Physiology of Flowering

25

Geetika Kalra and Manju A. Lal

About 90% of the ≈ 350,000 known plant species are the flowering plants. Flowering is the most enigmatic phase in the life of a plant. It provides a mechanism to plants for genetic outcrossing which provides a means of securing a greater variety of genetic recombination. Flowers are specialized structures which differ extensively from the vegetative plant body in form and cell types. Numerous physiological and biochemical changes take place within the shoot apex when it prepares itself for transition into floral bud. The precise time of flowering is important for reproductive success of the plant. Plants need to sense when to produce flowers so that fruit and seed development can be attained which will ensure its survival in the next season. Synchronous flowering is significant in outcrossing plants. Since long, people have wondered how plants are able to flower in a particular season. Plants possess the ability to anticipate and sense change of seasons. It has always been a fundamental question as to how environmental signals influence flowering and how these signals are perceived.

Transition from vegetative to reproductive development is generally marked by an increase in the frequency of cell divisions within the central zone of SAM. The process by which the shoot apical meristem becomes committed to forming flowers is termed **floral evocation**. SAM has an undifferentiated dome of cells at the center which, after the signal is perceived, triggers **quiescent** cells to enter into the phase of cell division and leads to transition from vegetative meristem to floral meristem. To some extent, timing of flowering can be influenced by external factors. The competence resides in the leaf, the shoot apex, or both. The regulation of competence to flower is complex and is variable across variable taxa. Floral induction requires both the perceptive organ and the shoot apex to acquire competence during plant maturation. Apical incompetence is a characteristic feature of the shoots of young trees, which are usually unable to transit into flowering even when grafted on to mature plants. This chapter deals with factors responsible for transition from vegetative to reproductive mode of differentiation and the associated physiological and biochemical changes, including present understanding about the role of genes in this process.

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Onset of flowering can be considered under three steps: first, the plant must acquire competence to respond to inductive signals; second, perception of the signal, which is then sent to shoot apex, resulting its transition from vegetative to reproductive phase; and third, the differentiation of shoot apex into floral organs.

25.1 Juvenile Phase

The internal developmental changes allow plants to obtain competence to respond to both external and internal signals that trigger flower formation. Such a transition is called as **heteroblasty** or **phase change**. Plants pass through a series of developmental stages starting from seed germination, root and shoot development, flower evocation and, finally, seed formation (Fig. 25.1). The transition between different phases is tightly regulated developmentally according to the integrated information perceived from the environment. Broadly, plant development is categorized into four phases: embryonic phase, postembryonic juvenile phase, adult vegetative phase, and reproductive phase. The main difference between the juvenile and the adult vegetative phase is that the latter is sensitive to various factors leading to development of reproductive structures. Distinct phases of juvenility and maturity can be identified in many species. Each phase is associated with a characteristic package of

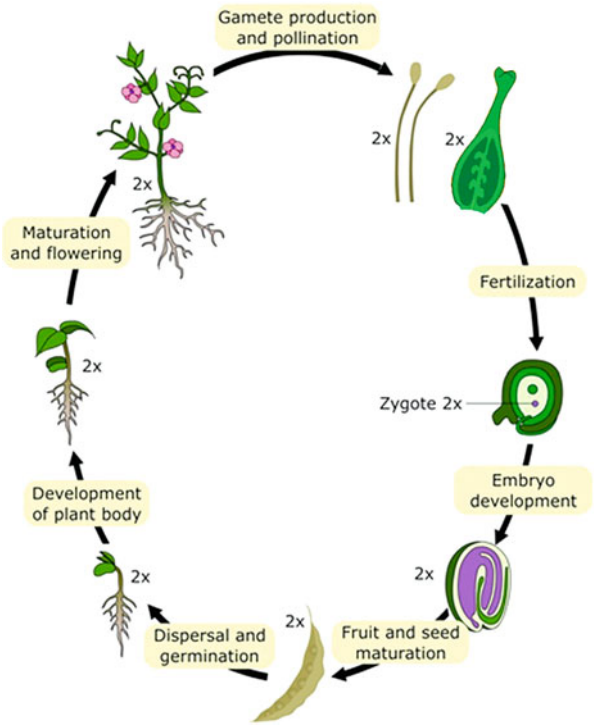

Fig. 25.1 Life cycle of an angiosperm showing various phases of development. Transition to each phase involves remarkable biochemical and physiological changes


Fig. 25.2 Characteristic features of ivy (*Hedera helix*) in its juvenile (nonflowering) and adult (flowering) phases of growth

Table 25.1 Distinguishing characteristics of juvenile to adult ivy

Character	Juvenile plants	Adult plants
Leaves	3–5 lobed, palmate	Entire, ovate
Phyllotaxy	Alternate	Spiral
Shoot apex	Relatively narrow with large cells	Wide apex with small cells
Rate of internode growth	High	Low
Stem	Hairy	Smooth
Habit	Climbing and spreading	Upright or horizontal
Shoots	Unlimited growth and lack terminal bud	Slow limited growth terminated by buds with scales
Flowering	Absent	Present
Adventitious roots	Present	Absent
Rooting ability of cutting	Good	Poor

morphological and physiological features (Fig. 25.2). For example, ivy (*Hedera helix*) exhibits characteristic juvenile and adult phases of life cycle (Table 25.1). In this plant numerous morphological changes take place with maturity, including leaf form and phyllotaxy, growth habit, and shoot and root development. During juvenile phase the plant will not flower even if exposed to suitable environmental conditions. Probably juvenile phase of the plant is required so that there are sufficient number of photosynthetically efficient leaves which are able to support flower development and seed setting later on. The transition of shoot apex from juvenile to the adult phase is affected by transmissible factors from the rest of the plant. Carbohydrate supply may play a major role in the transition from juvenility to maturity. Gibberellins are another important factor responsible for phase change. Treatments, like removal of roots, water stress, and nitrogen starvation, may result in accumulation of gibberellins in the plant. In *Arabidopsis*, carbohydrate is transmitted as a small signaling molecule—trehalose-6-phosphate—a disaccharide. This molecule

activates flowering pathway in the shoot apex. Factors such as exposure to low light conditions prolong juvenility or may cause reversal to juvenility. Transition from juvenile to adult phase (vegetative phase change) is influenced by environmental factors such as day length, light intensity, ambient temperature, and gibberellic acid. The signals that trigger phase change are perceived by leaf primordia. Transition from juvenile to adult phase shares some regulatory steps with reproductive phase transition. Two evolutionary conserved microRNAs (miRNAs), i.e., miR156 and miR172, and their targets have been identified as key components of genetic control mechanisms that underlie phase changes. miR156 targets the transcripts of the gene which have been found to promote transition from juvenile to adult phase. Level of miR156 declines with increasing age of the plant. miR172 targets the mRNA that encodes proteins which promote transition to flowering and floral development. Studies in *Arabidopsis* indicate miR156 promoting juvenile phase and delaying adult phase. Expression of miR172 appears to be under photoperiodic control.

After the plant has acquired the competence to flower, the perceived inductive signal is sent to the vegetative shoot apex which triggers transition of shoot bud to the reproductive form. Recently, genes have been identified that play crucial role in the formation of flowers, indicating that reproductive development in plants is genetically controlled. SAM becomes an inflorescence meristem when it produces structures like bracts and floral meristem instead of leaf primordia or stem. A cascade of gene expression leads to transition from SAM to floral meristem, which has been well established in the model plant, *Arabidopsis thaliana*. The *embryonic flower* (*EMF*) gene of *Arabidopsis* prevents early flowering. Mutant plants that lack EMF protein flower as soon as they germinate, indicating that wild-type allele is responsible for suppression of flowering. These observations suggest that flowering is a default state, and plants have evolved mechanisms to delay flowering. This delay allows plants to store more energy which may be allocated for reproduction at a later stage. Another example of inducing juvenile to adult transition comes from overexpressing a gene for flowering, namely, LEAFY (LFY). This gene was cloned in *Arabidopsis*, and its promoter was replaced with a viral promoter that results in high levels of LFY transcription. LFY with its viral promoter was then introduced to cultured aspen cells that were used to regenerate plants. When LFY is overexpressed in aspen, flowering occurs in weeks instead of years. Phase change thus requires both a strong promotive signal and the ability to perceive the signal. The final outcome depends on the increase in promotive signal or decrease in inhibitory signal in conjunction with production of adequate receptors on the shoots to perceive the signal.

25.2 Flower Induction

Transition to flowering involves major changes in the pattern of morphogenesis and cell differentiation at the shoot apical meristem (SAM), which is accompanied with reallocation of biomolecules and associated energy to the shoot tip to initiate floral differentiation. After the plant has acquired competence to flower, it is able to

perceive environmental or endogenous stimulus. The environmental factors which have been specifically monitored are day length and temperature. The endogenous factors include carbohydrates (nutrients), hormones, and circadian rhythm. The interaction of external and internal factors enables plants to synchronize their reproductive development with the environment. Some plants exhibit an absolute requirement for specific environmental conditions in order to flower. Such floral induction responses are referred as **obligate** or **qualitative** responses. If flowering is promoted by certain environmental conditions but eventually occurs in the subsequent absence of such conditions as well, the flowering response is said to be **facultative** or **quantitative.** There are plants which flower strictly in response to internal developmental factors and do not depend on any particular environmental condition. These are said to exhibit **autonomous regulation**, such as garden pea in which retuning of vegetative apex to form flowers is genetically determined. There can be early cultivars or late-flowering cultivars. Four genetically regulated pathways leading to flowering have been identified: (i) light-dependent pathway, (ii) temperature-dependent pathway, (iii) gibberellin-dependent pathway, and (iv) the autonomous pathway. Plants can rely on one of these pathways, but all the four pathways may be essential for floral induction.

25.3 Photoperiodism: The Light-Dependent Pathway

Flowering is so predictable in plants that it is used as a floral calendar. As we know that roses bloom in summer and chrysanthemums in winter. It is generally the length of day that gives the most reliable indication of advancing season. An organism's capacity to measure day length is known as **photoperiodism.** Initial experiments on photoperiodism were conducted by a French scientist J. Tournois in 1912. He observed that *Cannabis* plants flower vigorously when planted early in the spring but remain vegetative if planted in late spring or summer. He concluded that shortening of day length was not as important for early flowering as lengthening of night. At about the same time, George Klebs from Germany demonstrated that *Sempervivum funkii* could be induced to flower in winter in greenhouse when exposed to artificial light although normal time is June. First clear-cut hypothesis of photoperiodism was given by W.W. Garner and H.A. Allard from the US Department of Agriculture (Beltsville, Maryland) in 1920. They observed that *Biloxi* soybean flowers around same time in September/October even if it is germinated over a 3-month period from May to July, i.e., irrespective of how long they have been growing, they flower around same time. Garner and Allard hypothesized a seasonal timing mechanism in soybean. They also observed flowering response of tobacco (Maryland strain) which normally flowers in summer. A mutant of the plant called Maryland Mammoth was observed to grow up to the height of 3–5 m in summer without any flowering. The plants growing in green house under relatively short photoperiods flowered profusely in mid-December when the relative length of day was shorter than the length of the dark period. The mutants could be made to flower when exposed to short-day length next year in summer by placing the plants

in darkness after placing the plants in light equivalent to that of winters. These observations lead to the discovery of the phenomenon as well as for the coining of the term "photoperiodism" by Garner and Allard. These observations also lead to the fact that plants vary considerably in their response to day length.

25.3.1 Critical Day Length

On the basis of photoperiodic requirement for floral induction, plants have been classified under different categories. **Short-day plants (SDPs)** will flower if the day length is shorter than a critical photoperiod. Hillman (1959) showed that SDPs are capable of flowering even if kept continuously in dark provided with adequate sucrose. This shows that the SDPs require light only for carrying on photosynthesis. Examples of SDPs are soybean, poinsettia, potato, sugarcane, cosmos, chrysanthemum, etc. **Long-day plants (LDPs)** require a photoperiod of more than a critical length which varies from 14 to 18 h. The best flowering usually occurs in continuous light. A flash of light during a long dark period can induce flowering even under short- day conditions. Since dark phase has inhibitory effect on flowering, these plants can also be called as *short-night plants*. Examples of this category are spinach, lettuce, radish, alfalfa, sugar beet, larkspur, etc. The critical value of the photoperiod requirement is not absolute rather varies according to species (Fig. 25.3). In **day-neutral plants**, flowering is not affected by day length. For example, tomato, cucumber, cotton, pea, and sunflower. Within this category, there are obligate or facultative requirements for a particular photoperiod. Plants having absolute requirement for a particular photoperiod for flowering are called **qualitative photoperiod** types. For example, *Xanthium strumarium* does not flower unless it receives an appropriate short photoperiod. It is a qualitative SDP. In quantitative SDPs, flowering is accelerated by short days, e.g., *Cannabis sativa* (hemp) and *Helianthus annuus* (sunflower). Spring cereals, like *Triticum aestivum* (spring wheat) and

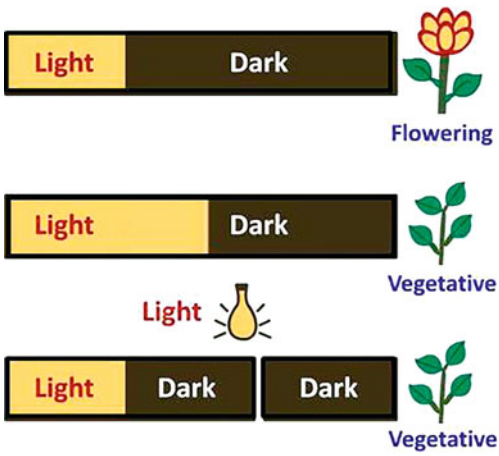

Fig. 25.3 Photoperiodic control of flowering. Decrease in critical dark period leads to vegetative stage. Also a flash of light during dark period inhibits flowering

Secale cereale (winter rye), are quantitative LDPs. They do flower under short days, but flowering is accelerated under long days. Qualitative LDPs include *Hyoscyamus niger* (black henbane) and *Arabidopsis thaliana*. Photoperiod requirement is often modified by external conditions like temperature. There are also other response types in which plants respond to long and short days in some combination. Thus, *Bryophyllum* is a **long-short-day plant**. It flowers when a certain number of short days are preceded by a specific number of long days. *Trifolium repens* exhibits a reverse condition of **short-long-day plant**. Some plants, like winter cereals, require a low temperature treatment before they become responsive to photoperiod, while others may have a qualitative photoperiodic requirement at one temperature but a quantitative requirement at another temperature. Some plants are **intermediate-day length** plants. They flower in response to day length of intermediate range but remain vegetative when the day is too long or too short. Interestingly, flowering is delayed in *Madia elegans* under intermediate-day length (12–14 h) but occurs under day length of 8 or 18 h. It may be noted here that this classification is based on whether a particular plant will flower when subjected to photoperiod that exceeds or is less than a critical length.

25.3.2 Critical Role of Dark Period

Plants neither measure relative length of day and night nor the length of photoperiod. They measure the length of dark period. This was demonstrated by K.C. Hammer and J. Bonner (1938) in experiments conducted with *Xanthium*. In a 24 h cycle of light and dark periods, *Xanthium* flowers only when dark period exceeds 8.5 h but remains vegetative when provided with 16 h of light followed by 8 h of dark (Fig. 25.4). Similarly, long-day plants require a dark period shorter than some critical maximum. In LDPs, a flash of light in the middle of an otherwise noninductive long dark period will shorten the dark period requirement to less than the maximum and permit flowering to occur. Measuring the time of dark period is central to photoperiodic time keeping.

25.4 Photoinductive Cycle

In nature, plants are exposed to photoperiodic cycles which consist of alternate periods of light and dark diurnally (Fig. 25.5). Any photoperiodic cycle which induces flowering in a plant is called **photoinductive cycle**. On the contrary the photoperiodic cycle which does not induce flowering in a plant is non-photoinductive cycle. A photoperiodic cycle consisting of 16 h light and 8 h dark period generally induces flowering in LDPs, while a cycle consisting of 8 h light and 16 h dark period induces flowering in SDPs (Fig. 25.6). The number of cycles required to induce flowering in a plant varies. One SD photoinductive cycle is sufficient to induce flowering in *Xanthium strumarium* and *Pharbitis nil*, while *Salvia occidentalis*, a SDP, may require at least 17 cycles. *Plantago lanceolata*, a

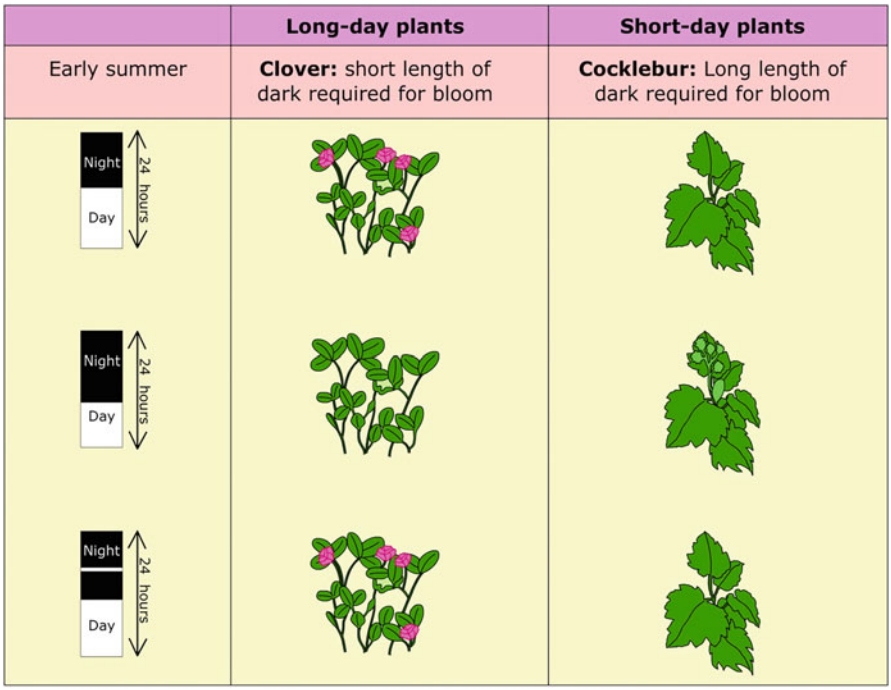

Fig. 25.4 Critical role of dark period in long-day and short-day plants

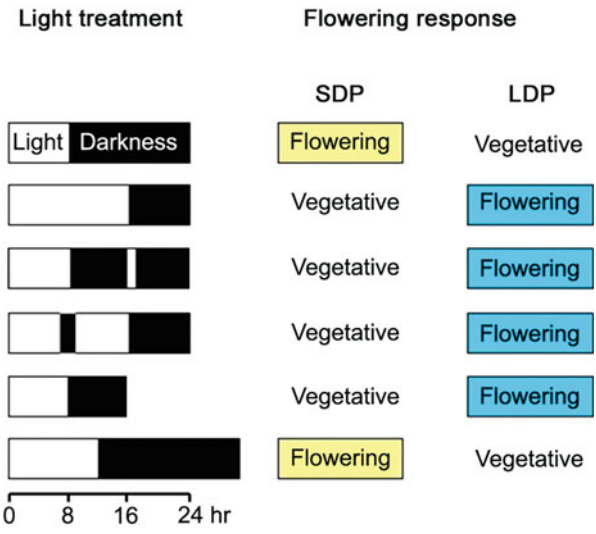

Fig. 25.5 Flowering response in LDP and SDP in 24 h cycle

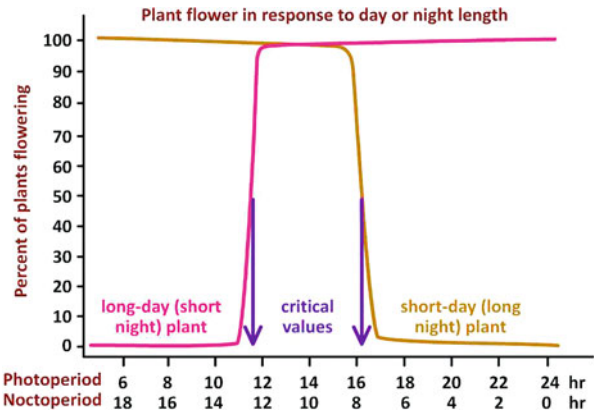

Fig. 25.6 Categorization of plants according to their response toward length of day and night

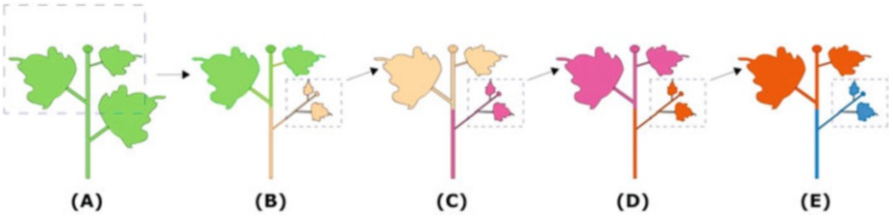

Fig. 25.7 Transmissible nature of florigen as depicted through grafting experiments in *Xanthium*. (**a**) Grown under short-day condition, (**b–e**) grown under long-day condition

LDP, requires 25 photoinductive cycles for maximum floral response. If the plant is returned to non-photoinductive cycle after ten cycles, it will not flower. However, if returned to photoinductive cycle, only 15 cycles are required. This indicates that some factor responsible for flowering response gets accumulated during inductive cycle.

25.4.1 Perception of Photoperiodic Signal and Florigen

The photoperiodic signal for floral induction is perceived by the leaves and not by SAM. This was demonstrated in experiments conducted by the Russian plant physiologist M. Chailakhyan in 1937. He reported flowering in *Chrysanthemum morifolium*, a SDP, when a leafy portion of the plant was subjected to short days and the apical meristem and defoliated portion of the shoot were subjected to long days. However, the plants remained vegetative when conditions were reversed, i.e., the upper defoliated portion kept in short days and the leafy portion in long days (Fig. 25.7). In another set of experiments, SDPs *Perilla* and *Xanthium* could be induced to flower even when all the leaves had been removed except one leaf which was kept in SD conditions. When leaves taken from the induced plants were grafted to non-induced ones, it resulted in induction of flowering in the non-induced ones.

Even the excised leaves of *Perilla frutescens* (SDP), when exposed to photoinductive cycle and grafted back to non-induced plants, induced flowering even when plants were maintained under noninductive long-day conditions. A rapidly expanding leaf is most sensitive to perceive the photoperiodic stimulus when it is half of its final size. Even when several *Xanthium* plants were joined to each other through grafts, all plants flowered even when only the first plant was exposed to short days. From these experiments Chailakhyan suggested that the floral stimulus might be a hormone which could diffuse through graft union. He called this stimulus as **florigen**. Grafting was also done in between the plants belonging to the same family but having different photoperiodic requirements, such as between SDP *Nicotiana tabacum* and LDP *Hyoscyamus niger*. *Hyoscyamus niger* flowers under short days if tobacco is kept under short days. Conversely, the grafted tobacco plants flower if *Hyoscyamus* is kept under long days. This experiment indicates that floral stimulus might be same in all photoperiodic classes. Chailakhyan proposed flowering stimulus to be a hormone, which he called **florigen**. He proposed **florigen** to be synthesized in leaves and transmitted to the shoot apex. Attempts to isolate and identify florigen remained unsuccessful until a protein encoded by **FLOWERING LOCUS T (FT)** was identified as a major component of the mobile signal in *Arabidopsis*. FT was found to contain phosphatidylethanolamine-binding domain which, in mammals, is involved in kinase signaling and mediates protein-protein interaction. In *Arabidopsis*, FTmRNA expression in the companion cells of the phloem in leaves triggers flowering when FT protein is transported to the apical meristem through phloem sieve elements where it interacts with bzip transcription factor encoded by **FLOWERING LOCUS D (FD)**, and it is responsible for the regulation of genes involved in the change of vegetative meristem to produce flowers. The florigen model was replaced by **nutrient diversion hypothesis**. According to this hypothesis, an inductive treatment stimulates the flow of nutrients into the apical meristem. A high level of nutrients has been found to stimulate flowering. This hypothesis is based on the observation that induction of flowering in white mustard (*Sinapis alba*), a LD plant, gives rise to a rapid and transient increase in the export of sucrose from leaves to the shoot apex. The third hypothesis, the **multifactorial hypothesis**, proposes that flowering occurs when a number of factors, including promoters, hormones, and nutrients, are present in the apex at an appropriate time and in appropriate concentrations. This hypothesis points at multiple genes that control flowering, out of which some genes respond to photoperiod and temperature, while others act independent of environment.

25.5 Circadian Rhythm

In addition to photoperiodism, plants also display other time measuring systems. Endogenous rhythms persist even when plants are placed in constant environmental conditions. They are based on a cycle of approx. 24 h and are known as circadian rhythms (circa = about, diem = day). Circadian rhythms are synchronized with the daily day-night cycle, which is known as **entrainment**. Erwin Bunning (1936)

proposed that daily rhythms consist of two phases, i.e., **photophil phase** (light-loving phase) and **skotophil phase** (dark-loving phase). Photophil phase is characterized by intensive photosynthesis and weak respiration (anabolic processes predominate). On the contrary, skotophil phase is characterized by intensive respiration. In this phase, hydrolytic activity increases, and decomposition of starch into sugars takes place (predominance of catabolic processes). According to *Bunning hypothesis*, the two phases alternate about every 12 h. Under constant environmental conditions, photophil phase would probably correspond to subjective day, while skotophil phase is equivalent to subjective night. The ability of light to promote or inhibit flowering depends on the phase in which light is given. When light signal is applied during light-sensitive phase of the rhythm, the effect is either to promote flowering in LDPs or to prevent flowering in SDPs. In an experiment, *Chenopodium rubrum* plants (a SDP in which exposure to single photoinductive cycle is sufficient to induce flowering) were shifted to 72 h. dark period after being exposed to a photoperiod. Two minutes of night break was given at different time intervals in the dark period before transferring the plant to continuous light. Inhibition of flowering was most effective if night break was given at 6, 33 or 60 h after the start of dark period. This is the time when the plant might have been in darkness in a normal 24 h cycle, i.e., skotophilous phase. However, night breaks do not result in inhibition of flowering if the night breaks are given near 18 and 46 h after the start of dark period. This is the time when plant would have been in light in a 24 h cycle, i.e., photophil phase. This indicates interaction of photoinduction with endogenous rhythm of the plant. Flowering in both LDPs and SDPs is induced when light exposure is coincident with the appropriate phase of the rhythm. Some kind of regulating mechanism is present which is called circadian regulator. Bunning's hypothesis has evolved into **coincidence model**. According to this model, a key regulator accumulates in LDPs and reaches a maximum concentration during LDs. The regulator also requires light for its activation, i.e., the presence of light coincides with the accumulated regulator, followed by cascade of events leading to flowering. In *Arabidopsis* (a quantitative LDP), the genes which have been identified and characterized as key regulators of flowering include GIGANTEA (GI), CONSTANS (CO), and FLOWERING LOCUS (FT). Isolation of a mutant (*co*) of *Arabidopsis*, in which flowering was delayed under LD but without affecting the response under SD, leads to identification and isolation of CO gene. The gene has been found to be a key regulator in photoperiodic control of flowering. In *Arabidopsis*, mRNA for CO (which encodes a nuclear zinc-finger transcription factor) starts accumulating and reaches a peak in LD and is translated in light. CO protein is stabilized by exposure to blue and FR light which is absorbed via the pigments CRY2 (cryptochrome) and PHYA (phytochrome), respectively. CO expression and activation of FT gene occur in the companion cells. As a result, FT protein is transported to the shoot apex. Thus, flowering in *Arabidopsis* occurs only when transcription and translation of CO gene coincide with exposure to light, which occurs under LD. There is an overlapping (coincidence) between CO mRNA synthesis and day light so that light can permit active CO protein to accumulate to a level that promotes flowering. Thus, rhythmicity of accumulation of COSTANS mRNA in photoperiod and its light-dependent

translation to CO protein provide the molecular basis for external coincidence model. Interestingly, FT is a target gene downstream of CO. FT is expressed in the companion cells. Thus, CO activity is mediated by the expression of FT. Movement of FT from the companion cells to the sieve elements requires ER-localized protein called FT INTERACTING PROTEIN (FTIP1). Once in floral meristem, FT protein enters the nucleus and forms a complex with bzip transcription regulator FD, which is encoded by the gene FLOWERING LOCUS D (FD). FT-FD activates expression of **floral meristem-identity** genes, the MADS box transcription factors, such as SUPPRESSOR OF OVEREXPRESSION OF CONSTANS-1 (SOC1) and AP1 (Fig. 25.9). These genes specify that the vegetative shoot meristem of the plant gets differentiated into floral meristem. Investigations have been undertaken on the flowering behavior of rice (SDP) plants. The major genes, i.e., CO and FT, which have regulatory function in *Arabidopsis*, are conserved in rice (SDP). However, their specific regulation has been altered by evolution to promote flowering under short days. The genes **Heading-date1 (Hd1)** and **Heading-date3a (Hd3a)** are homologous to *Arabidopsis* CO and FT, respectively. Similar to FT in *Arabidopsis*, overexpression of Hd3a in rice results in rapid flowering irrespective of photoperiod. Besides the expression of FT in *Arabidopsis* and that of Hd3a gene in rice, flowering is elevated during the inductive photoperiods, i.e., LD and SD, respectively. However, unlike in *Arabidopsis* (LDP), where coincidence of CO with light period promotes flowering in rice, coincidence of Hd1 expression with the light period suppresses flowering since Hd1 acts as the suppressor of Hd3a. The lack of coincidence between Hd1mRNA expression and day light prevents accumulation of Hd1 protein, which acts as a repressor of the gene encoding the transmissible floral stimulus, Hd3a, in rice. In the absence of the Hd1 protein repressor, Hd3a mRNA is expressed, and the protein it encodes is translocated to the apical meristem where it causes flowering. Under long days (sensed by phytochrome), the peak of Hd1 mRNA expression overlaps with the day, allowing the accumulation of the Hd1 repressor protein. As a result, HD3a mRNA is not expressed, and the plant remains vegetative.

25.6 Photoreceptors

Phytochrome and cryptochrome play important roles in photomorphogenesis of plants. One of the best studied SDPs in terms of effect of light on flowering is *Pharbitis nil*. It is a qualitative SDP in which 4–5-day-old cotyledonary photoresponsive tissue can receive the stimulus for floral induction when given a single photoinductive cycle. In experiments with this plant, night breaks given during photoinductive dark period, which prevent attainment of critical dark period, inhibit flowering. Night breaks were found to be most effective if red light was used. However, the effect was reversed if red light treatment was immediately followed by exposure to far-red light. The photoreversible effect of R/FR light suggested the role of phytochrome (Fig. 25.8). Phytochrome comprises of nuclear encoded proteins. The *Arabidopsis* genome encodes five phytochromes (PHYA to PHYE) that are

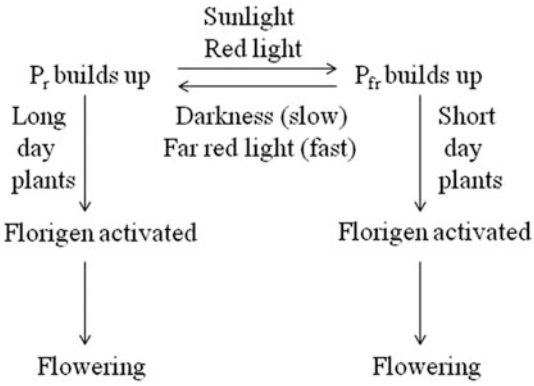

Fig. 25.8 Influence of phytochrome on flowering

involved in floral induction. Late-flowering mutants (phyA) are defective in genes that promote flowering, while early-flowering mutants (phyB) are defective in genes that ordinarily repress flowering.

Blue light promotes effect on flowering in LDPs, especially in members of family Cruciferae. Two members of cryptochrome gene family (CRY1 and CRY2) are present in *Arabidopsis*. Cryptochromes are flavoproteins that act as the blue light receptors. Both CRY1 and CRY2 function in stabilizing CO protein along with PHYA toward the end of light period, whereas in other plants this role is taken up by PHYA alone. CRY2 mutants of *Arabidopsis* flower later than the wild type under inductive long days. Under continuous white light exposure, phy1 mutant (any type of phytochrome cannot be synthesized in them because of defective enzyme, which is required for synthesis of chromophore of the pigment) plants flower similar to the wild types. This indicates that in continuous white light exposure, no phytochrome is required, and the blue light receptor is involved. Mutation in one of the cryptochrome genes (CRY2) causes a delay in flowering.

According to the coincidence model, CO gene is expressed during light period. The effect of light on CO stability further depends on the photoreceptor involved. In morning hours (after dark), phyB signaling enhances CO degradation, whereas in the evening (when CO protein accumulates after long day), cryptochromes and phyA antagonize this degradation and allow CO protein to build up. CO, a transcriptional regulator, promotes flowering by stimulating the expression of a key floral signal, FLOWERING LOCUS T (FT).

25.7 Vernalization

In many long-day plants, exposure to low temperature is critical for the acquisition of competence to respond to photoinductive conditions for flowering. This cold temperature requirement is called **vernalization**, which acts as a time computing mechanism that measures the passage of winter and ensures that flowering does not begin until the favorable conditions of spring arrive. The concept was introduced

by T. D. Lysenko (1920) who observed the ability of cold treatment to make the winter cereal behave as spring cereal. This could be of practical utility like: (1) crops can be harvested much earlier, (2) crops can be grown in regions where they are not naturally productive, and (3) plant breeding experiments can be accelerated. Generally, it is the stem apex which perceives the cold temperature signal. The dividing cells in plants perceive vernalization stimulus. Period of chilling can vary from few days to weeks and from plant to plant, but longer exposure to low temperature will be more effective for early flowering. Response due to vernalization decreases if it is interrupted by heat treatment. In contrast to photoperiodic effect, which leads to flower initiation, vernalization prepares plants for flowering. G. Melchers and A. Lang (1948) demonstrated that the biennial LDP *Hyoscyamus niger* (which requires a low temperature season before flowering unlike the annual type which flower in one season) should be at least 10 days old before becoming responsive to the low temperature treatment. However, Gregory and Purvis in 1930s suggested that hydrated seeds of Petkus winter rye (*Secale cereale*) may be vernalized making them sensitive to LD photoperiod. The cold treatment of the seeds reduces the number of photoinductive period required for flowering since the Petkus winter rye does not have obligate requirement for vernalization. That vernalization is an energy-dependent process was demonstrated in an experiment in which excised embryos were supplemented with carbohydrates and oxygen. Melchers had demonstrated that vernalization stimulus could be transmitted through graft union. He was the first to coin the term vernalin for the hypothetical active factor required for vernalization. It was observed that once a plant has been vernalized, it remembers the cold treatment throughout its life. The memory is maintained in cell derived from the induced cell through mitotic division but not the one which are derived through meiotic division. Lang stated a direct connection between vernalin and florigen.

$$\text{Low Temperature} \rightarrow \text{Vernalin} \rightarrow \text{Florigen}$$

One of the pathways for flowering is through vernalization, where low temperature treatment leads to accumulation of vernalin which in turn stimulates the flowering stimulus florigen. Vernalization affects competence of a plant to flower by bringing about stable changes in the pattern of gene expression in the meristem after cold treatment. Such changes are termed as epigenetic changes. Requirement of vernalization is conferred by two genes, FRIGIDA (FRI) and FLOWERING LOCUS C (FLC). FRI acts in upregulation of FLC. FLC encodes MADS- domain DNA-binding protein that functions as a repressor of flowering. Levels of FLC are the primary determinant of vernalization requirement in *Arabidopsis*. It is highly expressed in the shoot apical meristem of non-vernalized plants. It represses flowering by repressing the expression of **floral integrators**, such as FT, FD, and SOC1. Floral integrators are the genes that are involved in regulation of meristem-identity genes. These are so named because these integrate the floral stimulus which is due to some environmental cues and trigger the vegetative to reproductive transition. Binding of FLC with the promoters of SOC1, FD, and FT decreases the ability of the photoperiods to activate these integrators. During vernalization FLC is

epigenetically switched off for the rest of plant's life cycle. These are stable changes in gene expression that do not involve alterations in DNA sequence and which can be passed on to descendent cells through mitosis or meiosis. This is achieved by repressive changes in FLC which includes **chromatin remodeling**. This includes histone methylation of lysine-27 and lysine-9 residues which are characteristics of heterochromatin, and acetyl groups are removed from lysine-9 and lysine-14 of H3 which otherwise are characteristics of euchromatin. Thus, low temperature induces conversion of FLC from active to inactive form. The importance of histone modification was further clarified after mutants of *Arabidopsis* have been identified which do not respond to vernalization. These mutants included *vernalization insensitive* (*vin*) and *vernalization* (*vrn*) mutants. These mutants prevent vernalization and alter histone modifications. Thus, photoperiod pathway, vernalization pathway, and autonomous pathway form a regulatory network which converges to modulate the activities of a set of genes that integrate the floral stimulus and trigger the transition from vegetative to reproductive phase (Fig. 25.9).

25.8 Role of Gibberellins

Gibberellins play important role/s during transition of vegetative to reproductive meristem. This includes their role in competence, promotion of bolting, and flowering in *Arabidopsis* and many other long-day plants. Flowering in perennial species tends to be insensitive to gibberellins. In an interesting observation, when extract from photoinduced leaves of *Xanthium* is applied, it induces flowering in *Lemna* kept under noninductive conditions. However, the extracts need to be supplemented with gibberellin. The leaf extract alone or gibberellin alone has no effect. Extract prepared from spinach leaves grown under short days suggests that a critical step in GA biosynthesis is inhibited. Plants remain vegetative and rosetted under short days. This shows that gibberellin is partially responsible for flowering. There is a possibility that GA is a mobile signal that transmits the photoperiodic floral stimulus and its action is independent of FT, the phloem mobile protein that relays the floral induction signal from leaf to shoot apex. Expression of both SOC1 and LFY in *Arabidopsis* is promoted by GA via DELLA-mediated signaling mechanism. SOC1 is thus regulated in a multifactorial manner and integrates the autonomous, vernalization, and GA pathways (Fig. 25.10). Chailakhyan stated that vernalin hormone may be a precursor of gibberellin. Under long-day conditions, it is converted to gibberellin. Another hormone called **anthesin** is present in long-day plants which, along with vernalin, causes flowering in long-day plants. In short-day conditions, vernalin is not converted to gibberellin. Hence, flowering does not occur. Gibberellin treatment to long-day non-vernalized plants kept under long day leads to flowering as these plants possibly contain anthesin. Gibberellin is ineffective in flower induction in short-day plants as they lack anthesin. Auxin application induces flowering in pineapple and litchi. In pineapple, the effect of auxin may be due to stimulation of ethylene production.

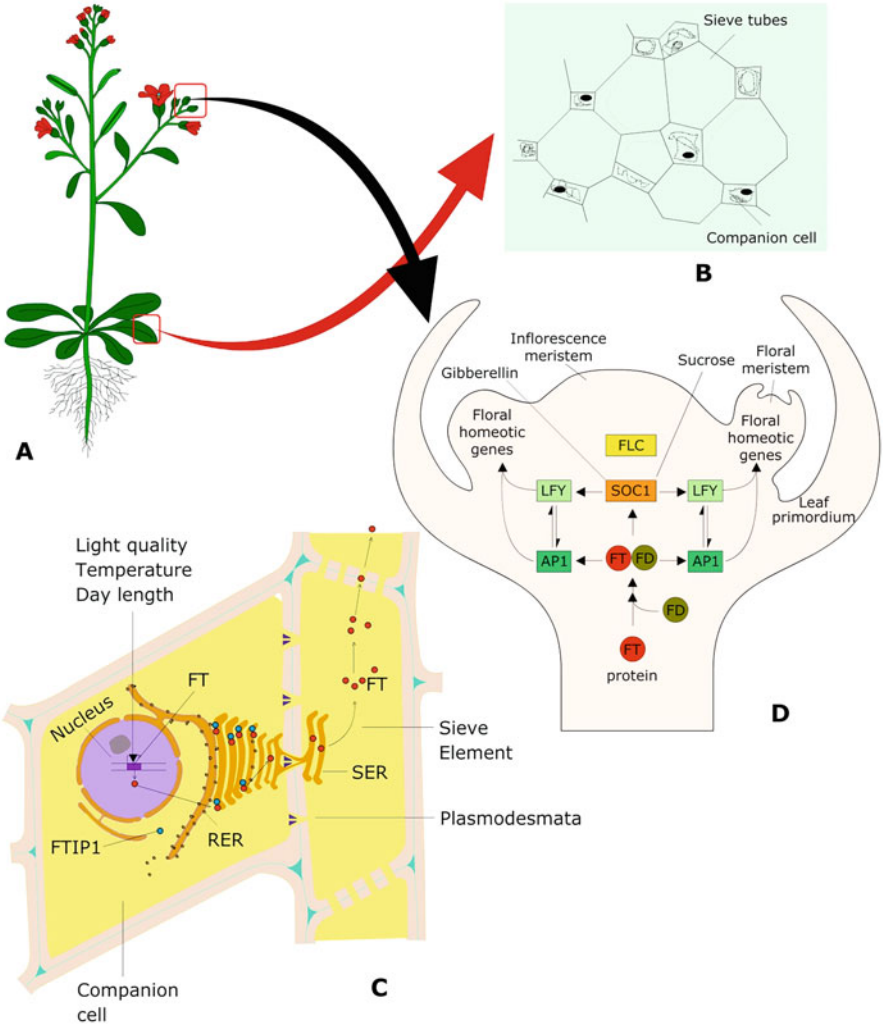

Fig. 25.9 Flowering is regulated by multiple factors in *Arabidopsis* (**a**); (**b**) FT mRNA is expressed in companion cells of leaf vein in response to multiple signals, including day length, light quality, and temperature; and (**c**) FTIP1 mediates through a continuous ER network between the companion cells and the sieve tube elements. FT moves in the phloem from the leaves to the apical meristem. (**d**) FT is unloaded from the phloem in the meristem and interacts with FD. Then FT-FD complex activates SOC1 in the inflorescence meristem and AP1 in the floral meristem, which triggers LFY gene expression. LFY and AP1 trigger expression of the floral homeotic genes. The autonomous and vernalization pathways negatively regulate FLC, which acts as a negative regulator of SOC1 in the meristem and as a negative regulator of FT in the leaves. *FD* FLOWERING LOCUS D, *FT* FLOWERING LOCUS T, *FTIP1* FT-interacting protein 1, *SOC1* suppressor of constans1, *AP1* apetella1, and *LFY* leafy

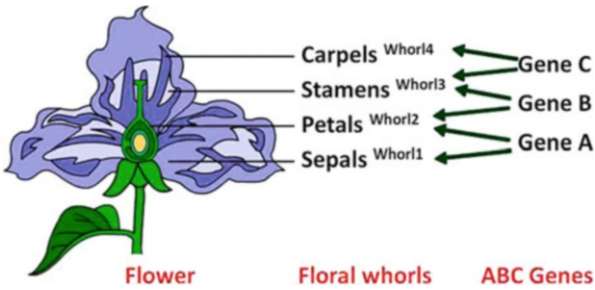

Fig. 25.10 ABC model whereby floral organ identity is controlled by three homeotic genes, namely, A, B, and C

25.9 Flower Development

Two categories of genes are responsible for flower development ,viz., floral meristem identity genes and floral organ identity genes. The floral meristem identity genes are responsible for the transition of vegetative meristem to floral meristem. In *Arabidopsis*, these genes include LEAFY (LFY), FLOWERING LOCUS D (FD), SOC1, and APETALA1 (AP1). LFY, FD, and SOC1 play a critical role in integrating the signals—both environmental and internal. These genes act as master regulators for the initiation of floral development. Floral meristems can be distinguished from vegetative meristem by its larger size. The transition from vegetative to reproductive phase is marked by an increase in the frequency of cell division within the central zone of shoot apical meristem. Four different types of floral organs are initiated in separate whorls, namely, sepals, petals, stamens, and carpels. They develop in concentric rings called **whorls**, numbered 1, 2, 3, and 4, respectively. Molecular basis of floral morphogenesis has been studied extensively in *Arabidopsis*. Floral organ identity genes were discovered in **homeotic gene** mutants. Homeotic genes encode transcription factors that determine the location where specific structures develop. Five key genes have been identified in *Arabidopsis* which specify floral organ identity, namely, APETALA1 (AP1), APETALA2 (AP2), APETALA3 (AP3), PISTILLATA (P1), and AGAMOUS (AG). Influence of organ identity genes on floral development in *Arabidopsis* can be understood by loss-of-function mutants of these genes. Mutations in these genes change the floral organ identity without affecting the initiation of flowers. The genes that determine the four basic whorls in flower have been grouped into three classes, A, B, and C. Each group does not necessarily represent a single gene. This view is expressed as **ABC model**. This model postulates that organ identity in each whorl is determined by a unique combination of the activities of three organ identity genes (Fig. 25.10). Type A gene alone specifies sepals, while A and B together are required for petal formation. Genes of B and C category are required for stamen differentiation, while type C genes are responsible for carpel formation (Fig. 25.11). According to ABC model, Class A and C genes are mutually repressive to each other. Loss of type A activity (encoded by AP1 and AP2) results in the formation of carpels instead of sepals in the first whorl and stamens instead of petals in the second whorl. Loss of

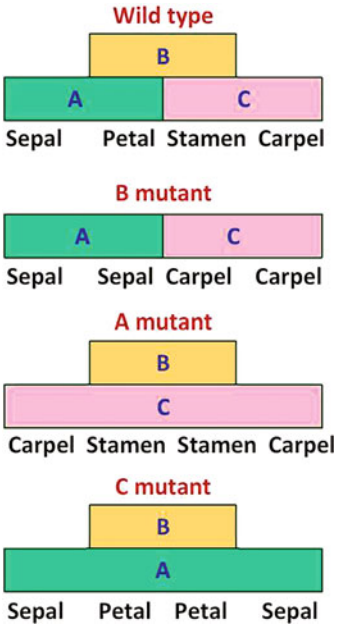

Fig. 25.11 ABC model that postulates when C-function is lost, A extends into whorl 3 and 4, leading to development of sepals and petals in whorl 3 and 4. Similarly, loss of A gene leads to extension of expression of C gene and formation of carpels and stamens in whorls 1 and 2

type B activity (encoded by AP3 and PI) results in the formation of sepals instead of petals in the second whorl and carpels instead of stamens in the third whorl since the genes belonging to this category control organ determination in the second and third whorl. Type C gene (AG) controls events in the third and fourth whorl. Loss of type C gene activity results in the formation of petals instead of stamens in the third whorl and replacement of fourth whorl by a new flower such that this whorl is occupied by sepals (Figs. 25.12 and 25.13).

These homeotic genes encode transcription factors which are consistent with their function in specifying organ identity. It is thought that each combination of A, B, or C class of transcription factors regulates a set of target genes required for the development of the corresponding organs. All homeotic genes, except AP2, encode MADS-domain transcription factors, which are characterized by a highly conserved, N-terminal DNA-binding domain unique to plants. Furthermore, expression of A, B, and C genes alone is insufficient for converting a leaf into floral organ. But, when they are expressed together with SEPALATA genes (SEP1, SEP2,SEP3), vegetative leaves are converted into floral organs dependent upon combination of A,B, and C class of genes co-expressed with SEP 1/2/3 (Fig. 25.14). The SEP genes are redundant, and only the triple SEP 1/2/3 are required for the normal development of petals, stamens, and carpels and are referred to as E class of genes. SEP genes also encode MADS-domain transcription factors that interact with other MADS-domain proteins. Thus, **ABCE** model was formulated based on genetic experiments in *Arabidopsis thaliana* and *Antirrhinum majus*. According to the ABCE model, carpel formation requires the activities of the C and E class of genes. However, a third group of MADS-box genes are required for ovule formation. These ovule-specific

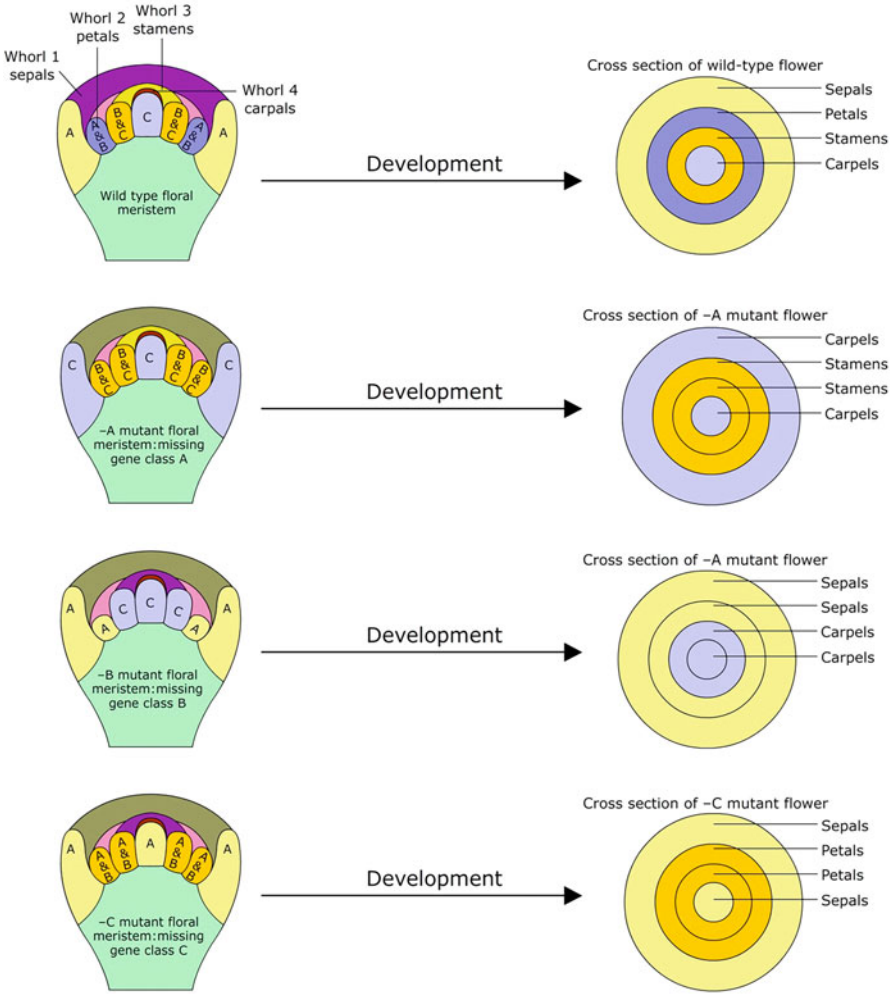

Fig. 25.12 Letters within the whorls indicate active genes. In case of loss of function of A, the role of C expands to the first and second whorls; in case of loss of B gene activity, the outer two whorls will have function of A; loss of function of C, A expands into the inner two whorls

genes are called class D genes. Since ovule is a structure within the carpel, class D genes are not basically organ identity genes. They were first discovered in *Petunia*. Silencing of two MADS-box genes is known to be involved in floral development in *Petunia*, FLORAL BINDING PROTEIN 7/11 (FBP 7/11), results in the growth of style and stigma in a location normally occupied by ovule. When FBP11 is overexpressed in *Petunia*, ovule primordia are formed on the sepals and petals. Thus, class D genes are required for normal ovule development.

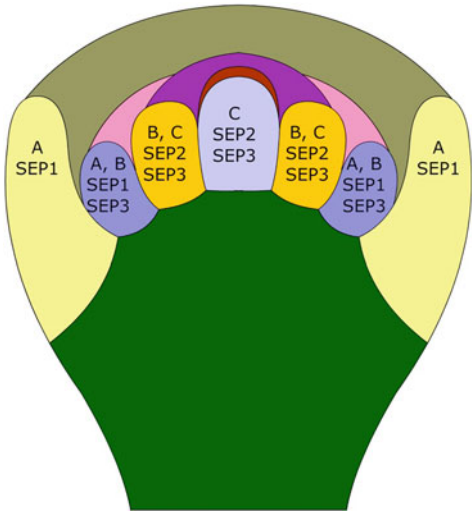

Fig. 25.13 Mutations in all three SEP genes lead to formation of four whorls of leaves. Class E genes are needed to specify floral organ identity

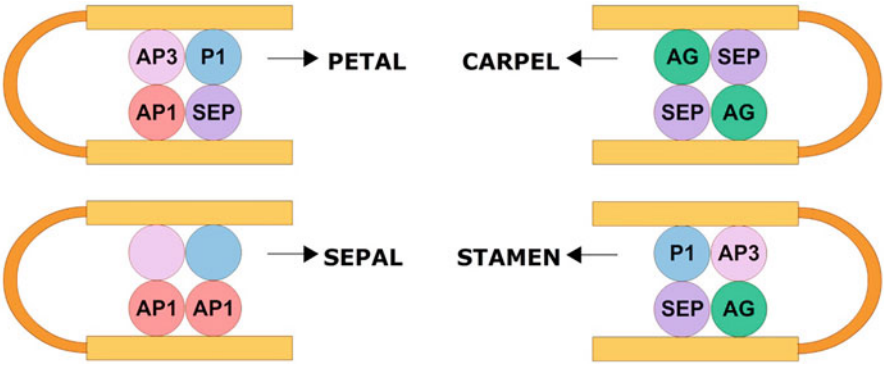

Fig. 25.14 The quartet model explains that floral organ identity genes function as heterotetramers, i.e., they form complexes consisting of four proteins

MADS-domain-containing transcription factors, encoded by floral organ identity, function as heterotetramers, i.e., they form complexes consisting of four proteins (**quartet model**). Each quartet consists of a set of MADS-domain proteins specifying a particular organ and controls a set of target genes required for the formation of specific organ. In other words, identity of different floral organs is determined by four combinations of floral homeotic proteins, known as MADS-box proteins. **MADS-box proteins** are transcription factors which operate by binding to the promoter region of target genes, which are activated or repressed for the development of floral organs. According to quartet model, two dimers of each tetramer recognize two different DNA sites on the same strand of DNA. These two sites are brought close by the bending of DNA. For example, the quartet

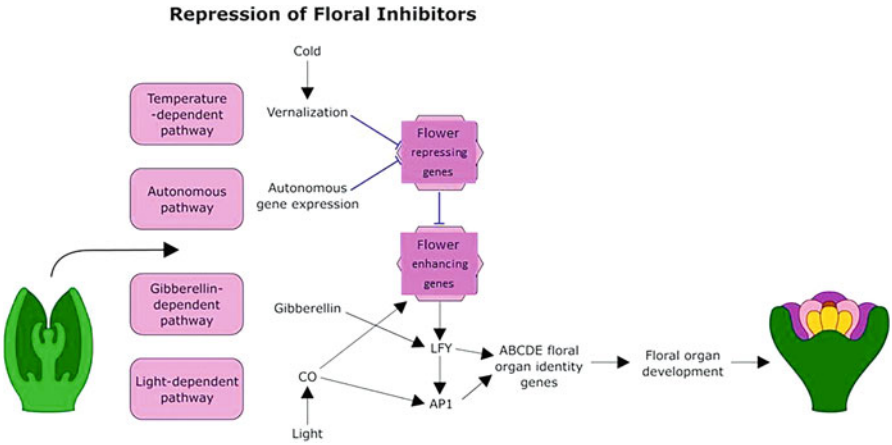

Fig. 25.15 Temperature, light, and gibberellin-dependent pathways work through repression of floral inhibitors for flower formation as well as by activating floral meristem identity genes

directing petal development would contain A-class protein- AP1, B-class protein-PI and AP3, and a SEP protein.

To sum up, photoperiodism and vernalization facilitate plants to synchronize their life cycle with the time of the year. It is clear that the process of flower formation is an interplay of various transcriptional networks that regulate organ-specific gene expression. Such altered expression of floral homeotic genes also explains the floral diversity that we observe in our daily life. Flowering plants constitute an enormous range which needs to be explored with reference to gene networks that regulate floral development. Future challenge is to explore the variability found in nature which is due to gene network regulating the floral development (Fig. 25.15).

Summary

- Flowering of plants depends on three basic requirements. Plants must be able to respond to an inductive signal. The signal is perceived in leaves and transmitted to the shoot apical meristem. The meristem responds by changing from vegetative phase to reproductive phase. Best understood factors that trigger flowering are duration and timing of light and dark periods (termed as photoperiodism) and temperature.
- Plants are classified into three classes according to their requirement of photoperiod, viz., long-day plants, short-day plants, and day-neutral plants.
- It is observed that leafless plants do not produce flowers. Leaf is the site of perception of photoperiodic signals. This means that some chemical agent (a flowering hormone) is synthesized in leaves and passed to the flowering apex. This hormone was named as florigen**.** This was later identified as FT (FLOWERING LOCUS T) which is a small globular protein which moves via phloem from leaves to the shoot apical meristem under inductive photoperiods. In

the shoot meristem, FT forms a complex with the transcription factor FD to activate floral identity genes.

- Vernalization is defined as the method of inducing early flowering in plants by pretreatment of their seeds or young seedlings at very low temperature. Apical buds are the sites of vernalization.
- Plants have distinct adult and juvenile phase, and only plants in adult phase are competent to flower. Gibberellins are important in regulating the phase change from juvenile to adult.
- The photoperiod, vernalization, and an autonomous pathway, which is independent of light, operate together to control the expression of key genes that trigger the switch from vegetative to reproductive development.
- CO (Constans in *Arabidopsis*) and Hd1 (Heading-date 1 in rice) regulate flowering by controlling the transcription of floral stimulus genes. CO protein is degraded at different rates in the light vs the dark. Light enhances the stability of CO, allowing it to accumulate during the day, and it is rapidly degraded in the dark.
- Flowers are made up of floral parts arranged in concentric whorls, with sepals and petals surrounding the inner reproductive parts. Formation of floral meristem requires active floral meristem identity genes, such as SOC1, AP1, and LFY, in *Arabidopsis*. Mutations in homeotic floral identity genes alter the types of organs produced in each of the whorls. The ABC model suggests that organ identity in each whorl is determined by the combined activity of set of three organ identity genes. A quartet model has been described to explain how transcription factors act together to specify floral organs. ABCE model with certain variations explains the diversity of angiosperm flower structure. MADS-box genes closely related to class C genes are required for ovule formation.

Multiple-Choice Questions

1. Induction of flowering by low temperature treatment is termed as:
   (a) Vernalization
   (b) Photoperiodism
   (c) Cryopreservation
   (d) Defoliation
2. A gene that represses flowering in Arabidopsis:
   (a) LFY (Leafy)
   (b) FLC (Flowering locus C)
   (c) CO (Constans)
   (d) CRY2
3. The phenomenon of photoperiodism in plants was discovered by:
   (a) Chailakhyan
   (b) Borthwick and Hendricks
   (c) Skoog and Miller
   (d) Garner and Allard

4. Which of the following pigment plays a role in induction of flowering as identified by Chailakhyan:
   (a) Cytochrome
   (b) Vernalin
   (c) Florigen
   (d) Xanthophyll
5. According to ABC model in *Arabidopsis*, B and C genes are required for:
   (a) Petals
   (b) Stamens
   (c) Sepals
   (d) Carpels

Answers

1. a 2. b 3. d 4. c 5. b

Suggested Further Readings

Davies B (2006) Floral meristem identity genes. In: Jordan BR (ed) The molecular biology and biotechnology of flower. CABI Publishers, Cambridge, MA, pp 81–99

van Dijk ADJ, Molenaar J (2017) Floral pathway integrator gene expression mediates gradual transmission of environmental and endogenous cues to flowering time. Peer J 5:e2724v1