3) in A. thaliana shows that they play a significant role in the flower development processes. Single or double sep mutants fail to exhibit a dramatic phenotype in floral development; whereas, the triple sep mutants give sepal like structures of indeterminate nature. This shows that these three genes are required to maintain the determinate type of inflorescence and they also function redundantly to specify petals, stamen and carpel. Thus, on the basis of SEP gene family, Theissen and Saedler in 2001 have postulated the modified form of ABC model and called it ABCE model, wherein they named these SEP genes as E class genes. This revised ABCE model postulates that sepals are specified by A activity alone; petals by A, B and E; stamens by B, C and E; and carpels by C and E.

(e) MCDK Model of Flowering

In 1994, Martinez-Zapater et al postulated MCDK model, which states that floral repressors are expressed during the vegetative phase and when plants receive proper endogenous and exogenous signals after attaining the competence, these floral repressors are down regulated, resulting in the flowering of plants. It is also authenticated by the presence of miRNAs—the RNAs of approximately 21-nucleotide length—whose function is to down regulate the mRNAs of their complementary sequence and they are never translated to protein. They are different from siRNAs. miRNA172 that targets AP2 gene for early flowering was also identified.

On the basis of different models discussed above, it can be said that the entire floral development process takes place in four stages.

I. Floral Induction Stage

It is the first stage of flower development and occurs only in those plants that possess competence. A cell or group of cells is said to be competent if it can respond in an expected manner especially for flowering. They may cause floral induction based on the levels of exogenous (environmental) and endogenous factors (signals). The clonal analysis in corn indicated that about four cells of the meristem at the dry-seed stage are committed to produce the terminal male inflorescence or tassel. So, in all plants, except corn, shoot meristem are characterized by the absence of the permanent apical initials and the central zone of relatively inactive cells, found in many vegetative meristems, is not generally a meristeme d’atente—the germ line in plants that was composed by a group of quiescent cells in SAM—which was placed in reserve during vegetative growth. Some genes that have been identified for competence are SN and DNE—the genes that expresses themselves in leaves—and their activity is decreased as the plant ages. Thus, these two genes maintain the juvenility up to a certain time and then their activity gets reduced. Once, their action gets reduced, plants start flowering. Gene HR blocks the decline in the activities of SN and DNE genes as age proceeds and, hence, lengthens the juvenile phase. The best example is the production of the vegetative growth in the older stems that have competence to flower due to the activation of HR gene that maintains juvenility by blocking the degradation of the SN and DNE gene activity. Gene LF acts at the apex and influences its competence, which responds to the balance between floral promoters and inhibitors. Once competence is achieved, the floral evocation or induction starts taking place by means of exogenous and endogenous factors. The most important exogenous (photoperiod, vernalization, stress, nutrients) and endogenous (florigen/antiflorigen concept, electrical signal concept and multifactorial control by PGR) factors that influence the flowering are:

(a) Photoperiod

A detailed study of photoperiod in relation to flowering was undertaken through homeotic mutants
and from which more than 40 mutants from 12 loci are identified and are grouped into three phenotypic classes\textsuperscript{31}. The first class of mutants is sensitive to both photoperiod and vernalization. The mutants identified under this class are \textit{fca}, \textit{fpa}, \textit{fve}, \textit{fy}, and \textit{ld}. The second class of mutants is insensitive to both and they are \textit{co} and \textit{gi}. They are assumed to be responsible for flowering in the day neutral plants. While the last class of mutants is sensitive only to photoperiod and insensitive to vernalization. The mutants identified in this class are \textit{fe}, \textit{fd}, \textit{fhc}, \textit{ft}, and \textit{fwa}. Later on, attempts were made to clone all the late flowering genes by chromosome walking or tagging\textsuperscript{32}.

(b) Vernalization

Vernalization is also one of the major and important exogenous controls of the flowering in plants. In case of long day plants, cooling causes flowering; whereas, in case of short day plants, warm temperature causes flowering. Till now only one gene related to vernalization has been identified, i.e. \textit{VERNALIZATION INSENSITIVE (VRN)}\textsuperscript{33}.

(c) Stress

Stress causes flowering in an indirect way, e.g. the water stress causes the plant to produce a protein called osmotin that acts as an endogenous signal for flowering\textsuperscript{34,35}. In a nutshell, the genes involved in the exogenous control and their respective mutant phenotype are given in Table 1.

(d) Florigen/Antiflorigen Concept

In photoperiod sensitive plants, all photo-induced leaves types are supposed to produce florigen and, in contrast, antiflorigen in non-induced leaves\textsuperscript{8}. Similar florigen, if not identical, has been found out in all plants tested from eight families, like \textit{Solanaceae}, \textit{Crassulaceae}, etc.\textsuperscript{36}. Floral induction occurs when the balance of florigen and antiflorigen at the meristem is shifted in favour of florigen. Recently, An \textit{et al} have showed that the gene \textit{CONSTANS (CO)} regulates the level of florigen that is produced as a systemic signal in the leaves and has been transported from leaves to meristem through phloem\textsuperscript{37}. Total ethanol extract from leaves of short day and long day of tobaccos, both grown in short day, consistently caused flower formation when sprayed on the seedlings of SDP \textit{Chenopodium} \textit{sp.} kept in long day\textsuperscript{38} and concluded that florigen is composed of two complementary materials, \textit{viz}. a hypothetical anthesis produced in short day by both short day and long day plants, and \textit{GA} produced in long day by both short day and long day plant.

(e) Electrical Signal Concept

When various stimuli such as wounding, etc., are made to one part of the plant, it exerts an effect on

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<tr>
<th>Genes</th>
<th>Mutant Phenotype</th>
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<tr>
<td>Late flowering</td>
<td></td>
<td></td>
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<tr>
<td>Luminidependens (\textit{ld})</td>
<td>Delayed flowering</td>
<td>31,43,75,76</td>
</tr>
<tr>
<td>Constans (\textit{co})</td>
<td>Delayed flowering</td>
<td>43,70,77</td>
</tr>
<tr>
<td>Vernalization insensitive (\textit{vrn})</td>
<td>Delayed flowering</td>
<td>69,78,79</td>
</tr>
<tr>
<td>Late flowering in SD</td>
<td></td>
<td></td>
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<tr>
<td>Gibberellin insensitive (\textit{gai})</td>
<td>Delayed flowering</td>
<td>80,81,82</td>
</tr>
<tr>
<td>Gibberellin requiring (\textit{ga 1})</td>
<td>Delayed flowering</td>
<td>80,81,82</td>
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<tr>
<td>Early flowering</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early flowering - 1, 2, 3 (\textit{elf - 1, 2, 3})</td>
<td>Early flowering</td>
<td>83</td>
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<tr>
<td>Embryonic flowering - 1, 2 (\textit{emf - 1, 2})</td>
<td>Inflorescence meristem without vegetative growth</td>
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<tr>
<td>Long hypocotyl - 1, 2 (\textit{hy - 1, 2})</td>
<td>Causes early flowering and long hypocotyl</td>
<td>85,86,87</td>
</tr>
<tr>
<td>Phytochrome b (\textit{phy b})</td>
<td>Early flowering</td>
<td>85,86,87</td>
</tr>
<tr>
<td>Spindly (\textit{spy})</td>
<td>Early flowering</td>
<td>88</td>
</tr>
<tr>
<td>Early flowering in SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abscisic acid deficiens (\textit{aba})</td>
<td>Early flowering</td>
<td>89</td>
</tr>
<tr>
<td>Abscisic acid insensitive - 1 (\textit{abi - 1})</td>
<td>Early flowering</td>
<td>89</td>
</tr>
<tr>
<td>Photoperiod Insensitive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phytochrome a (\textit{phy a})</td>
<td>Long hypocotyl in continued far-red light.</td>
<td>87,90-94</td>
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other parts of the plant by transport of chemical through symplast. In some cases, a signal is found to propagate waves of electric depolarization by means of the fast moving electrophysiological signal. However, numerous attempts to detect this transmission have been failed39.

(f) Multifactorial Control by PGR

In this concept, the floral induction was caused due to the action of the PGR, like gibberellins and cytokinins40. Though all PGRs cause the floral induction8, the above-mentioned two PGRs have a significant role in the process of floral induction. The action of gibberellins in the flowering of corm species, *Polianthes tuberosa* L. (cv. Double) has been elucidated41. Whereas the endogenous gibberellins are isolated and identified by HPLC, bioassay, and GC-MS. Gibberellins, GA1, GA19, GA20 and GA53 are quantified at three stages, viz. vegetative, early flower initiation and flower developmental stages, and was found that an increase in GA1 and GA20 with a simultaneous decrease in GA19 levels coincided with the transition from the vegetative phase to the phases of floral initiation; whereas, GA53 stayed at constant level in all the stages. Thus, it shows that, during flowering in *P. tuberosa*, there is a conversion of GAs in the decreasing order of GA53>GA19>GA20>GA1.

In *Lolium temulentum*, 13-hydroxyl group GAs enhance the inflorescence initiation, whereas 3 β-hydroxyl group GAs enhance stem elongation42. The presence of cytokinin-kinetin directs the flower formation in plants43. This was shown by using thin cell layer (TCL) explants and preparing cDNA library using the floral bud day 7 (FB-7), i.e. using explants after seven days of culture on the medium. These represent transcripts expressed abundantly in mRNA of vegetative shoot day 7 (VS-7) explants. In the cDNA of FB-7, till now 52 clones have been identified and isolated, and they are grouped into six gene families by sequence homologies and are designated as FB7-1 to FB7-634. These gene families encode gene products that are also induced by environmental and physiological stress. Most of FB7 gene expressions are observed in non-floral tissues and their highest levels are found in the roots (Table 2). β-1,3-glucanase is also expressed with chitinase during pathogenesis. They also show acidic and basic properties similar to chitinase with the same chemical and cellular properties. Of all these four (chitinase, osmotin, extensin and β-1,3-glucanase), extensin is induced by kinetin and others are cytokinin induced34. Various molecular changes that are involved during transition stage of flowering are:-

1) Increase in the levels of sucrose, ATP levels, invertase activity, mitochondrion number and the energy charge44,45. 2) 2,4-DNP (dinitro phenol), which inhibits floral induction44. 3) There is an increased rate of cell division46.

II. Floral Initiation

It is the second stage in the flowering process. It occurs only in plants where they produce inflorescence meristems. In plants where there is no inflorescence as in sunflower, they directly form the floral meristem and determination of the floral organs. This step is the one wherein the inflorescence meristems are developed to floral meristem by means of the inflorescence cycle. As the cells divide, the inflorescence goes towards terminal end and they complete the inflorescence cycle once the activity of genes that suppress inflorescence gets activated.

The most important gene involved in specifying the floral meristem identity from inflorescence meristem is *LFY* identified in *Arabidopsis thaliana*47. This gene is responsible for the conversion of the inflorescence shoots to floral meristems. Its homologue in Snapdragon is *FLORICAULA* (*FLO*)48. The next important gene that enhances the activity of *LFY* is *AP1*49,50 and its homologue in snapdragon plants is *SQUA*10. So, the double mutants *lfy* and *ap1* will produce inflorescence shoots with bracts alone and some irregular flowers due to the reason that *LFY* gene involves at floral meristem identity itself.

Another gene that acts with *LFY* and enhances its activity is *CAULIFLOWER* (*CAL*), as the mutants of this gene cause floral meristems behave like the inflorescence meristems and, as a result, they produce the massive proliferation of meristems giving the view similar to cauliflower. Gene *CAL* is 76% identical to *AP1*49. Other genetic locus that plays a minor role in the floral meristem identity is *AP2*20. An *AP1, AP2* double mutant causes the indeterminate inflorescence. The homologue of this gene in

<table>
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<th>Gene family of FB7</th>
<th>Protein produced</th>
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<tr>
<td>FB7-1</td>
<td>Chitinase</td>
<td>34,95</td>
</tr>
<tr>
<td>FB7-2</td>
<td>Osmotin</td>
<td>96</td>
</tr>
<tr>
<td>FB7-3</td>
<td>Extensin</td>
<td>34</td>
</tr>
<tr>
<td>FB7-4</td>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>FB7-5</td>
<td>β-1,3-glucanase</td>
<td>34</td>
</tr>
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*FB7 = 7 day old floral bud*
snapdragon is called as OUV\textsuperscript{10}. Another gene of minor action is CLAVATA1 (CLV1) that under mutation produces enlarged meristems\textsuperscript{49}. Hence, it causes extra organs in the resulting flowers. The homologue for LFY in pea plant is UNIFOLIATA\textsuperscript{2} (UNI).

The gene TERMINAL FLOWER1 (TFL1)\textsuperscript{51} identified in A. thaliana has the role of maintaining the inflorescence and it prevents the formation of floral meristem \textsuperscript{52,53}, as observed in the mutant tfl1 that produce floral meristem in place of inflorescence meristem. The homologue of this gene in snapdragon is CENTRO RADIALIS\textsuperscript{10}. At first, TFL1 gene acts for the formation of inflorescence meristem through inflorescence cycle\textsuperscript{54} and once when LFY acts, floral meristem is formed than the inflorescence meristem as explained by biophysical theory.

At the end of this stage, an important gene gets activated, which is identified in A. thaliana and called UNUSUAL FLORAL ORGANS (UFO)\textsuperscript{55}. It is used to mediate the developments from meristem identity to organ identity-and is involved in the transition of second to third stage of development. The homologue of this gene in snapdragon is FIMBRIATA (FIM)\textsuperscript{10}. Thus, FIM acts after FLO and SQUA but before DEF and PLE, wherein the former two are meristem identity genes and the latter two are organ identity genes\textsuperscript{10,56}. The mutant of this gene affects the second, third, and fourth whorl causing sepaloïd in the second whorl and the petaloid in the third whorl. The proof that FIM expresses before DEF and PLE is that the expression of DEF and PLE in the fim mutant is weak and altered. Whereas, the expression of FIM in the def and ple single mutants showed no difference in its expression\textsuperscript{10}. Thus, UFO and FIM lead the flower ontogeny towards organ identity stage that involves determination of floral organs\textsuperscript{52}.

According to the ABC model, the region A gives the organ identity to sepals (AP1, AP2); A with B gives the organ identity to petals (AP2 and PI, AP3); B with C gives the organ identity to stamens (PI, AP3 and AG); and C exclusively gives organ identity to carpel (AG)\textsuperscript{6}. Another important aspect in this organ identity determination is that the perfect regulation of the gene activities of A, B, and C (ABC model) without interrupting the other regions. This was maintained by a gene called SUPERMAN (SUP). This gene prevents the B region to interrupt with the C region\textsuperscript{11,12}. Recently, Takeda et al have identified a gene RABBIT EARS (RBE) that encode for the expression of the gene SUP, which in turn regulates petals\textsuperscript{57}. But in case of male flowers this gene suppresses the whorl that develops the carpel and, as a result, it causes the meristem to produce the male flower in case of monoecy or dioecy. Similarly, the gene called LEUNIG (LUG) that prevents the A region (petals) to be interrupted from the genes of C region under the case of producing the female flowers and suppresses the growth of the stamens\textsuperscript{6}. As these two genes SUP and LUG have the capacity to keep the boundary under the normal conditions, they are called cadastral genes. Those genes that are involved in the formation of floral meristem, like LFY, AP1, CAL, etc., are called as meristem identity genes. While those are involved in the formation of floral organs, like AP2, AG, PI, AP3, etc., are called floral organ identity genes\textsuperscript{12}.

**IV. Identification of Organ Specificity**

It is the fourth stage of the floral development, wherein the determined cells get differentiated and then later they form the floral organ, which is mediated by the cascade of the transcriptional regulation\textsuperscript{9}. Though the MADS box genes (MCM 1 from yeast; AG and DEF A from plants and SRF from human) from plants have various functions, namely lateral root elongation; meristem identity; flowering time; floral organ identity and determination; pollen fertility and ovule development; and fruit tissue identity, ripening and dehiscence\textsuperscript{68}. The MADS box genes consist of transcriptional factors that are involved in the floral organ identity. Some important genes involved in the organ specificity are BICAUDAL (BIC), controlling pistil development\textsuperscript{59}, FIDDLEHEAD (FDH), ontogenic fusion process\textsuperscript{60}, MALE STERILE (MS), microsporogenesis\textsuperscript{61,62}, and antherless (at), mutant having stamen devoid of anthers\textsuperscript{62,63}. During the stage of organ specificity, MS

**III. Identification of Organ Primordia**

It is the most important stage of flower development because once the plant attains this stage, it should complete the flowering process. The fate of the cells at the floral meristem is decided in this stage and the whorls are formed over the meristem. This is the stage wherein the miniature of the flower is determined but not yet differentiated\textsuperscript{9}. Till previous stage, they have provision to revert back from the floral meristem to vegetative meristem; as they are not yet determined to form a flower, which may give a clue towards the mechanism of floral reversion.
genes are activated during the time of microsporogenesis, whereas AT genes get activated during the time of the differentiation and growth of the stamen. MADS-box genes are those set of genes that play a central role in flower development and consists of all the genes involved from the meristem identity to the organ identity. This shows that each of the MADS-box genes may be evolved from the common ancestral gene. The consensus region of the gene product is of 56 amino acid length DNA-binding domain localized at the amino terminus and generally begins immediately after the initiator methionine codon.

The consensus DNA sequence upon which MADS-domain proteins bind is “CC(A/T)GG” and was shortly known as CArG box. The MADS-domain containing proteins have additional regions with moderate sequence similarity. One is K-domain, which shows similarities in the keratin protein and has the ability to form amphipathic α-helices. Its predicted role is to mediate protein-protein interactions. In addition, plants MADS protein also have two divergent regions namely I domain (Inter domain) that lies between MADS and the K-domain and the other one is the C-region that contribute to the most C-terminal position of the protein. Some genes of MADS-box family enhance the flower development are AG, AP1, CAL, SQU, DEFA, etc.

Location of Genes

Most of the genes of the model plant A. thaliana have been sequenced. Of these, genes that are related to the flower development are also identified and mapped on the chromosomes. Lists of genes that have been located and mapped on the chromosomes of A. thaliana are listed below:

- Chromosome 1: UFO, CAL, GAI, GA4, CLV2, CLV1, and AP1.
- Chromosome 2: CLV3.
- Chromosome 3: SUP3, SPY, and MS2.
- Chromosome 4: GAI, and VRN2.
- Chromosome 5: TFL1, TFL2, EMF1, GA3, and LFY.

This list shows that almost all the chromosomes have at least one gene that is responsible for the flower formation or flower development in the model plant.

Conclusion

A study on the molecular basis of flower initiation reveals that though the flowering plants are highly diverse, the basic pattern of flowering (sepal, petal, stamen and carpel) and the mechanism of flowering are highly conserved. Even though angiosperms and gymnosperms are discrete, their genes show high percentage of homologue sequences that gives newer insights on the study of evolution to bridge out the gap in the evolutionary pathway from gymnosperms to angiosperms. Another important aspect is the property of redundancy in single mutants for flowering. So, whichever may be the mutant, they at least flower irregularly, showing that each step in the process of flowering is decided by more than one gene. It further implies that more than one gene is involved for every character in the flowering mechanism. Another important thing to be noted is that invariably in all plants, either determinate (annuals) or indeterminate (perennials), the inflorescence is of determinate type only as proved by the SEP genes. This makes a new way to reason out the possible genetic mechanisms or interactions between the genes so that the nature distinguishes some as determinate ones and the others as indeterminate ones. If that secret of natures is disclosed, then it will be possible to produce trees of rice or corn or wheat that will yield sustainably in a perpetual manner. Though landmark progress has been made in the field of the flowering mechanism, there is still mystery surrounding floral reversion, i.e. some plant species have ability to switch over from vegetative meristem to a floral meristem and vice versa. Once solved, it will open a new gateway in the field of the flowering mechanism.

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