The Sensory Biology, Communication, Memory and Intelligence of Plants

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TABLE OF CONTENTS

DETAILED SUMMARY OF CONTENTS

Chapter 2

EXTRACORPOREAL COMMUNICATION IN PLANTS

- Immunology in response to the touch of herbivores

Plant-plant chemical communication
- Host-parasite plant recognition
- Communication among competing plants
- Positive allelopathic interactions

Acoustic communication in plants
- Plants have phonotropic responses
- Acoustic plant-animal communication
- Plants as sound reflectors

Electrical communication in plants
- Plant-animal electrical communication
- Plant-plant electrical communication

Olfactory communication in plants
- Volatile compounds and defense communication
- Volatile compounds action mechanisms
- Volatile compounds and pollination by sexual deception
- Various effects of communication by volatile compounds

Plant-plant communication via mycorrhiza
- Plant-to-plant nutrient transfer via mycorrhiza
- Allelochemicals plant-to-plant transfer via mycorrhiza
- Plant-to-plant defense signaling via mycorrhiza

Plant-plant communication by light
- How do plants predict shade?

Chapter 3

INTRACORPOREAL COMMUNICATION IN PLANTS

Intracellular communication
- Chloroplast-mitochondria-nucleus communication
- Chloroplast-peroxisome communication
- Endoplasmic reticulum-Golgi communication
- Endoplasmic reticulum-peroxisome communication
- Endoplasmic reticulum in intracellular communication
- Calcium in intracellular communication
- Organelle movement and intracellular communication
- Photoreceptors in intracellular communication
- Retractable projections of organelles in intracellular communication

Chapter 4

PLANT MEMORY

Chapter 5

INTELLIGENCE IN PLANTS

INTRODUCTION

This book sets out to explore cognitive abilities in plants. Although nominally a theme that has only been consistently addressed in the last two decades, science has in fact been investigating some of its conceptual bases for a long time, as in the case of studies of plants' perception and communication skills. In their condition as organisms with no central command structures, such approaches necessarily center on general and individual cellular capacities, avoiding metaphors or analogies with living beings endowed with canonical nervous systems and brains. The intention is that reading this work should help to dispel the prevalent idea that plants are passive organisms and, despite their long evolution in interaction with the environment, only capable of responding to stimuli, an unlikely condition for any living being.

At one time, given their sessile condition, plants were considered to be asexual simply because they lacked the means of moving around in search of a partner, a role associated to sexual beings. Even though today we consider such ideas groundless, nevertheless, to some extent we perpetuate the attitude when we consider that because plants lack the sensory organs that typify big animals, they must be insensitive to stimuli such as sounds, smells and touch, in spite of the fact that their sensitivity to gravity and light is well known. Although the effects of many stimuli that plants respond to have been explored, the corresponding sensory apparatuses are still little known as, for example, in the case of responses to temperature, relative humidity, barometric pressure, atmospheric gases, water, salts, magnetic and electrical fields, all of which are addressed in the first chapter.

Plants are not just skillful at detecting the presence and realizing the importance to their development of various elements in the environment, but they are also capable of recognizing themselves and their relatives, of memorizing, learning, communicating and even deliberately modifying the environment to their own benefit and that requires intelligent behavior. Proof of those plant skills set out in this book is evidence that cognitive abilities are not an exclusive characteristic of specific cells of immunological or nervous systems and reveal hitherto unexplored potentialities of cells which are apparently unspecific and occur in various plant tissues and organs.

Given the importance of this theme, I felt impelled to write this book which in spite of being essentially based on studies in the field of plant biology is of common interest to all professionals, students and persons imbued with curiosity in the various areas of life and life behavior sciences. Despite the considerable knowledge associated to the themes addressed here, there is still a long way to go and that will certainly require the concerted efforts of professionals in a variety of segments.

It is my fervent wish that the fascinating world of plants should become more attractive as we show, in the course of the chapters, that they are in fact active, dynamic and ultramodern living beings even capable of wireless communication and of discerning the difference between true information and fake news.

—The author

CHAPTER 1

SENSORY BIOLOGY IN PLANTS

Plants are organisms responsive to environmental stimuli and many excellent books on plant biology are dedicated to the exploration of plant-environment interactions. However, there is a gap in the knowledge of what happens between the signal and the response that concerns the plant's perception of the stimulus; only the sensory apparatuses for light and gravity are widely known.

This chapter is dedicated to exploring the elucidated or putative mechanisms of plant perception that link the stimulus factors present in the environment to the responses of plants. Light perception will be explored within another sensory context, namely, how plants perceive time. That is because light is the most important stimulus for the synchronization of plants to different time cycles and a factor responsible for several phenomena that coincidentally manifest themselves at specific times of the 24-hour cycle, in specific months of the year, or even according to the exact sum of many annual cycles.

Different metabolic and developmental events, widely known in plant biology, will be used to contextualize plants' perception of CO_2, O_2, O_3, cold, heat, water, relative humidity, nutrients, salt, atmospheric pressure, gravity, sounds, electric and magnetic fields and touch.

The sensory apparatus of plants is not limited to inanimate elements alone. There are interactions between plants and other living organisms that require sophisticated perceptions, often integrated into a complex ambit of recognition and communication. Those aspects will be explored together in the chapter "Extracorporeal communication in plants". Similarly, the perception of intracellular or intercellular communication signals will be explored in the chapter "Intracorporeal communication in plants".

Light Perception

The sensory apparatus of plants for light is known. Many photoreceptors have already been identified, as well as the effects they mediate. This section will study how the perception of light patterns by plants is involved in plant phenomena synchronized to different time cycles.

Light is a necessary factor for plants, not only as a source of energy, but of information. Its intensity, quality, duration and direction are aspects perceived by plants, triggering changes in their metabolism and development. The perception of light patterns is fundamental for plants to respond to the condition of day and night, of sun and shade, to the times of the day, to the months of the year and even to certain other time cycles.

We often come across phenomena revealing that plants are synchronized to different cycles of time. For example, the flowers of the queen of the night cactus *Epiphyllum oxypetalum* (Figure 1.1A) are closed during the day and only open at night; the strong exhalation of perfume by the night-scented jessamine, *Cestrum nocturnum* flower (Figure 1.1B), is typically a nocturnal event. We also know that some plants only bloom in specific months of the year, such as the false Christmas cactus, *Schlumbergera truncata* (Figure 1.1C); we can also observe an effect of the time of day, such as in the moss rose or eleven o'clock plant, *Portulaca grandiflora* (Figure 1.1D), which in tropical regions of Brazil, opens its flowers around 11:00 am. Naturally, for plants to respond to those variables, they need to perceive the condition of the environment, and for that purpose, light is the most important signal they use.

How are plants synchronized with time?

Light is the most important environmental signal for the synchronization of plant behaviors with time cycles. Light integrates electromagnetic radiation emitted by the sun, characterized by a specific wavelength range. In addition to waves, electromagnetic radiation is characterized by particles known as photons, which have different quantities of energy depending on the different wavelengths. The intensity and proportion of photons of different energy quantities (quality of light), the direction in which light touches on a plant organ, and its duration are aspects that, isolated or combined, are used by plants as signals of environmental condition. The plant has a large apparatus of biomolecules called photoreceptors that act in the perception of light. The interpretation of the light signal by the plant depends, in principle, on the physical state assumed by the photoreceptors after being exposed to light.

The term light is widely used in photobiology studies; nominally it represents the electromagnetic radiation emitted by the sun that sensitizes our ocular photoreceptors, allowing us to see them as colors. However, in addition to this part of the radiation visible to the human eye, plants also perceive and absorb non-visible radiation in the ultraviolet spectral band (Figure 1.2).

Figure 1.1 Plants with floral behaviors synchronized to temporal variables. 1.1A. *Epiphyllum oxypetalum*, an example of flowers that only open at night. 1.1B. *Cestrum nocturnum*, an example of flowers that exhale a strong perfume at night. 1.1C. *Schlumbergera truncata*, an example of a plant that flowers during a specific month of year. 1.1D. *Portulaca grandiflora*, an example of a plant that opens its flowers at a specific time of the day. Credits: 1.1A Image by luismagroarq from Pixabay. License to use at https://pixabay.com/en/photos/dama-da-noite-porta-casa-flor-1824024/ 1.1B Image by Asit K. Ghosh from Wikimedia Commons. License to use at https://commons.wikimedia.org/wiki/File:Jasmine_NightBloomimg2_Asit.jpg 1.1C Image by TuJardínDesdeCero from Pixabay. License to use at https://pixabay.com/en/photos/cacto-de-natal-cacto-flores-4662167/ 1.1D Image by Meriele Oliveira Pereira Mel from Pixabay. License to use at https://pixabay.com/en/photos/natureza-planta-flor-folha-jardim-3304689/

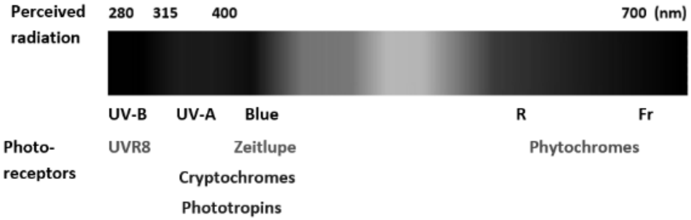

Figure 1.2. Radiation perceived by plants. The phytochrome photoreceptor absorbs radiation in the red (R) and far-red (Fr) band; components of the zeitlupe family absorb in the blue band; phototropins and cryptochromes absorb in the bands of blue and ultraviolet A (UV-A); UVR8 absorbs in the ultraviolet B (UV-B) band.

Plant photoreceptors are molecules that perceive and absorb light. They are made up of proteins linked to a light-responsive molecule, the chromophore. The activation of the photoreceptors depends on the intensity and quality of the light that reaches the plant. As shown in Figure 1.2, phytochromes absorb radiation in the red (R) and far-red (Fr) band, cryptochromes and phototropins absorb radiation in the blue and ultraviolet A (UV-A) bands, some members of the zeitlupe family absorb in the blue band, and UVR8 absorbs in the ultraviolet B (UV-B) band.

There are several genes in plants that encode for photoreceptor proteins, resulting in several phytochromes (phyA, phyB, phyC, phyD, phyE, phyF), three cryptochromes (cry1, cry2, cry3), two phototropins (phot1, phot2), three members of the zeitlupe family (ZTL, FKF1 and LKP2) and the UVR8. Considering the photoreceptors coming from multigenic families, within each class there are some that are more sensitive to the intensity of radiation in the spectral band that they absorb and some that are less sensitive. The different phytochromes, in addition to their different sensitivity in terms of radiation intensity, are also sensitive to the proportion of R/Fr that reaches the plant, intermediating several photodependent responses (Gavassi et al., 2017; Melo et al., 2009; Melo et al., 2011).

Based on the intensity, quality, direction and duration of the light reaching the plant, different photoreceptors are activated, triggering events that culminate in the plant's responses to different time cycles.

How do plants distinguish day and night?

The most remarkable difference between day and night is the intensity of light that reaches a surface of the Earth. Naturally, the intensity of light that reaches the plants during the day is much greater because the main natural emitting source is the Sun. The plant realizes that it is daytime due to the large number of photoreceptors biologically activated by the light that they absorb. The activation of the photoreceptor occurs due to the conformational change of its structure or simply by the elevation of the energy state of some of its components, promoting autophosphorylation, and triggering, from then on, a cascade of interdependent cellular signals that culminate in the manifestation of daytime events. When the active photoreceptors act by inhibiting events, then those events can occur at night when their inhibitors are deactivated. Thus, we typically have daytime or nighttime events in plants.

A phytochrome is a molecule with a characteristic that is rare in biology, photoreversibility. Looking at Figure 1.3, we observe a *cis* isomeric form, called Pr, which is biologically inactive. Pr absorbs red light (R), which is abundant during the day, and thus assumes a *trans* isomeric form, called Pfr, which is biologically active. Pfr, in turn, when absorbing the far-red light (Fr), relatively abundant at dusk, assumes a Pr isomeric form. Therefore, the fact that a plant has a high percentage of phytochrome in Pfr form in its body is indicative of day and it is this form that will trigger phytochrome-dependent events that only occur during the day, as well as inhibiting those that only occur during the night. On the other hand, a high percentage of Pr is indicative of the night period, and the events that were inhibited by Pfr during the day will be released from the inhibition, and the events that happen at night will occur.

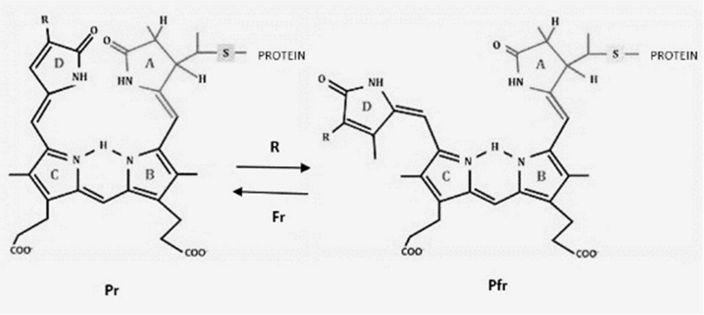

Figure 1.3. Phytochrome photoreversibility. In the presence of red light (R), the phytochrome takes on a biologically active Pfr form, and in the presence of far-red light (Fr) it takes on a biologically inactive Pr form.

During the day, 88% of a plant's phytochromes can take the Pfr form and during the night 97% take the Pr form. 100% conversion does not occur because the Pr also absorbs a little in the Fr spectral band and the Pfr also absorbs a little of R, which means that what regulates phytochrome-dependent events is actually the ratio of Pfr to Pr in the body of the plant.

It is important to note, however, that phytochromes are not the only photoreceptors that participate in the plant's perception of the day or night condition. The others also contribute to day or night metabolisms. However, they are not photoreversible; they are only activated or deactivated depending on the presence or absence of their respective absorbable radiation.

To sum up, plants are synchronized with day and night because during the day there is a high ratio of Pfr/Pr phytochromes in their bodies, as well as several other photoreceptors in their activated forms, acting as positive regulators of the metabolisms that culminate in daytime events. On the other hand, at night there will be a low Pfr/Pr ratio, and the other photoreceptors will be in their inactivated forms, triggering metabolisms that culminate in nocturnal events, such as the opening of the *Epiphyllum oxypetalum* flowers (Figure 1.1A) and the strong release of perfume by the flowers of the *Cestrum nocturnum* (Figure 1.1B).

Plants perceive the conditions of full sunlight and shade

More sophisticated than being synchronized with the condition of the day or night, the plant senses and responds to the condition of full sunlight and shade during the daytime. This is important, for example, for them to arrange their leaves so that they are not shaded by one another. It is also important for shaded branches to increase their growth rates to reach direct sunlight as soon as possible. These are especially important events for heliophyte plants.

To perceive a refined level of light patterns, such as direct sunlight/shade, photoreceptors with different sensitivities to radiation intensity are important and some must be photolabile and others photostable. Although phyA absorbs light in the R band, cry2 and phot1 absorb in the blue and UV-A bands, high radiation intensities promote loss of their activities and may even cause photoreceptor destruction. The remaining photoreceptors, phyB-F, cry1 and 3 and phot2, are photostable, with high activity performance under high radiation intensities. Therefore, if it is daytime and a branch has a high ratio of active photolabile photoreceptors to active photostable photoreceptors, it is indicative of shading, triggering in plants events that promote an increase in the growth rates of the branch, so that it exposes its leaves to full sunlight as soon as possible. When that objective has been achieved, the intense incident radiation leads to the destruction of the photolabile photoreceptors, which, consequently, culminates in curbing the accelerated growth. This behavior of different speeds of growth due to shade and full sunlight is easily observed in young heliophyte plants which tend to grow faster in shade conditions. In that context, the ratio between photolabile and photostable photoreceptors active at a given moment serves as a code regarding the ambient light condition that the plant organ interprets.

In conclusion, a high proportion of photolabile photoreceptors active in relation to photostable photoreceptors active in a plant organ is the way in which the plant synchronizes its metabolism with the shade condition, which triggers the appearance of typical events, such as the increase in the rate of stem growth, while the opposite proportion of photoreceptors is indicative of full sunlight.

Plants perceive the direction of light

Clear evidence that plants perceive the direction of light is the phototropism (Figure 4.7), an event observed when the young region of a stem curves towards the light source. An even more notable example is the heliotropism that occurs in the young stems of sunflowers, which move according to their alignment with the sun throughout the day, and therefore from East to West. Figure 1.4 displays an image of the positioning of young sunflowers as a function of heliotropism.

Phototropic or heliotropic movements are events mediated by phototropins, which, upon perceiving and being activated by blue light shining laterally on a stem, trigger responses of radial redistribution of the hormone auxin in the stem region close to the apex, culminating in asymmetric cell growth of the opposite sides. Although there is East-West orientation in the movement of sunflowers during the day, near dusk they turn to the East. It is a period in which there is loss of phototropin activity, so the return to the East does not seem to depend on the activated phototropins, showing that this could be a movement dependent on circadian regulation (the object of study of the next topic). That was confirmed by Atamian et al. (2016), who observed that even the movement from East to West has circadian regulation. As part of the mechanism of this regulation there is different expression of genes, regulated by the circadian clock, which act on the cellular sensitivity to auxin, and whose expression during the day is greater on the side of the stem facing East and at night is greater on the side facing West.

A factor that influences the responses of plants depending on the direction of light is the angle of inclination of the radiation that affects a plant organ, which is due to the positioning of the emitting source and the receiving organ. Different angles cause changes in the intensity and quality of incident radiation; aspects used by plants as informative signs of the environment. In addition, although the effects are not known, both phytochromes and phototropins are anisotropic to light and can alter their connection with the plasma membrane according to the angle of the incident radiation (Hughes, 2013).

Figure 1.4. Synchronous and uniform positioning of young sunflowers according to the position of the sun (heliotropism). Credits: on the left, image by Siggy Nowak from Pixabay. License to use at https://pixabay.com/en/photos/girass%C3%B3is-natureza-campo-3250317/ On the right, image by Erwin Nowak from Pixabay. License to use at https://pixabay.com/en/photos/campo-girass%C3%B3is-flores-agricultura-4388347/

As will be discussed in the section “Electric Field Perception”, there is evidence of the participation of plant photoreceptors intermediating electrical signaling in plants. In the context of phototropism there is also evidence of electrical stimuli, mediated by photoreceptors, influencing the response of plants. One such piece of evidence appeared in the work developed by Volkov et al. (2004) using soybean plants illuminated with white light. When the light source illuminating the upper part of the plant from above was moved to its side, there was a depolarization of the plasma membrane, creating an action potential with an amplitude of 60 mV for 0.3 ms. However, when isolating the light spectrum bands, this action potential only occurred when there was incidence of light in the 400-500 nm and 650-730 nm bands, with no action potential being generated in the 500-630 nm range. This is indicative that the blue (400-500 nm) and red (650-730 nm) photoreceptors are involved in creating the action potential, and so, as is typical of “all or nothing” reactions, because those photoreceptors are not sensitive to other spectral bands, they did not induce the generation of the action potential in the 500-630 nm band.

In short, the direction of light can cause phototropic and heliotropic movement in plants. The degree of inclination of the radiation reaching the surface of the plant organ interferes in its intensity and quality, characteristics used by the plant as informative signs of the environment. In addition, intermittent and regular positions of light can generate information that feeds movement responses regulated by the circadian clock. Although the importance of anisotropy itself for photo-dependent events is not known, it is known that phytochromes and phototropins alter their interactive positions towards the plasma membrane according to the angle of the incident light. There is also evidence of the generation of action potential, dependent on the action of photoreceptors, influencing the phototropic response.

How are plants synchronized with the time of day?

The internal cellular signaling triggered by the activity of the photoreceptors commonly leads to the production or activation of positive or negative factors of gene transcription. A large part of plant genes is regulated by light, which in rice and *Arabidopsis thaliana* (arabidopsis) corresponds to 20% of the genome (Jiao et al., 2007). In a 24-hour period, there is a fixed schedule in which there is greater or lesser expression of photo-dependent genes, or even non-expression. The products of those expressions consequently trigger events whose peaks of manifestation always occur at a certain time of the day or night.

The eleven o'clock plant or moss rose, *Portulaca grandiflora*, Figure 1.1D, is a plant that often open its flowers at a specific time of day, 11:00 am in tropical regions, and an example of the influence of the time of day on plant behavior. Let us say that dawn today occurred at 06:00 am. This means an increase in R radiation in the environment and, consequently, an increase in Pfr phytochromes in the plant, as well as in other activated photoreceptors, including photolabile ones. From dawn on, the active forms of these photoreceptors trigger the activity of some cell signaling routes that lead to the production or activation of repression factors or the transcription of light-regulated genes.

As the day goes on, the products of expression of the light-dependent genes that started activity at 06:00 am accumulate. Likewise, the products of the genes that have been suppressed (whether consumed or degraded) decrease. This means that at every moment there will be changes in the balance of different cellular products; it means, there are changes in the proportion of one product in relation to another. The photo-dependent responses occur according to the accumulated quantity of products (or their ratios). For example, at 7:30 am, a response will be triggered by the accumulation of these products reaching the threshold necessary to trigger an event, an accumulation that will only be attained 1.5 h after dawn.

It can be expected that as the day goes on there will be an increase in light intensity and that at a certain moment the photolabile photoreceptors will become inactive. For example, if a gene is positively induced to expression by the action of Pfr-phyA, and this photoreceptor is destroyed at 9:00 am, from that time on there may be stagnation or decrease in the quantities of products dependent on the expression of that gene. The product begins to accumulate in the cell at 6:00 am and has its maximum accumulation at 9:00 am. This behavior will be repeated in 24 hours, since the next day it is expected that dawn will again occur at around 6:00 am, a time that provides the light and photoreceptor activity conditions necessary to repeat the event. The same reasoning applies to genes that have been suppressed by the presence of light, which, when expressed during the night, result in an accumulation of products necessary for events whose peaks occur at specific times of the night.

Flower opening will be one of the results of the start of gene expression or repression at 6:00 am, due to the time when the minimum limit of accumulation (or balance) of biomolecules necessary for floral opening will be reached. From 6:00 am on, there can also be inhibition of genes that result in products that inhibit floral opening, and with the consumption or alteration in the balance between different products, at 11:00 am they reach a threshold that no longer represses the opening of the *Portulaca grandiflora* flowers (Figure 1.1D). Since tomorrow's dawn will be at a time close to today's, once again the same photo-dependent events will occur at the same time. The same reasoning applies to nighttime events, since at twilight there is an enrichment of Fr light in the environment inducing the conversion of Pfr to Pr, and, at dusk the other photoreceptors are also inactivated by the absence of light.

According to the approach above, there is a rhythmic synchrony between different times of the day or night and the photo-dependent events during the 24 h period, revealing an effect of the circadian clock. It is also evident that there are two moments of synchronization of that clock, dawn and dusk.

Some events are very dependent on momentary light patterns, so that, if the day is cloudy, a photo-dependent response may occur later than usual, or not occur, since changes in the intensity and quality of radiation alter the responses dependent on photoreceptor activity. In that case, the flower that should open at 11:00 am could delay or simply not have a floral opening. It is important to note that, even on bright days, other environmental factors, especially temperature changes, can modulate the photo-dependent responses, causing some variation in the time of their occurrence.

Not all photo-dependent events are sensitive to momentary light patterns. The production of chlorophyll and leaf movements are examples of events that do not change their time of occurrence, even if the start and end time of the 24-hour light/ dark period changes, and, furthermore, they are not modulated, or are little modulated by temperature changes. This non-change in the time of events, in this case, events regulated by a circadian clock, results from the regulation of genes by their own products. As an example, consider that a gene, A, starts to express at 6:00 am, and the high quantity of products resulting from its expression, at 6:00 pm, causes inhibition of the gene itself, or that this quantity of “A” products at 6:00 pm triggers the expression of a gene “B”, resulting in products that inhibit the expression of gene A. Thus, we will have a change in events dependent on the expression of these two genes every 12 hours. Even considering that gene A is photo-dependent but regulated by the circadian clock, a sudden change in the start time of the light period, on a certain day, would not cause a change in the usual times of its expression and, therefore, there would be no changes in events dependent on the expressions of genes A and B. However, if that change extends for many consecutive days, the circadian clock will be resynchronized according to the change that the new dawn time represents for the plant, but it will take several daily cycles for the resynchronization to take place and, after that, the times for gene expressions will be changed according to the resynchronization setting of the clock.

The regulation of events by the circadian clock is very important for plants, so that they are not over-sensitive to light variations, maintaining many of their photo-dependent events even on cloudy or rainy days, a mechanism contributing to the adaptive success of plants in the course of evolution.

Many genes are known to participate in the oscillator of the circadian clock. As seen in Figure 1.5, taking *Arabidopsis thaliana* as an example (Harmon et al., 2018), light-activated photoreceptors, especially phytochromes and cryptochromes, induce the expression of the genes *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) and *LATE ELONGATED HYPOCOTYL* (*LHY*), which are fundamental to the rhythmic maintenance of the oscillator. The product of their expressions induces the expression of the diurnal genes *PSEUDO-RESPONSE REGULATOR* (*PRR7* and *PRR9*) and suppresses nocturnal genes such as *TIMING OF CHLOROPHYLL A/B BINDING PROTEIN 1* (*TOC1*), which is also essential to the rhythmic maintenance of the oscillator, as well as the regulation of other nocturnal genes such as *EARLY FLOWERING 4* (*ELF4*), *EARLY FLOWERING 5* (*ELF5*), *GIGANTEA* (*GI*) and *FLAVIN-BINDING KELCH REPEAT F-BOX 1* (*FKF1*).

The greatest accumulation of *CCA1* and *LHY* products occurs early in the day. As the day goes on, the products of the *PRR7* and *PRR9* genes accumulate and they are repressors of the *CCA1* and *LHY* genes. That leads to less accumulation of those gene products at the end of the day. Once the repressive action of CCA1 and LHY has decreased at the end of the day, nocturnal genes that were repressed, such as *TOC1*, *ELF4*, *GI*, *PRR3*, *PRR5*, are released from repression, and the product of their expressions reaches maximum accumulation during the nocturnal period. The *TOC1* product, through some unidentified factor, acts as a positive factor for the expression of *CCA1* and *LHY* at dawn, thereby closing the 24h circuit.

The main loop (circuit) of the clock is in the *TOC1*-*CCA1*/ *LHY* interrelation (Figure 1.5). Several other genes and biomolecules, in addition to those already mentioned, participate in the regulation of the circadian clock and may even be involved in secondary loops. However, the idea presented here will be enough to understand the role of the circadian clock in different plant responses to be explored in the course of this book.

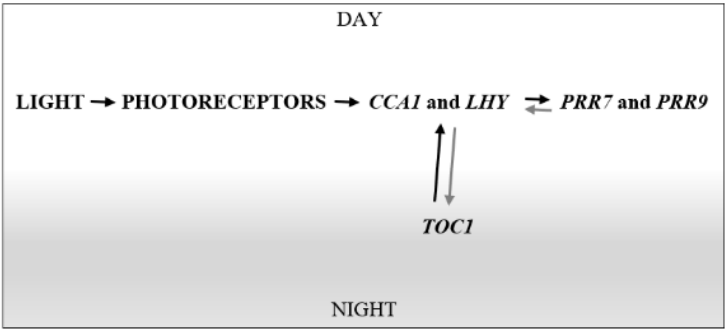

Figure 1.5. Main circuit of the circadian clock. Black arrows indicate signs of stimulus and grey arrows indicate signs of inhibition. At dawn the light activates the photoreceptors, which promote the expression of the *CCA1* and *LHY* genes, whose products induce the expression of *PRR7* and *PRR9*, which, in turn, gives rise to products that suppress the expression of *CCA1* and *LHY* at the end of the day, resulting in the release of repression of the nocturnal genes that they inhibit, such as *TOC1*. The accumulation of *TOC1* products at night promotes the expression of *CCA1* and *LHY* at dawn, completing the circuit.

It is important to note that not all responses triggered by the activation of photoreceptors culminate in gene expression. Some can occur very quickly because light, mediated by the photoreceptors, is only needed to activate or deactivate certain pre-existing molecules in the response circuit. As an example, it is known that in the opening of stomata, the role of light is to promote the activation of phototropins which occurs due to their autophosphorylation resulting from the elevation of the chromophore's energetic state. This last is the initial event for the activation of the signaling cascade that leads to the opening of stomata in a matter of seconds, although the circadian clock also influences stomatal movement.

In short, plants are synchronized with the time of day because their distinct behaviors over 24 hours depend on the cyclic expression of many genes that are positively or negatively regulated by light. At dawn, there is an increase in activated photoreceptors that trigger the expression of daytime genes, as well as repression of nighttime genes. At twilight/dusk, nocturnal genes are released from suppression concomitantly with no expression for the diurnal ones. Many genes are regulated by their own products or by the product of other genes, regulation dependent on a threshold or a balance of accumulated products. This threshold is reached at different times, starting from sunrise or from sunset. Since the time of dawn and dusk does not vary much between nearby days, different events resulting from the expression of the photo-dependent genes tend to have rhythmic peaks or valleys, that occur at the same times every day. The intermittent and constant rhythm of gene expression can establish a memory inherent to the circadian clock, which will guarantee that even if there is a sudden variation in the radiation pattern on a certain day, there will be no change in the rhythmicity of the response, unless that variation persists for several consecutive days, inducing a resynchronization of the clock.

How are the plants synchronized with the months of the year?

In Figure 1.1C we can see the *Schlumbergera truncata,* whose flowering is an example of phenomena that occur once a year in a specific month. The number of plant species that bloom at specific times of the year is abundant in nature. The phenomenon is associated to the photoperiod/nictoperiod of the geographic region where these plants are located.

Due to the Earth's translation movement, the lengths of the day and the night are different at different times of the year because of the position of the planet in relation to the Sun. Since the same positioning will only occur again after one year, the duration of the day and the night in a given geographic area will be the same on the same dates of all the years to come, with little change, due to the annual cycle being completed in exactly 365 days and 6 hours.

It is exactly the different lengths of day and night that are perceived by plants in a specific geographic region, resulting in responses, such as flowering, in specific months. Not all plants are sensitive to the photoperiod/nictoperiod and some of them can bloom at any time of the year. The flowering of photoperiodic-sensitive plants can be modulated by other environmental factors, especially temperature and water availability, and thus, there may be an anticipation or delay of their response in relation to the reference month.

How do plants measure time?

As an example of measuring the photoperiod/nictoperiod, consider that a plant in the adult-vegetative phase is photoperiodic-sensitive and is stimulated to bloom in an environment that has 13 hours of light or more, and consequently, less than 11 hours of darkness. It would be a species that, in the geographical location of Brasília-DF, Brazil, would be induced to bloom between November and February, which is when this environmental condition occurs. If in this same location there is a plant that only blooms with days that last less than 11 hours, and consequently, nights longer than 13 hours, it would be a species that would only be induced to bloom between June and July.

Since the light period induces the activation of photoreceptors and these commonly activate cell signaling pathways that culminate in the expression or repression of genes, then if the day has 13 hours of light there will be a certain amount of products resulting from 13 hours of expression of certain genes. If at least 13 hours of gene expression is required to reach the minimum amount of substances to stimulate the flowering of a plant, after 13 hours of light this plant will be induced to flower, and consequently, in months of the year in which days have less than 13 hours of light this plant will not be induced. For that situation, in which flowering is induced above a critical photoperiod in number of hours, the species is called a long-day plant.

There are plants that require long and uninterrupted nights to induce flowering, the so-called short-day plants. This means that, if exposed to long days, the product generated by the daytime expression genes will exceed a maximum threshold for floral induction, becoming an inhibitory amount after a certain accumulation, or it is possible that it depends on large quantities of products due to the nocturnal genes expression. For floral induction, those amounts of gene products may result from a single 24-hour cycle, or from the accumulation from several successive daily cycles. It is important to note that when making inferences about products of gene expression, it does not necessarily mean that their effect on events depends directly or exclusively on the proteins to be formed, but on the resulting metabolism.

In short, the plants are synchronized to the month of the year because the products of the daytime and nighttime genes have variable accumulation depending on the duration of the light or dark period; temporal periods that vary in the different months of the year depending on the position of the

Earth in relation to the Sun. Because accumulation of these products can positively or negatively regulate different photoperiod-dependent events, we can have different phenomena occurring in plants in specific months of the year, and that will be repeated in the same month of next year, when the same Earth-Sun positioning will occur. It is still possible that some events depend not only on the duration of a single day/night cycle but also on products accumulated by the plants on several consecutive days of the same photoperiodic duration.

Can plants add time cycles?

A very intriguing event in plants is the synchronization of flowering and of death of all plants of some bamboo genotypes, regardless of the geographic space in which they are located. Unlike most bamboo genotypes which may vary the years between flowerings, all *Phyllostachys pubescens* (Moso Bamboo) plants bloom at intervals of 67 years (Isagi et al., 2004), even if they are in places with different edaphoclimatic conditions, including photoperiodic conditions. In that sense, the accounting of time cannot be associated to current environmental factors alone, but rather, it is closely related to the biological clock of the genotype in question (Franklin, 2004). This means that this clock may be unresponsive to changes in the environment for its resynchronization, or, even if resynchronized, it will not be completely resynchronized, so some memory of the oscillator remains unchanged, preserving a primordial synchrony. There are many possibilities for the purpose of synchronized death and synchronized flowering. It is argued that such flowering would increase the chance that the propagules are not all foraged due to the large amount in the environment, thereby increasing the chance of some of them developing, while synchronous death would favor fires that would eliminate aerial pests or open necessary clearings for the growth of new bamboos. It is important to note, however, that this synchrony is not found in all bamboo genotypes.

Synchronized death is characteristic of a programmed event. However, little is known about the control of the biological clock over programmed cell death. Nevertheless, it is known that the methylated form of jasmonic acid, or its precursors in the pathway of oxylipines (pathway of oxygenation of polyunsaturated fatty acids), act as factors, which, by themselves, can trigger the accumulation of reactive oxygen species (ROS) in mitochondria, whose ROS promote the activation of routes that accelerate cell death (Reinbothe et al., 2009). ROS formed in chloroplasts can also act as promoters of greater synthesis of jasmonic acid, which, in interaction or not with ethylene, acts as a positive signal of metabolic pathways that culminate in the expression of the *EXECUTOR* genes involved in cell death (Figure 1.6).

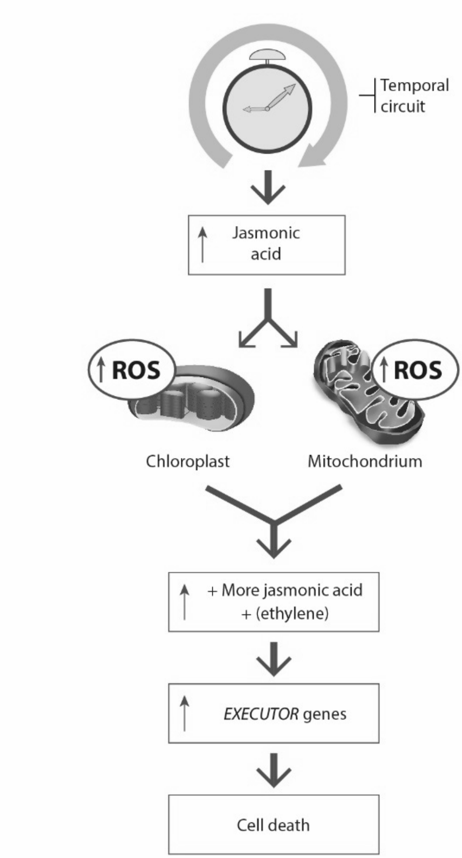

Figure 1.6. Putative signaling pathway for programmed and synchronized cell death induced by the biological clock in bamboo. The time between the beginning of plant development and death is the result of the sum of daily or annual cycles performed by the biological clock, or the closure of a single (unknown) time circuit by the oscillator. When the circuit is closed or the cycles are completed, the clock itself will induce an increase in the synthesis of jasmonic acid which will elicit the production of reactive oxygen species (ROS) in chloroplasts and mitochondria, which, in turn, will induce a second increase in jasmonic acid synthesis, accompanied or not by the increase in ethylene, which will induce the expression of the *EXECUTORS* genes, which will act on the metabolism that will lead the cell to death.

Evidence of programmed cell death influenced by the biological clock was observed in the work developed by Nitschke et al. (2016), in which it was found that circadian stress effects, induced by sudden and frequent changes in light/dark duration, promoted, at the same time, a reduction in the expression of *CCA1* and *LHY* and activation of the jasmonic acid synthesis pathway, whose increase was fundamental to cell death due to stress, showing, therefore, an intimate relationship between the circadian clock and jasmonic acid. This relationship was proven later, when Zang et al. (2019) observed the reciprocal regulation between jasmonic acid synthesis and circadian activity. Based on that evidence, it is assumed that high synthesis of jasmonic acid is triggered by the biological clock after the closure of a long but unknown timing circuit, or by the sum of several circuits (annual or daily) started at the same time at the beginning of the bamboo's development. It is, however, a hypothesis that still needs to be proven. In Figure 1.6 we find a putative model involving the biological clock and the programmed death of bamboo.

In short, the accounting of time cycles by the plant depends on circuits completed by the biological clock oscillator. The circuits can be counted in scales that vary from daily to annual, but there can also be circuits of unknown times. It is not known how the memory of the circuit or the sum of the cycles is stored, however it can be extremely accurate, to the point of triggering synchronized events even after more than one hundred years in some genotypes from the start of timing.

Light perception considerations

Knowledge about the perception of light by plants has already resulted in many technological advances, to the point that we can currently achieve effective plant cultivation in an environment devoid of sunlight, using only artificial lighting. In addition to cultivation, it is possible to modify the quality of the desired products. Examples include manipulating the increase in the synthesis of a secondary metabolism compound for pharmacological use, inducing fruit development with better nutritional quality or increasing the production of an organ of interest through manipulating the intensity, quality and duration of the light provided for plants.

A widespread, technological and high precision use derived from this knowledge is to induce the opening of flowers on the desired day, according to market demand. These technological possibilities we now have are due to the knowledge of how plants perceive light, because today we know that different photoreceptors are activated by different patterns of luminosity which trigger different metabolisms. When we know that an event that we want will, for example, be triggered in a plant subjected to the action of Pfr-phyB for 14 hours daily, for 4 consecutive days, under radiation above 1200 μmol of photons m^2 s^{-1}, we can simply seek to artificially enrich the environment with red light and in the required patterns to achieve the desired effect, although it can be eventually modulated by other factors. Therefore, knowing the mechanisms by which plants perceive light, in addition to allowing us to understand how they are synchronized over time, enables the development of technologies that can be used in their cultivation.

Temperature Perception

There are many known phenomena in plants triggered by low or high temperature. In a cold environment, among several effects commonly observed are loss of chlorophyll, leaf abscission, flowering and the dormancy or dormancy release of propagules. In warm environments, the most notable temperature-related phenomena are decrease in growth and production of reproductive organs, reduction in the rates of net photosynthesis, alteration in the partitioning of photosynthates, among other effects. Although the responses are evident, the mechanisms of perception of this environmental factor are not yet widely known, albeit some elements have already been elucidated and they will be explored throughout this section.

Plants can count hours of cold

Evidence of the counting of cold hours by plants can be observed for the apple tree, which needs a minimum accumulation of hours below a certain temperature to induce flowering. Regarding this phenomenon, it is known that both cold-responsive genes and genes involved in the biological clock oscillator are involved. An example of gene regulation in *Arabidopsis thaliana* (Amasino, 2005), whose flowering depends on the cold, can be seen in the action of the *FLOWERIG LOCUS C* (*FLC*) gene, which acts as a repressor of a set of floral genes. The expression of the *FLC* is stimulated by the products of the *FRIGIDA* gene (*FRI*), which, in turn, is inhibited by the cold, and therefore, in the cold season there is no effect of the floral repressor FLC. Photoperiodic-sensitive plants may not flower in the cold condition, even though they have been exposed to a sum of sufficient hours of cold, if they are not under an inducing photoperiod (Salathia et al., 2006). However, if after vernalization, even if they are exposed to a warm condition, but with an inducing photoperiod, they will flourish, a phenomenon that refers to a memory built from the sum of the hours of cold. Surprisingly, *FRI* also participates in the regulation of the biological clock, and its action