Florigen
and
Theory of flowering

by

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Preface

It is a matter of great satisfaction that this text book “Florigen and Theory of flowering” will be received by the teachers and students.

For quite sometime I was receiving requests, suggestions from many students to prepare a text book of “Florigen and Theory of flowering” to cater the requirement of some Universities.

Besides fulfilling the need of undergraduate and post-graduate students for whom this book is primarily meant, this book will also be of great use to research scholars.

In chapter 2, The Biochemistry of flowering are explained. Several attempts have been made to extract and characterize the floral stimulus from flowering plants or photoinduced plants. Transport of the floral stimulus are explained in chapter 3. Chemical nature of the florigen are described in chapter 8. Among the numerous theoretical interpretations of photoperiodism and plant flowering which have been proposed, are explained in chapter 11.

It is expected that present book shall fulfill the requirement of students and they will be greatly benefitted with this book. Valuable suggestions for the improvement of the book will be thankfully acknowledged.

I am extremely grateful to my publishers for their keen interest and kind Co-operation in publication of this book.

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Dr. Indu Kumari
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1. Introduction

When a plant reaches a certain stage of vegetative growth, profound changes take place in its structure and function and it starts flowering. The apex of the branch on plant, is induced to form a flower instead of other branches or leaves. Significant changes take place at cellular and metabolic levels, during these modification. All these changes are finally manifested into morphological changes, the vegetative bud is transformed into a reproductive or floral bud.

A flower is nothing but a compressed, determinate shoot bearing sepals, petals, stamens and carpels as lateral organs instead of leaves.

The flowering in plants is influenced by various environmental and nutritional factors. In most cases, a specific environment is necessary for flowering to occur. However the specific environment acts as a floral stimulus, only when the plant has reached a certain stage of maturity. Very young plants as being in juvenile phase, cannot flower, even if all other factors are favourable. They are receptive to floral stimuli when they have reached puberty.

Among various environmental factors, the day length, the quality and intensity of light and temperature are the most important variables affecting the timing and intensity of flowering. It has been found that photoperiod is a major factor determining the time of flowering in some plants, while temperature is a major factor for others. With some plants, a certain combination of the two is a necessary factor.
2. The Biochemistry of flowering

Julius Sachs in the 19th century was probably the first person to support the idea that ‘flower-forming substances’ are present in flowering plants. The plant which are sensitive to photoperiods, a definite chemical stimulus is produced in the leaves, when the plants are exposed to the desired photoperiod. The stimulus is then translocated to the shoot apex where it evokes flowering. As far as the biochemistry of flowering is concerned, it can be divided into two steps-

1. The production of floral stimulus and
2. Evocation of flowering.

(1) The production of floral stimulus

In the 1930s, Mikhail Chailakhyan in Soviet Union grafted induced plants to noninduced plants held under noninducing day lengths and observed that the flowering stimulus would cross a graft union, causing the noninduced plant to flower. Chailakhyan named the hypothetical stimulus florigen (Latin flora, “flower” and greek, genno, “to beget”).

In such grafting experiment, donor and receptor plants may either be of the species or of two different species or genera. In an extreme case, the two partners belonging to different photoperiodic groups (SDP, LDP and DNP) can be used as donor and receptor. These results support the concept of a transmissible floral stimulus which seems to be physiologically similar in all photoperiodic plants. In grafting experiments, in which the donor is either the stock or the scion, the movement of the floral stimulus may be in both the upwards and downwards directions (Figure-1).
In 1937, Melchers reported transmission of a flower stimulus in biennial *Hyoscyamus* formed as a result of low temperature (vernalization) and called it ‘vernalin’ as the product of vernalization. However there was no evidence of transport of a stimulus formed as a result of cold treatment. Melchers assumes that vernalin is the physiological precursor of florigen.
3. Transport of the floral stimulus

Evans and Wardlaw (1966) obtained a velocity of the floral response is 1 to 2 cm/h in the LDP *Lolium*, whereas King et. al. (1968) obtained a much higher value of 30 cm/h in SDP *Pharbitis*.

Regarding the pattern of transport, Lang (1965) has indicated that the floral hormone moves only through living tissue. Initially the movement probably occurs from cell to cell through the mesophyll of the leaf blade until loaded into the phloem. The phloem tissue is the path of further transport in the petiole and stem. Assimilates are known to move in the phloem by mass flow from sources to sinks. Florigen in thought to move passively with assimilates and can move both acropetally and basipetally, i.e., its movement is non-polar as is the flow of assimilates.

Several attempts have been made to extract and characterize the floral stimulus from flowering plants or photoinduced plants. However, attempts have met with limited success only. K.C. Hamner and J. Bonner (1938) tested some 246 different kinds of extracts from short-day, long day and day neutral plants, but none had any flower evoking effect in non-inductive plants. Nevertheless, florigenic acid, gibberellins, sterols, phenolic acids and several other compounds have been shown to act as a flower stimulus in many species.
4. Florigen

The Pr and Pfr forms of phytochromes pigments are the products of photoperiodic stimulus and they are responsible for inducing flower formation in the plant. So the respective pigments elicit certain responses in the plants which probably produce some kind of a substances which are responsible for transforming vegetative shoot into reproductive shoot. Production of such substances by plants has been suspected by many plant physiologists long ago. But so far, no one has succeeded in isolating or identifying such compounds. In spite of it, the presence of substances responsible for flower induction has been proved by different methods and by different investigators.

**Grafting experiments suggesting the existence of florigen**

The existence of substances responsible for flower induction has been proved by the grafting experiments. The grafting experiments conclusively prove the presence of flowering substances. In such grafting experiment, donor and receptor plants may either be of the species or of two different species or genera. In an extreme case, the two partners belonging to different photoperiodic groups such as - SDP, LDP and DNP can be used as donor and receptor. These results support the concept of a transmissible floral stimulus which seems to be physiologically similar in all photoperiodic plants. In grafting experiments, in which the donor is either the stock or the scion, the movement of the floral stimulus may be in both the upwards and downwards directions.

What is this substance is it a protein or a chemical signal such as cAMPs like or is it a mRNA. Any substance that is induced and produced in leaf cells has to be transported to long distances and should be stable. It is possible that the substance
produced should cross through the cell wall of mesophyll cells into sieve tube cells, and then it has to be transported to stem apex meristems (SAMs). There again it has to cross cell wall barriers to reach a whole mass of cells. So this substance should be a small molecule that can be easily transportable and easily induce signal transduction that is capable of spreading.

Chailkhyan, a noted Russian botanist named such flower inducing elusive hormone as ‘Florigin’. Attempts to isolate such a substance have failed. In fact, people have made attempts to collect the substance from the donor plant to a receiver plant through a water jacket, but failed to obtain any stable compound which could induce flowering in other plants.
5. Florigen as a physiological concept

Julius Sachs (1865) may be considered the father of the flower hormone concept. He worked on partially darkened *Tropaeolum majus* and *Ipomoea purpurea* plants. From his well-known experiments, he concluded that leaves in the light produce flower-forming substances in very small amounts, which direct the assimilates to form flowers in darkened shoots. However, more convincing evidence in support of flower-forming substances did not appear until after the discovery of photoperiodism, the response of plants to the relative length of day and night by Garner and Allard, 1920.

A finding with photoperiodically sensitive plants was that daylength is perceived by the leaves, whereas flower formation takes place in the shoot apical meristem (Knott, 1934). This finding implies that a long-distance signal moves from an induced leaf to the shoot apex. Later, it was shown that this signal can also be transmitted from a flowering partner (donor) through a graft union to a nonflowering partner (receptor).

Chailakhyan (1936) introduced the term “florigen” (flower-former) for this floral stimulus, which he defined as specific substances with a regulatory function. Grafting experiments between related species, but of a different photoperiodic response type (e.g., a short-day plant (SDP) and a long-day plant (LDP)), provided evidence for exchangeability of florigen among different response types.

This earlier work showing that floral stimulus substance - florigen is functionally conserved in different species has been extensively reviewed (Lang, 1965; Zeevaart, 1976). The *Crassulaceae* family has representatives of SDPs, LDPs, long-short-day plants (LSDPs; require long days (LDs) followed by short days (SDs) to flower), and short-long-day plants (SLDPs; require short days followed by long days to flower), which are all graft-compatible and can transmit the floral stimulus in every
possible graft combination (Figure-2,3 and Figure-4). Thus, the
dogma emerged that florigen is universal in plants (at least in
closely related species and different photoperiodic response
types). Despite numerous attempts to extract florigen and
several reports of extracts with flower-inducing activity, which
all turned out to be nonreproducible, florigen remained a
physiological concept rather than a chemical entity. As a result,
the florigen hypothesis fell into disrepute, and a rival
hypothesis, proposing that flowering would be induced by a
specific ratio of known hormones and metabolites, gained favor
(Bernier, 1988; Bernier et al., 1993).

Figure-2  Flowering is induced in a noninduced Scion by transmission of
florigen from a Florally Induced Stock. The appropriate photoperiodic
conditions were used to induce flowering of the donor plant, whereas the
receptor was in a noninductive photoperiod. The stock (below the graft
union) is the donor and the scion (above the graft union) is the receptor.

Four examples from the Crassulaceae-

(A) The SDP Kalanchoe blossfeldiana as donor for the LDP Sedum spectabile
    as receptor (Zeevaart, 1958)
(B) The LDP Sedum spectabile as donor for the SLDP Echeveria harmsii.
(C) The LSDP Bryophyllum crenatum as donor for the LDP S. spectabile.
(D) The LSDP B. daigremontianum as donor for the SLDP E. harmsii.
What is the nature of the flower signal?

Grafting experiments by Chailakhyan (1936) showed clearly that the signal was a chemical substance that he called **florigen** which is made of anthesin and gibberellin.
6. Isolation

Problems with isolating floral stimulus substances - florigen and the inconsistent results acquired led to the suggestion that florigen does not exist; rather, a particular ratio of other hormones must be achieved for the plant to flower. However, more recent findings indicate that florigen does exist and is produced, or at least activated, in the leaves of the plant and that this signal is then transported through the phloem to the growing tip at the shoot apical meristem (SAM) where the signal acts by inducing flowering.

This substance was active & hence identical in LDP, SDP & day-neutral plants. Probably, the suspected florigen may be an extremely unstable, labile and sensitive compound, which could not withstand the most simple extraction methods.

Recent experiments however involving solvent extraction methods indicate that florigin might be a compound similar to a labile protein or sterols or mRNA-mRNP complex. But there are many plant physiologists who suspect the very existence of such a compound because they feel that some of the known growth promoting hormones by themselves may bring about flower induction by some complex interactions. Most of the known hormones are small molecules, that are easily transportable and bind to receptors and induce signal transduction. Either such hormones and their binding proteins or the combined complexes may be involved.
Where does the signal for flowering come from?

Figure- 5 Signal for photoperiodic stimulation comes from leaves.

Experiments clearly show that the signal for photoperiodic stimulation comes from the leaves and transfers from there to the shoot apical meristem (SAM) (Figure-5).
7. Translocation of florigen

The transport of floral stimulus substances has been found to be through sieve tubes, but the rate of translocation in short day plants and long day plants varies. In short day plants, the rate of transportation is about 45-50 cm/hr. but in long day plants, it is about 2-2.5 cm/hr. The rate of translocation of the flowering substance is found to be 40-100 times slower than the rate of transportation of sucrose, though the components involved in transportation are the same. It is suspected that the different rates of transportation observed in short day plants and long day plants to be due to the presence of two different substances. It is also known that sieve tubes translocate different substances at different rates because of specific carriers involved in the translocation process.

It is not known that whether the florigen is one compound or a complex of compounds. If it is one compound, then the flowering substance produced by both the short day plants and the long day plants should be the same. If there are two compounds, the rates of translocation may differ. But question is - why should they differ?

The probable explanation is that one of the compounds is synthesized and such substances reach their destination earlier and the other compound that is synthesized when it is subjected to photo inductive conditions reaches the destination later. However, for the inductive action, both compounds are required, but it is difficult to visualize whether these compounds elicit their action in complexed form or independently at one or two different sites.

S. Imamura and A. Takimoto (1955) studied the rate of translocation this stimulus. In Japanese morning glory, the rate of translocation was observed to be slow, 2.5 to 3.0 nm per hour as compared to more than 200 nm per hour for most sugars. Thus florigen was not just a sugar synthesized in photoinduced leaves. In other studies also, exogenous application of sugars or
amino acids did not induce flowering in non-induced plants. This strengthened the concept that the floral stimulus substances - florigen was not just a general type of synthetic output of the photoinduced leaves.

Figure – 6 The pathway through which the photoperiodic signal is translocated from the receptor leaves (L) to the shoot apical meristem (SAM) and the leaf primordia (P).
8. Chemical nature of the florigen

In search for the chemical nature of the florigen, plant physiologists focused their attention to find out the effect of plant hormones on flowering. Several plant hormones have flower promoting effects, although none of hormones can qualify as a florigen because of its limited effect. Gibbrellins induce flowering in most long day plants, but not in short day plants. It is also definite that the gibbrellins alone do not constitute the ‘florigen’, but it is usually held that the gibberellins are in some way connected with the overall process of flowering in plant.

Although all attempts by researchers to isolate and identify florigen have been unsuccessful because of the lack of a reliable bioassay. There is an indication that florigen might be an isoprenoid or a streoid like compound.
9. Recent view

Recent studies reveal that plants produce the elusive substance (florigen), which is synthesized in leaves and translocated through sieve cell and reach the base of SAM. The complex of substance now called the actual “Florigen”, is a “Molecule of the century” has been identified as FT (flowering time protein correctly Flowering locus T), not the FT mRNA suspected earlier. Actually FT is synthesized in response to constans (CO). It is now believed that it combines with another protein called FD, together activate AP1, SOC1 in the apical Meristem, they in turn activate the expression of LFY. Then AP1-LFY triggers the expression of floral homeotic genes. This just explains light induced components, but the flower initiation is also due to GA, sucrose, vernalization and in a large number of plants it is autonomous. There is a kind of confluence of the products of these effects ultimately responsible for triggering the floral homeotic genes.

Some researchers have identified this signal as mRNA coded by the FLOWERING LOCUS T (FT) gene, others as the resulting FT protein, in Arabidopsis thaliana.
10. Gibberellins and the Florigen Concept

It is very well known that gibberellins induces bolting and flowering in rosette leaved long day plants, but not in short day plants (with some exceptions). In long day plants, GA not only stimulates the elongation the condensed internodes, but at the same time, it also promotes the formation of factors needed for flowering. Thus GA treatment substitutes photoperiodic treatment in long day plants. Added to this complexity, application of high concentrations of gibberellins and cytokinins to the callus, obtained from *Arachis hypogea* (peanut plant), results in the induction of flowers directly from the callus.

A model based on the role of phytochrome, GA and florigen in the photoperiodic induction of flowering can be proposed. Based on gibberellin’s promotive effect on long day plants and its failure on short day plants, Brain and his colleagues (1958-59) came out with a working model to explain the action of photoperiods on flower inducing substances. According to this model, during photo inductive red light treatment a precursor gets converted to Gibberellin or Gibberellin like substance. The same substance is believed to undergo decaying back to the precursor either in dark or under far-red light treatment. Photoperiodic reactions involving assimilation of CO$_2$ causes the build up of a precursor of GA or GA like hormone. This precursor may be converted to GA.

\[
\text{CO}_2 \rightarrow \text{Precursor (P)} \rightarrow \text{Gibberellin – like hormone.}
\]

According to their concept it is assumed that Gibberellin like substances have to be maintained at high concentrations in long day plants to be effective in producing the elusive compound called ’florigen’. But in short day plants, according to Brain, et. al. GA like substances are effective only when their concentration is very low, for higher concentration of GA is
inhibitory. That is why when GA is applied to short day plant there is no effect in terms of flower induction.

Nevertheless, GAs seem to play an important role in flowering. It may be directly involved in the formation of florigen or any such floral stimulus.

Even today, with all the knowledge of molecular biology of the exact processes involved in inducing flowers are not known. Still, it is very important to understand the model proposed by Chailkhyan, a great Russian plant scientist. He worked his entire life time to understand this process and his proposed model is very worth understanding. After the discovery of effect of gibberellins on flowering Chailakhyan modified his florigen concept. Cajlachjan has assumed that the florigen formation takes place at two levels but in two steps. Further, florigen is not one substance but it is a complex of two substances, i.e., gibberellins and Anthesins.

He suggested that there are two steps involved in the flowering process, the first flowering process mediated by gibberellin and the second flowering process by one or more flowering factors called anthesins. Together, gibberellin and anthesins constitute the true florigen. He proposed that long day plants could produce anthesin under any day length but gibberellins only under long days, that short day plants could produce gibberellins under any day length but anthesin only under short days, and that day neutral plants could produce both under any day length. In presence of both gibberellin and anthesin ,a plant could flower. But again anthesin is just as hypothetical and elusive as florigen has been, until additional experimental evidence becomes available.

Sachs (1978) and Sachs and Hackett (1983) questioned the florigen concept and suggested that photoperiodic induction causes a diversion of nutrients (sucrose and so on) within the plant, leading to floral initiation. In Bougainvillea, Flowering was correlated with soluble solid percentages (Ramina et al.,1979) and was promoted by added sucrose in the quantitative LDP Brassica campestris grown in sterile culture (Friend et al.,1984). A GA4/7 mixture strongly promoted “Flowering” in
Pinus radiata and caused a significant reallocation of dry matter within the terminal buds to developing long shoot primordial (Potential seed-cone buds; Ross et al., 1984).

It seems clear that transport pattern can change during photoperiodic induction as Sachs and Hackett emphasized, but it is not clear that this causes flowering rather than being a result of it. The defoliation and grafting experiments outlined previously are specially difficult to explain by nutrient-diversion hypothesis.

GA can induce or promote flowering in many LDPs that grow as a rosette in SD. However, all rosette plants can not be induced to flower by GA, although applied GA always causes stem elongation. GA does not induce flowering in SDPs grown in noninductive LD conditions. Because results of grafting experiments indicate that florigen is exchangeable between LDPs and SDPs, it was concluded early on in work on the role of GA in flowering that GA cannot be florigen (Zeevaart, 1983). In the LDP Lolium temulentum, GA causes floral initiation without first causing stem elongation, and GAs, especially GA5 and GA6, are endogenous signals plant hormone transmitted from an induced leaf to the shoot apex. These GAs have been assigned a role as florigen in grasses (King and Evans, 2003), but this role appears to be restricted to a certain group of plants, temperate grasses, just as the flower-inducing effect of ethylene is limited to the family of the Bromeliaceae (Zeevaart, 1976, 1978). Florigen was meant to indicate a universal flower hormone. At present, FT-regulated flowering appears to be widespread, and it would be preferable, therefore, to restrict the term florigen to the FT-induced transmissible signals.

The effect of plant hormone - GA on flowering process raises the question about the relationship between GA and FT expression. In Arabidopsis, GA activates the floral meristem identity gene LEAFY (LFY) (Blazquez et al., 1998) but does not regulate expression of FT (Moon et al., 2003). In support of separate GA and FT flowering pathways, King et al. (2006) also found that an increase in FT mRNA in L. temulentum in LD
occurred independently of GA. LFY is conserved in plants (Maizel et al., 2005), so that with respect to the GA response pathway the question is: What is the effect of GA on expression of *LFY* in LDPs and SDPs that do not flower in response to applied GA?
11. Theory of flowering

Sachs's hypothesis on plant-forming and especially on flower-forming substances, which was first published in 1880, was the earliest concept of the physiological nature of plant flowering. Klebs's theory of the role of the ratio of nutrients appeared much later (in 1918). In 1918, Gassner found that winter forms require cold and that they differ in this requirement from spring forms; he discovered the phenomenon of thermo induction in plants while in 1920 Garner & Allard discovered photoperiodism in plants.

Besides treatment with an appropriate photoperiod, several other environmental as well as chemical treatments induce flowering. Sometimes these treatments are absolutely essential, but in other cases, they just hasten or increase the flower formation. All present theories and concepts of flowering are theories and concepts of photoperiodism since flowering or its absence are criteria of photoperiodism. Among the numerous theoretical interpretations of photoperiodism and plant flowering which have been proposed, the following are the most widespread and interesting: theory of endogenous rhythms and phytochrome theory; Applied plant hormones and growth – regulators theory, Florigen Theory, Floral inhibitor theory, C/N Relationship theory, Trace Element Nutrition theory, Plant Age theory, Water Stress theory and the less popular hypothesis of the ratio of rates of light and dark reactions, and the recently offered conception of two-phase flowering.
i) Theory of endogenous rhythms

The study of various aspects of life in daily and other cycles corroborated the correct suggestion that plants possess endogenous rhythmicity. Bunning's experiments were the earliest in this direction; they showed the transposition of phases of daily periodicity of leaf motions in long-day and short-day species; in the former leaves are erect in the evening and in the latter, in the morning.

According to the theory, the metabolism of plants oscillates between two separate phases-

1. Photophile (light loving) and
2. Skotophile (dark loving) phase.

In photophile phase, light increases the various processes of plant including flowering. In skotophile phase, light inhibits flowering and other plant processes. The photoperiodism is executed by an endogenous free running oscillation between these two phases at a regular interval of approximately 12 hours.

Currently Bunning puts forward a conjecture that daily endogenous rhythms consist of two half-cycles: the first, an approximately 12 hour period, coincides with the light period and is characterized by the dominance of synthetic processes; the second half-cycle is characterized by the activation of decomposition processes. Plants of both photoperiodic groups are distinguished for higher sensitivity. In long-day species one reaction from close to the skotophilous phase, takes place in darkness and in light; the second reaction, close to photophilous, depends on light and in this respect, is connected with the oscillator, i.e., with the supposed intracellular biological time-recording mechanism. In short-day species the reaction, close to the photophilous phase, is the reaction which takes place in darkness and in light; the second reaction close to
the skotophilous depends on darkness and is connected with the oscillator.

Bunning's theory of endogenous rhythms, as a foundation for the photoperiodic reaction of flowering, is of phenomenological nature for it is based on admission of the existence of the hypothetical central oscillator (auto-fluctative process) and is not easily explained from the point of view of metabolic transformations. Moreover, presently there are data that plant flowering can proceed at the same rate under different proportions of light and darkness not only in 24-hour but also in 13- and 16-hour cycles. At the same time it is quite admissible and useful to study the photoperiodic reaction of flowering considering historically formed and hereditarily fixed endogenous rhythms and the internal coordination of physiological processes.

ii) Phytochrome theory

The discovery of phytochrome came as a result of the study of the action spectrum of the visible light effect on morphogenesis, especially on plant flowering; it was carried out by Borthwick, Parker, Hendricks and others showed that in short-day species of cocklebur soybean, Amaranthus, and Chrysanthemum red light given in the middle of the dark period prevented plant flowering, but after the subsequent treatment with far-red light these plants started flowering; in long-day species - barley and henbane-red light, on the contrary, stimulated flowering and the subsequent effect of far-red light stopped it. This phenomenon was used as an argument that there is a special pigment - phytochrome with two interconvertible forms.

It is supposed that the P 730 form of phytochrome, which suppresses flowering of short-day species and hastens flowering of long-day species, is physiologically active. The duration of the transformation of P720 into PGGO in darkness is
equal to the critical length of night; therefore the formation of metabolites necessary for flowering and for flowering hormones does not take place until the transformation of P 730 to P 660 is accomplished, i.e., until the accomplishment of its transformation into a form able to absorb red rays.

Under long-day conditions phytochrome retains the P 730 form for longer time, and this stimulates flowering of long-day species and oppresses flowering of short-day species. Under short-day conditions phytochrome retains the P 660 form for longer time, and this stimulates flowering of short-day species and inhibits flowering of long-day species. The isolation of pure phytochrome from seedlings of different plants grown in darkness was a great achievement.

It was found that phytochrome is a substance whose chromophoric group is closely linked with protein; phytochrome differs from chlorophyll in its absorption spectrum, especially in the relatively weak absorption of blue rays. The effect of phytochrome on plants is exerted through an effect on metabolism, respiration and oxidative phosphorylation, synthesis of anthocyanins and chlorophyll, activity of enzymes as well as through the content of growth-promoting substances. The many-sided effect of phytochrome on plants proves that it is connected with some general metabolic link or links and is not a specific system which regulates photoperiodic reactions.

Phytochrome is probably one of the pigment systems which participate in the perception of the photoperiodic stimulus and in subsequent transformations of primary metabolites in plants, and this explains its importance for plant flowering.
iii) **Applied plant hormones and growth –regulators theory**

**Auxin**

It has been found that the formation of auxins in the top parts of stems of all plants, regardless of the nature of their photoperiodic reaction, is not connected with flowering; it is directly connected with day length, being more intensive in long-day species. There are no experiments where treatment with auxin solutions would have resulted in flowering of annual plants under unfavorable day-length conditions. But as soon as the daylength conditions became favorable and provided that there was induction with optimal photoperiods, flowering of long-day species became slightly hastened while flowering of short-day species became slightly inhibited. At the same time such growth inhibitors as triiodobenzoic acid and maleic hydrazide have proved able to stimulate flowering and to sharply inhibit growth of short-day species like cocklebur, soybean, and Perilla.

These experiments show that auxins and synthetic growth-promoting preparations which function as auxins have indirect influence and do not play a decisive role in plant flowering; they cannot be considered direct regulators of flowering.

In pineapple and cocklebur, applied auxins cause production of ethylene, which itself influences flowering in the same way as auxins (inhibition in SDPs, Promotion in bromeliads).

**Kinins**

Kinetin strongly affects growth and differentiation of cells, influences the translocation of substances in plants, and inhibits tissue aging. Attempts to isolate kinins or, more
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precisely, phytokinins from plants were successful, 6- N-dimethyl-amine-adenine (named zeatin) being isolated from corn seeds (Letham, Schannon & Me Donald, Miller). However, there are no definitive data on the effect of phytokinins on plant flowering. There is no doubt that this group of substances - kinins or phytokinins and metabolites of nucleic acid metabolism - plays an essential role in flowering processes, especially in connection with the role which belongs to deoxyribonucleic and ribonucleic acids in the storing and translating of genetic information. the introduction of kinetin and metabolites of nucleic metabolism hastens flowering of short-day species and inhibits flowering of long-day species.

Gibberellins

The discovery of gibberellins was a great achievement of plant science because it enabled men to control growth and development of plants (Kurosawa; Yabuta & Sumiki, Yabuta & Hayashi; Stodola; Brian). Numerous studies by scientists in different countries helped to discover the general function of gibberellins, i.e., stimulation of growth processes in all tested plants, and their specific effects, i.e., induction and stimulation of flowering of many long-day species under short-day conditions but absence of induction and hastening of flowering of short-day species under long-day conditions.

The discovery of gibberellins arose an interest in discovering the physiological nature of vernalization processes. Studies of the effect of gibberellin preparations showed that under long-day conditions they induce stem and flower formation in unvernalized one-year-old seedlings of biennial species- henbane, carrot, parsley, turnip, chicory, cabbage, and European goldenrod (Solidago virga-aurea)- as well as in seedlings of winter forms Arabidopsis thaliana, rape, and lettuce. In contrast to them the treatment of winter forms of cereals-rye, wheat, and barley with gibberellins resulted only in the formation and growth of stems and in the slight stimulation
of the development of rudimentary spikes but did not induce heading and flowering of these plants.

Gibberellins affect the beginning of the formation and growth of flower stems; the result is the beginning of flowering in long-day species which possess a qualitative reaction and the stimulation of flowering under short-day conditions in species which possess a quantitative reaction.

It is interesting that some gibberellins affect flowering while others do not; for example, in an experiment on *Myosotis alpestris* gibberellin Ag influenced only stem formation while gibberellin A7 stimulated both stem formation and flowering. It is quite natural that such a strong effect of gibberellins on the morphogenetic process is possible only through their effect on plant metabolism.

Gibberellins are the most effective plant hormone in inducing flowering. It substitutes for long day requirements and also the cold treatment requirement in several species. Some examples of long day plants which can be induced by gibberellins under non-inductive (short day) photoperiods are: *Arabidopsis thaliana, Cichorium endivia, Crepis parviflora, Hyoscyamus niger, Nicotiana sylvestris, Spinacea oleracea* etc.

Examples of some cold requiring biennials which can be induced to flowering by GA are: *Beta vulgaris, Brassica oleracea, Daucus carota, Viola tricolor, Myosotis alpestris* etc.

Gibberellins usually do not induce flowering in short day plants under non-inductive conditions, although they may cause stem elongation.

Auxins are also known to induce flowering in pineapple and they have been used commercially for this purpose, ever since their this effect was discovered in 1942. They have also been found to be effective on long day plants, Winter barley and *Hyoscyamus niger*. However, in most other species, either they have no effect or inhibit flowering. It is possible that the inhibitory action of auxin on flowering is mediated via ethylene production, which is stimulated by auxin treatment.

Exogenous application of cytokinins is also known to induce flowering in many species, under non-inductive
photoperiods, including *Chrysanthemum, Lemna paucicostata, Pharbitis nil, wolffia* etc. However, they are known to inhibit flowering in *Chenopodium*.

Abscisic acid has no effect under non-inductive photoperiods, but increases flowering under inductive photoperiods in *Chenopodium* and *Pharbitis nil*.

Salicyclic acid and some other phenolic acids have also been shown to induce flowering in *Lemna* and several other plants. But most other plants do not have an obligate requirement of salicyclic acid for flowering. The salicyclic acid has other morphogenetic effects also, and it has been suggested that it acts like a plant growth regulator.

Ascorbic acid is also known to induce flowering in some plants such as *Lemna*, *Trigonella* and *Brassica*. 
iv) Florigen Theory

Chailakhyan (1937) proposed that the signal generated in the leaf is a substance of hormonal nature and named it florigen. Results of grafting experiments support the concept of a transmissible floral stimulus in which a previously induced donor plant may transmit the floral stimulus to the receptor plant in a graft union.

In 1937, Melchers reported transmission of a flower stimulus in biennial Hyoscyamus formed as a result of low temperature vernalization and called it ‘vernalin’ as the product of vernalization. There was, however no evidence of transport of a stimulus found as a result of cold treatment alone indicating lack of mobility of the product of vernalization. Melchers assumes that vernalin is the physiological precursor of florigen.

The physical and chemical nature of the postulated flowering hormone florigen is not known. In 1964, Lincoln and co-workers prepared a crude extract from flowering branch of Xanthium but their efforts to purify the active principle did not meet with any success as it led to loss in activity during the purification process. The active material is highly water-soluble containing a carboxylic acid and so it has been referred to as ‘florigenic acid’. Cleland (1974) could identify the flower – inducing principle as salicylic acid but failure to induce flower initiation with this chemical makes it unlikely that this compound is the floral hormone.

To identify the nature of florigen, a number of known chemicals such as cyclic AMP, prostaglandin, DNA and plant hormones, particularly GA have been tested on vegetative plants but the work is inconclusive as no single compound exhibits universal florigenic activity.
v) Floral inhibitor theory

It is the counter-theory of the floral hormone theory. Plants grown in conditions unfavourable for flowering produce floral inhibitors. Some experiments indicate that flowering in plants is controlled by the presence of a flowering inhibitor, either independently or in the presence of the floral stimulus. The inhibitor is apparently present in non-induced plants and either disappears or diminishes when the plant is kept on an inductive cycle.

Existence of a specific transmissible inhibitor has been demonstrated in non-induced leaves of *Coleus, Fragaria, Lolium, Rottboellia* and tobacco. Experiments on numerous amino acid analogues showed that only ethionine (an inhibitor of the transmethylation process) inhibited cocklebur flowering during the dark period, this inhibition being removed only with methionine, a donor of methyl groups; however, the removal of the inhibiting effect of ethionine with methionine in another plant-Ipomea-was not observed.

Like florigen, the chemical nature of the inhibitors is unknown. Only a few attempts have been made to extract and isolate a floral inhibitor. It is however, suggested that either gallic acid or ABA is the inhibitor produced in leaves kept in non-inductive condition (Zeevaart 1976). R. J. Pryce (1972) isolated gallic acid from the short day plant *Kalanchoe blossfeldiana* growing in long days, which acted as a floral inhibitor.
vi) **C/N Relationship theory**

G. Klebs (1913) and E. Kraus and H. R. Kraybill (1918) had proposed that C/N ratio of the plant was an important determinant in flowering. The role of nutrients in flowering—sugars formed in the process of photosynthesis and nitrogenous compounds introduced through plant's roots—was considered of primary importance in Klebs's theory. Increase in flowering due to increased C/N ratio was subsequently observed in many species including *Pharbitis nil* and *Lemna paucicostata*. It was found, for instance, that the deficiency of nitrogen in nutritive substrates stimulates flowering of long-day species like barley, oat, mustard, and others, and inhibits flowering of short-day species like millet, corn, tobacco, chrysanthemum, and others; a surplus of nitrogen, on the contrary, stimulates flowering of short-day species and inhibits flowering of long-day species. Thus there is a regular correlation between the nitrogen-floral reaction. Some short day species of *Lemna* can be induced to flower even under continuous light by culturing them on a nitrogen free medium. Day-length independent flowering is also induced by several chemicals, including ferricyanide which inhibit nitrogen assimilation by the plants. On the other hand, treatment with ammonium ions, nitrite, glutamine or a mixture of amino acids, inhibit day length independent flowering.

Likewise, exogenous supply of sucrose, which would increase C/N ratio in the plant, increases flowering in many species including *Anagalis arvensis, Pharbitis nil* etc.

Klebs enunciated the carbohydrate/Nitrogen (C/N ratio) relationship theory which indicates that a high endogenous C/N ratio is essential for flowering and vice versa. However, the later worker believed that Klebs theory was too simple to explain the complexity of the flowering process and many subsequent observations have failed to confirm Klebs theory which was soon discarded.
vii) Trace Element Nutrition theory

Trace elements, particularly copper and iron are critically involved in photoperiodic induction in duckweeds and other plants. Hillman (1962, 1967), studied the flowering behaviour of *Lemna* species and proved that the plants behave as SDP when copper is eliminated from the growing medium. Likewise, addition of copper to the medium leads to the loss of SD requirement and the plants behave as DNP. Hillman postulated that Cu acting as a SH-inhibitor interferes with phytochrome action, possibly by influencing some metal-sensitive membrane system.

Iron is also involved in photoperiodic induction. Hillman pointed out that flowering in *Lemna* is inhibited by reducing the iron supply. The role of iron deficiency in the inhibition of flowering process may be related to the role of iron in general metabolism.

Changes of phosphorus and potassium content in the substrate affect flowering to some extent but their effect is less significant and is not connected with the nature of photoperiodic reaction.

Flowering of cocklebur was also suppressed when cocklebur leaves were treated with dinitrophenol solution which uncoupled oxidative phosphorylation. This suppression was observed mostly under the exposure of plants during the second half of the dark period; and in cocklebur leaves, treatment with cobalt ions inhibited flowering only during the first half of the dark period; it was then removed by the application of ascorbic acid, cysteine, or glutathione.
viii) Plant Age theory

For most plants vegetative growth proceeds for some time. This is the juvenile or maturation phase through which the plant attains a minimal size till the onset of reproductive development. In the juvenile phase, the plants are not able to show photoperiodic response, whereas at the end of the juvenile phase, they become adult and sensitive to conditions that promote floral induction.

There are many reasons to account for the inability of the young plant to flower even when subjected to favourable conditions. First, insufficient leaf area has been found to delay or reduce flower initiation which may be related to the supply of photosynthetic products to the shoot apex. Also the rate of thermoinduction increases with increase in leaf number and area. Immature leaves may act by producing floral inhibitors while mature leaves produce floral stimuli. Obviously, floral initiation should depend on an optimum ratio of immature (young) leaves to mature leaves. A second reason for juvenility is the relative insensitivity of the young leaves to favourable day length conditions. The third is the inhibitory influence of the root system on flower initiation in the aerial shoots. A close proximity of the lateral buds to the root system prevents floral initiation and prolongs the juvenile phase. The fourth reason supports the idea that juvenility is probably located in the meristems and not in the leaves. It has been shown that the meristems of juvenile scion when grafted on mature plants bearing flowering shoots remain in the juvenile phase as they are unable to respond to stimuli from mature leaves.
ix) **Water Stress theory**

Brenchart (1963) demonstrated that a period of water shortage is absolutely required for flower initiation, in an experiment on *Geophila renaris*, a perennial herbaceous plant of the tropical rain forest. This observation suggests that limitation of water supply during certain developmental period may have a direct action on flower formation. This phenomenon of “Xero-induction” has also been shown in *Cichorium intybus* (Chicory) and *Chenopodium polyspermum* in which application of excess water promotes regeneration of vegetative buds but proves to be inhibitory to flowering (Bonnials, 1974; Sotta, 1978). An opposite effect of water stress has been observed in the SDP *Pharbitis* and *Xanthium* and the LDP *Lolium*. In these plants, water shortage prevents flower formation which is due to a stress-induced inhibition of translocation of floral stimulus from the induced leaves.
x) Hypothesis on the relation of rates of light and dark reactions

An hypothesis on the role of the activity of physiological processes during the period of light and dark reactions was first formulated by Lubimenko & Scheglova thirty years ago. Their experiments showed that the respiration energy in long-day species is considerable as compared with that of photosynthesis, this ratio in short-day species being much lower. In other words, the rate of dark reactions in long-day species is relatively higher and the rate of light reactions is relatively lower than in short-day species.

The comparison of the nature of light and dark photoperiodic reactions, as affected by temperature and aeration conditions, also led much later to the conclusion that light reactions result in the full decomposition of specific products of photosynthesis in short-day species and their slight decomposition in long-day species, while dark reactions, on the contrary, result in the full decomposition of products of photosynthesis in long-day species and slight decomposition in short-day species, i.e., light reactions pass more quickly in short-day species and dark reactions pass more quickly in long day species.

Presently this hypothesis is supplemented with additional data on the effect of temperature and inhibitors of oxidative metabolism during the treatment of plants with light and darkness. Under long-day conditions low temperature in the light inhibits or suppresses flowering of long-day species and hastens flowering of short-day species while the decrease of temperature in darkness has no effect. Under short-day conditions, on the contrary, the decrease or considerable increase of temperature in light has little effect and in darkness suppresses flowering in short-day species and stimulates it in long-day species. The same results are produced by naerobiosis, and respiration inhibitors-cyanide, sodium azide, and dinitrophenol. When oxidative processes are suppressed during the long-day light period, there is an inhibition of flowering of
long-day species and a stimulation of flowering of short-day species; when oxidative processes are suppressed in the short-day dark period, on the contrary, there is an inhibition of flowering of short-day species and a stimulation of flowering of long-day species. The effects of anaerobiosis and respiration inhibitors are barely observed, if at all, in darkness under long-day conditions and light under short-day conditions.

It is necessary to remember that the nature of light and dark reactions is gradually being established. They are connected with such principal physiological processes as photosynthesis and respiration so we may hope that basic data on the nature of individual reactions and on the transformation of primary products of photoperiodism into metabolites necessary for plant flowering will be obtained through photoperiodic studies.
xi) Conception of two-phase flowering

The conception of two-phase flowering is based on available data on trophic and hormonal factors. In summing up all cited data it is possible to draw the general picture of the role of trophic and hormonal factors of plant flowering.

Long-day species flower under long-day conditions; flowering is hastened provided that the carbohydrate content increases and the content of nitrogenous compounds is relatively low; flowering is stimulated by those segments of the respiratory process which are activated by light and which are connected with the activity of enzymes containing heavy metals; at the same time flowering is hastened provided that the auxin content of stem buds increases; a decisive effect is exerted by an increased gibberellin content in leaves. Flowering of short-day species, on the contrary, takes place under short-day conditions and proceeds more rapidly with increase of nitrogen compounds and decrease of carbohydrates; flowering is stimulated by those parts of the respiratory process which are activated in darkness and are connected with intensification of the activity of enzymes of residual respiration; at the same time flowering is inhibited by increase of the auxin content in stem apices and is stimulated on increase of the content of metabolites of nucleic acid metabolism; a decisive role in flowering of short-day.
12. CONCLUSION

Flowering is an important stage in a plant's life cycle because the transition from vegetative growth to generative development involves essential changes in metabolism and translocation of nutrients and aro
essential formative processes which are connected with the development of organs of sexual reproduction and alteration of generations. In this field it is especially urgent to study the whole system of interrelations which exist in the plant as a whole and consequently to use widely methods of the differential application of physical and chemical factors, surgical methods, isotope methods, methods of the extraction of endogenous substances from plants and methods of biological tests and reactions. One must also make wide use of summarizing constructions and schemes which, being only physiological and not exact chemical representations, all the same help to sum up results of numerous studies and give the general characteristics of the principal regularities of plant flowering. At the same time many phenomena of plant flowering can be studied with the help of accurate and elaborate methods and gradually become translated into the precise language of physics and chemistry. There is a trend to study complex reactions which take place in cells and subcellular structures, connected with deepest aspects of their functions.

This broad approach to research methods in order to study all-from the physiology of cells and subcellular structures to the physiology of the whole plant-is a reliable basis for the further understanding of all life processes, including flowering of plants.

The discovery of the role of trophic and hormonal factors is not enough to draw the overall picture of such many-sided processes as flowering and interrelated photoperiodic and vernalization processes.

The same effect obtained as a result of the photoperiodic induction with long-day conditions and chemical induction with gibberellins or the same effect of vernalization with lower
temperatures and the chemical vernalization with gibberellin does not mean that chemical induction and chemical vernalization fully reproduce these processes but only testifies to the fact that we have found some principal expression of those metabolic transformations which result in plant flowering under environmental conditions where they have not been observed before. At this point scientists face an important task - to connect studies of the physiology of flowering with structural transformation, especially with the transformation of apices in which new tissues and organs are born.

It should be stressed that the discovery of the roost intimate relations which develop between metabolic changes and the earliest transformations in meristems of apices, which result in the development of leaf and flower rudiments, is one of the most important problems of present-day biology.

The further study of flowering processes inevitably brings us to problems of a genetic nature. The evolution of all known photoperiodic groups and their adaptation to environmental conditions is connected with changes in the hereditary apparatus of developing species; some properties remain tightly fixed; others fall beyond the direct control of hereditary information, and are controlled by environmental factors. And if physiology of plants, especially physiology of flowering, for many years did not want to deal with problems of physiology of heredity and genetics, now this task becomes the most urgent for the proper understanding of the numerous phenomena of plant life, including flowering processes.

Flowering is a decisive stage in plant life; it attracts scientists' attention because it precedes fruiting connected with the yielding ability of crops. The united efforts of scientists of many countries were required to solve the problem of yields to arrive at those great achievements which were made during recent decades; they helped to solve many mysteries of plant life and gave main efficient methods for the control of plant growth and development. The old truth is again true—there is nothing so practical as a thoroughly elaborated theory.
Abbreviations

We will use the following abbreviations:

CO == Constans

FT == FLOWERING LOCUS T (FT)

GA == Gibberellins

LD(s) == Long day(s),

LFY == LEAFY

LDP(s) == Long-Day Plant(s),

LSDP(s) == Long-short Day Plant(s),

SAM == Shoot apical meristem

SD(s) == Short day(s),

SDP(s) == Short-Day Plant(s).

SLDP(s) == Short-long Day Plant(s).
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