Chronopharmacognosy

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ABSTRACT

This study aims to review the concept of biological rhythms in medicinal plants. Dictionariesgenerally define pharmacognosy as the subject of the study of crude drugs of plant and animal origin. The name is derived from the Greek words *pharmakon* (drug) and gnosis (knowledge). Today pharmacognosy is also defined as the study of physical, chemical, biochemical and biological properties of drugs, drug substances, or potential drugs or drug substances of natural origin, as well as the search for new drugs from natural sources. Also, another important phenomenon to be taken care of in the production of therapeutic compounds in medicinal plants is the use of circardian clock. The circardian clock is studied by chronobiology, which can be defined as a field of science that examines periodic (cyclic) phenomena in living organisms and their adaptation to solar and lunar related rhythms. Thus, it is the scientific study of the effect of time on living systems and of biological rhythms. Also rhythmic oscillations in plants lead to the enormous production of particular compounds in plants at particular time, which may or may not produce any therapeutic effect in humans. Thus, the study of chronobiology and pharmacognosy can be put together as chronopharmacognosy

Key words: Chronobiology, circadian rhythm, melatonin, pharmacognosy

INTRODUCTION

Pharmacognosy is a general term describing the study of medicinal plants. During ancient times, it was a dream of mankind to prolong life, which could be realized through chronobiology. Scientifically, chronobiology can be defined as a field of science that examines periodic (cyclic) phenomena in living organisms and their adaptation to solar and lunar related rhythms. Thus, it is the scientific study of the effect of time on plants and includes the study of biological rhythms. A rabbit in a meadow experiences a very different environment during the day, during the night, and during the moonlit night. The same meadow is very different in winter from what it was last spring, summer or fall. An organism needs to evolve biochemical, physiological and behavioral adaptations to all disparate environments, as well as switches that turn these adaptations on or off at appropriate times, often very quickly. Because these switches have to act so fast, many of them have evolved to act independently of environmental triggers. The environmental cycles like day and night, tides, moon phases and seasons, are very predictable, thus a switch can get started in advance of the environmental changes, thus rendering the organism "ready" for the new environment just in time for its appearance. Even if the organism is removed from the cyclical environment, the switches keep going on and off, and the physiological state of the organism keep oscillating on its own, becoming a timer: A biological clock. The mechanism of such oscillations, as well as the various uses of the biological clock in different organisms, is studied by chronobiology.[1]

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Rhythmicity is one of the "constant" characteristics of life, which expresses itself at all the levels of organization, from a unicellular system to complex multiorgan man. Rhythmic phenomena is the changes in the physiology, development and behavior of all living systems over lengths of time and periods, and can range from fractions of a second to hourly, daily and even annual cycles.[2-4] The study of such rhythmcity in plants can be termed as chronopharmacognosy.

Glossary of terms commonly used in chronobiology

Circadian

Literally, 'about a day' (24 hours).[5]

Clock

Generally refers to the entire circardian system, although it is sometimes used to mean the oscillator.

Clock gene

It is a rhythmically expressed negative element of a transcription - translation feedback loop. This term is also used to refer to a gene that encodes any oscillator component.

Entrainment

It is the setting of the oscillator to match environmental cycles of light and dark, or of temperature.

Input pathways

It is the sequence of events via which information from the environment, such as changes in light and temperature, is transduced to the oscillator.

Oscillator

It is the cell- autonomous timekeeper responsible for generating self-sustained rhythmicity, Also called the pacemaker.

Output

These are the pathways linking the oscillator with the various biological processes it controls.

Pacemaker

This term is also used to describe a central oscillator that is coupled to and can entrain an array of peripheral oscillators; for example, the suprachiasmatic nucleus in the brain of mammals, which controls multiple peripheral clocks in cells of other tissues and organs

Phase

It is the relationship of some point in a rhythm to a marker, such as another rhythm. For instance, the relationship of the peak expression of a gene to daybreak during a day-night cycle, is an example of phase.

Rhythm

It is the regular oscillations of a process.[5]

HISTORY

The first writings, at least in the western canon, to recognize diurnal rhythms come from the fourth century BC.[6] The study of biological rhythms has a hoary scientific tradition. It drew into its fold many brilliant scientists in the 18th, 19th and 20th centuries.[7] The first recorded circadian rhythm was for the sleep movements of the leaves of the tamarind tree by the Greek philosopher Androsthenes. The sleep movements of the leaves of the *Tamarindus indicus* were first observed on the island of Tylos (now Bahrein) in the Persian Gulf during the marches of Alexander the Great. It took more than two millennia for this to be experimentally tested. The scientific literature on circadian rhythm began in 1729 by when the French astronomer de Mairan, who carried out a blemishless experiment in the modern scientific mode and established that the 'sleep' movement of the 'Touch-me-Not' plant (*Mimosa pudica*) was endogenous. He also moved the plants into the perpetual darkness of deep caves and demonstrated that their sleep rhythms were related to the sleep rhythms of bedridden humans.[7] It was only after 30 years that de Mairan's observations were independently repeated. Till 1903, these studies excluded temperature variation as a possible zeitgeber driving the leaf movement rhythms.[6]

JC Bose wrote significant papers on his findings on diurnal movements of leaves of plants and discovered the entrainment of movement of darkness, light cycles and observed free running periods in continuous light and continuous darkness as early as 1919. This work was carried by Bunning (1958) in his first monograph on the subject of biological rhythms.[8] Semon (1905,1908) argued in favour of the genetic inheritance of circadian rhythms.[9,10] This Dutch botanist working with the leaf movements of the large jack been *Canavalia ensiformis* reported entrainment light. In 1930, Bunning and Stern stressed that the periods of rhythms under constant conditions deviated from 24 hours, thus justifying the use of the term circadian.[7]

It was then realized that the leaf movement rhythm was only one among the many plant rhythms that included germination, growth, enzyme activity, stomatal movement and gas exchange, photosynthetic activity, flower opening and fragrance emission.[6]

As early as 1880, Charles and Francis Darwin suggested the heritability of circadian rhythms as opposed to the imprinting of a 24 hour period by exposure to diurnal cycles during development. There are several genes which are responsible for the control of circadian clock. *Arabidopsis* exhibits many circadian rhythms. It exhibit myriad rhythmic outputs or "hands" of the clock. Like many plants, *Arabidopsis* displays rhythmic cotyledon and leaf movement, although this rhythm in *Arabidopsis* is based on differential growth and thus differs from the rhythmic turgor-driven expansion and contraction of the pulvinus that underlies rhythmic leaf movement in legumes, including *Tamarindus* and *Mimosa*. Also there is a circadian rhythm in the elongation rate of the abaxial and adaxial cells of the petiole that confers an oscillation in position of cotyledons and leaves. *Arabidopsis* also exhibits a circadian rhythm in the rate of hypocotyl elongation and in the elongation rate of inflorescence stem.

Although the study of circadian rhythms has focused on constant conditions, it is important to remember that plants in nature grow in a changing world. In plants grown in diurnal cycles, there is an important interaction with sugar metabolism that strongly influences cycling gene expression. In addition, recent data make it clear that the circadian clock modulates the ability to respond to abiotic stresses, such as cold. Clock modulation of response to biotic stresses has been the subject of speculation, but remains to be established.[6]

Types of rhythms

The biological rhythms are related to the sunlight.[1] The earth rotates on its axis every 24 hours with the result that any position on the earth's surface alternatively faces towards or away from the sun, giving rise to day and night respectively. That the metabolism, physiology and behavior of most organisms changes profoundly between day and night is obvious to even the most casual observer. These biological oscillations are apparent as diurnal rhythms. It is less obvious that the most organisms have the innate ability to measure the time. Indeed, most organisms do not simply respond to surprise, but rather anticipate the dawn and adjust their biology accordingly. When deprived of endogenous time cues, many of these diurnal rhythms persist, indicating their generation by an endogenous biological clock.[6] Thus, based on the biological clock, chronopharmacognosy is broadly classified into three types. They are:

1. Ultradian
2. Infradian
3. Circadian[1]

Ultradian

The ultradian rhythms are rhythms that have a period shorter than 24 hours.[1] For example, seasonal changes in the plant growth, flowering and seed production. This depends on the control system which perceives environmental signals such as daylength, temperature and humidity of the soil. If this environment-reactive pathway reports the right condition, flowering is induced.[11]

Infradian

Seasonal changes are quite spectacular and evident especially at higher latitudes. They are the result of the 23° tilt of the rotating planet earth in its orbit around the sun. These rhythms have a period greater than 28 hours. Climate, the environment and organisms are profoundly affected by it.[12] In their natural environment, plants develop under daily cycles of light–dark and high–low temperatures. The change of seasons is associated with characteristic fluctuations in day length, and fluctuations in the phase relationship between the photo and thermoperiod. In spring and winter, light and temperature change in parallel, in a so-called radiation climate. In summer and early fall, the coldest point in the day is around sunrise. At sunset, however, the light "goes off" while the temperature slowly decreases until sunrise. Changes in temperature and light intensity are therefore not synchronous at sunset. Plants have adapted their development to such environmental conditions by the evolution of photo and thermoperiodic responses.[13] The basis of such responses is endogenous changes in sensitivity to environmental light- and temperature signals.[2,14] The temperature and daylength both correlate with the seasons, but the latter is a more reliable indicator for us to establish the time of year. Based on a method to distinguish between days getting longer (spring) or shorter (fall), daylength would be a precise calendar, provided the timing device is independent of the environmental temperature. An alternative calendar for an organism would be an internal annual clock. This is indeed realized in quite a number of organisms, also in plants (e.g. seed germination, Bünning;[15] water uptake, Spruyt and De Greef;[16] stomatal movement of bean seeds, Seidman and Riggan[17]). However, annual clocks must be synchronized, usually by the photoperiod. Without synchronization, after a few years an annual clock would no longer match the physical year because its period is not exactly 12 months.[12]

Circadian

A circadian rhythm is an approximate daily periodicity, a roughly 24 hour cycle in the biochemical, physiological or behavioral processes of living beings, including plants, animals, fungi and cyanobacteria.[1] The term "circadian", framed by Franz Halderg,[7] comes from the latin circa, "around ", and diem or dies, "day", meaning literally "approximately one day".[1] These rhythms allow organisms to anticipate and prepare for precise and regular environmental changes. There are clear patterns of hormonal production, cell regeneration and other biological activities linked to this daily cycle. Circadian rhythm is regulated by endogenous pacemakers, whose activity is modulated by environmental cues, primarily the daily light – dark cycle. All living organisms, act as 'Biological clock'. Plants may produce leaves or flowers only at certain seasons, and flower may open and close at particular times of day.[1] Plants fit to their different ecological niches not only by occupying different parts of habitat, but also by occupying it at different times, dividing up the resources they compete for, temporally as well as spatially. To do so, they all have evolved in the form of 'clocks' and hence, they timetable themselves and their activities round the year, across the day, or through the rise and fall of the tides. Ayurvedic experts knew since long time, that many components in flowers and leaves are present only at the certain times of the day; if collected at wrong time, they cannot produce effective medicine. For example, rose petals plucked before sunrise will have more scent than those plucked at 2 p.m. Sunshine and winds are responsible for setting in seasons [Figure 1].[1]

General circardian clock in plants

a. The expression of several genes shows circadian rhythms. Two examples are the genes encoding the light-harvesting chlorophyll-*a*/*b*-binding proteins (*Lhcb* or *CAB*) and nitrate reductase (*NIA2*). Many of these genes are associated with photosynthesis and its related biochemical and physiological activities. It is possible that the timing of expression of such genes (for instance, the predawn rise in *Lhcb*), indicates a role for the clock in the coordination of metabolism to maximize photosynthetic yields. The use of fluorescent differential display[19] and high density Deoxyribonucleic acid (DNA) arrays to monitor global expression profiles should give us

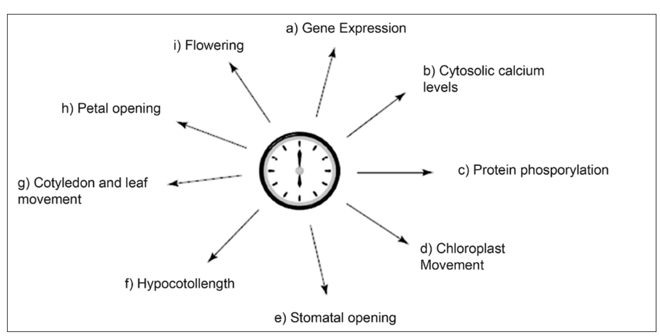

Figure 1: Plant clock controls a plethora of biological processes[18]

an indication of the range of the genes showing circadian control.

b. Cytosolic concentrations of free calcium have been shown to oscillate with a circadian rhythm in Arabidopsis. Considering the importance of calcium as both a secondary messenger and a cofactor for many enzymes, this might be a means by which the clock regulates a variety of cellular process.
c. The clock regulates the phosphorylation of some proteins. The best-studies example is in *Kalanchoe fedtschenkoi,* which exhibits circadian activity of a kinase that phosphorlates phosphoenol pyruvate carboxylase.[20] At a higher level of organization, chloroplast movement is also regulated by the clock.[21]
d. Stomatal opening,[22] Hypocotyls elongation,[23] and cotyledon and leaf movements[22] in *Arabidopsis* all show circadian rhythms. In *Kalanchoe,* petal opening shows a circadian rhythm.
e. The clock is also vital for synchronizing developmental process such as flowering time.
f. Indeed, mutations in all the putative clock-associated genes cause altered photoperiodic control of flowering. The clock's role in the control of flowering has been extensively reviewed.[7,24]

Schematic representation of input, oscillator and output rhythms

This simple model includes an input pathway (from light and/or temperature) to a circadian oscillator. The oscillator generates signals that are transduced via output pathways to produce overt circadian rhythms (output). The output is depicted as two idealized rhythms (red and green lines) with different phases. Yellow and grey boxes represent light and dark (diurnal) intervals, respectively. Under diurnal conditions, the period of the oscillator (the time between comparable points in the repeating cycles) matches the period of the entraining cycles. Under constant conditions, the clock oscillates with an endogenous period close to 24 hour. Amplitude is half the distance between the peak and trough [Figure 2].[7]

Factors influencing the growth of plants

Plants are very sensitive to changes in their environment, and respond to such changes with altered growth and development. Most plants have a daily rhythm set by environmental conditions such as light. The many external factors exert great influence on the plant growth and development of plants. The most two important factors are:

a. Intensity and duration of the light
b. The temperature of the air and soil around the plant

Intensity and duration of the light

The light influences the growth of individual organs or of the entire plant in less direct ways, expecting the effect through photosynthesis. The most striking effect can be seen between a plant grown in normal light and the same kind of plant grown in total darkness. The plant grown in the dark will have a tall and spindling stem and leaves fail to expand, and both leaves and stem are pale yellow due to lack of chlorophyll. Such a plant is said to be *etiolated.*[26]

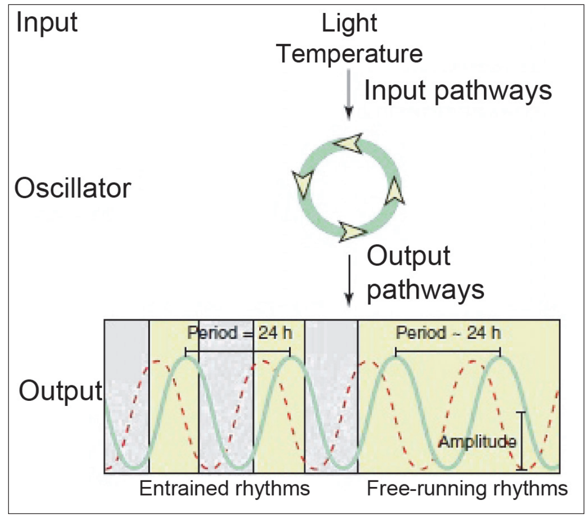

Figure 2: Components of a circadian system[25]

Plants grown in shade instead of darkness show a different response. Moderate shading tends to reduce transpiration more than it does photosynthesis. Hence, shaded plants may be taller and have larger leaves because the water supply within the growing tissues is better. With heavier shading, photosynthesis is reduced to an even greater degree and it results in small and weak plants.[26] The intensity and duration (length) of light both shows different and characteristic effects upon plant growth and development. The length of the daylight period may have a striking effect upon vegetative growth and reproductive activities of plants. The reaction of plants in relation to the length of the day is called *photoperiodism.*[26]

Examples of photoperiodic reactions in plants: the first is for a unicellular alga. The second example is the succulence of leaves in *Crassulaceae*. The third is tuber formation in potatoes. Finally, flower induction in short- and long-day plants is described. In the examples chosen, the level of complexity increases from a photoperiodic event in a single cell, to that in an organ such as the leaf, in underground stolons with remote perception of photoperiodic light in the leaves, and in the apex of stems, again with light perception in the leaves.[12]

According to photoperiodic effect the plants of temperate region are divided into three categories and they show a difference in their vegetative growth and reproduction methods.

Short – Day plants

These plants grow vegetatively during the long days of summer and do not produce flowers until the days become shorter as in late summer and early fall. Examples for short-day plants are poinsettias, most aster and goldenrods, the ragweeds, chrysanthemums, sorghum, and many others. Many short – day plants are extremely sensitive to light exposure during the night. In some types, even vehicle headlights or the light from a flashlight can prevent flowering. *Pharbitis nil* (Chois) cv *violet* and *Chenopodium rubrum* (L.) are examples for short-day plants. (in Evans 1969a).[12]

Long – Day plants

These plants will give flowering only under extended periods of illumination and produce only vegetative growth when the photoperiod is long. Examples for long – day plants are hollyhock, radish, garden beet, spinach, iris, and clover. *Lolium perenne* is another example of a long-day plant (in Evans 1969a).[12]

Day – Neutral plants

These plants are not particularly sensitive to the length of day. Included in this group are the bean, tomato, vetch, cyclamen, nasturtium, roses, snapdragon, carnation and many other common weeds.[26]

Light quality

Light quality refers to the color or wavelength reaching the plant's surface. A prism (or raindrops) can divide sunlight into respective colors of red, orange, yellow, green, blue, indigo and violet. Red and blue have the greatest impact on plant growth. Green light is least effective (the reflection of green light gives the green color to plants). Blue light is primarily responsible for vegetative leaf growth. Red light, when combined with blue light, encourages flowering. Light quality is a major consideration for indoor growing. For flowering plants that need more red light, use broad spectrum fluorescent bulbs. Incandescent lights are high in red and red-orange, but generally produce too much heat for use in supplementing plant growth.[27]

Light intensity

The more the sunlight a plant receives, the higher the photosynthetic rate will be. However, leaves of plants growing in low light readily sun scorch when moved to a bright location. Over time, as the wax content on a leaf increases, it will become more sun tolerant. In hot climates, temperature is often a limiting factor related to shade.[26] Light intensity is measured in *lux* or *foot-candles* [Figures 3 and 4].

Landscape plants vary in their adaptation to light intensity. Many gardening texts divide plants into sun, partial sun and shade. However the experienced gardener understands the differences between these seven degrees of sun/shade:

Full sun – direct sun for at least 8 hours a day, including from 9 a.m. to 4 p.m.

Full sun with reflected heat – Where plants receive reflected heat from a building or other structure, and temperatures can be extremely hot. This situation significantly limits the choice of plants for the site.

Morning shade with afternoon sun – This southwest and west reflected heat can be extremely hot and limiting to plant growth.

Morning sun with afternoon shade – This is an ideal site for many plants. The afternoon shade protects plants from extreme heat.

Filtered shade – Dappled shade filtered through trees can be bright shade to dark shade depending on the tree's canopy. The constantly moving shade pattern protects under-story plants from heat. In darker dappled shade, only the more shade tolerant plants will thrive.

Open shade – Plants may be in the situation where they have open sky above, but direct sunlight is blocked during the day by buildings, fences and other structures. Here, only more shade tolerant plants will thrive.

Closed shade – The situation where plants are under a canopy blocking sunlight is most limiting. Only the most shade tolerant plants will survive this situation, like under a deck or covered patio.[27]

Light duration

Light duration refers to the amount of time that a plant is exposed to sunlight. Travelers to Alaska often marvel at the giant vegetables and flowers that grow under the long days of the arctic sun even with cool temperatures.[27]

Temperature

In general, growth is promoted when temperature rises and is inhibited if the temperature falls. However, the growth rate does not continue to increase indefinitely with temperature rise. High – temperature injury due to *desiccation* (drying) and a runaway metabolic rate eventually occurs. Temperature affects growth through its effect on metabolic activities. Also, high temperatures increase transpiration and thus reduce turgor and growth, especially during the day. Each species has a minimum temperature, below which it fails to grow; an optimum at which

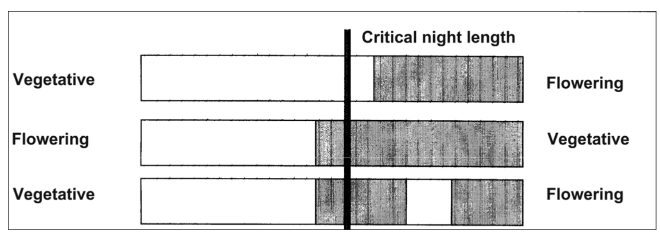

Figure 3: Intensity and duration of light[27]

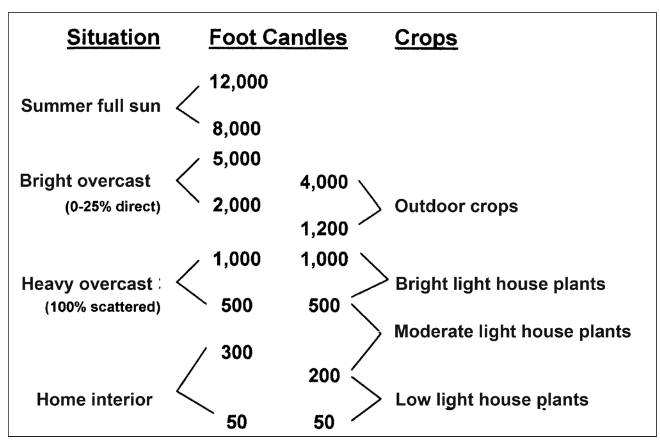

Figure 4: Light Intensity for various situations[25,27]

the growth rate is highest; and a maximum, above which, growth comes to an end. The optimum temperature may vary with each stage of development, and with the length of time the temperature prevails. Temperature affects not only the rate but also the type of growth. The photoperiod seemed the most important. It is now known that the light response for many plants is modified by temperature. The suitable photoperiod alone may be insufficient to bring about flowering unless it is accompanied by suitable temperatures. Temperature also plays a major role in the cycle of activity and inactivity known as *dormancy* in plants of temperate climates. Dormancy is especially prominent in woody plants; the leaves drop in autumn, the tree is inactive during the winter, and with the coming of spring, activity and growth are renewed. The length of the dormant period varies, and for many species a period of low temperatures is required to break the dormancy and permit growth to resume. Most deciduous fruit trees, such as the apple, peach, and cherry, require extended winter rest periods and therefore can be grown only in temperate climates. While some plants require freezing temperatures to break the rest period, others need only low temperatures above freezing. Most bulbs, tubers, and other underground stems require at least a short rest period.[26]

Seasonal timing of flower induction

In order to flower and produce seeds at the right time, plants use several control systems to evoke the transition from the vegetative to the reproductive stage of the apex [Figure 5]. One of these control systems depends on the developmental stage: The plant has to go through a juvenile phase and has to reach a certain age before flowering is induced. This is the autonomous pathway of flowering. Another part of the control system perceives environmental signals such as day length, temperature and humidity of the soil. If this environment-reactive pathway reports the right conditions, flowering is induced. Whether the autonomous or the environment-reactive pathway prevails depends on the plants in question, and varies strongly. [28] Recently, the molecular mechanism of flower induction has been clarified a great deal by using forward and reverse genetics, genomic technologies[29] and new techniques such as microarray analysis.[30] In the following experiment, the photoperiodic induction in the environment-reactive pathway will be treated. It is at the same time the best studied photo periodically controlled event in plants.[12]

The photoperiod Light/Dark (*L/D*) is perceived by photoreceptors in the leaves. Under long-day conditions, Flowering locus T (FT) is produced and transported to the apex. There it combines with Flowering locus D (FD) and activates flower meristem identity genes *ap1*, *ap2*, *cal* and *lfy*, thereby initiating flower development. There are other pathways, besides the photoperiodic one (*P.P.*), which control flowering, such as an autonomous pathway (*A.P.*, which eventually leads to flowering even under non-inductive photoperiodic conditions), a light quality pathway (Q.P., which accelerates flowering under shading conditions), a vernalization pathway (*V.P.*, a treatment where low temperature is necessary for flowering to occur) and a gibberellic acid pathway (G.P., in which Gibberallic acid (GA) will induce flowering). The A.P. and V.P. pathways inhibit *flc*, thereby removing the blockade of FT production by Flowering locus C (FLC), or activate *ft* (Q.P. via PFT1, which is not shown) or (G.P.) activate Suppressor of over expression of constans1 (*soc1*) and the flower meristem identity genes directly. There exists also an activation of FLC (*activating*). The photoperiodic reaction is due to rhythmic control of co-expression (left curve at bottom, Constans messenger Ribonucleic acid - (CO- mRNA) by the circadian clock, Flavin-binding kelch repeat F-Box (FKF1) protein functioning as a photoreceptor, and the fact that CO is stabilized by Crypto chrome (CRYs) and Phyto chrome A (PHYA) under light conditions, but is unstable in darkness. PHYB antagonizes the activity of CRYs and PHYA especially in the morning. It takes some time in light (a long light period, *left bottom curves*) until sufficient CO has accumulated to induce FT expression, which is necessary for flower induction. In short days, there is not enough time under light to produce the CO protein (*right bottom curves*).AP1- Apetala1[12]

Circadian clock and the photoperiodic control of flowering

Circadian system is often divided into three general parts. The general oscillator is the core of the system and generates the 24 hour rhythm. The oscillator is synchronized or entrained to daily cycles of night and day through light and temperature signaling pathways, which are often referred to as input pathways. Output pathways are controlled by the oscillator, and represent range of biochemical and developmental pathways. Control of flowering by day length may be triggered by such an output pathway, and the activation of this pathway by day length may be caused by a requirement that the time at which the activity of the pathway peaks, coincides with a time at which the plant is exposed to light. In *Arabidopsis*. mutations that alter the functions of the circadian clock have been isolated

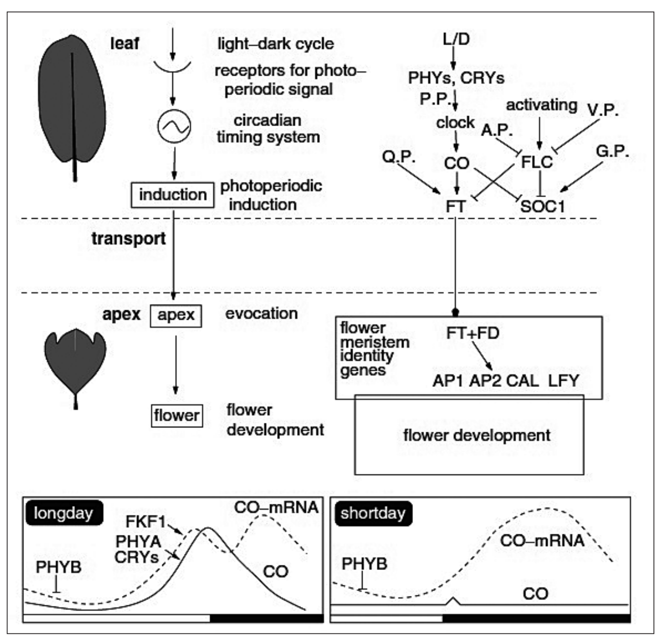

Figure 5 : Scheme of flower induction of a long-day plant[12]

either by using luciferase marker gene to follow rhythms in output pathways or by identifying mutants that exhibit altered flowering time, a trait that is regulated by the circadian clock [Figure 6]. These approaches have identified mutants in which the duration or period length of output rhythm is altered. These experiments have demonstrated that the distinctions between these three parts of the system are not often clear cut. For example, feedback loop in which output pathway regulate input to the oscillator, mean that some gene is simultaneously acting on both parts of the system.[31]

Model of the circadian system of Arabidopsis and its relationship to the flowering-time gene Constans (CO): Phytochromes and cryptochromes act additively or redundantly as photoreceptors for the light resetting of the circadian oscillator. Late Elongated Hypocotyl/ Circardian Clock Associated1 (LHY/CCA1) and Timing Of Cab Expression1 (TOC1) form a negative-feedback loop in the circadian oscillator. LHY/CCA1 proteins suppress the expression of TOC1, and act as a positive regulator for CCA1 and LHY. The circadian oscillator generates multiple circadian rhythms, including that of the expression of CO, which acts as on output for the regulation of flowering time. CO activity may also be regulated directly by light signals in a post-transcriptional manner, allowing CO to activate under long days and to induce the expression of FT, a gene that functions to promote flowering. ELF3 expression is also regulated by the circadian clock, and acts on light input into the clock to suppress or 'gate' the light signals. This allows the circadian to be reset by the dawn signals, and to cycle even under constant light. Zeitlupe (ZTL) and Gigantea (GI) also act on light input into the clock, and ZTL function in the input is accompanied by its interaction with PhyB. The transcript levels of ZTL are not regulated by the circadian clock.[31]

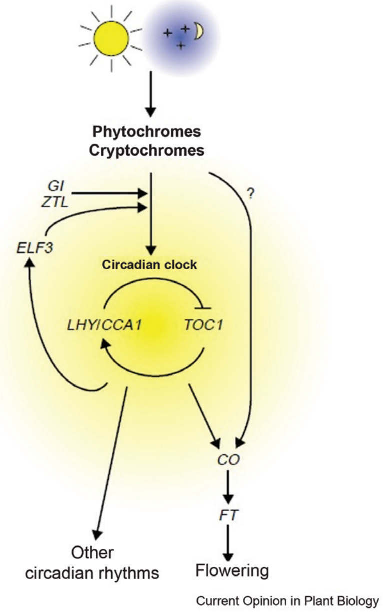

Figure 6: Regulation of circadian-clock function in Arabidopsis[31]

How plants tell the time

Time is closely monitored in nature as many biological processes are coordinated both, within each plant and in relation to the environment. To function as a circadian clock, the oscillator must be entrained to daily light and temperature cycles so as to match biological time with solar time. As light is an important environmental cue for the entrainment of the circadian clock, a long-standing goal has been the identification of the specific photoreceptors that are responsible for resetting the oscillator.[32,33] Little is known about the molecular basis of plant circadian oscillators; however, both the Coupland group[34] studying flowering time and the Tobin group[35] focusing on light-regulated gene expression have recently come very close to characterizing the clock mechanism. They have identified the genes *Late Elongated Hypocotyl* (*Lhy*) and *Circardian Clock Associated* (*Cca1*). *CCA1* functions in the light regulation of gene expression as well as affecting the circadian oscillator[36] as do the white *collar* (*wc*) genes in *Neurospora*.[37] *CCA1* could be a component of a light signaling pathway providing the molecular link between the phytochromes and the timekeeper. Rhythmic phosphorylation is part of at least one circadian output pathway in plants that mediates the circadian control of certain enzyme activities[38,39]

Melatonin in plants Influenced by circardian rhythm

Melatonin (N-acetyl-5-methoxytryptamine) (MEL) is an indole-amine isolated for the first time in 1958 from the bovine pineal gland by Lerner and co-workers from Yale University.[40] In animals, MEL is involved in the regulation of the circadian rhythm. It was called as the hormone of darkness as its highest level is observed at night and during the day it decreases to the hardly detectable level. A similar pattern of MEL synthesis is observed in some Dinoflagellates and in some photosynthesizing plants.[41] However, very little is known about the physiological role played by MEL in plants[42,43] although inconclusive attempts have been made to seek a role for this indolic compound as a photoperiodic and circadian regulator.[44-46] Endogenous chronobiological regulator - biological clock,' the phenomenon correlated with cyclic and rhythmic changes of different bioprocesses in agreement with the day/night rhythm is the basis of plant reactions on photoperiod. In plants, MEL seems to be involved in the regulation of circadian changes of physiological processes.[47] MEL plays an important role in the regulation of the endogenous circadian rhythm in unicellular photosynthesizing *Gonyaulax polyeda* from the phylum *Dinophyta*.[48] Bioluminescence observed in *Gonyalux polyedra* is the classic example of circadian changes and this organism emits strong light at night while daylight decreases it significantly. It was shown that the level of MEL changed accordingly with the maximum at night and minimum during the day.[48] Photoperiodic impulse induces a chemical factor in plant leaves, which migrates to a shoot and triggers flowering processes. Photoperiodic reactions are mediated by phytochrome that regulates the genes responsible for the metabolic changes leading to flowering. Thus short-day plants may be made to blossom if short day is followed by a long night. Light flesh interrupting darkness inhibits flowering. It also inhibits MEL synthesis.[49] In 15-day old *Chenopodium rubrum* L., seedlings growing at 12 hours/12 hours

Table 1: Melatonin (MEL) contents in some plant organs

Common names	Scientific names	Organ	MEL(ng g/1)
Banana	*Musa paradisiaca* (L.)[53]	Fruit	0.002
Onion	*Allium cepa* (L.)[54]	Bulb	0.03
Pineapple	*Ananas comosus* (Stickm.) Merill[54]	Fruit	0.04
Apple	*Malus domestica* (Borkh)[54]	Fruit	0.05
Carrot	*Daucus carota*[54]	Root	0.06
Tomato	*Lycopersicon esculentum* (Mill.)[53]	Fruit	0.05
Ginger	*Zingiber officinale* (Roscoe)[54]	Root	0.06
Rice	*Oryza sativa japonica* (L.)[54]	Seed	1
Corn	*Zea mays* (L.)[54]	Seed	2
Cherry	*Prunus cerasus* (L.)[55]	Fruit	18
Fennel	*Foeniculum vulgare* (Gilib.)[56]	Seed	28
Sunflower	*Helianthus annuus* (L.)[56]	Seed	29
Almond	*Prunus amygdalus* (Batsch)[56]	Seed	39
Black Mustard	*Brassica nigra* (L.)[56]	Seed	129
White Mustard	*Sinapis alba* (L.)[56]	Seed	189

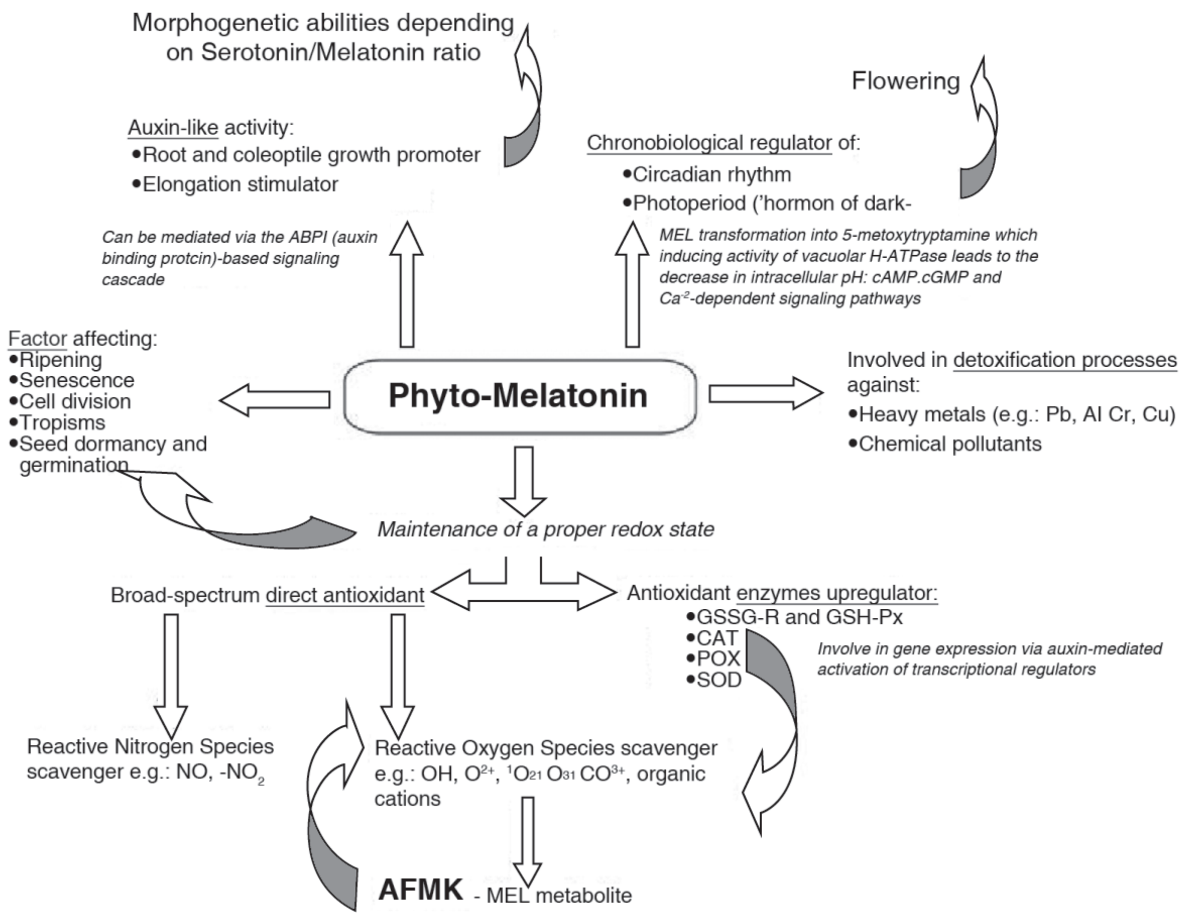

Figure 7: The phyto- melatonin role and mechanism of action[51, 52]

photoperiod, and the level of MEL is hardly detect- able during the light/day and reaches its maximum 4–6 hours into the dark/night period.[45] The moment of MEL maximal concentration depends on the length of the darkness period.[46] The longer the darkness period, the later the MEL peak appears [Figure 7].[50] MEL is believed to mediate the photoperiodic response in higher plants including the photoperiodic induction, but the mechanisms are still unknown.[41,50]

Some important medicinal plants and their melatonin levels are described in Table 1.

CONCLUSIONS

Our knowledge of the chronopharmacognosy system has increased significantly in recent years. Biological clock is important in producing number of rhythm in plants and produces numerous metabolic activities. Growth of the plants is influenced by intensity of the light, duration of light, light quality, temperature of the air and soil around the plant. Flower induction in the apex is induced through the light and temperature received by the leaves of the plants, thereby inducing circadian clock, producing oscillation and inducing flower at the apex in a right time. CCA1 gene functions in the light regulation of gene expression as well as affecting the circadian oscillation. It also provides molecular linkage between the phytochromes and the time keeper. Melatonin synthesis is observed in *Dinoflagellates* and in some photosynthesizing plants. This involves the regulation of circadian changes of physiological processes. Photoperiodic impulse induces a chemical factor in plant leaves, which migrates to a shoot and triggers flowering process. Light flesh interrupting darkness inhibits flowering and it also inhibits melatonin synthesis. Melatonin maximum concentration depends on the length of the darkness period. Melatonin mediates the photoperiodic responses in higher plants. In medicinal plants such as white mustard and black mustard, a very high melatonin level is found in the seeds. The diversity of chronopharmacognosy demonstrates the potential importance of perceiving many wavelengths in order to run the daily timekeepers. By studying chronopharmacognosy, we are able to find the time at which the medicinal plants produce more therapeutic agents and the time to extract a particular therapeutic agent from the plant.

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