The Sensory Biology, Communication, Memory and Intelligence of Plants

Hyrandir Melo

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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
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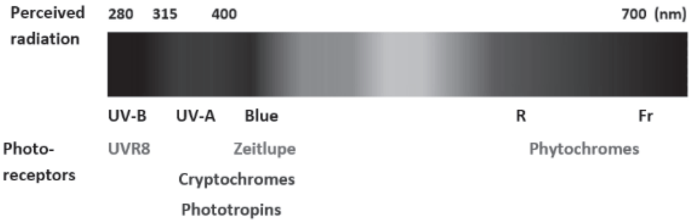

Figure 1.2. Radiation perceived by plants. The phytochrome photoreceptor absorbs radiation in the red (R) and far-red (Fr) band; components of the zeitlupe family absorb in the blue band; phototropins and cryptochromes absorb in the bands of blue and ultraviolet A (UV-A); UVR8 absorbs in the ultraviolet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

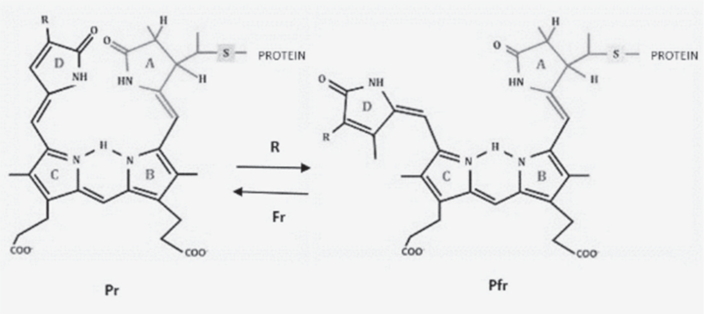

Figure 1.3. Phytochrome photoreversibility. In the presence of red light (R), the phytochrome takes on a biologically active Pfr form, and in the presence of far-red light (Fr) it takes on a biologically inactive Pr form.

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.

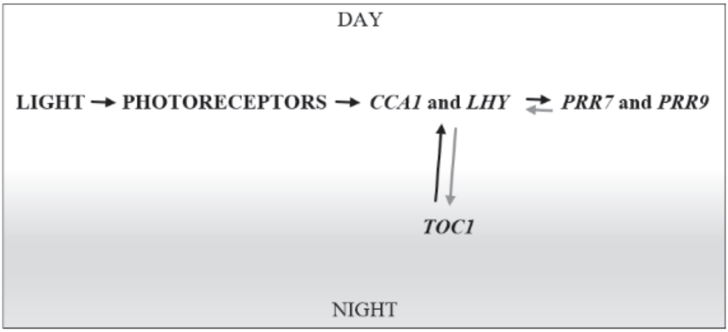

Figure 1.5. Main circuit of the circadian clock. Black arrows indicate signs of stimulus and grey arrows indicate signs of inhibition. At dawn the light activates the photoreceptors, which promote the expression of the *CCA1* and *LHY* genes, whose products induce the expression of *PRR7* and *PRR9*, 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).

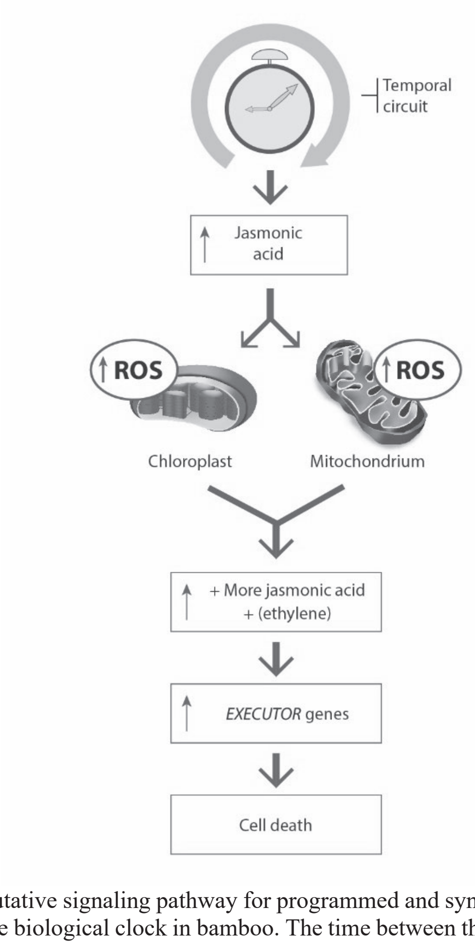

Figure 1.6. Putative signaling pathway for programmed and synchronized cell death induced by the biological clock in bamboo. The time between the beginning of plant development and death is the result of the sum of daily or annual cycles performed by the biological clock, or the closure of a single (unknown) 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 on the clock remains active in the cold condition, unlike floral regulation. There is no evidence of *FLC* direct participation in the main clock circuit as it is not expressed rhythmically, however, it is believed that it regulates the action of other secondary genes that participate in the oscillator.

Although the mechanism by which the cold modulates responses related to the biological clock is not known precisely, its role in the expression of genes involved with the oscillator is known and new discoveries have shed some light on this question. In their research, Dantas et al. (2019) observed alternative splicing mechanisms related to some genes of the biological clock in sugarcane. There was production of alternative transcripts of the mRNA from the *LHY* gene which integrates the central clock oscillator, and that such production was higher in the cold season of the year than in the hot season. That response indicates how cold temperature can act in the modulation of the clock. Alteration of transcripts not only of *LHY*, but of *TOC1*, under very cold temperatures, has also been observed in chestnut trees (Ramos et al., 2005).

It can be inferred that products accumulated from the expression of genes that were expressing in the cold period, to the detriment or not of others that were repressed, lead the plant to attain a quantitative threshold of products necessary to trigger a response, such as flowering, whose threshold coincides with the number of cold hours added up. From then on, the plant would be determined to bloom when exposed to an additional inducing condition, such as a specific photoperiod. We cannot forget, however, the modulating interference of the cold on the oscillator of the biological clock, which can promote changes in the demand for the time necessary for an event, and, therefore, can lead to changes in the sensitivity of plants to the photoperiod.

Cold can alter the photoperiodic sensitivity of plants

Based on the previous topic, the effect of cold on the biological clock oscillator, as expected, promotes tangible effects, such as altering the photoperiodic sensitivity of plants. This phenomenon is already known for several species, such as the ornamental *Lysimachia mauritiana* (Im et al., 2020) and wheat (Lee, 2019). In a study by Lewandowska-Sabat et al. (2017) carried out to observe the effect of altitude on the flowering of *Arabidopsis thaliana*, it was found that, in a higher altitude environment, and therefore, colder, the plants were less sensitive to photoperiodic regulation of flowering than those of lower altitude environments. Although the effect was of altitude on flowering, which involves a change in several factors of the environment, the one most likely to alter the photoperiodic sensitivity is the temperature difference.

The sum of high temperature hours as a predictor of the phenological stage

The sum of hours of high temperatures has been used to predict the phenological stage. In agriculture there is a term called degrees days, which refers to a sum of degrees Celsius, from a base temperature for a crop, used to predict the stages of plant development and the ideal time for harvest. It is a reference used as a calculator based on an environmental factor for a response to manifest itself in the plant. However, unlike cold, the counting of hours of heat is not linked to the biological clock, but to the accumulation and consumption of organic carbon, that means, the net photosynthesis. In that sense, it is an indirect reference related to the radiation that reaches the plant, since the production of photosynthates depends on it. Reducing the intensity of the incident radiation, but maintaining the photoperiod, and even with an increase in the thermal sum, there was a delay of several days in the time required for the maturation of grapes in plants grown under cover (Chavarria et al., 2009). Observing its nuances, the thermal sum, although still used, is not an accurate instrument for predicting the phenological phase.

How do plants perceive temperature?

When we study the importance of temperature in plant development, it is customary to first target its effect on metabolic activity, since that can affect the kinetics of many enzymes, as well as interfering in the expression of several genes.

Despite the relevance of the metabolic aspects, the responses of plants to temperature are much more than simple events observed because of thermal variation, and they require the existence of a prior perception mechanism.

When considering the existence of biological temperature sensors, their perception must be analyzed by components upstream of the cell signaling cascade triggered by the thermal effect (Plieth et al., 1999; Ruelland and Zachowski, 2010). As a reference component for thermal sensitivity, there is membrane fluidity, which fatty acid desaturase enzyme acts on directly and which, in turn, is responsive to temperature changes. Depending on the fluidity state of the membrane there may be an early triggering of the activity of metabolic pathways in relation to the temperature of the protoplasm necessary for the action, which also means that genes can be induced to be expressed at temperatures different from those inducing expression in the protoplasm. Conformational changes in heat stress response proteins have also been observed due to membrane fluidity. In that case, the fluidity state of the membrane is the cell reference for temperature even before cooling or heating rates occur in the protoplasm. Another change that also precedes cascades of events downstream of changes in heating/cooling rates is the depolymerization of microtubules and actin microfilaments, whether under cold or heat; an event that may be associated with the detection of temperature from components integrated into the cell wall, such as the formin proteins involved in the polymerization of actin filaments. A diagram integrating the most probable temperature sensors can be seen in Figure 1.7.

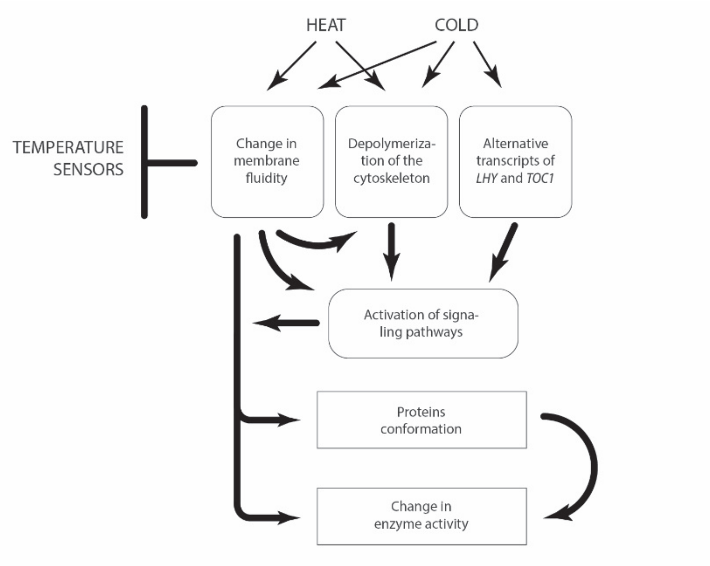

Figure 1.7. Cellular perception of temperature. Heat and cold trigger changes in membrane fluidity and promote depolymerization of the cytoskeleton. These two phenomena act as sensory temperature mechanisms, triggering thermo-dependent events that anticipate the effective change in the cooling or heating rate of the protoplasm. The change in membrane fluidity induces changes in protein conformation and enzyme activity, whose activity can also occur due to the conformational change itself. The depolymerization of the cytoskeleton and the alteration in the production of alternative transcripts of genes that participate in the biological clock oscillator (seasonal sensors, with an action independent of the effective change in the momentary cooling rate of the protoplasm) activate cellular signaling pathways that are added to the changes promoted by membrane fluidity changes.

Plant cells' response to temperature change does not take into account the absolute temperature, such as that obtained with thermometers. Since the sensitive apparatus is biological, what matters most is the rate of cell cooling or heating.

An example of responses that occur from the perception of temperature is the phenomenon of thermonastism seen in the closing of *Mimosa pudica* leaflets as the temperature goes down (Figure 1.8C), as well as the decumbence of the leaves of *Rhododendron* sp. in low temperature conditions (Figure 1.9C). The triggering of leaf movement depends on the differentiated turgor in pulvinus cells, part of the petiole. The influx of Ca^{2+} from the apoplast to the cytoplasm precedes the movement, implying the participation of that ion in thermal signaling, with its transmembrane channels being considered by some researchers as integral elements of the temperature-sensory apparatus (Mittler et al., 2012). As previously discussed, the temperature causes a structural change in the membrane, which implies altering its permeability to some chemical compounds, and that, in turn, can lead to changes in electrical gradients between the symplast and the apoplast, necessary for the activation of the voltage-dependent calcium channels.

Figure 1.8. Thermotropic response of *Mimosa pudica*, which closes the leaflets according to the temperature of the environment. 1.8A. Fully open leaflets, typical condition of high temperature (35 °C). 1.8B. Semi-closed leaflets, typical condition of low temperature (12 °C). 1.8C. Completely closed leaflets, typical condition of very low temperature (5 °C). Credits: 1.8A Image by Pixy. License for use at https://pixy.org/4812910/ 1.8B Image by Johan from Wikimedia Commons. License to use at https://commons.wikimedia.org/wiki/File:Mimosa-pudica-red-leaves.jpg 1.8C Image by Pancrat from Wikimedia Commons. License to use at https://commons.wikimedia.org/wiki/File:Mimosa_pudica_Feuille2.jpg

Figure 1.9. Thermotropic leaf response in *Rhododendron*, a plant genus whose leaves droop according to the temperature of the environment. 1.9A. Leaves not decumbent, typical of the temperature condition at 35 °C. 1.9B. Half-decumbent leaves, typical of the temperature condition at 25 °C 1.9C. Decumbent leaves, typical of the temperature condition at 5 °C. Credits: 1.9A Image by cocoparisienne from Pixabay. License to use at https://pixabay.com/photos/rhododendron-bud-plant-blossom-737406/ 1.9B Image by Pezibear from Pixabay. License to use at https://pixabay.com/photos/rhododendron-plant-nature-green-715017/ 1.9C Image by RococoNeko from Pixabay. License to use at https://pixabay.com/photos/rhododendron-spring-flowers-107713/

Thermotropic movement is observed in the roots of several species. Maize roots curve positively in response to temperatures between 9 and 25 ºC and negatively in response to temperature above 39 ºC. In fact, temperature can modulate the growth angle in relation to the gravitational vector (Fortin and Poff, 1991).

For thermotropism to occur, it is possible that there are sensory factors in addition to those already discussed in terms of upstream modifications of thermo-dependent events. We have seen that structural modification of the membrane, the depolymerization of the cytoskeleton and the production of alternative transcripts of mRNA from the *LHY* and *TOC1* genes can be considered thermo-sensing mechanisms, but they only trigger a signaling if the plant is exposed to a certain thermal condition. For the thermotropism that has been observed in roots, the question requires an even more sophisticated sensory apparatus, since it is a growth directed into a thermal gradient.

As a proposal for other mechanisms that may be involved in the thermotropic response, phytochrome B and phototropins should be considered. They are photosensors-thermosensors due to the structural or functional modification they suffer according to temperature, and those modifications precede the signaling that results in responses. Temperature can reverse the isomeric form of phytochrome B in an inverse way to light (Dai Vu et al., 2019; Fujii et al., 2017), just as temperature can reverse the action of blue light on the activation of phototropin (Rockwell et al., 2006). There is evidence of how photoreceptors perceive the direction of light; the angle of radiation between the emitter and the receiver alters the intensity and quality of the incident radiation, which is related to the anisotropic interaction positioning between phytochrome/phototropin-plasma membrane. If the photoreceptors do also act as thermosensors, then their sensitivity when exposed to a certain temperature is understandable, but there is still no evidence that allows us to fully understand how they could act as thermal gradient direction sensors.

In addition to the possibility of the photoreceptors acting as thermal direction sensors, there is the possibility that they could be sensitive to electric fields. Considering that temperature interferes in electric fields, and that electric fields can alter responses induced by photoreceptors (Sidaway, 1969), it is therefore suggestive that the sensitivity of roots to the direction of temperature gradients in the soil may be associated with photoreceptors-thermosensors/electrosensors. More about photoreceptors as electrosensors will be addressed.

Summary

Plants can add up hours of cold by the progressive accumulation of products resulting from expression of genes positively regulated by low temperatures and by the action of genes that participate both in the biological clock oscillator and cold-dependent responses. In the latter case, exposure to cold can modulate the photoperiodic sensitivity of plants. A mechanism for this interference probably results from the synthesis of alternative transcripts of the *LHY* and *TOC1* genes, which integrate the main oscillator of the circadian clock, and whose transcripts increase in low temperature conditions. Alternative transcripts together with the depolymerization of the cytoskeleton add to changes in membrane fluidity according to the cold or heat, promoting the anticipation of metabolic events that would only occur after an effective increase in the heating or cooling rates of the cellular protoplasm. It is believed that there are more elements in the thermo-sensory apparatus of plants, since those mentioned are not yet sufficient to account for the thermotropism observed in the roots of some species. Photoreceptors, which are also responsive to changes in temperature, are potential candidates for the perception of thermal gradients, just as they are for the direction of light and electric fields.

Perception of Atmospheric Gases

Atmospheric gases can be essential or harmful to plant development. Although many gases are present in the environment, we will consider in this section only those most important for plant development, as well as those most studied regarding their perception by plants, namely, O_2, O_3 and CO_2.

Oxygen perception

Oxygen is an atmospheric gas that plants require for fundamental events in their metabolism, such as respiration, in which it acts as a final electron acceptor in oxidative phosphorylation. It is also important for the activity of some enzymes, such as Ribulose 1,5-bisphosphate carboxylase oxygenase (RUBISCO), in this case, when it acts with its oxygenase function, triggering, from then on, the photorespiration pathway. Although this gas is produced by the plants themselves during the photochemical phase of photosynthesis, some environmental situations, such as flooding or soil compaction, can decrease their availability to plants and promote hypoxia stress. Despite its importance, little is questioned about how it is perceived by plant cells. We will see that, albeit still under study, many biomolecules can act as oxygen sensors in plants.

The perception of oxygen by cells has been widely studied in animals, in which the sensory apparatus is well known, and has served as a reference for the still incipient studies in plants.

In addition to enzymes that require oxygen, other biomolecules have sites of sensitivity to this gas, as some photoreceptors do, and that leads us to consider their possible actions as oxygen sensors. Although classic plant photoreceptors have recently been revealed to be molecules with multiple functions, including being involved in the modulation of responses to various stresses (Carvalho et al., 2013; D'Amico-Damião and Carvalho, 2018; Gavassi et al., 2017; Zhang and Shimazaki, 2005; Liu et al., 2020), there is still a lack of specific studies that reveal their performance in the primary sensory apparatus for the detection of stress factors or whether they only act downstream of the perception in the signaling pathways, which include stress by hypoxia.

In the case of hypoxia stress, it is suggested that phototropins or members of the zeitlupe family participate in the perception of oxygen concentration in the internal cellular environment, since they are molecules that have an oxygen sensitivity domain, the LOV (light, oxygen and voltage). This also applies to other molecules with domains of sensitivity to oxygen, such as the gene transcription factors ETHYLENE RESPONSE FACTORS (ERFs), also considered to be potential oxygen detectors in plants. As well as being potential sensors for various signals from the environment, transmembrane channels are also used for oxygen. The potential of channels as sensors is mainly due to their membrane location, facilitating more direct contact with the conditions of the external environment. As will be discussed at various other moments, cell wall protein kinases anchored in plasma membranes have also been considered in the context of perception of various environmental factors and their sensory performance can be facilitated by their localization.

Ethylene plays a central role in triggering plant responses to hypoxia. In that context, ERFs are involved in the regulation of proteinases for the formation or expansion of aerenchyma, the formation of ROS and even in the regulation of membrane transporters, in a time scale of seconds, and thus, they have been considered strong candidates for being oxygen sensors in plants (Wang et al., 2017). It could be thought that the ERFs were not sensors but merely participative in the signal transduction route, if it were not for the presence of the oxygen sensors PLANT CYSTEINE OXIDASES (PCOs), integrating the ERF structures.

Based on chains of amino acids conserved from ancestors and present in oxygen receptors already characterized in several organisms, the transmembrane channels TWO-PORE CHANNEL 1 (TPC1), ARABIDOPSIS K^+ TRANSPORTER (AKT2) and TANDEM-PORE K^+ CHANNEL 4 (KCO4), found in plants, are highly homologous with those known in animals. Probably, TPC1, a transporter of Ca^{2+} and monovalent cations, acts as a sensor in endo-membranes since it is not found in the plasma membrane. AKT2 and KCO4, K^+ transporters, are the strongest candidates found in the plasma membrane for external oxygen perception. In Figure 1.10 it is possible to see the set of the most likely O_2 receptors in plant cells.

To sum up, there are many biomolecules with potential to act as oxygen sensors in plants. Among them, photoreceptors with an oxygen sensitivity domain; gene transcription factors sensitive to ethylene, which contain oxygen sensitivity domains in their structures; and transmembrane channels with a high degree of homology with conserved amino acid chains already known in oxygen receptors of other organisms.

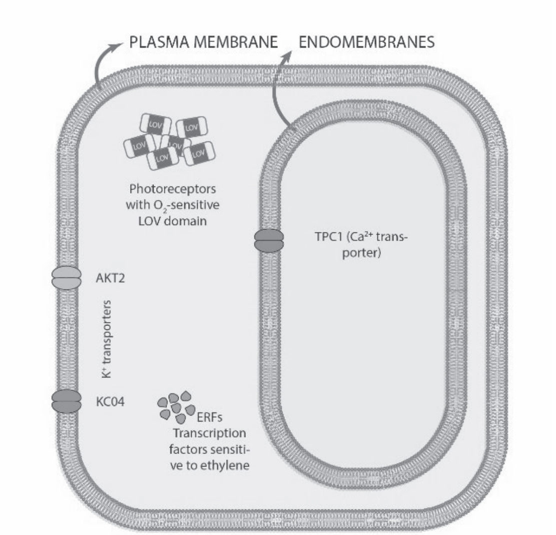

Figure 1.10. Main putative O_2 sensors in plant cells. Among many candidates, there are the photoreceptors phototropins and members of the zeitlupe family, which have the LOV domain (sensitive to light, oxygen and voltage); the K^+, AKT2 and KCO4 transporters, present in the plasma membrane, and the Ca^{2+} TPC1 transporter, present in endo-membranes which, has highly conserved amino acid chains with a high degree of homology to other O_2 sensors already known in several other organisms; ethylene-sensitive gene transcription factors, ERFs, have domains of cysteine oxidases, also considered O_2 sensors.

Ozone perception

Among air pollutants, tropospheric ozone (O_3) is considered the most phytotoxic and the one that causes the most damage to agriculture. Effects such as inhibition of photosynthesis, alteration in the partition of photosynthates, promotion of senescence and change in reproductive patterns have been reported as consequences of the action of O_3 in plants.

Plants respond to ozone by triggering typical signaling pathways to defend against stress. However, sensitivity varies among genotypes and organs. The increase in the synthesis of WRKY gene transcription factors, typically produced under conditions of abiotic stress, is observed after plant exposure to ozone. WRKYs usually promote increased expression of genes encoding MAPK protein kinases, which are commonly involved in metabolic pathways that promote increased tolerance to biotic and abiotic stresses (Marchica et al., 2019).

The oxidizing action of ozone acts on lipids and proteins, which can give rise to different ROS or stimulate the production of other substances, such as jasmonic acid and salicylic acid, which act in signaling pathways involved in cell defense. According to Evans et al. (2005), the accumulation of cytosolic Ca^{2+} and the activation of protein kinases, after plant exposure to ozone, precedes the expression of several genes that encode for the antioxidant proteins that are part of the plants' tolerance responses to stress but the ozone reception apparatus is not well known. It is known, however, that the response of calcium in the plant varies according to the speed of exposure and the concentration of ozone and can be perceived in less than a minute, showing that this ion is involved in the ozone signaling pathway in a position very close to its reception. Furthermore, some researchers consider that the membrane peroxidation itself, induced by O_3, is part of the perception. It is assumed that cysteine residues from plasma membrane proteins are involved in reception, since this amino acid may undergo modifications in the presence of ROS and nitrogen. Cysteine can be present in the transmembrane calcium transporters themselves, or in associated proteins that are distinctly activated by the presence of ozone and H_2O_2, although both are ROS. This selective perception is evidenced by the different set of genes expressed according to the ROS elicitor of the response.

In addition to the rapid response of ozone perception, evidenced by the increase in cytosolic calcium, there seems to be another, apparently slower pathway, based on the oxidative breakdown of some components of the cell wall by the presence of ozone, generating apoplastic elicitors that can be perceived by membrane proteins (transporters or associated proteins) that trigger a specific signaling pathway for the expression of ozone-responsive genes. That path, triggered by the apoplast, begins similarly to that to be explored in detail in the section “Touch Perception”. The deduction that this pathway is slower than that of calcium comes from the fact that a change in the composition of the cell wall is only detected after two hours of the plant’s exposure to ozone.

In short, the perception of ozone depends on a rapid transient increase in cytosolic calcium, the accumulation of which differs (in quantity, speed, time, transporters or cytoplasmic interactions) from the increase caused by other reactive oxygen species. The importance of calcium in the sensory apparatus is due to its performance being upstream of the metabolic pathways typical of oxidative stress, such as the production of antioxidant proteins, which suggests that its membrane transporters act as probable receptors. It has also been observed that ozone triggers cross-routes with other stressors by inducing the production of salicylic acid, a typical route of plant defense against pathogens, and of jasmonic acid, a typical plant defense against herbivory. Apparently, there is more than one cell apparatus for O_3 perception, since a response pathway is activated in less than one minute, probably associated to membrane transporters or associated proteins, as well as membrane peroxidation, and another pathway occurs after two hours, which depends on the formation of cell wall elements eliciting the action of ozone-responsive metabolic routes.

Carbon Dioxide Perception

CO_2 is an atmospheric gas essential to plants, since, during the photosynthetic process, the carbon of this inorganic molecule is used to produce organic carbon skeletons, necessary for the structuring of all other organic molecules. Although the importance of this gas to plants is widely acknowledged, yet little has been explored about its perception by the plant cell.

CO_2 sensors are expected to be present in the stomatal apparatus since this is the main door of its entry into plants. As we will see, many biomolecules involved in the perception of this gas are already known, but the understanding of the whole mechanism still needs further studies.

The opening of the stomata, even though the plant is experiencing water deficiency, and therefore, needing to save water, is indicative of the existence of a cellular perception mechanism of the low availability of CO_2 that imposes the need to open the stomata to acquire this gas, even if that will increase transpiration rates. Additionally, there is a tendency for stomatal closure when the CO_2 concentration inside the leaf is very high, which further highlights the need for internal leaf perception regarding its availability.

It is suggestive that the CO_2 leaf sensory apparatus is linked to the perception of abundance or scarcity of biomolecules resulting from changes occurring in carbon metabolism, such as the malate anion, which interferes with the opening of ion channels that are also related to CO_2 perception, as we will see later. The most curious thing, however, is the tendency to decrease the opening of the ostiole under increased CO_2 in the atmosphere, as observed in several species (Mott, 1990), which indicates a cellular perception of CO_2 from the external environment, and that, in turn, encourages us to think of a perception apparatus that is sensitive not only to products of carbon metabolism but to the CO_2 molecule itself.

Other evidence of the perception of external CO_2 is that isolated protoplasts of guard cells respond to variations in the concentrations of that gas, inflating and deflating (Fitzsimons and Weyers, 1983), as illustrated in Figure 1.11. That cellular behavior shows the perception of CO_2 by the cell, which serves to trigger the closing or opening of stomata in the leaves, since the turgor state of the guard cells is what determines the appearance or not of the ostioles, as well as the degree of stomatal opening.

The perception of CO_2 availability interferes in gene expression. The *HIC* (*HIGH CARBON DIOXIDE*) gene encodes a negative regulator for stomata development, which is responsive to the concentration of CO_2 in the atmosphere, once again showing the plant's perception of the external fluctuation of this gas. This information provides a basis for understanding the negative effect of increasing CO_2 on the development of stomata observed in several species.

As expected, the sensitivity of cells to CO_2 is not restricted to the gas itself. The activity of carbonic anhydrase catalyzing the reaction of CO_2 to bicarbonate, or even the actual concentration of bicarbonate itself, is involved in the internal signaling, which, downstream of perception, will trigger the stomatal closure induced by CO_2, and which occurs independently of the action of abscisic acid (ABA), the most well-known pathway in promoting stomatal closure. However, it is expected that the signaling for stomatal closure via the CO_2 pathway will at some point be crossed with the ABA signaling, because the deficiency of ABA down-modulates the action of the CO_2-pathway.

Transmembrane anion channels in the guard-cells, important to osmotic regulation that acts on the opening or closing of stomata, such as bicarbonate sensitive SLAC1 (SLOW ANION CHANNEL1), participate in CO_2 perception, probably due to the involvement of two tyrosine residues exposed on the membrane surface (Yamamoto et al., 2016). This protein can activate stomatal opening repressors that integrate the CO_2 pathway. The most interesting thing is that the sensitivity to CO_2 does not depend on the N or C-terminal side of the transporter (Gray et al., 2000), indicating that SLAC1's performance as a CO_2 sensor can occur on both sides of the membrane.

Through studies on defective mutant plants, it is known that in addition to SLAC1, the anionic channel ALMT12 / QUAC1 (ALUMINUM - ACTIVATED MALATE TRANSPORTER 12 / QUICKLY ACTIVATING ANION CHANNEL1), as well as the protein kinases HT1 (HIGH LEAF TEMPERATURE 1) and OST1 (OPEN STOMATA 1), PATROL1-type proteins (PROTON ATPASE TRANSLOCATION CONTROL 1) Munc13 and the RHC1 MATE (RESISTANT TO HIGH CO_2 MULTIDRUG AND TOXIN EXTRUSION) transporter also interfere in stomatal movement regulated by the CO_2 pathway (Engineer et al., 2016). Although many points still need to be clarified and interconnected, there are now many known factors influencing the perception of CO2 by the plant cell.

It is believed that the protein kinases are important for phosphorylation of SLAC1 anionic channels, and that the activation of some kinases is calcium-dependent since this element interferes with the CO_2-dependent stomatal movement response.

For a simplified summary of cellular perception of CO_2, see Figure 1.11.

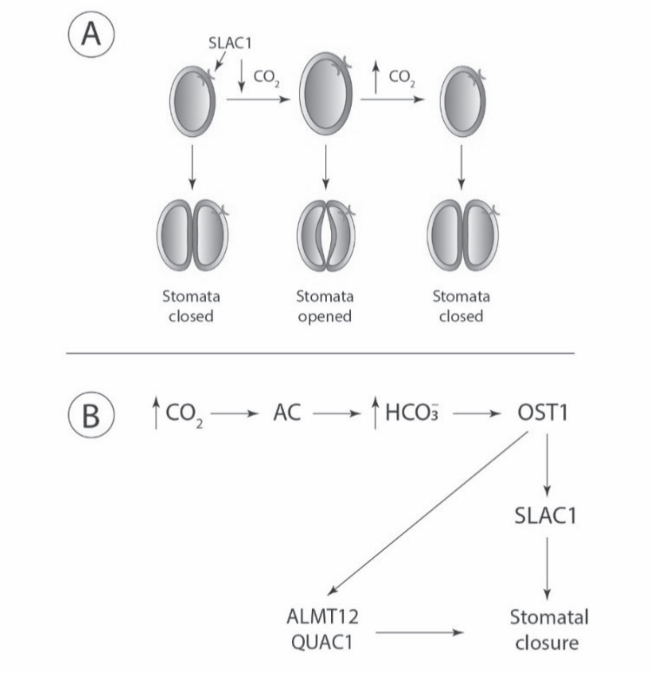

Figure 1.11. Evidence of cellular perception of CO_2. A) isolated guard cell protoplasts inflate and deflate due to the external concentration of CO_2, showing the presence of cellular sensors. SLAC1 (SLOW ANION CHANNEL1), a transmembrane anion channel, is an example of a sensor candidate for having bicarbonate-sensitive amino acid residues on both the C and N-terminal sides. The increase in volume of the protoplasts represents the gain of turgor necessary to open the stomata in the leaves, and the loss of volume represents the loss of turgor of the guard cells in the leaves that leads to the closure of the ostioles, independently of abscisic acid. B) Scheme representative of the changes caused by the increase of internal CO_2 in the leaves that culminate in the closure of stomata in an independent manner from abscisic acid. The increase in CO_2 concentration promotes the activity of carbonic anhydrase (AC), which will result in a higher content of bicarbonate (HCO_3^-). That induces the activity of kinases such as OST1 (OPEN STOMATA 1), which, when phosphorylating SLAC1 or ALMT12/QUAC1 (ALUMINUM – ACTIVATED MALATE TRANSPORTER 12 / QUICKLY ACTIVATING ANION CHANNEL1) triggers the activation of these channels for extrusion of anions to the apoplast, resulting in the closure of stomata.

Relative Humidity Perception

The relative humidity of the air has been widely explored in studies of water relations in plants because it is a key factor in establishing a vapor pressure gradient between the leaf and the atmosphere that directly interferes in transpiration rates. It is important to highlight the presence of an aerial continuum in plant organs formed by air spaces, where there are high percentages of relative humidity due to the high water content of the cells present in the tissues. Since the cell wall itself is a hydrophilic environment and retains water in its liquid form, the idea of having sensors for relative humidity in the internal tissues of plant organs is not plausible. However, its presence at the plant-environment interface is expected.

It is a factor that also participates in the regulation of the circadian clock, a phenomenon that necessarily evokes its perception by plants, but by mechanisms still unknown. Its input to the clock is known because high humidity promotes increased expression of *CCA1*, but not *LHY*, thus showing a divergence between the way the oscillator is regulated by light and by the relative humidity of the air. Not only the percentage of humidity, but its fluctuation throughout the day seem to be synchronized with the circadian clock events. Its effect on the clock promotes different responses, such as promoting increased cellular resistance to some specific pathogens inoculated overnight in plant cells, an event also associated with circadian control of the jasmonic acid pathway.

In a study conducted by Mwimba et al. (2018), changing the fluctuation of relative humidity in the air over 24 hours, they observed that the fluctuation interfered in the cyclical rhythms of the expression of central clock genes, thereby acting as a regulating factor of the oscillator. The failure to identify a sensory system for moisture detection led researchers to investigate whether the cyclical responses adjustable to humidity fluctuations were not due to temperature changes, and, using temperature-insensitive biological materials, they observed that it was an independent effect. It is assumed that the perception of moisture by the plant is associated with the structure involved in transpiration.

The idea that aquaporins are involved in the perception of the relative humidity of the air is not discarded, since many current studies have revealed their participation not only in the transport of water, but also of some ions and gases, depending on their position in the plant, in addition to the fact that the relative humidity of the air itself influences the expression of some genes that encode for specific aquaporins.

Drought and Water Perception

Plants are not only responsive to the presence or absence of water, they can also perceive its abundance or scarcity. Water deficiency affecting plant development is a contemporary and central theme in the debate on food availability security in the world, due to the tendency to progressive drought in agricultural areas. Many scientific research works are being dedicated to studying the effect of water on plants, although few are dedicated to exploring how plants perceive that resource.

How do plants perceive soil water deficiency?

The morphological and physiological consequences of water deficiency in plants are diverse (Melo et al., 2007; Schneider et al., 2019). It is very common to observe in studies of salt stress and water deficiency that, after the perception of the stress signal by the plant, there is an increase in the synthesis and/or action of abscisic acid, a hormone that regulates a wide range of metabolic events, including the expression of stress-related genes that are linked to morphophysiological changes that contribute to the greater tolerance of plants to stressful conditions.

Considering that stress due to water deficiency results from the lowering of the soil water potential, the most likely feature to be expected from a cell perception system is that it is related to the tension generated at the cell wall-plasma membrane interface of the root cells because of the lowering of water availability of its surroundings. In research by Osakabe et al. (2014) and Takahashi and Shinozaki (2019), they observed a protein present in the arabidopsis plasma membrane, called ATHK1, with a histidine kinase function and that has two transmembrane domains. Considering the similarity to prokaryotic osmo-sensors, it is suggested that this protein acts as a plant sensor, so that, based on the perception of a threshold of water potential, phosphorus transfer occurs, activating other proteins which, directly or indirectly, act in gene expression. In that perspective, Ca^{2+} channels, such as REDUCED HYPEROSMOLARITY-INDUCED CA^{2+} INCREASE1 (OSCA1) are activated for a rapid influx of calcium from the apoplast that will act in the protoplasm as a secondary messenger in triggering responses to water scarcity.

Although in various stresses, there is at some point a greater synthesis of, and sensitivity to abscisic acid, the perception of different stressors is also different. In addition to abscisic acid, the presence of Ca^{2+}, calmodulin, protein kinases, phosphatases or even ROS are recurrent factors in signaling after the perception of different stressors, including that caused by water deficiency. Furthermore, there is evidence of the action of some common membrane proteins that are sensitive to various stresses, such as those of the RLK (Receptor-Like Kinase) family of kinases, projected on the cell wall, which are involved in the perception of osmotic stress of the apoplast or of cell turgor pressure. Probably, those proteins also participate in the perception of water stress, but it is still a hypothesis and lacks clarifying studies.

How do plants perceive water?

The growth of roots, especially of plants that grow in an environment with water scarcity, towards regions of the soil with greater water potential is a phenomenon well known in plant studies. It shows that the roots perceive water availability and that their growth's being directed to those more favorable regions is not a purely interceptive factor. However, the sensory mechanism is still poorly understood.

The cell wall-plasma membrane region is sensitive not only to low water availability but also to positive pressures imposed by cell turgor. The pressure suffered by the plasma membrane because of the turgor can cause the opening of mechanically sensitive ion channels (Figure 1.12). Five families of these pressure-responsive channels are known, including the MID1-COMPLEMENTING ACTIVITY (MCA) for Ca^{2+}, and the presence of a transient quantitative threshold of this ion in the protoplasm can trigger far-reaching signals in the plant. This type of pressure on plasma membrane has also been observed altering the activity of some aquaporins mechanically regulated.

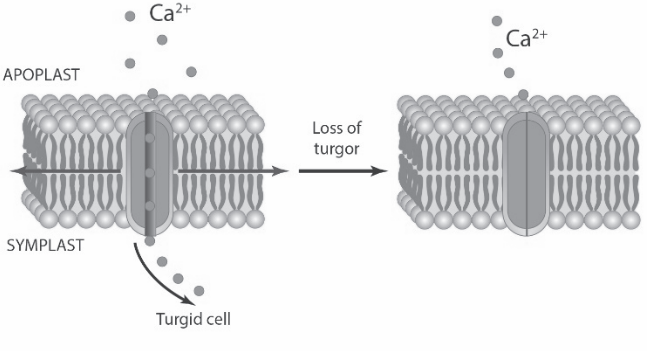

Figure 1.12. Example of mechanically sensitive Ca^{2+} channels, which open or close depending on cell turgor. In turgid cells there is pressure in the opposite direction on both sides of the channels (arrows inside membrane) inducing their opening (left). When the cell loses turgor, the pressure is diminished and the channel closes again (right).

As mentioned in the previous topic, it is important to note that plasma membrane protein kinases projected on cell walls are also responsive to turgor changes.

Even more intriguing and sophisticated than the fact that the roots perceive gradients of water availability to adjust the direction of their growth is the hydrotropism that occurs due to the perception of acoustic vibrations of water in movement. In the research carried out by Gagliano et al. (2017) with peas grown in inverted Y-shaped tubes (Figure 1.13), they observed that plants respond preferentially to moisture gradients rather than acoustic vibrations of water when assuming a root growth direction. However, in a very dry environment, the tendency is for the root to grow towards the source of acoustic vibrations produced by the movement of water. Although it is known that sound is a mechanical wave and therefore perceived by plants, the way in which they identify specific sound frequencies is not yet understood. In that research, the growth of the roots in the opposite direction to the water source was also observed when the sound of the movement was recorded and emitted by sound devices, differently from the natural one, which possibly happened due to different waves emitted by the sound devices, suggesting a negative magnetotropism.

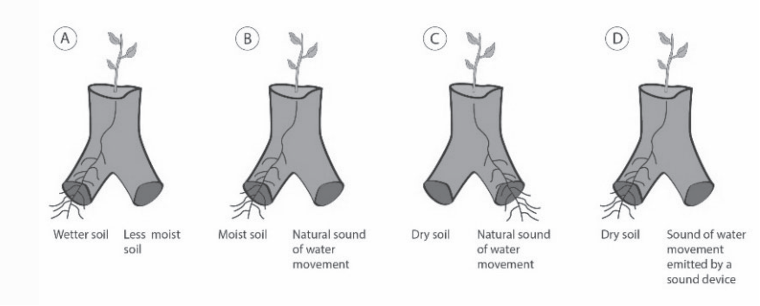

Figure 1.13. Schematic representation of the experiment carried out by Gagliano et al. (2017), in which the directional growth of the pea roots to the side of greater water availability was proven (A); increased root growth towards wet soil instead of the natural sound of water movement (B); the growth of the roots in the direction of the natural sound of water movement instead of the side where the soil had very low water availability (C); the directional growth of the roots avoiding the side where the sound of the water movement was recorded and emitted by a sound device (D).

To sum up, plants' perception of water or its deficiency is very likely to be associated with the pressures existing in the plasma membrane-cell wall, which undergoes changes due to cell turgor or plasmolysis, and with the opening or closing of mechano-sensitive transmembrane channels, which involves some aquaporins and some calcium channels. The transient increase in Ca^{2+} in the cytoplasm acts as a sign of low water availability and that drives the activity of the response pathways downstream of perception. Other membrane transporters whose activity depends on phosphorylation can be activated by kinases present at the cell wall-membrane interface, which are also responsive to the pressures of the environment. The directional growth of the roots in favorable water gradients proves the perception of water availability by plants. However, it is not clear which elements participate in hydrotropism. Nevertheless, it is known that in critical situations of water deficiency the roots tend to direct their growth to places where there are acoustic vibrations inherent to the movement of water.

Gravity Perception

Gravity is known for directly interfering in plants in the direction of root growth, although it also interferes with the angle of inclination of aerial organs in relation to the soil. Its perception mechanism in plants has already been fairly well elucidated.

Gravitropism and phototropism are fundamental in the architectural projection of plants. How they perceive and respond to the direction of light has been explored in the first section of this chapter, however, the way they perceive and respond to a gravitational vector is another, very intriguing, event (Chen et al., 1999; Vandenbrink and Kiss, 2019).

For the gravitropic response of the plants to occur, it is necessary that they have a vector perception of a gravitational center. In the case of positive gravitropic growth of the roots, a sensory point is the deposition site of statoliths, dense amyloplasts in sensor cells called statocytes present in the columella of the root cap. Statoliths act as susceptors, something that moves due to the gravitational pressure suffered by the body. The sedimentation of statoliths acts in triggering the transmission of chemical or electrical signals that should reach the region of root elongation so that there is an asymmetric growth of cells in this region that enables the curvature of the root apex (Figure 1.14).

Studies of starch mutant plants have concluded that amyloplasts present in the endodermis of aerial organs influence the normal directional growth angle of those organs in relation to the gravitational vector, which shows the importance of starch for negative gravitropism as well.

It is known that, by causing the displacement of statoliths in root apexes, the direction of root growth is modified in relation to the new positioning they assume, which will determine the growth of the root in the direction of the sedimentation of the statoliths, while in aerial organs growth is directed in the opposite direction to the sedimentation of the statoliths. The tension that occurs at the base of the cell due to the compression exerted by statoliths on the plasma membrane and cell wall has been considered a factor in the perception of the gravitational vector.

Many gravitropism-signaling elements have been identified, although the mechanism of action is not always fully understood. One of those elements is the cytoskeleton, whose role is due to its interconnection with statoliths. In the region where there is a greater degree of depolymerization of the actin filaments, there is an increase in the gravitropic curvature rates. In addition, the movement of statoliths in cells, irrespective of cyclosis, seems to be restricted by the cytoskeleton network in association with chaperones that act on the actin-statolith filament interconnection. The compression of the plasma membrane by statoliths, or when they compress the endoplasmic reticulum, stimulates the activation of calcium channels, and calcium increases in the cytoplasm, either from the apoplast or from that stored by the endoplasmic reticulum itself, acting as a secondary messenger in this signaling, so much so that calcium channel blockers, calmodulin or Ca^{2+} -ATPase inhibitors inhibit gravitropism.

From the increase of calcium at the point of sedimentation of statoliths in cells of the root cap, there is a signaling, of a type not yet known, between the cap and the root elongation region, which induces differentiated growth between opposite sides in the root elongation region, and which requires a non-homogeneous radial distribution of auxin. As discussed in the topic “Plants perceive the direction of light”, there is also asymmetric expression of auxin response genes on opposite sides of the root elongation region after gravitational stimulation. In that case, the greatest cellular increase/elongation due to auxin occurs in the upper region of the roots, so that the asymmetry in the growth of the organ promotes downward curvature in the direction of the gravitational vector (Figure 1.14). However, considering the stem, in the case of curvature of aerial organs in relation to the gravitational vector, the increase in auxin content occurs on the basal side, promoting greater growth of the basal cells and driving an upward curvature, opposite to the direction of the vector (Figure 1.14). For the radial and differentiated distribution of auxin, although the mechanism is not precisely known, it is known that there is participation of the cellular transporter for auxin influx AUXIN1 (AUX1) and the transporter for auxin efflux PIN-FORMED2 (PIN2), because its mutants are affected. It has also been found that there is an increase in the synthesis of PIN2 transporters in sensory cells (statocytes) after the sedimentation of statoliths.

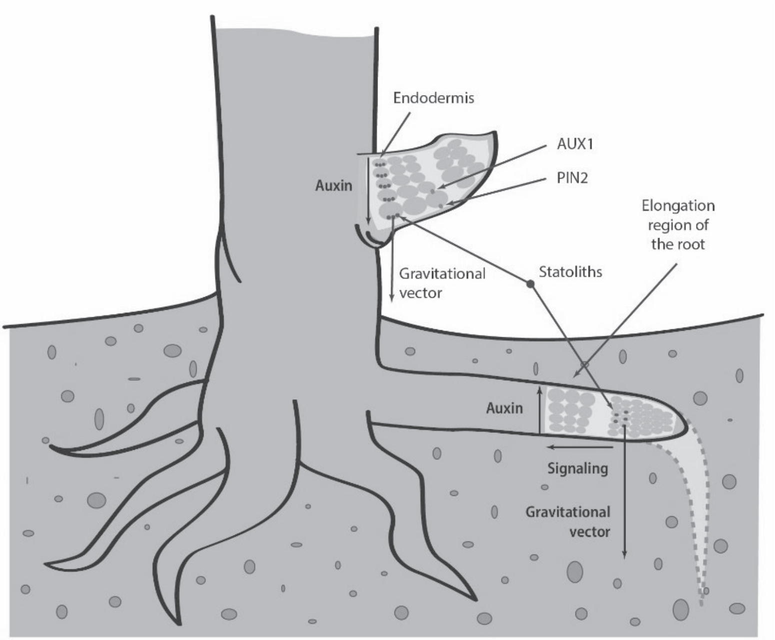

Figure 1.14. Sensory mechanism of plant perception of gravity. In the root cap, statocytes are present with the sedimented statoliths at their base. The pressure exerted by statoliths on the plasma membrane and cell wall acts as an indicator of the gravitational vector. A communication occurs between the root cap and the cells in the elongation region of the root, which leads to a radial and asymmetric distribution of auxin via the AUX1 and PIN2 transporters, resulting in greater accumulation of auxin in the root region opposite the gravitational vector, which triggers greater growth of those cells, culminating in the curvature of the root apex in the direction of the gravitational vector. In young stems, the difference is that the deposition of starch in the endodermis acts as an indicator for greater accumulation of auxin in the basal cells of the organ, culminating in its growth in the opposite direction to the gravitational vector.

In short, the location of statolith positioning in the cells of the root cap acts as a gravitational vector indicator, directing the positive gravitropic growth of the roots. For that, a communication will need to occur from the root cap towards the root elongation region, so that there can be a radial and asymmetric redistribution of auxin in the elongation region, through AUX1 and PIN2 which favors greater accumulation and effect of auxin in the cells of the upper region of the organ, leading to their increased growth. That, in turn, will culminate in the curvature of the root apex in the direction of the gravitational vector. For stems, the positioning of the starch in the cells of the endodermis will act in the indication of greater accumulation of auxin in the cells of the basal region, culminating in the growth of the organ in the opposite direction to the gravitational vector. There is evidence of the participation of Ca^{2+} and the cytoskeleton, integrating the sensory apparatus of the gravitational vector.

Altitude and Atmospheric Pressure Perception

Altitude is a condition of the environment that induces changes in the flora or in the behavior of plants. There are many aspects that differ in the environment due to this factor, which, individually or in interaction, can alter some aspects that interfere in plant development, such as water and oxygen availability, temperature, sensitivity to gravity and light patterns. As these are aspects addressed individually throughout this chapter, this section will emphasize the importance of atmospheric pressure, which is greatly altered according to the altitude.

Multiple factors to be perceived at different altitudes

The variation in altitude implies differences not only in the availability of oxygen and water, but in temperature, light patterns and atmospheric (barometric) pressure. As previously described, light and temperature are involved, respectively, in the synchronization and modulation of the biological clock. In a study by Lewandowska-Sabat et al. (2017) they observed that *Arabidopsis thaliana* plants occurring at higher altitudes were less sensitive to the photoperiod than those occurring at lower altitudes; however, the study only considered the light and temperature patterns, without considering other aspects.

In a study by Paul et al. (2017), isolating the plants in chambers, but without any variation in light, temperature, oxygen and water availability, only atmospheric pressure was changed in the cultivation of arabidopsis. It was observed that pressure changes caused differentiated expression in more than three thousand genes, many of which had increased expression under low atmospheric pressures, a condition typical of higher altitudes. Most of the genes that had increased expression are already known for their involvement in cell wall remodeling, which implies changes in the support for cell turgor and tension in tracheal elements. Another set of genes that have increased expression under hypobaric condition is related to cellular sensitivity to abscisic acid, a key hormone in the response of plants to various stresses. There was also an increase in the expression of genes known to be involved in stress caused by water deficiency, oxidative stress, temperature stress, and, mainly, by hypoxia, revealing common gene expression pathways among hypobaric stress and other stresses. The above-cited research also proved that the roots are more sensitive to changes in pressure than the other organs, and that could cause changes in the perception of gravity by plants.

Based on the above, plant responses to different altitude conditions do not only result from changes in light, temperature, oxygen and water patterns, but also, either in an integrated or an isolated way, from barometric pressure. However, there are still no studies that identify the biological sensors upstream of the responses of plants under pressure change.

There are no studies that link barometric pressure to the regulation of the circadian clock that would justify changes in the photoperiodic sensitivity of arabidopsis at different altitudes, as reported above. On the other hand, the common relationship between genes involved in stress due to hypobaric condition with those involved in several other stresses and their cross-relationships with the regulation of the biological clock are already well-known (Sanches et al., 2011), as are the interrelationships of various stresses involving phytochromes (Gavassi et al., 2017) that are important for the regulation of the biological clock. Apart from barometric pressure, there is evidence that cold, which can occur at different altitudes, is a very important factor in changes in photoperiodic sensitivity, as reported in the topics "Cold can change the photoperiodic sensitivity of plants" and "Winter memory", Chap. 4.

Plant behavior and rainfall forecasting

People from various communities use plant behaviors linked to the biological clock, barometric pressure and relative humidity to predict rainfall. The folding of dandelion and clover petals, as well as the curling of oak leaves are examples of indicators of short-term rain. Bioindicators are also used to predict rainfall fluctuations throughout the annual cycle, such as flowering patterns of *Nyctanthes arbor-tristis*, which even serves for some Asian communities to predict monsoons. However, it is important to note that these plant bioindicators do not have satisfactory degrees of reliability, since the responses observed in plants commonly depend on multiple interacting, endogenous and or exogenous factors for them to manifest themselves.

Many plant behaviors used as bioindicators of rainfall variation are linked to environmental factors that modulate the synchronization of the biological clock, such as the anticipation or postponement of the flowering of plants of some species throughout the year, due to changes in temperature, relative humidity or water availability.

Other predictive bioindicators for short-term rainfall, such as leaf or petal folding (Figure 1.15), are likely to be associated to responses by plant organs to changes in barometric pressure and or relative humidity, which usually increase prior to precipitation.

Figure 1.15. Closing of dandelion and clover petals as bioindicators used by traditional communities to predict rain, a phenomenon probably due to changes in barometric pressure and or relative humidity. 1.15A. Open petals of dandelion, bioindicator of rainless day. 1.15B. Closed dandelion petals, bioindicator of rain. 1.15C. Open clover petals, bioindicator of a rainless day. 1.15D. Closed clover petals, bioindicator of rain. Credits: 1.15A Image by aixklusiv from Pixabay. License to use at https://pixabay.com/en/photos/dandelion-abelha-n%C3%A9ctar-3342976/ 1.15B Image by Marco Centenaro from Pixabay. License to use at https://pixabay.com/en/photos/taraxacum-officinale-abelha-flor-3489530/ 1.15C. Image by Erich Westendarp from Pixabay. License to use at https://pixabay.com/en/photos/flor-do-trevo-trevo-de-montanha-3429074/ 1.15D Image by Станислав Березкин from Pixabay. License to use at https://pixabay.com/en/photos/trevo-flor-natureza-ver%C3%A3o-ograma-4889170/

In short, there are many environmental factors that vary according to altitude. The complexity of those factors, in interaction, results in the changes observed in plant development. Although much is known about water, temperature, radiation and oxygen in regard to the development of plants, and how each one is perceived through its respective sensory apparatus, little has been studied about the importance of the atmospheric/barometric pressure factor, which also alters according to the altitude. Plant sensors for barometric pressure are not known, although there is clear evidence of their perception, since it causes changes in the expression of thousands of genes, many of which have a cross-action with different stresses, and especially that caused by hypoxia. Some plant behaviors used as morphological bioindicators to predict rainfall, such as the curling of leaves and petals in some species, are likely to manifest themselves because of changes in barometric pressure and relative air humidity perceived by plants.

Salt Perception

Halophyte plants have many salt resistance mechanisms and so they can grow in saline environments without necessarily undergoing stress, unlike non-tolerant plants. In addition to environments typically abundant in salts, such as mangroves, the indiscriminate use of agricultural fertilizers has been the cause of salinization of new areas. To study the sensory apparatus of plants regarding salt, it is necessary to consider the differences in their chemical composition. When considering nutritious salts, as we will see in a later section, perception depends on various cell transceptors. This section is specifically dedicated to the perception of NaCl.

How do plants perceive NaCl?

When in high concentration, saline elements, such as NaCl, lead the plant to stress, impairing its development, causing morphological damage or causing losses in yield (Jiang et al., 2019; Steinhorst and Kudla, 2019).

The reception of NaCl by the plant cell involves membrane lipids. Ca^{2+} signaling is essential to the cellular perception of salt but in mutants of a protein called INOSITOL PHOSPHORYLCERAMIDE GLUCURONOSYLTRANSFERASE 1 (IPUT1), central to the formation of membrane sphingolipids, there is a low Ca^{2+} signaling performance in plants exposed to high concentrations of Na^+. IPUT1 catalyzes the synthesis of sphingolipid glycosyl inositol phosphorylceramide (GIPC), which is abundant in the outer layers of plant bio-membranes. Likewise, mutants of GIPC do not have a Ca^{2+}-mediated signaling response. It happens because the change in polarization caused by the accumulation of Na^+ in the apoplast leads to the binding of this ion to the GIPC, which is necessary to activate

Ca^{2+} channels triggering cellular defense events associated to salt stress. When calcium enters the cytoplasm, it binds to the SALT OVERLY SENSITIVE 3 (SOS3) protein, which, when decoding the calcium signal, activates the SOS2 protein, and that in turn activates SOS1, an antiport Na^+/H^+ membrane transporter, which acts in the extrusion of Na^+ from the cytoplasm, reducing its negative effect on the osmoregulation of the protoplasm, as well as the future metabolic consequences caused by saline stress. As a summary of this mechanism, an illustrative scheme can be seen in Figure 1.16.

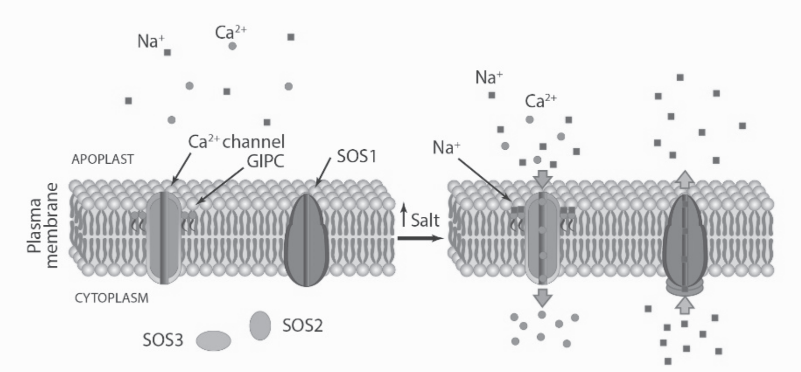

Figure 1.16. Mechanism of cellular perception of NaCl. The increase in NaCl in the apoplast leads to the binding of Na^+ to sphingolipids glycosyl inositol phosphorylceramide (GIPC), whose binding is necessary for the activity of Ca^{2+} channels essential for cellular signaling for Na^+. The increase in Ca^{2+} in the cytoplasm is a necessary signal for the binding of the SALT OVERLY SENSITIVE 3 (SOS3) protein to SOS2, which, when bound to SOS1, a transmembrane Na^+ transporter, will trigger its activity, resulting in the extrusion of Na^+ from the cytoplasm to the apoplast.

Nutrients Perception

The plant cells' perception of nutrients is already well known, although the term itself is little used. The input of nutrients to the cell symplast tends to be selective, through different membrane transporters. Obviously, in the absence of ion recognition by the carrier there would be no selectivity which necessarily requires a sensory apparatus. The perception is not limited to identifying the transportable nutrient ion, but also to its availability in the soil, which causes an increase or decrease in the synthesis or activity of its transporter.

How do plants perceive nutrients?

Transmembrane nutrient transporters are protein structures affected by different factors of the environment, such as magnetic flux, pH, electrical potential gradients and mechanical forces, and their synthesis or activity depends upon the availability of the nutrient to be transported. There is a wide approach in the scientific literature to nutrient transporters, but little emphasis is focused on the initial perception of the nutrient by the plant cell, which is associated with the transporter itself.

When the membrane structure has both the role of receptor and transporter it is called a transceptor. Transceptors effectively recognized in the reception/perception and transport of nutrients are those for nitrogen, boron and iron. However, considering that the transporters of most nutrients, whether macro or micronutrients, respond to the quantum of specific nutrients present in the soil, it must inevitably be considered that they participate in the perception apparatus. Furthermore, transporters can select the specific elements to be transported. Some mechanisms already known for recognizing ions by their specific transporters consider the ionic ray, the ionic condition as a cation or anion and the electrovalence of the ion. This section will address, not only the already known nitrogen, boron and iron transceptors, but also the other potential nutrient transceptors.

NITROGEN

For nitrogen, the transceptors not only detect the presence of NO_3^- and NH_4^+ in the soil, but also their availability, since the available quantum favors or disadvantages the synthesis of high or low affinity transceptors (Ho and Tsay, 2010; Xuan et al., 2017).

Several families of genes encode nitrate transceptors, namely, NPF (NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER FAMILY), NRT1 (NITRATE TRANSPORTER1) and NRT2, while for ammonium they are the AMT1 (AMMONIUM TRANSPORTER 1) and AMT2 families.

The membrane transceptors for nitrogen, cation or anion, are sensitive to the pH of the cellular apoplast. For absorptive transport of the anion, which normally occurs symportically with H^+, low pH in the cell wall is required, and for the cation, higher pH is required, transport that normally occurs in the antiporting process with H^+. That behavior of the transceptors can be expected for the other nutrient ions due to the need to maintain negative cytoplasmic electro-homeostasis. The pH gradient itself between the apoplast and the cell's symplast can also generate a transmembrane potential that modulates the activity of some proteins, such as NRT2.

The perception of nitrate is impaired in mutant plants by some nitrogen-associated gene transcription factors such as NLP7 (NIN-LIKE PROTEIN), NRG2 (NITRATE REGULATORY GENE 2) and TCP20 (TEOSINTE BRANCHED1/CYCLOIDEA/ PROLIFERATING CELL FACTOR1-20), which suggests that perception is not an event exclusively mediated by the transceptors.

A regulating factor in the synthesis of transceptors are the inter-organ signal-communicating molecules such as HY5 (Elongated Hypocotyl 5), which at the same time that it acts in the regulation of photo-dependent responses, where it is widely known, also acts as a transcription factor for NRT2 genes. As seen in Figure 1.17, HY5 participates in endogenous signals that regulate the reception and transport of N, acting on the aerial-root communication. In the same Figure, we can also see the peptide that signals the lack of N, the CEP (C-TERMINALLY ENCODED PEPTIDE), and the peptide that signals N satiety, the CLE (CLAVATA3/EMBRYO-SURROUNDING REGION). Downstream signaling of those peptides results in the presence of molecules that act as factors of activation or deactivation of the transceptors.

A protein that is also associated with both nitrate and ammonium transceptors is CIPK23 (CBL-INTERACTING PROTEIN KINASES 23), which participates in the phosphorylation processes necessary for its functionality.

Some plants absorb organic nitrogen in the form of amino acids and, since there are different transporters for different amino acids, there is inevitably a need for recognition sensors, although the mechanisms are unknown. The LHT1 (LYSINE/ HISTIDINE TRANSPORTER1) and AAP1 (AMINO ACID PERMEASE 1) receptors are sensitive to acidic and neutral amino acids and AAP5 has specificity for L-arginine and L-lysine (Figure 1.18). Other transporters such as AAP2 and AAP3 do not integrate the plasma membrane of cells of the epidermis, being restricted to internal tissues (Näsholm et al., 2009). In mycorrhizal plants, the absorption of amino acids is preferably carried out by the fungus, which uses other transporters.

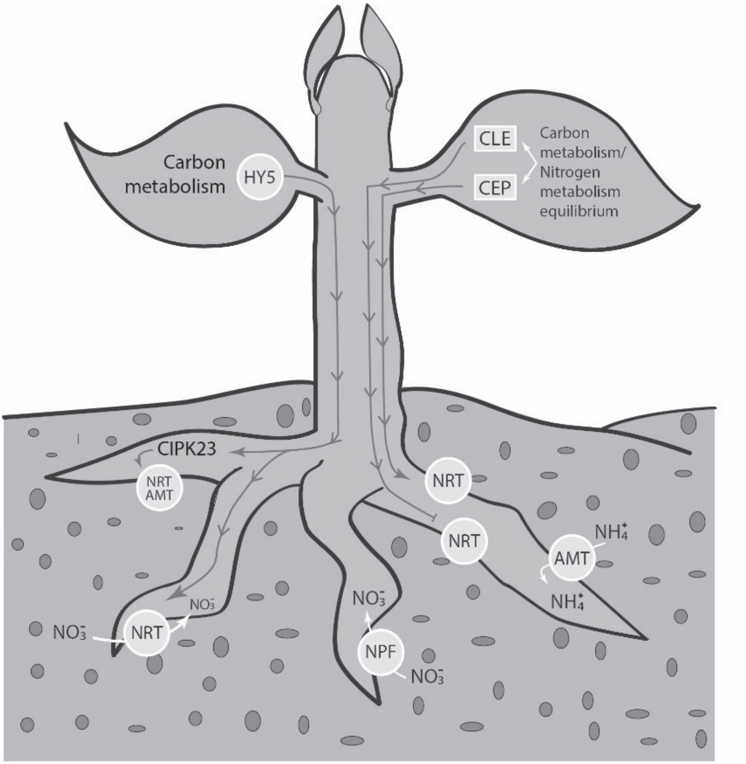

Figure 1.17. Root nitrogen transceptors (sensors/receptors and transporters) and their regulation by the aerial part. NRT (NITRATE TRANSPORTER) and NPF (NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER): NO_3^- transceptors; AMT (AMMONIUM TRANSPORTER): NH_4^+ transceptors. Depending on the availability of organic carbon in the leaves, necessary for the assimilation of nitrogen, signaling molecules such as HY5 (Elongated Hypocotyl 5) are transported from the leaves to the roots, where they will stimulate the root absorption of nitrogen. Similarly, depending on the leaf C/N balance, the peptide CEP (C-TERMINALLY ENCODED PEPTIDE), an indicator of N "hunger", when acts in signaling towards the roots, induces greater nitrogen absorption. The CLE peptide (CLAVATA3/EMBRYO-SURROUNDING REGION), an indicator of N satiety, acts to inhibit N absorption by the roots. Another way of regulating the activity of the transceptors is their phosphorylation mediated by the protein kinase CIPK23 (CBL-INTERACTING PROTEIN KINASES 23), which is also positively regulated by the action of HY5.

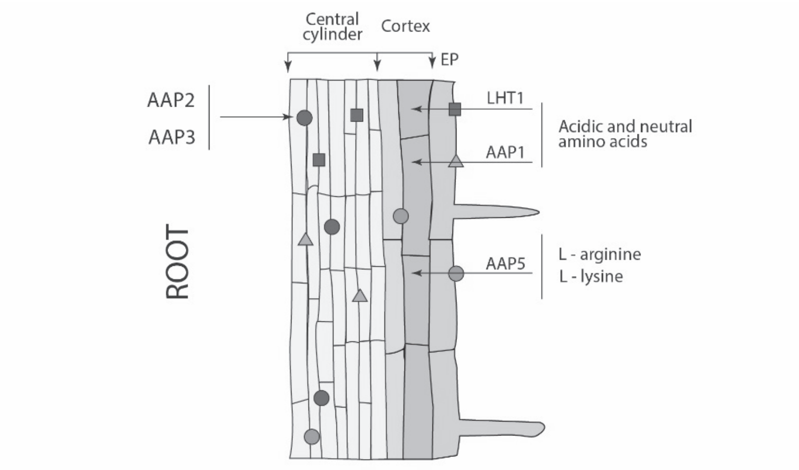

Figure 1.18. Cellular amino acid transporters. LHT1 (LYSINE/HISTIDINE TRANSPORTER1) and AAP1 (AMINO ACID PERMEASE 1), transporters of acidic and neutral amino acids, are present in the plasma membrane of the cells of the epidermis (EP) and of the innermost tissues of the roots; AAP5 transporters of L-arginine and L-lysine, are present in the plasma membrane of EP and in the innermost tissues of the root; AAP2 and AAP3 are present only in the plasma membranes of tissues of the central cylinder, mainly of the sieved elements, participating in the loading of the phloem.

In general, it can be seen that the regulation of nitrogen sensors present in the plasma membrane occurs both due to external conditions, such as the type of ion, its availability, pH, and, due to factors inherent to the cell itself, such as intermembrane gradients or even long-distance factors that regulate perception and absorption, such as HY5 and peptides that signal scarcity or satiety of the nutrient.

POTASSIUM

Potassium transporters will be considered here as the preferential sensors for this element. In addition to the transport function, transmembrane proteins are of high or low affinity, which indicates their participation in the sensory apparatus of plants in detecting the availability of K^+ in the soil. The AKTs channels (K^+ inward rectifying channel AKT) and the transport proteins HAKs (HIGH-AFFINITY K^+) and HKTs (HIGH-AFFINITY POTASSIUM TRANSPORTER) are high affinity transporters for the ion.

The calcium-dependent protein CIPK23, the same one that acts in nitrogen transceptor phosphorylation processes, also acts on the AKT1 protein, promoting its phosphorylation. There is evidence that the AKT1 transporter has altered perception when connected to SNARE (Soluble N-ethylmaleimide- Sensitive Factor Attachment Protein Receptor) receptors, which are involved in membrane fusion and vesicular transport.

PHOSPHORUS

Phosphorus transporters will be considered here as the most likely receptors for the nutrient. The root apex can detect the presence of phosphate. The expression of phosphorus-sensitive genes PDR2 (PHOSPHATE DEFICIENCY RESPONSE), LPR1 (LOW PHOSPHATE ROOT) and LPR2 occurs in the apical meristem of the roots, which perceives phosphate through high affinity H^+- symportic transporters, such as PHO84 and PHO89, or through low affinity ones, such as PHO87 and PHO90, in addition to those of the PHT family (PHOSPHATE TRANSPORTER), and others present in endo-membranes. Similarly to what occurs with nitrogen, the gene expression of phosphorus transporters is regulated by components of the PHR1 (PHOSPHATE STARVATION RESPONSE 1) family, which can act as transcription factors, and whose regulation occurs by phosphorus both outside and inside the cell (Chiou and Lin, 2011; Nussaume et al., 2011; Del-Saz et al., 2018).

Due to the variation of phosphorus availability in the soil, not only do phosphate sensitivity factors in the cells change, but also high affinity sensors/transporters such as PHT1 and PHT2 have their synthesis reduced in high phosphate concentrations, probably to avoid excessive absorption and that, naturally, requires a refined level of perception by the cell. The sensitivity of different transporters in relation to the availability of phosphorus, internally or externally, as well as some of its regulators, strengthen the idea that these biomolecules compose the sensory apparatus of plants for this nutrient.

SULPHUR

Following the perception patterns of the other nutrients, the synthesis of the high affinity sulphur transporters, SULPHATE TRANSPORTERS, SULTR1;1 and SULTR1;2, is regulated upwards due to the low availability of sulphate in the cells or in the external environment, while the SULTR1;2 is also under high availability (Maruyama-Nakashita et al., 2004), which again shows the function of those transporters as sensors.

Apparently, acetylserine acts as a positive regulator of the SULTR1 genes since both exogenous application and its natural accumulation in cells with sulphate deficiency induce the expression of these genes. As observed for N and P, the C/N balance affects the expression of sulphate transporters, by a mechanism not yet identified.

CALCIUM

Due to the wide variety of metabolisms that have calcium as a secondary messenger, the plant cell is expected to have quite refined sensory mechanisms for the perception of this nutrient, especially because the countless signals involving cytoplasmic calcium waves culminate in different metabolic pathways, depending on the factor and the intensity of the stimulus, which means that not only the presence and concentration of calcium in the cytoplasm must be perceived by the cell but also the speed of accumulation, the duration of the wave, the transporters that act in the influx, the molecules that act in interaction with the ion and the origin of the ion, that is, whether it is apoplastic or from internal vesicular stocks of the cell. In that sense, it is believed that the sensory apparatus that involves calcium must be complex and that it has its transporters as participants.

Calcium has a mechanism for rapid entry or exit of cellular compartments, which occurs through the transport channels. The importance of Ca^{2+} signaling is so wide that there are specific transport channels activated in the most diverse physical and chemical conditions of the apoplast. The membrane permeability to this nutrient, through its channels, may require an electric gradient promoting the depolarization of the membrane; in that case they are called DACCs channels (Depolarization- Activated Ca^{2+} Conductance Channels); and when membrane hyperpolarization is required to activate the channels, they are called HACCs (Hyperpolarization-Activated Ca^{2+} Conductance Channels). Independent-voltage channels are called VICCs (Voltage-Independent Ca^{2+} Conductance Channels). VICCs are the most widely used form for the acquisition of Ca^{2+} by the roots. In addition to the channels, there is the Ca^{2+}-ATPase electrogenic pump used by the cells, including for the extrusion of the protoplasm ion. The activation of some of the calcium channels may depend on ligands, such as inositol-3-phosphate (IP3).

MAGNESIUM

Magnesium has receptors from the Mg^{2+}-transporters (MGTs) family, with two transmembrane domains. As for other nutrients, transporters have different affinities, which shows their participation in the mechanism of quantitative perception of the ion. MGT6 is an example; its synthesis drastically increases when the concentration of the nutrient is very low in the soil.

MICRONUTRIENTS

The process of perception of micronutrients by plants does not seem to differ from that seen for macronutrients, except that it is more common for transporters to act on more than one nutrient. A member of the YSL (Yellow-Stripe Like) family has been reported to transport five types of metallic micronutrients. The fact that micronutrient transporters are also sensitive to high and low ion concentrations suggests that they participate not only in transport but also in the cellular sensory apparatus.

Boron can be perceived and transported in the form of borate or boric acid by BOR1 (BORON TRANSPORTER 1), just as boric acid can be transported through the NIP5;1 channel (NODULIN 26-LIKE INTRINSIC PROTEIN 5;1). The BOR1 transporter is degraded when the boron content is high in the protoplasm and thus toxicity is avoided (Takano et al., 2010), which proves the biomolecule's transceptor condition. The destruction of the protein shows a stricter control than just increasing or decreasing the synthesis of transporters by the cell, or regulating the action of the transporter by phosphorylation, as observed for some macronutrients. This greater rigidity in the control of absorption is necessary since the amplitude of the content that can cause toxicity to the cell is less for micronutrients than for macronutrients.

Molybdenum has MOT1;1 (MOLYBDATE TRANSPORTER) and MOT2 which act as high affinity molybdate transporters, and cell absorption is greatly affected by the pH of the medium. SULTR5;1, although it is known to transport sulphate, also transports molybdate, especially in tonoplasts (Huang et al., 2019). As we will see later, other transporters, of less importance to molybdenum, can also act in the transport of this nutrient.

Iron and other metals are absorbed by membrane transporter ITR1 (IRON-REGULATED TRANSPORTER 1) which is synthesized in the epidermis of the roots or, alternatively, in the outermost layers of the root cortex. Other transporters for one or many metals can also transport iron, but they apparently cannot replace the action of ITR1 for the necessary acquisition of this nutrient by plants.

In addition to iron, ITR1 acts on other metals, such as cadmium, cobalt, manganese and zinc, especially when the iron concentration is low. ITR1 itself, when perceiving excessive accumulation of other non-ferric metals in the cytoplasm, triggers signaling involving ubiquitination by IDF1 (IRON DEFICIENCY-RESPONSIVE ELEMENT-BINDING FACTOR 1) ubiquitin-ligase, for its own destruction (Figure 1.19), thus preventing damage to the cell by toxicity, an effect that proves its condition as a transceptor. Its phosphorylation by protein kinase CIPK23 is part of the signaling process for the destruction of ITR1 in the vacuole (Dubeaux et al., 2018), after endocytosis.

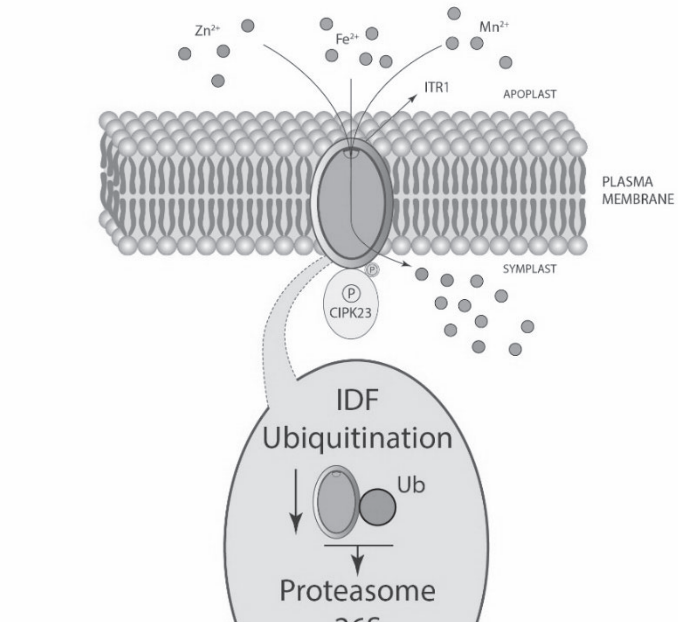

Figure 1.19. Regulation of the ITR1 transceptor (IRON-REGULATED TRANSPORTER 1). ITR1 is the main cellular transporter of Fe^{2+} present in the epidermis of the roots, but it also transports several other divalent metal cations. When the accumulation, especially of other metals, is becoming elevated in the cytoplasm, and thus, increasing the risk of cell toxicity, ITR1 itself is destroyed to preserve cell homeostasis. For destruction, ITR1 is phosphorylated by the protein kinase CIPK23 (CBL-INTERACTING PROTEIN KINASES 23), a condition that favors both the vacuole-endocytosis of ITR1 and its ubiquitination by IDF1 (IRON DEFICIENCY-RESPONSIVE ELEMENT-BINDING FACTOR 1) ubiquitin-ligase, making ITR1 ubiquitinated, and therefore the target of the action of the 26S proteasome, which will promote its destruction.

The main copper transporters are from the COPT (Copper Transporter) family, sensitive to the divalent cation, or even to the monovalent ion when the divalent one is scarce in the soil, but it can also be perceived and transported by the metal transporters of the ZIP families (Zinc-Iron Permease or ZRT, IRT-like Proteins), YSL and NRAMP (Natural Resistance Associated Macrophage Protein 1). There is evidence that its absorption does not depend on a change in membrane polarization, although the mechanism for transporter activation is unknown (Sanz et al., 2019).

As previously mentioned, receptors/transporters can act on more than one nutrient, according to their physical-chemical characteristics. These transporters have been observed for some macronutrients and many essential or beneficial micronutrients such as zinc, iron, manganese, cobalt, cadmium, calcium, copper, lithium, sodium, potassium, magnesium and nickel, distributed in different transporter families such as CAX, CCX, BICAT, PAM, CCHA, CMT, MTP, ECA, LCA, VIT, MEB, ZIP, YSL and NRAMP (Vert et al., 2002). It is important to note that although nutrients such as manganese, copper, calcium, iron, potassium and magnesium can be transported by components of some of these families, the main transporters have already been described when we mentioned the specific nutrients.

Cation-Cl^- co-transporters (CCC), in symport with potassium or sodium, seem to be important for the acquisition of chlorine by plants, justifying an important role of this anion, which is the negative electro homeostasis of the cytoplasm, when it is absorbed simultaneously with those cations (Colmenero-Flores et al., 2007).

Electrogenic calcium and hydrogen pumps

For the stabilization of cellular homeostasis or the perception of cellular state transition due to a stimulus during the activation of nutrient sensors/transporters, there may be a need for H^+ or Ca^{2+} ions to enter or exit through H^+-ATPase or Ca^{2+}-ATPase activation. H^+-ATPase activation requires phosphorylation. The kinases that usually promote phosphorylation can be activated by different signals, such as the blue light that activates phototropins, and they transfer phosphorus to protein 14-3-3, an important secondary messenger in plants, which in turn, promotes the phosphorylation of H^+-ATPase, resulting in membrane hyperpolarization, an essential condition, for example, for stomatal opening. In that case, the activity of this transporter can be a secondary consequence of a signaling caused by an endogenous or exogenous factor. On the other hand, the calcium binding to calmodulin, making it active, can cause its binding to Ca^{2+}-ATPase, activating it. Regulation of the activity of this electrogenic calcium pump has also been reported by the phosphorylation process. Usually, this electrogenic pump dynamics occurs because of the perception of a signal that may be external or internal to the cell (Bækgaard et al., 2005).

Plants perceive the location of nutrients

The movement of plants towards a chemical compound is called chemotropism. When we study plant-environment interaction, it is pertinent to analyze whether the roots have chemotropic movement towards nutrients, since their spatial distribution in the soil is not always homogeneous. It is already well known that the architecture of the roots in the soil is influenced by the nutrient content in the different layers.

A study carried out with *Nicotiana attenuata* (Ferrieri et al., 2017) observed that the number and positioning of roots responded to the amount of nutrients present, either as a seeking or avoidance response, depending on their concentration in the medium. The apices of the lateral roots responded chemotropically when bending to seek for suggested points in a culture medium with adhesives containing nutrients. The chemotropic response was more effective for the presence of Cu^{2+} and Fe^{3+}.

The regulation of root curvature induced by chemotropism appears to be similar to that already known for several other curvature events in plants, such as the growth of roots influenced by gravitropism (see section "Gravity Perception"). Mutants defective in ethylene and jasmonic acid had impaired chemotropism, possibly because of the content and radially asymmetric action of auxin and ethylene inducing the differentiated cell growth necessary for curvature. Jasmonic acid, on the other hand, has crossing points with auxin during cell signaling as well as regulating the expression of several genes related to the auxin biosynthesis pathway, in addition to regulating the distribution of auxin transport PIN proteins, mandatory for radial hormone movement in the organ that is to bend.

Again, as has been reported for water, it is evident that ion-root contact does not occur only by mere interception during root growth. There is growth and root curvature guided by the perception of what is to be absorbed. For the case of nutrients, the mechanisms by which roots perceive their location have not yet been elucidated. It is possible that with the advance in the studies of electrotropism in roots, the movement in relation to electric fields, which will be examined later, future research will find points in common with chemotropism because of the ionic characteristics of the great majority of absorbable nutrients.

Magnetic Field Perception

The influence of geomagnetism on plants has been a central object of various research projects for decades. For a time, however, such studies became increasingly neglected in the field of plant science, but currently, with the revived interest in the subject, those former research efforts have been revisited, and in addition to the stimulus-effect, the possible mechanisms of vegetal perception of this environmental factor have been studied.

Knowledge about the perception of geomagnetic fields by plants is still under construction, based mainly on the different aspects that precede their effects on plant development. On Earth, the geomagnetic field tends to be static, close to the Equator line at a flow of approximately 35 μT (T: tesla) and at the poles, approximately 70 μT, quantities considered relatively weak (Zhadin, 2001) but, even so, sufficient for, and fundamental to the manifestation of phenomena observed in the field of biology, as in the case of directing the long-distance migration of animals, whose perception is mediated by cryptochromes, biomolecules also known in plants as photoreceptors.

In plants, sensitivity to geomagnetic fields has also been associated with cryptochromes (cry), since cryptochrome-induced genes have altered expression due to the magnetic flux. It is known that the cryptochrome takes on a singlet state under blue light during its activation, resulting in pairs of radicals. The movement of radicals in response to the magnetic field has been discussed as a possible mechanism of magnetoreception and plant perception of its position in the Earth's geomagnetic field. Under high magnetic flux there is an increase in cry1 and cry2 phosphorylation, resulting in the activation of these photoreceptors, while under low flux there is no decrease in activated cry1, but there is a decrease in activated cry2. It has also been observed that the dephosphorylation of cryptochromes in the dark was slowed down under a high magnetic flux and accelerated under a very low flux, indicating a modulating action of magnetism on the activity of those biomolecules, including at night, when their action as a photoreceptor is not known. Even in the dark period, but with intermittent light, there is still the action of cryptochromes (Pooam et al., 2019). All those observations show that cryptochromes are a strong candidate for plants' perception of magnetic flux.

The influence of the geomagnetic field on the movement of radicals in molecules in a singlet state is not restricted to cryptochromes, but also involves other molecules, such as chlorophyll. It is already known that the increase in magnetic flux, depending on its magnitude, also increases the formation of reactive oxygen species (ROSs) from the overexcited chlorophylls.

Evidence that plants are sensitive, and some even extremely sensitive to magnetic fields, is, for example, in the fact that magnetized water or magnetic storms, which alter the strength of the geomagnetic field by only 1%, actively promote nuclear and chromosomal anomalies in onion and broad bean meristem cells. In several species, the orientation of the seeds according to the magnetic poles, alters the germination percentage, or the magnetic field can even induce changes in the orientation of the growth of the roots. Under weakened magnetic fields, altered cell division rates in meristems, changes in mitochondrial morphology, reduced ferritin content in chloroplasts and altered cytosolic calcium content, are recurrent effects in several species. Further details on the effects of magnetic fields on plants can be seen in the review by Galland and Pazur (2005) and Maffei (2014).

Geomagnetic fields can modulate the activity of ionic currents in biomembranes, as well as electrical signals to stimulate osmoregulation. They also promote changes in the movements of cyclosis and cause torque effects in ferrimagnetic particles. Due to the many modifications caused in plant cells by magnetic flux, its effects on the organism as a whole are not surprising and can change the time of floral transition, plant growth and agricultural productivity.

In addition to the perception by cryptochromes, other possibilities can be considered regarding the potential sensory mechanisms of geomagnetic fields by plants. The participation of another photoreceptor, phytochrome, has been observed in studies of magnetotropism. Perceptual responses to the direction of the magnetic source have been positively associated with plant responses to red light, although phytochromes have only been mentioned indirectly as a potential receptor. There is also evidence of a sensory mechanism linked to that related to the perception of gravity, since plants that are mutant in starch and consequently insensitive to the gravitational vector are also insensitive to geomagnetic fields.

The geomagnetic field influences the entry of the circadian clock, since, when the magnetic flux goes down to almost zero, there is a significant increase in the daytime genes *LHY* and *PRR7*, unlike the nighttime *GI* gene. That event leads to the forcing of daytime metabolisms in the 24-hour cycle, independently of light. Based on the above discussion of cryptochromes photoreceptors, it is believed that this entry in the clock happens due to the action that the magnetic flux has on those photoreceptors (Agliassa and Maffei, 2019).

In conclusion, there are many influences of geomagnetic fields on plant behavior, however, the possible forms of their perception by plants are still putative, but with strong evidence of the involvement of cryptochromes. In addition, other apparatuses have been taken into account, such as phytochromes, ion transporters, ferrimagnetic molecules, chlorophyll and starch, as well as components of the circadian clock.

Electric Field Perception

The soil is a large complex of electrically charged structures. Colloids, nutrients, organic compounds, biota and roots are elements rich in electrical charges, which can potentially alter the electrical state of the soil. It is believed that the effects of electric fields on the development of plants, especially roots, are many and that they interfere with nutrient-induced chemotropism. In the Chapter on extracorporeal communication in plants, we will see the importance of those fields in influencing the plant-microbiota, plant-pollinators and even plant-plant interaction.

The ionic exchanges that occur in the roots change the electrical fields of the rhizosphere all the time, and cause changes in the polarization of cell membranes, and consequently, they change the electrical gradients necessary for the activation of transmembrane structures of perception and transport of different substances. Controlled experiments regarding the non-harmful voltage/cm for root tissues, considering salt and pH control, have shown that the tendency of healthy and intact roots of wild genotypes is to grow towards the cathode, while decapitated roots or agravitropic mutants curve towards the anode (Ishikawa and Evans, 1990; Stenz and Weisenseel, 1993). Those observations lead to the conclusion that the sensory system of the roots to electrical poles involves root apex interference.

The signaling of the electrotropic curvature involves ion channels, since their chemical blockers interrupt tropism. The Ca^{2+} channels are decisive in the response. However, it is not possible to say that they act in the initial perception of the stimulus, since they can participate only in secondary signaling. Knowing that photoreceptors trigger calcium-mediated signaling, and that phytochromes and phototropins are already known as direction recognizing molecules, it is plausible to consider them as possible sensors involved in electrotropism.

Among some studies that show the participation of photoreceptors as sensors of electrical direction, there is the one developed by Tanada (1968), in which it was observed that barley roots with decapitated apexes have electrically polarized development, in a light-dependent and photo-reversible manner to radiation V and VE, necessarily implying an action regulated by phytochromes. Similar responses were seen in beans and oats (Jaffe, 1968; Newman and Briggs, 1972).

The phototropins and photoreceptors of the zeitlupe family have the LOV domain, which is voltage sensitive, and phototropins are already known to mediate the perception of photo-direction (Hughes, 2013). The overall set of information about these photoreceptors suggests the participation of phototropins and phytochromes as sensors of electrical direction.

Touch Perception

Plants are organisms sensitive to touch. Some respond to the stimulus by closing leaflets or traps on a time scale of seconds, while others develop drastic changes in their development, visible over the days. The sensory apparatus of plants to touch is linked to the perception of mechanical impacts, whose sources of stimulus are quite varied, such as a manual touch or with objects, contact with herbivores, wind or even sound.

Quick touch responses

Knowledge of electrophysiology has been instrumental in understanding the rapid trapping of some insectivorous (carnivorous) plants or the closing of leaflets in touch-sensitive plants, as well as for ultra-fast communicative signaling over long distances between organs and tissues whose speed is beyond chemical communication capacity.

The venus flytrap (*Dionaea muscipula*) (Figure 1.20) has been the central object of studies concerning rapid movement in plants. That is because its modified leaf, which acts as a trap, can close in less than one second. The trap closes in response to the touch of insects on mechanosensory trichomes that act as a trigger inside the organ. In a study developed by Volkov et al. (2009) it was observed that even if the trigger is not touched, the trap closes due to a threshold of electrical charge accumulated in the central midrib. From this threshold, there is a charge transmission between the positive pole, which is the midrib, and the negative pole, which is the lobe, culminating in a response that can occur in a time scale of just 0.3 s.

Figure 1.20. Venus flytrap (*Dionaea muscipula*). In the central part of the modified leaf (left) it is possible to observe the presence of mechanosensory trichomes, indicated by the arrow. On the right, the general aspect of the plant is observed. Credits: Left: Image by Noah Elhardt from Wikimedia Commons. Use license at https://commons.wikimedia.org/wiki/File:Venus_Flytrap_showing_trigger_hairs.jpg Right: Image by David Stang from Wikimedia Commons. License to use at https://commons.wikimedia.org/wiki/File:Dionaea_muscipula_33zz.jpg

From the touch on a mechanosensory trichome, a potential receptor is generated that generates an electrical signal, which acts as an action potential that spreads through the tissues. In the species in question, in order to achieve a charge capable of provoking action, two touches on the trichomes are necessary, generating two cumulative electrical charges, but the second touch should not exceed a time limit of approximately 30 s, in relation to the first. The behavior is linked to an electrical memory. The propagation of the action potential can be stopped by blockers of aquaporins or voltage-gated transmembrane channels. In this case, the sensor is linked to the trichome and the signal transmission is linked to membrane components.

In regard to the voltage-dependent membrane components, the activity of the K^+ channels is fundamental for the response to occur; that is to be expected due to its central role in the osmotic regulation necessary for water to enter through aquaporins (and also via plasmodesms) in the outer cells of the leaf lobes, culminating in the closure of the trap. For the closure to be very fast, it is necessary that the plants keep the cells inside the lobes with high turgor pressure, while opened. When the electrical signal for closure reaches the lobe, a rapid flow of water is triggered to the outside of the lobe, culminating in the trap closing.

Another widely explored example of rapid movement in plants is a thigmonastic one, observed in the closing of *Mimosa pudica* leaflets (Figure 1.8), which close in response to touch (and also temperature, water availability, lighting patterns, wind, injury, vibrations and electro-stimulation).

Like the venus flytrap, the movement of mimosa leaflets depends on voltage-dependent membrane ion channels, such as those for Ca^{2+}, K^{+}, Cl^{-} as well as aquaporins, so that there is rapid movement of water in the secondaries pulvinus, to close the leaflets. As expected, the higher the turgor pressure in the upper layer of the pulvinus, the greater the opening of the leaflets. The maintenance of this high-water pressure is important for a fast closing from a stimulus, where the water will move to the basal part of the pulvinus, increasing the turgor pressure in this region, favoring the closing of the leaflets (Volkov et al., 2010).

When analyzing the response events to the touch, as well as its elements, it is significant that touch sensors in the venus flytrap are found in the mechanosensory trichome; both in the venus flytrap and in the mimosa, it is possible that the transmembrane channels for water or ions integrate the sensory apparatus. That is because aquaporins (and plasmodesms) must not favor the movement of water between the inner and outer sides of the leaf lobe or between the upper and lower portions of the pulvinus when the trap of the venus flytrap and the mimosa leaflets are open. The flow of water from the side where the cells have high turgor pressure to the opposite side has to be very fast and, in this case, touch acts as a trigger for the activation of aquaporins as well as ion channels for osmotic control.

Mechanosensory elements

When addressing how plants perceive temperature, depolymerization of actin filaments has emerged as a potential sensor, as it occurs upstream of the response signaling cascade. Again, those filaments reappear as a possible member of the sensory apparatus, because in thigmonastic movement, when phosphorylated, they are positively related to the flexibility of the pulvinus. In the case of a relatively strong mechanical impact, it is likely that the filaments that make up the cytoskeleton in the protoplasm can act as sensors regardless of cell wall or plasma membrane signaling, since the entire cell is affected by the impact at the same time. However, in situations of tension or subtle or more superficial mechanical impacts, it is expected that some wall or membrane elements act as the sensors that first perceive the stimulus factor, due to their more external positioning in the cell, but it still do not exclude the cytoskeleton because its points of interaction with the plasma membrane.

The cell wall of the plant's epidermis is the first structure to come into contact with mechanical forces and stresses from the external environment, which is why it is important that its components should receive special attention in the study of mechanosensory aspects of the cell.

Fruleux et al. (2019) propose putative mechanosensors taking into account the visco-elastico-plastic nature of the cell wall (it returns to its initial organization after a tension has ceased, but can suffer irreversible deformation if the tension exceeds a certain threshold). It is formed of various compounds, such as structural carbohydrates covalently linked to proteins like the HRGPs, which are glycoproteins rich in hydroxyproline, and to rapid alkalinization factors (RALFs), which are peptides non-covalently linked to other wall components. The mechanical impact on the wall can generate a signal to be perceived by the sensors. This can happen due to a pressure that is sufficient to alter connections of wall components with protein domains, or to break connections between components that may leave peptides loose, which, in that condition, can be perceived by membrane sensors. Another involvement of the wall in the perception of mechanical impacts results from the affinity of remodeling or hydrolysis enzymes when their activities depend on the degree of tension of their substrates and can lead to the release of particles, such as oligosaccharides, to be perceived by the membrane receptors/sensors (Figure 1.21).

Although the mechanical impact is felt preferentially by the cell wall, the mechanosensory perception is primarily attributed to the membrane, even though it is less mechanically affected. In the topic on the perception of the calcium nutrient, it was reported that the activity of some of its channels, the VICC channels, is voltage-independent so that they can be responsive to other stimuli to their activation, such as, for example, to mechanical changes felt by the membrane. From this membrane perception a whole cascade of signal transduction is triggered, like the one responsive to atmospheric pressure, covered in the section “Altitude and atmospheric pressure perception”, which regulates the expression of more than three thousand genes, including many involved with the cell wall remodeling that takes place under mechanical pressures. In addition to VICCs, there are many transmembrane channels that respond to mechanical pressures felt by the plasma membrane, such as MSL (MECHANOSENSITIVE CHANNEL OF SMALL CONDUCTANCE-LIKE), MCA (MID1-COMPLEMENTING ACTIVITY), TPK (TWO PORE POTASSIUM), OSCA (HYPEROSMOLALITY- GATED CALCIUM-PERMEABLE CHANNELS) and DEK1 (DEFECTIVE KERNEL1).

The perception of cell wall components loosened by mechanical impact, such as peptides, as well as the perception by the plasma membrane of the pressures imposed on the wall, requires connection elements existing between these two structures, including the protein kinases anchored in the membranes, but which are projected on the walls, as already reported in plants’ perception regarding oxygen, water deficiency and the presence of water. In addition to the anchored ones, it is also likely that other transmembrane kinases that interact with the cell wall act as mechanosensors, such as RLKs (RECEPTOR-LIKE KINASES), previously reported for detecting the tension imposed by water deficiency at the cell wall-plasma membrane interface, RLPs (RECEPTOR-LIKE PROTEINS), WAKs (WALL-ASSOCIATED KINASES), PERKs (PROLINE-RICH EXTENSIN-LIKE RECEPTOR KINASES), LECTIN RECEPTOR-LIKE KINASES, the formins (proteins involved in the polymerization and accelerated growth of actin filaments), GPIs (GLYCOSYLPHOSPHATIDYLINOSITOL), or even the proteins of the COBRA family. Glycosyl-inositol-phospho-ceramides, which are glycosylated lipids that bind the membrane to the cell wall, may also be involved in the perception or signaling of mechanical pressures. A simplified scheme of the mechanical sensors can be seen in Figure 1.21.

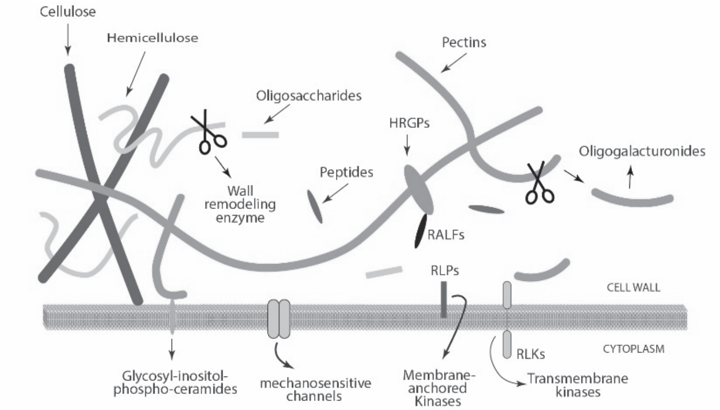

Figure 1.21. Simplified scheme of the cell wall-plasma membrane components involved in the perception of mechanical impacts. Wall carbohydrates, such as pectins, covalently linked to HRGPs proteins (glycoproteins rich in hydroxyproline, usually linked to RALFs, which are peptides that act as rapid alkalizing factors), depending on the degree of tension of impact can suffer deformations that may even be irreversible. Wall remodeling (hydrolysis) enzymes sensitive to the degree of tension of their substrates can act on structural carbohydrates giving rise to oligosaccharides, such as oligogalacturonides, which, when released, can activate sensors present in the plasma membrane. Both the tension of the mechanical impact of the wall on the membrane and loose oligosaccharides can activate transmembrane mechanosensitive channels such as VICCs, MSL, MCA, TPK, OSCA, DEK1, or activate transmembrane or membrane-anchored protein kinases such as RLKs, RLPs WAKs, PERKs or others like formins and GPI. The channels and kinases, in addition to the glycosyl-inositol-phospho-ceramides, which are glycosylated lipids that bind the membrane to the wall, are the most likely mechanical sensors, which, after being activated, will trigger the signals that occur in the cell symplast. Modified from Fruleux et al. (2019).

The cell wall appears as a plasma membrane screen to the pressures imposed by the mechanical impact, such as touch, but one that can act in the triggering of the mechanosensors present in the cell wall-plasma membrane interface.

To evaluate the apparatus for perceiving mechanical impacts on plants, it is important to consider the force of the impact, which can result, for example, from the tread of a cow or the touch of a fly's leg on a mechanosensitive trichome. If we consider that the whole cell is affected at the same time, it can be expected that the external and internal sensory mechanisms will be activated at the same time. However, if the mechanical impact is subtle, it can be expected that the perception occurs from the outside to the inside of the cell, initially activating the cell wall/plasma membrane sensors, triggering a chemical and or electrical signal towards the protoplasm, or even, as in the case of the venus flytrap, the signal can spread throughout the organ.

Thigmomorphogenesis

Not all responses to touch are as quick as those previously reported for venus flytrap or mimosa. Some responses, such as plant growth, can be seen only after a few days of touch treatment, although on a cellular scale some responses occur within seconds or minutes after stimulation. The term thigmomorphogenesis is used for the formation and growth of plants influenced by touch, which can, for example, lead to stunted growth and delayed flowering.

Thigmomorphogenesis responses depend on the dose, which can be both the pressure exerted and the frequency of the stimulus. The effect can be visualized not only in the place touched, but in the whole plant and that requires a systemic dissemination of the signals from the stimulus, which can occur via chemical substances or electric currents.

The term “touch” corresponds to a mechanical stimulus, which may be due to various sources, such as insects, rain, winds or even acoustic vibration. Whatever the source, it has been perceived that calcium plays a central role in signaling responses, and its transporters or proteins attached to them can act as mechanosensors, or at least are very close to some sensor that precedes them. Of the various touch sensitivity genes (*TCHs*), at least twelve encode for proteins linked to calmodulin (*CMLs*), which together with the calmodulin itself, act in cytosolic signaling due to touch (Chehab et al., 2009).

Touching also triggers the formation of lipoxygenase transcripts, from which jasmonic acid (JA) is derived. The increase in this hormone is closely related to a greater defense of plants against attack by herbivores, and its increase is also related to both the foraging of leaves and the vibrations of herbivore bites, as will be discussed in the next chapter. The touch of a herbivore can trigger not only greater synthesis of JA, but also the action of its reserve present in glandular trichomes damaged by mechanical impact. Touch-induced JA increase is likely to be the negative regulator of plant growth. Such evidence highlights JA as a central indicator for responses to mechanical impacts. Other hormones, such as ethylene, seem to have a regulatory role after the JA, and the enzyme ACC synthase, essential to ethylene biosynthesis pathway, has increased upward regulation by mechanical stimulus.

Another suggestion for intracellular signaling in thigmomorphogenesis is that Hechtian strands, which are extensions of the plasma membrane connected to the cell wall, in interaction with actin microfilaments, microtubules, endoplasmic reticulum and RGD peptides (which contain arginine, glycine and asparagine) play an important sensory role. These peptides are an integral part of membrane proteins that connect to the cell wall through the cytoskeleton. When disturbances occur in this cell wall-plasma membrane-cytoskeleton set, not only the opening of mechanosensitive calcium channels, independent of voltage may occur but also internal signaling due to the changes undergone by the cytoskeleton, which in this case, would act as a sensor. Although its performance is not known in detail, we have already seen that the cytoskeleton participates in internal cellular signaling due to its polymerization or phosphorylation.

How do plants perceive the wind?

The wind is yet another source of mechanical stimulus, and therefore the plant is expected to perceive it through a touch-like mechanism. Its influence on plant development is known regarding transpiration rates and production of supporting tissues and it can lead to decreased plant growth, a typical effect of thigmomorphogenesis.

When they exposed *Nicotiana plumbaginifolia* plants to the wind, Knight et al. (1992) observed an immediate increase in cytosolic calcium, which apparently, when observed by luminescence technique, came from the cell's internal stores in the endoplasmic reticulum and the vacuole. They also verified the existence of a positive relation between wind speed and calcium accumulation and that prolonged wind stimulation rendered the plant cells refractory to further response to calcium signalling. Other studies have reported that half an hour after submitting plants to the wind, there was an increase in the production of calmodulin.

Touch perception summary and considerations

Touch is a mechanical stimulus that, depending on the pressure exerted, triggers apoplastic and symplastic signaling pathways concomitantly, or initiated in the apoplast and ended in the symplast. The apoplastic perception of this stimulus involves physical changes to many structural components of the cell wall and plasma membrane. The opening of transmembrane channels of water or mechanically sensitive ions channels, independent of voltage, can occur due to the pressure suffered by the membrane, and they can also be activated by peptides that are released or small fragments formed in the cell wall because of touch, such as polysaccharides that are released due to the action of wall remodeling enzymes, activated by the degree of tension of their substrate. These loose structures can activate kinases present at the wall-membrane interface and trigger symplastic signaling. It is known that transient waves of cytosolic calcium, as well as alteration of the cytoskeleton structure are dependent on symplastic signaling. Very fast responses, in a time scale of seconds, such as closing traps and leaflets, can occur through mechanical stimulus. In this case, the stimulus triggers the formation of an electrical action potential, which acts as a long-distance communication signal for rapid activation of transmembrane channels.

Drastic and slower morphological responses to the touch can be observed during the growth of the plants, studied in the scope of thigmomorphogenesis, which has even been the object of technological use in agriculture to form shorter seedlings less liable to lodging or more tolerant to pests, due to the touch trigger increase in the synthesis of jasmonic acid.

A very intriguing subject, which is linked to mechanical stimuli in plants, is their responsiveness to sound, since it is a mechanical wave, however this theme will be approached in the next chapter and addressed in perception/communication contexts.

CHAPTER 2

EXTRACORPOREAL COMMUNICATION IN PLANTS

Plants are organisms that interact with other different beings and they often depend on such interaction for reproductive success, for the dispersion of their propagules, as well as for their nutritional and sanitary maintenance. The population control of plants and the harmony among the flora species of an area also depend on intraspecific or interspecific interactions.

In many situations, recognition between the organisms that interact with each other is a fundamental part of the communication process to be established. It has been observed that the recognition codes among the communicators can be of different origins, such as patterns of sounds, light, chemical substances or electrical signals.

The term "communication" has been widely discussed in the literature regarding its suitability for use with plant organisms. Regardless of whether there is intentional action by plants to communicate (a subject to be addressed in the last chapter), it will be used here for situations in which the signs present in biotic interactions, when perceived/recognized by organisms, cause changes in their behavior.

In this chapter, interactions will be approached within stimulus-response contexts, some of which are already well known in plant biology. However, the focus will not be on exploring the effects themselves, but on the signals and mechanisms used in communication.

Plant-Microorganism Communication

The plant-microorganism interaction has been the subject of intense research for many decades. The most recent discoveries have revealed that its benefits to plants go beyond the well-known nutritional favoring promoted by the symbiosis between roots and diazotrophic bacteria or mycorrhizal fungi; it is also useful to increase tolerance to various different stresses. On the other hand, plants, in their natural habitat, are in frequent contact with pathogenic organisms, needing to develop and activate defense mechanisms. In that context, the plant needs to be able to recognize the organisms to trigger cooperative or defensive action. The following items will explore the mechanisms by which plants recognize microorganisms.

Plants recognize microorganisms

The existence of a sensory apparatus in the cell wall and in the plasma membrane is essential for plants' recognition of microorganisms enabling their cells to trigger mechanisms that facilitate or hinder the organism's entry into the protoplasm, depending on whether the species is beneficial or harmful to plant development. It also allows the cell, when necessary, to trigger metabolic defense pathways in advance of the effective invasion of the cell symplast. The recognition involves, in an isolated or combined manner, the perception of chemical and electrical signals.

Plant-Microorganism Recognition by Electrical Signaling

Electric fields present in the rhizosphere can act in the attraction or repulsion of microorganisms, which is evidenced by the electrotaxis observed in swimming zoospores of pathogenic oomycetes (van West et al., 2002), a phenomenon that can even replace the interactive affinity established by chemical compounds. Interactive stimuli are not similar between different species, and it is expected that environmental factors, such as pH, can influence electrical interaction. It was observed that *Phytophthora palmivora* was attracted to the anode region in the rhizosphere, even though the cathode region was the one where there was protrusion of lateral roots, which could facilitate the infection, while *Pythium aphanidermatum* moved towards the cathode region and *Phytophthora sojae* was attracted by both the anode and the cathode. Those observations indicate that plant-bacteria communication may depend on the state of electrical polarization of the interacting organisms.

The interest in knowing more about plant-bacterial interactions is not restricted to pathogens, but also to beneficial ones. There have been many studies of interaction of diazotrophic bacteria and plants, which, in symbiosis with the roots, manage to fix atmospheric nitrogen, N2, and make it available to plants in assimilable forms in exchange for photosynthates. However, little attention has been focused on electrical factors that permeate this symbiotic interaction. It is known, however, that the biological N fixation (BNF) performed by diazotrophic bacteria is positively affected by polarized biofilms, as compared to non-polarized ones. Some bacteria only express the *Nif* genes, encoding enzymes involved in BNF, when electro-stimulated, which recently resulted in the appearance of the term "bioelectric nitrogen fixation" (Rago et al., 2019). It is expected that the interference of electrical factors directly related to the communication or recognition between plants and diazotrophic bacteria will occur in a similar way to other interactions already known, such as those mentioned above in the plant-pathogenic bacteria interaction.

Another well-known symbiotic interaction occurs between plants and mycorrhizal fungi, which provide nutrients to the roots in exchange for photosynthates. Both in fungi and in roots, electric currents are carried mainly by symplast-apoplast proton efflux or influx movement and in plants this movement occurs preferentially in the root elongation zone, generating high electrical currents in the rhizosphere due to the activity of membrane H^+-ATPases, which are important for orienting the growth and branching of mycorrhizal fungi hyphae (Sbrana and Giovannetti 2005; Fromm and Lautner 2007; Ramos et al., 2008). The fact that fields or electric currents generate electrotropism in some fungi makes its importance to communication evident. Electrotropism also shows that fungi have electrical sensors, and it is already known that, like plants, they have proteins with an LOV domain, sensitive to light, oxygen and voltage, which putatively must participate in electrotropism.

In laboratory conditions, the presence of root extract promotes an increase in the metabolic activity of mycorrhizal fungi, inducing hyperpolarization in the fungus membrane and increasing the electric current, a moment associated with an increase in the growth of their structures, thus showing the interference of plant substances in fungal bioelectric events. Another piece of evidence suggestive of electrical signaling participating in the communication is observed in the penetration of the hypha at the root, which occurs in specific regions where the electric current tends to enter the point of infection. It is assumed that this current is induced by the fungus involving the participation of sensors present in the cell wall and plasma membrane of the plant cell. The electrical current established during the infection involves movement of Ca^{2+}, K^+, and especially H^+, and, once these cations enter, depolarization of the plasma membrane of the plant cell occurs.

Although the sensory apparatus for electrical stimuli between plant and fungus has not yet been elucidated, based on other known signals, the plasma membrane ion channels or pumps are the strongest candidates for being sensors for the plant cell to detect signals. Once the electrotropic capacity of fungi is known, they are expected to have biomolecules that sense the direction of electric fields that attract them or not to the roots.

Plant-Microorganism Recognition by Chemical Signaling

Plant-diazotrophic bacteria recognition

In addition to the electrical aspect, organisms are recognized by the perception of chemically (in)compatible signals and that has been the most studied mechanism. When analyzing the interaction between roots and biological nitrogen fixation (BNF) bacteria, a very refined system of perception and chemical recognition between the organisms can be discerned. The plant has an active molecular apparatus for suppressing symbiosis and nodule development when it is growing in an environment rich in nitrogen. Furthermore, when using symbiosis, this mechanism is also kept active concomitantly with the development of nodules from pre-established interactions to avoid excess nodulation, and, consequently, an excessive and unnecessary expenditure of photosynthates. In other words, the plants inhibit processes that lead to future nodulations, but although activated the mechanism cannot inhibit nodulations that have already been initiated (Kosslak and Bohlool, 1984). This is a very intricate and complex level of perception that needs to be processed by the plant and which apparently requires a computing and prediction capacity (topics to be explored in the last chapter) involving the C/N balance (Carbon/Nitrogen). For that, an efficient communicative signaling is necessary between the roots and the aerial part of the plants, so that they make decisions that do not disadvantage them in the future. In short, the plant needs a self-perception regarding its C/N balance prior to the establishment of communication with the diazotrophic bacteria.

The discussions presented in the section “Nutrient Perception”, Chap. 1, made it clear that plants have the capacity to perceive and respond to different concentrations of nitrogen in the soil, which is fundamental for making stimulus decisions or ensuring the inhibition of infection of its roots by diazotrophic bacteria. It is already widely known that the high availability of NO_3^- or NH_4^+ in the soil is a factor that inhibits root-bacteria symbiosis, justified by the fact that the plant does not waste photosynthates unnecessarily, since symbiosis can result in an expenditure of 12 to 17g of carbon by the plant in exchange for 1g of nitrogen from the BNF, and that is considering the interaction that results in nodules which has the best cost-benefit ratio. Figure 2.1 illustrates a nodulated root.

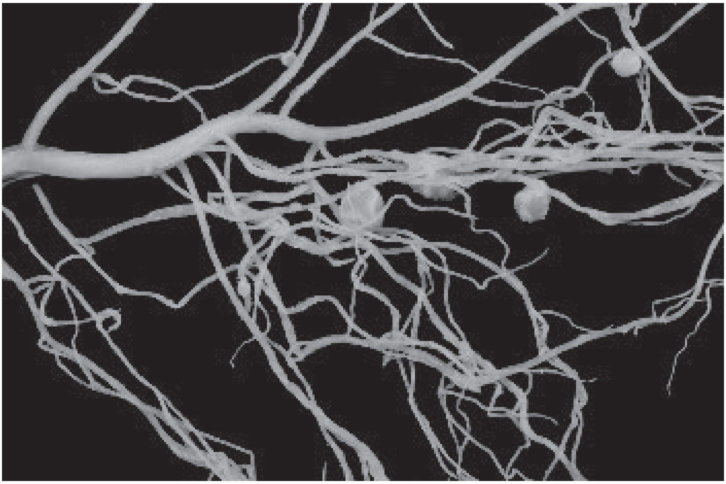

Figure 2.1. Nodulated *Robinia pseudoacacia* roots. Credit: image by Ninjatacoshell from Wikimedia Commons. License to use at https://commons.wikimedia.org/w/index.php?curid=27530472

The decision of the plant for nodulation based on the availability of nitrogen in the soil precedes the establishment of the factors of recognition and stimulus to infection, the Nod factors, which means that the nodulation can be independent of stimuli originating from the microorganism and can therefore be induced by nitrogen deficiency in the soil. This is possible because nodular self-regulation genes such as *SUNN* (*SUPER NUMERIC NODULES*), in the aerial part, and *RDN1* (*ROOT DETERMINED NODULATION 1*), in the root, in *Medicago truncatula* (Kassaw and Frugoli, 2015), are only responsive to nitrogen availability. However, the participation of bacterial nodal factors can also stimulate nodulation or interfere with nodule development.

The plasma cell membrane nitrogen transceptors (receptors and transporters) are expected to be sensors or integrate the sensors that act in triggering the signaling that will lead to nodulation or not and that has been confirmed in *Medicago truncatula* (Bagchi et al., 2012) for high affinity transceptors for NO_3^- belonging to the NRT1 family, the NIP/LATD (NUMEROUS INFECTIONS AND POLYPHENOLICS/ LATERAL ROOT-ORGAN DEFECTIVE) involved in nodulation. In addition, the high synthesis of peptides, whose formation is induced or inhibited by nitrate and which are involved in signaling nitrogen satiety or deficiency, Figure 1.17, also participate in the regulation of nodulation (Murray et al., 2017). CLE peptides, satiety indicators, inhibit the formation of nodules by the plant, while CEP peptides, deficiency indicators, induce the formation of nodules. It is assumed that these peptides can also act in regulating the production of flavonoids by the plant, compounds that regulate the expression of several genes responsible for the nodulation process, in addition to acting as chemical attractants for bacteria.

For the nodules to be or become effective, signaling is required to attract the bacteria to the plant. After the plant's perception of nitrogen deficiency, signaling leads to the process of exuding substances from the roots that may attract nitrogen-fixing bacteria. Different exuded flavonoids are perceived by different bacteria, a process that involves a mediating gene called *NodD*. Flavonoids act to attract these bacteria (Figure 2.3) and stimulate the transcription of their nodulation genes (*nod*). Depending on which *nod* genes are expressed, bacterial exudates produced from this expression are known as Nod factors and are formed of chito-oligosaccharides with variations in the side residues of sugars, acetyl, carbamoyl, or the presence of sulfated molecules, which are important for recognition among related genotypes (Long, 2001; Liu and Murray, 2016). Some, but not all, Nod proteins act as enzymes, which are specific for their host's specific lipo-oligosaccharides and that is also an important factor in the recognition between organisms and the entry of bacteria in the plant cell. On the other hand, plants have hydrolytic enzymes as Nod factors that can act against those that are not compatible or act at times that are not conducive to infection.

The Nod factor, when adhering to the plant cell to be infected, promotes alkalinization of the apoplast and depolarization of the plasma membrane, indicative of the entry of H^+ into the cell symplast. In addition to hydrogen, there is a rapid influx of Ca^{2+} into the cytoplasm and that will influence the transduction of signs of infection, which includes a rearrangement of the cytoskeleton for morphological changes in the host cell. It is not possible to say, however, that membrane transporters are the primary sensors in the detection of Nods. Although they participate in signaling, there is evidence that their activities occur after an initial perception by another element. At least for one Nod factor, its previous perception by the G protein is known and, in that case, the activation of the calcium channel was found to be a consequence of that perception (Pingret et al.,1998). It is also already known that the receptor kinases NFR1 (NOD FACTOR RECEPTOR 1) and NFR5, which contain three lysine residues facing the plant cell apoplast, are fundamental in the reception of Nod factors (Wong et al., 2019). Other receptor kinases such as LYK3 (RECEPTOR-LIKE KINASE 3) and LYK4 also act on the reception of Nod factors (Limpens et al., 2003). In that sense, it is plausible to consider that, although nitrogen transceptors possibly act as sensors that trigger signals of infection and nodulation, the perception of Nod factors is likely to occur initially by other molecules, such as the receptors of NFR, LYK and G protein family.

There is also evidence that the perception of Nod factors may occur by lecithin proteins, which bind to carbohydrates. In mutant plants defective of the carbohydrate binding site in these proteins, there was neither infection nor development of nodules in several species, even with the presence of the stimulating Nod (D’Haeze and Holsters, 2002).

Although the focus of perception of signals here is on plants, it is known that diazotrophic bacteria adjust their motility to the attraction or repulsion of chemical signals, which shows the existence of sensors in the form of chemoreceptors in these organisms. The regulation of this movement occurs downstream of the reception of the signal exuded by the plant, such as the production of the proteins CheW, CheA, and CheY, which act in signaling the flagella so that they move so as to take the bacteria in the direction of encounter with a particular chemical signal or in the opposite direction. The existence of more than 80 types of chemical receptors involved in chemotaxis in diazotrophic bacteria has been reported. Two of the several sensors in *Azospirillum brasiliense*, the TLP1 and AERC of chemotaxis were studied in the interaction with wheat by O'Neal et al. (2019), and TLP1 was also necessary for root colonization, which is a sensor that on the C-terminal side has a PilZ domain conserved in bacterial chemotaxis sensors, anchored on the outer side of the membrane, and that binds to the secondary messenger cyclic diguanylate monophosphate (c-di-GMP), whose metabolism can be regulated by various factors of the rhizosphere, such as oxygen gradient and nitrogen availability. The AERC, on the other hand, seems to bind to the FAD cofactor, acting as an oxidation reduction sensor, monitoring the flow of FAD/$FADH_2$ in the bacterial cell due to the cell's metabolic response to a gradient of root exudates, as well as oxygen gradients.

Plant-mycorrhizal fungal recognition

Many elements of plant-diazotrophic bacteria signaling resemble the plant-mycorrhizal fungus association. The importance of mycorrhizal interaction for the plant is the increase in the acquisition of nutrients, especially those with little mobility in the soil, such as phosphorus, which, when deficient, becomes the most prevalent factor in symbiosis. In this case, the plasma membrane phosphorus receptors/transporters are presumably the pioneering molecules in the perception of the availability of this element, causing a signal that leads the plant to determine the infection. The plant responds to phosphate deficiency in the soil by decreasing the growth of primary roots and increasing the number and length of lateral roots, which favor arbuscular mycorrhizal symbiosis.

The fungi spores are induced to germinate by the presence of root exudates, including many flavonoids, which have their exudation increased by the roots in a condition of low phosphate availability in the soil. However, it is believed that other components of the exudates, identified or not, are more important than flavonoids in stimulating the hypha branching. In some species, strigolactones exuded by the roots (Figure 2.3) not only stimulate spore germination but also the branching of hyphae, fungal metabolism and the activation of calcium channels. Currently, this plant hormone is considered the most important chemical signal perceived by mycorrhizal fungi. The receptor for strigolactones in fungi is not yet known, and there are no homologues for those known in plants. In Figure 2.2 it is possible to observe the visual aspect of mycorrhizal roots.

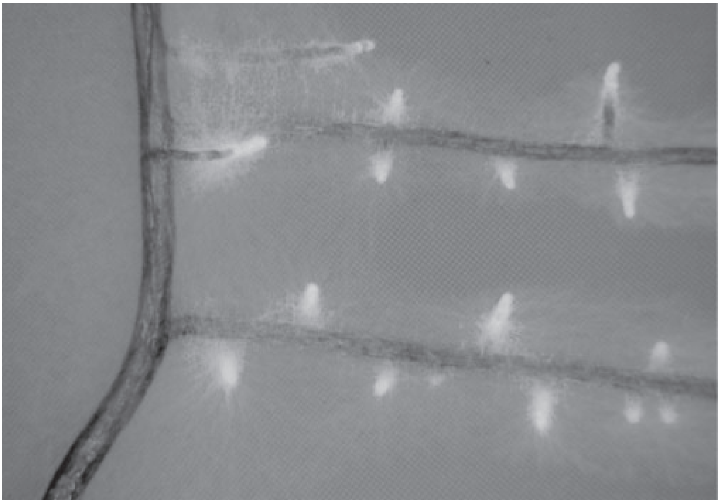

Figure 2.2. Mycorrhizal interaction between *Picea abies* roots and the fungus *Piloderma croceum*. Credit: image by Ingrid Kottke from Wikimedia Commons. License to use at https://commons.wikimedia.org/wiki/File:Mykorrhizen_Kurzwurzeln.tif

Cutin monomers and hydroxylated aliphatic acids, whose biosynthesis depends on a biomolecule called RAM2 (REQUIRED FOR ARBUSCULAR

MYCORRHIZA) have also been recognized as plant exudates necessary for the differentiation of fungal structures in the formation of the hyphopodium, which is necessary for the fungus to enter the roots. Mutant plants defective in RAM2 demonstrate its importance both for the formation of cutin and the formation of hyphopodia.

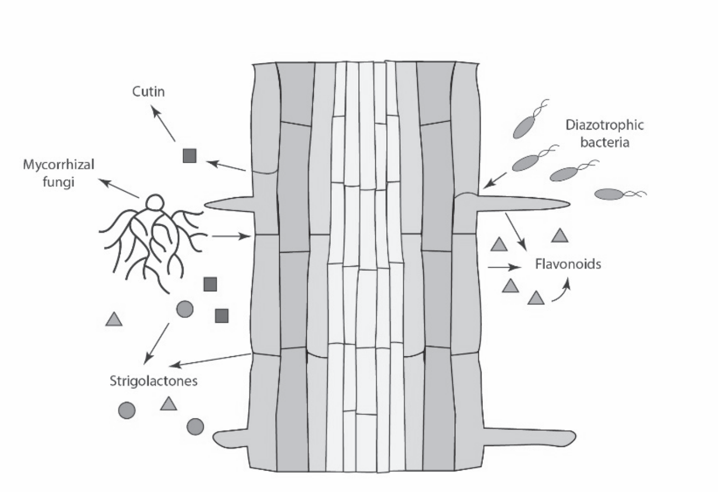

Figure 2.3. Attractive exudates of roots necessary for the germination or differentiation of symbiotic microorganism structures. Flavonoids have a predominant action on diazotrophic bacteria and strigolactones on mycorrhizal fungi. Alternatively, flavonoids, cutin monomers and hydroxylated aliphatic acids also influence fungi reaction.

In several plant species, the expression of the *ENOD11* gene (*EARLY NODULIN 11*) is common prior to infection in response to the presence of specific exudates of mycorrhizal fungi (Harrison, 2005), which shows the plant's perception of the fungus before the infection occurs. As seen in the interaction of diazotrophic bacteria-plant, (lipo)-chito-oligosaccharides exuded by fungi are essential for their recognition by plants, prior to infection (Wang et al., 2012; Bonfante and Genri, 2015). Apparently, these molecules are involved in the activation of transmembrane calcium channels, an ion that tends to enter the cell symplast in their presence.

According to Gough and Bécard (2016), the Nod factors NFP (NOD FACTOR PERCEPTION), DMI1 (DOES NOT MAKE INFECTIONS), DMI2, DMI3 and NSP2 (NOD SIGNELING PATHWAY) are important in the control of symbiosis, showing aspects common to mycorrhizal fungi and diazotrophic bacteria. The (lipo)-chito-oligosaccharides exuded by the fungi stimulate the symbiotic transcriptomics of the plant. Based on studies of mutants, plant recognition of these reference substances depends on receptors of the Lys-RLK family (LISIN RECEPTOR LIKE-KINASE). Another possible receptor is the LYK3 (LysM-CONTAINING RECEPTOR-LIKE KINASE 3) from arabidopsis, or its homologues, which receives Nod factors from both diazotrophic bacteria and mycorrhizal fungi.

Many orchids require mandatory mycorrhizal fungi infection for germination and seedling development. In this interaction, in addition to exchanging NH_4^+ for carbohydrates, there are situations in which the fungus appears to require organic nitrogen from the host in exchange for inorganic nitrogen. In addition to the nutritional needs of very small seeds and seedlings of orchids, fungal infection contributes to the tolerance of plants to stress conditions often experienced by epiphytic orchids. The fungus induces the transcription of genes related to antioxidant enzymes and thus relieves oxidative stress resulting from other stresses, such as nutritive and water ones, in addition to the fact that endophytic fungi can produce substances toxic to pathogenic fungi, such as fusaricidin.

There are many genes and proteins involved in the symbiotic signaling between fungus and orchid, among them, SYMRK (SYMBIOSIS RECEPTOR-LIKE KINASE), which acts as a kinase receptor, necessary for the later activation of the CASTOR and POLLUX ion channels of the nuclear envelope, which, in turn, make up complexes of the nuclear pore NUP85, NUP133 and SEC13 HOMOLOG1 (SEH1) NENA. Other proteins are fundamental to the perception and interpretation of symbiosis-linked calcium signals, such as kinase-dependent calcium-calmodulins (CCaMK), calcium signal decoding proteins (CYCLOPS), E3 ubiquitin ligase CERBERUS and the GRAS family of gene transcription factors. The importance of these proteins involved in signaling is detected in studies using mutants.

Although the identified genes are in symbiosis with orchid mycorrhiza, it is assumed that should be a similarity in transcriptomics when symbiosis occurs with arbuscular mycorrhizae since both types of fungi are endophytic.

Probably, several hormones, produced by fungi similar to those produced by plants contribute to the germination of orchid seeds (Favre-Godal et al., 2020), including gibberellin, known to be a key hormone in triggering germination.

Plant-pathogen recognition

Plant-microorganism interactions are not always beneficial, such as those previously described for diazotrophic bacteria and mycorrhizal fungi. They can occur with pathogenic organisms, which requires sufficient perception and recognition mechanisms from the plant to distinguish between beneficial and harmful organisms.

Plants have an innate basal immunity. There is an apparatus for the recognition of pathogenic microorganisms by plants formed by transmembrane proteins, some with kinase function, known as pattern recognition receptors (PRRs), which recognize a wide range of molecules present in pathogens. Pathogen-associated molecular patterns (PAMPs) are common in different pathogens and are molecules that trigger plant defense mechanisms because they are already recognized in the cellular apoplast by PRRs (Parker, 2003; Dodds et al, 2010; Tang et al., 2017).

PAMPs are composed of several types of molecules, such as lipids, carbohydrates, proteins and viral nucleic acids. An example of them is Pep13, an avirulent peptide fragment of a cell wall glycoprotein from the pathogen *Phytophthora sojae*. Pep13 can be recognized by plants, including those that are non-hosts for this pathogen, through PRRs, which maintain a constant state of attention to the presence of PAMPs (Figure 2.4). This is because Pep13 is present in all species of *Phytophthora*, and plants, whether they are hosts or not, have receptors that recognize it. After recognition in the cellular apoplast, immunity signal transduction pathways are triggered, normally with calcium as a secondary messenger and triggering the expression of several genes involved in the synthesis of phytoalexins. Because PAMPs usually participate in the pathogen's indispensable metabolism, they cannot be removed, even though they signal to the host cell the pathogenic potential of the organism they are present in. On the other hand, they benefit the plant cell, which upon recognizing them can trigger its defense mechanisms.

For a cell to succumb to the attack of a pathogen, pathogenic virulence factors must overcome the cell's basal immunity during infection. Another way is for pathogenic effectors to deceive PRRs, as, for example, when the pathogen secreting effector proteins impede the recognition of PAMPs by PRRs, or even secrete substances that mimic host cell hormones.

Based on the above context, PRR proteins are assigned the function of PAMP recognition sensors within the basal immunity of cells. However, specific defenses may require other ways of perception. In a study developed by Rezzonico et al. (2017) it was observed that in tomato leaves infected with three distinct pathogens, dozens of genes were distinctly induced by each pathogen, thus demonstrating the unique signaling pathways triggered by the individualized recognition of each one. This means that, in addition to the recognition of PAMPs common to different pathogens, it is assumed that there are specific recognition sensors in the apoplast, as well as the fact that the sensorial pathway of the symplast can be different for the different pathogens.

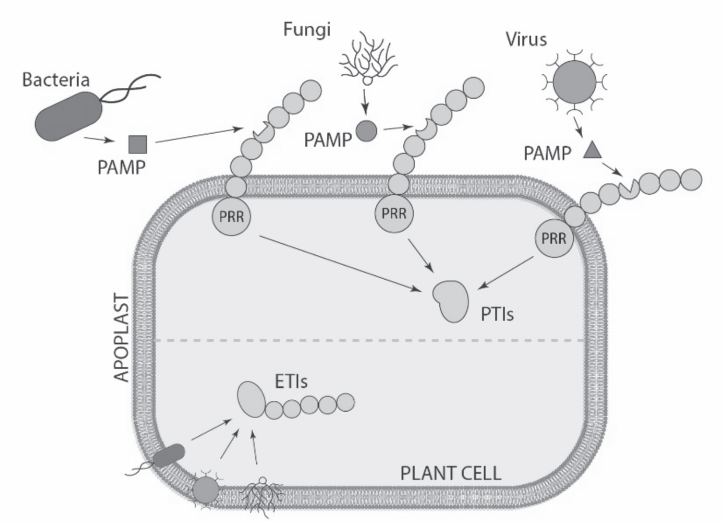

Figure 2.4. Plant-pathogen recognition. Pathogen-associated molecular patterns (PAMPs) will first be recognized by the pattern recognition receptors (PRRs) in the apoplast. After recognition, pathogen-triggered immunity factors (PTIs) come into activity to stop the invasion of the cell symplast. If the invasion still occurs, which will be recognized by the endogenous immune receptors, a second immune barrier is formed by the Effector-triggered Immunity factors (ETIs).

Plant-Microorganism Olfactory Recognition

Some volatile compounds released by the roots are metabolized by some soil microbes, such as bacteria that metabolize umbelliferone, salicylic acid and 4-hydroxybenzoate (Parales and Harwood, 2002). An important aspect of this phenomenon is that some volatiles from plants released in the soil can recruit beneficial microorganisms from the soil to the rhizosphere, including diazotrophic bacteria. In a study with maize, Neal et al. (2012) observed that benzoxazinoids such as DIMBOA, a heteroaromatic metabolite, are participants in the chemical defense of plants known for their toxicity against aphids, caterpillars and some pathogenic fungi and, when released by the roots, they act as an allelochemical against pathogenic microbes or competing plants and also attract beneficial microorganisms to the rhizosphere.

Those authors observed that the presence of DIMBOA induced the expression of several genes related to the tolerance of heteroaromatic compounds and chemotaxis of the beneficial bacterium *Pseudomonas putida*, increasing its colonization in the rhizosphere, as well as contributing to the attraction of diazotrophic bacteria. Naturally, this implies a complex network of signals and receptors, resulting in the response of attraction or chasing away some organisms when they perceive the compound. In the above case, the *P. putida* bacterium had DIMBOA hydrolysis mechanisms, which promoted its transformation into less toxic metabolites.

Several studies demonstrate olfactory trophic interactions involving more than two organisms. In a study by Ali et al. (2010), using an olfactometer, it was observed that citrus roots attacked by *Diaprepes abbreviates* larvae released more volatile compounds than non-attacked or mechanically damaged roots, and that the roots attacked by the insect produced four terpenoids not produced by the other roots. Furthermore, it was precisely the roots attacked by the insect that most attracted entomopathogenic nematodes (*Steinernema diaprepesi*) to their surroundings, showing the action of volatile compounds released by the plant that act in the attraction of natural enemies of its pests. A similar effect was observed in a study carried out in a laboratory with roots of *Carex arenaria* (Schulz-Bohm et al., 2018), which normally release aromatic compounds and esters, but after infection by *Fusarium culmorum* there was an increase in the release of monoterpenes to attract more genera of bacteria that repress the growth of the fungus.

Although a plant-microbe interaction is on the agenda, apparently the sensory apparatus for olfactory perception is required from the microbe. In a later section, however, situations will be addressed in which we will see that this apparatus should also occur in plants.

Summary

Plants have control over the establishment of symbiotic interactions with diazotrophic bacteria and mycorrhizal fungi. The determination of plants to interact with microorganisms depends on their perception of the sufficiency of nutrients to meet their demand. For that to occur, the nutrient transporters present in the plasma membrane participate in the sensory apparatus.

Chemical communication between plants and symbiotic microorganisms is initially stimulated by root exudates, with flavonoids being the most important in attracting bacteria and strigolactones in attracting fungi. The exudates are received and interpreted by the microorganisms, which respond by moving towards the roots. They are recognized as beneficial or harmful organisms by plants, especially because of the different chemical residues present in their (lipo)-chito-oligosaccharides, which will facilitate or not infection and symbiosis. There are many proteins that participate in the establishment of symbiosis, involving receptors and co-receptors of different substances, protein kinases, gene transcription factors and transmembrane ion transporters, especially for calcium, which participates in intracellular signaling.

Part of the extensive sensory apparatus involved in the recognition among symbiotic organisms is already known, and, given the necessity for the plant to determine symbiosis, as well as the specificity commonly found in interactions, it is evident that the phenomenon does not occur by mere chance, and therefore, that it depends on a refined communication system which is affected by physical-chemical signals between the symbionts.

The plant-microorganism interaction can occur in the sense of approach or avoidance. For both situations there is a need for one organism to perceive and recognize the other. For pathogenic infection, the mechanisms of recognition by plants must be overcome by the pathogens. In any event, the recognition between the organisms depends on electrical and/or chemical signals, initially detected by elements of the cell wall or plasma membrane, structures that act as the doors that can facilitate or hinder the entry of the microorganism into the cell symplast, in addition to triggering downstream signals that determine the degree or manner of interaction between the organisms.

Plants Have a Sense of Danger

Plants are organisms surrounded by danger in the form of herbivores, pathogens, parasitic plants or other plants that compete for natural resources. On the other hand, there are many beneficial interactions that plants have with organisms of the same classes as those that cause damage. To maintain good interspecific relationships, it is therefore necessary that the plant perceives when it is in danger in order to use defense mechanisms. For that, there is a non-specific basal immune system, based on the molecular patterns associated with potentially dangerous agents, recognizable by plants, as already described in the previous section, exemplified by the PAMPs/PRRs model of pathogen recognition by the plant cell.

Constitutive immunology

PRRs are a great example of a constitutive mechanism that plants have to perceive danger associated to pathogens (Chen and Nuñez, 2010; Gust et al., 2017). In addition to PRRs, other constituent endogenous molecules participate in the perception of danger associated to the most varied factors, even in the absence of a real danger factor. In animals, they are responsible for what is called "sterile inflammation" when there is recognition of a danger pattern that does not come directly from a pathogen. As in animals, there are molecular patterns associated with damage in plants (DAMPs), typical substances eliminated by damaged cells that stimulate the action of PRRs, which, in turn, act as sensors of perception of the dangers' indicator substances, activating the immunological pathways. PRRs, in turn, are perceived in the cell cytoplasm by NOD-Like Receptors (NLRs) present in nucleotide oligomerization domains. Many molecules are recognized as DAMPs by plant cells, such as the high-mobility proteins of the Box group associated with chromatin (HMGB), mitochondrial proteins, purine metabolites such as ATP, NAD(P)(H), heat shock proteins, hyaluronic acid or even particles that can cause damage to the membrane.

Some pathways are already triggered by the perception of the DAMPs ATP and NAD(P)(H), molecules that induce a transient increase in cytosolic calcium, thus promoting the depolarization of the plasma membrane and the activity of some transmembrane transporters. The ATP induces metabolic pathways regulated by jasmonic acid. It should also be considered that DAMPs, made up of small particles, can be part not only of PAMPs, but also of mechanical damage caused by touch or processes that trigger cell wall tensions (Figure 1.21).

After the cells recognize the DAMPs/PAMPs that trigger immunity, through the PRR sensors, which occurs in the apoplast, there is a triggering of the immune activation factors (PTIs). For the pathogen to be able to colonize a cell, it needs mechanisms to overcome the PTIs. In the case of infection, and therefore, from its presence in the symplast, as a second immune barrier, the effector patterns of infection (ETIs), which are recognized by endogenous immune receptors (Figure 2.4), will emerge.

In the case of mechanical damage to the plant cell promoted by herbivory, naturally there will be exposure of DAMPs at the damaged site, as well as polygalacturonases or pectin methylesterases, whose hydrolytic actions on the cell wall will result in pectin fragments known as oligogalacturonides (OGs) (Figure 1.21), which also act as DAMPs. In turn, OGs, as well as PAMPs, represent strong signals for the damaged tissue to trigger the activation of its defense system against infection by pathogens facilitated by mechanical damage. In addition to OGs, fatty acid monomers derived from cutin and cellobiose, a cellulose derivative, act as triggering signals for immunity (Figure 2.6).

A common mechanism triggered by many DAMPs and PAMPs is the production of reactive oxygen species (ROSs), which can cause damage to undesirable microorganisms. Other mechanisms induce: the production of volatile compounds that are directly harmful to specific microbes or induce subsequent immune responses; production of peptides eliciting immune responses, such as systemin, which can trigger activation of metabolic pathways regulated by jasmonic acid that culminate in the production of substances toxic to insects or act to release volatile compounds; proteases, such as phytaspases, which act in the processing of the systemin; and activation of rapid alkalinization factors (RALFs). An illustrative scheme related to DAMPs and immune responses can be seen in Figure 2.5.

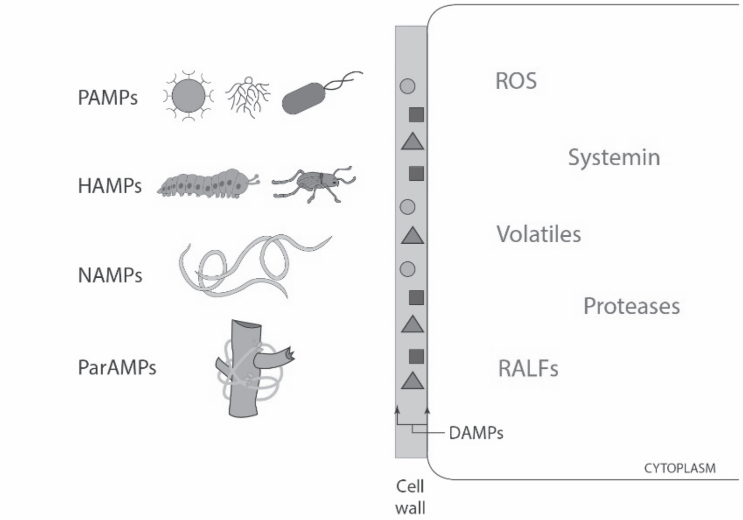

Figure 2.5. Factors that trigger immune responses in plant cells. Molecular patterns associated with damage (DAMPs) such as oligogalacturonides, cutin monomers and cellobioses are formed on the cell wall due to mechanical stresses or hydrolysis occurring on the wall or even due to the presence of molecular patterns of pathogens (PAMPs), herbivores (HAMPs), nematodes (NAMPs) and parasitic plants (ParAMPs). From the perception of cells to DAMPs, as a plant defense mechanism, there is production of reactive oxygen species (ROSs), volatile compounds, peptides eliciting immune responses such as systemin, proteases such as phytaspases, as well as the activation of rapid alkalinization factors (RALFs).

Potential receptors for DAMPs include a lecithin kinase receptor DAMP1 (DOES NOT RESPOND TO NUCLEOTIDES 1) for the perception of ATP from cell disruption. The eliciting peptides are recognized by the PEPR1 (PEP1 RECEPTOR1) and PEPR2 receptors, found in arabidopsis, but with several homologues in different species. There are several factors that trigger the expression of *PEPR1* and *PEPR2*, such as wounds, PAMPs, infection by various microbes or herbivory. RALFs belong to a family with cysteine-enriched peptides, some of which come from the action of proteases in the apoplast after the secretion of their propeptides, whose accumulation can be induced by various factors such as fungal infection, PAMPs and stresses. RALFs, in addition to their pro-immunological effect, have also been observed in immune suppression responses when linked to the FERONIA receptor (FER).

A model of endogenous danger signaling from different origins in a plant cell, which can be mediated by PRRs, attributes it to phyto-cytokines (a term derived from their similarity of action to metazoan cytokines – evidence of the common ancestry of the immunological apparatus of various organisms-), a wide range of small peptides such as stomagen, PSK (phytosulfocins), CLE (CLAVATA3/EMBRYO-SURROUNDING REGION), IDA (INFLORESCENCE DEFICIENT IN ABSCISSION), ROT4/DVL1 (ROTUNDIFOLIA4/DEVIL1), PIPs (PATHOGEN-ASSOCIATED MOLECULAR PATTERN-INDUCED PEPTIDES) and RALFs. The release of these peptides by cells is involved with several plant development processes (not always associated with damage), and very small amounts trigger actions, including immunological autocrine and paracrine actions. Those characteristics have led these peptides to be considered potential plant hormones, although they are not found in all plants.

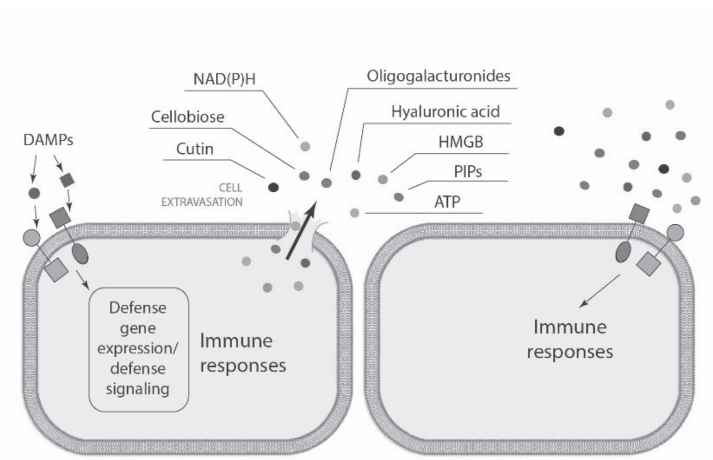

Figure 2.6. Scheme of autocrine response (left) of immune response-stimulating signaling. The presence of DAMPs (damage associated molecular patterns), when perceived by plasma membrane receptors (PRRs), trigger signal transduction pathways that result in defense responses. When physical damage that compromises the integrity of the membrane enables the extravasation of substances such as ATP from the protoplasm, reducing power (NAD(P)H), chromatin-associated proteins (HMGB), and PIPs (PATHOGEN-ASSOCIATED MOLECULAR PATTERN-INDUCED PEPTIDES), those substances, whether together with the DAMPs or not already present in the apoplast (cutin, cellobiose, OGs), in a paracrine way (right), promote the activation of membrane receptors that trigger immune responses in neighboring cells not yet attacked by the initial damage-promoting agents.

Induced immunology

In addition to the constitutive immune apparatus, immune responses can be induced when the plant defense mechanism is triggered by a specific factor (Karban, 2020). In the topic “Plant-pathogen recognition”, the case of different pathogens triggering different signal transduction pathways in plant cells was mentioned, culminating in the different synthesis of dozens of genes involved in specific defenses for each pathogen. It is a situation that highlights what is called induced immunology, although we are not yet aware of the details about the mechanism of individualized perception by the cell.

The triggering of immunological pathways caused by specific pathogens requires the plant to have a risk evaluation capacity since it needs to decide whether it is worth triggering a defense action, as well as which action to trigger and that even requires an ability of the plant to predict; a theme to be addressed in the last chapter. This is because the cost of defense needs to be compensatory, and it is not worthwhile to use expensive procedures for low risk of damage factors, since the resources, instead of being allocated to growth or other more important defenses, or even investment in metabolites of ecological importance, such as volatiles and attractive pigments for pollinators or dispersers, would be destined unnecessarily for defense. The existence of basic constitutive defense mechanisms could be a way of saving resources since the metabolic machinery for induced defense would only be activated if the basal immunity were suppressed or ineffective in combating the damage agent.

Decision making by the plant regarding its defense needs to consider the degree of reliability of the stimulus, because fake news is also present in the world of plants. An example of this situation is when substances present in the oviposition site of the butterfly *Pieris brassicae* provoke, in arabidopsis, the attraction of salicylic acid, which regulates the defense system against fungal and bacterial pathogens, and which is in the beginning antagonistic to the defense regulated by jasmonic acid, exactly the hormone that would be effective in the positive regulation of the defense mechanism against herbivory. In that way, oviposition undermines the plant's defense against herbivory by sending a false informational signal. This also occurs in response to the plant's contact with different fluids of herbivores, which often carry microbes with pathogenic potential (Bruessow et al., 2010; Wang et al., 2016), inducing the plant to trigger vias of defense against pathogens instead of herbivores. For cases of this type, the idea is that the plants need to make decisions for specific combat after identifying more than one clue indicating the agent, which, in turn, may appear at different times, and thus, will require a plant response dependent on plant memory, a theme to be addressed in the penultimate chapter.

When comparing the advantages and disadvantages of innate and induced defense systems, it is necessary to consider that a resistance induced by the production of a specific secondary metabolite would be more efficient in combating a certain invader. However, this could make the plant more susceptible to other invaders. On the other hand, if the plant's resistance mechanism is of an overly broad spectrum, it can inhibit beneficial interactions with other organisms.

As seen in the topic “Olfactory plant-microorganism recognition”, a compound, even if ineffective for an invader, can trigger the attraction of its natural enemies, or serve as a deterrent to invasion by other invaders because others will realize that they would have to spend more energy to access their target, due to having to compete with their peers. All of this demonstrates how complex the context for plant learning has been in the course of evolution, especially regarding defense decision-making, whether constitutive or induced.

Hormones and immunology

Because of the importance of jasmonic acid and salicylic acid in plant defense, and the recurrence of the subject throughout the chapters, there now follows a brief description of their synthesis and their action as regulatory signal pathways for defense against herbivores or pathogens.

The importance of jasmonic acid for the defense of plants against herbivory stems from its action in the expression of several genes, through a complex signal transduction pathway. For those genes to be expressed, MYC transcription factors are required, which are repressed by a family of proteins called JAZ (JASMONATE ZIM-domain). The bioactive jasmonate, jasmonoyl-L-isoleucine, binds to its nuclear receptor COI1, which is a component of the E3-ubiquitin-ligase complex. That link is necessary for the destruction of the JAZ repression factors by the proteasome 26S, thus releasing the defense genes from their repression (Erb and Reymond, 2019).

The damage caused by herbivores, in addition to activating the action of the jasmonic acid already present, such as that contained in glandular trichomes destroyed by touch, also triggers the synthesis of more jasmonic acid. After the damage caused, substances formed, such as H_2O_2, stimulate the synthesis, which is initiated in the plastid membranes by a reaction catalyzed by 13-LIPOXYGENASE. It inserts oxygen into the C13 of linolenic acid, forming the 13-hydroxiperoxide of linolenic acid which is processed by ALENE OXIDE SYNTHASE and ALENE OXIDE CYCLASE to form 12-oxophytodienic acid (OPDA). OPDA is then transported to the peroxisomes, where it is reduced by a 12-OXOPHYTODIENOATE REDUCTASE 3 (OPR3). After submission to three stages of β-oxidation, (+)-7-isomeric jasmonic acid is formed, which is transported to the cytoplasm, where isoleucine will be conjugated by JASMONOIL AMINO ACIDO SYNTHASE, JAR1. Jasmonoil-L-isoleucine is the biologically active canonical form of jasmonic acid that will ultimately bind to its nuclear receptor COI1 (Peiffer et al., 2009; Erb and Reymond, 2019).

Salicylic acid has been found to be effective in regulating the defense system induced by biotrophic pathogens and sucking herbivores, while jasmonic acid acts as a positive regulator defense for other herbivores and for necrotrophic pathogens. These two hormones can act synergistically or antagonistically in the immune regulation. Although salicylic acid triggers mechanisms antagonistic to jasmonic acid at the beginning of a herbivore attack, it is synthesized hours after the attack without harming the jasmonic acid pathway (Costarelli et al., 2020). Other plant hormones also participate in the modulation of defense responses, but they do not have known well-established patterns for their influences.

Salicylic acid also acts on the expression of defense genes. The pathogenic signs perceived by the plant provoke the conversion of chorismate to isocorismate in the chloroplast, mediated by ISOCHORISMATE SYNTHASE 1/SA INDUCTION DEFICIENT 2 (ICS1/SID2). Isocorismate is then transported to the cytoplasm by ENHANCED DISEASE SUSCEPTIBILITY 5 (EDS5) and converted to isocorismate-9-glucose by AVRPPHB SUSCEPTIBLE 3 (PBS3). The conversion of this molecule to salicylic acid occurs spontaneously or catalyzed by ENHANCED PSEUDOMONAS SUSCEPTIBILITY 1 (EPS1). Its plant receptors that mediate positive immune responses are NONEXPRESSOR OF PATHOGENESIS-RELATED GENE 1 (NPR1) and NPR2, and when mediated by NPR3 and NPR4 they trigger negative immune responses. The positive responses culminate in the expression of several genes that participate in the biosynthesis pathway of secondary metabolites that act as antimicrobial phytoalexins or in the production of proteins such as chitinases and glucanases that act in the hydrolysis of the microbial cell wall, as well as promoting the strengthening of the plant cell wall promoting greater impregnation of lignin and callose (van Butselaar and Van den Ackerveken, 2020).

It is important to note that both salicylic acid and jasmonic acid, central regulators of immune responses in plants, can promote a decrease in plant growth when in excess. Therefore, for their use for technological purposes, it is necessary to find a balance between their stimulating immunity and their effects on plant growth.

Summary

Molecular patterns present in different damage agents (DAMPs), such as pathogens (PAMPs), are recognized by pattern recognition receptors (PRRs), which will trigger immune responses in cells, such as the production of immune activation factors (PTIs), which, in turn, will act against the invasion of cell protoplasm by pathogens. If there is no success, effector patterns of infection (ETIs) will serve as recognizable signs for endogenous immune receptors. Although these patterns are part of a basal and constitutive immune system, it is known that there is a sensory apparatus for specific recognition of some pathogens by the plant cell, a mechanism yet to be elucidated.

As elements recognizable by cells, PAMPs can trigger the production of cell wall compounds similar to those of other DAMPs, such as oligogalacturonides, cutin monomers and cellobioses, which, when perceived by membrane receptors, will be considered by cells as danger factors, triggering the production of different defense substances, such as ROS, proteinases, peptides, volatile compounds and RALFs. In addition to the DAMPs present in the cell wall, when there is leakage of liquid matrices from the protoplasm, some substances such as ATP, NAD(P)(H), HMGB and hyaluronic acid are also recognized as danger factors, and, when perceived by neighboring cells, unaffected by the damage agents, will trigger their immune defense pathways before they are effectively damaged.

The most important plant hormones for plant defense are salicylic acid and jasmonic acid. The action of these substances comes from their influence on the expression of genes that participate in the defense pathways. Salicylic acid is very effective in regulating the defense system induced by biotrophic pathogens and sucking herbivores, while jasmonic acid acts as a positive defense regulator for other herbivores and for necrotrophic pathogens.

Plant-Herbivore Communication

Plants, in their natural environment, are exposed to attack by herbivores, which can lead them to an unfavorable condition for their development and reproduction. In average percentages, it is considered that the plants can support a loss of leaf area by herbivory of up to 18% without serious prejudice to their development. However, the impact of this loss of photosynthetic area is highly variable among species, with some tolerating no more than 10%, while others tolerate up to 25% without serious damage (Strauss and Agrawal, 1999).

The fact that the plants are foraged by herbivores does not mean that they are passive organisms or that they do not have defense mechanisms to prevent the attack or reduce the damage, when attacked.

Plant defense against herbivores

When considering DAMPs, molecular patterns associated with damage, derived from herbivores (HAMPs) (Figure 2.7), plants can distinguish them from others by recognizing saliva, oviposition, regurgitation, feces, or even their own plant products derived from a specific metabolic action induced by a herbivore (Erb and Reymond, 2019). This demonstrates a selective perception by the PRRs towards a class of potentially harmful organisms. In addition to the non-specific constitutive immune barrier for pathogens, discussed in the previous section, specific microorganisms trigger signals that lead to the selective expression of a set of genes. This same observation is valid for the plant-herbivore interaction.

The current focus in research on induced immunology is to identify the biologically active molecules of the organisms that cause damage and the specific receptors for them in plants, as well as the signaling pathways triggered by that recognition. For some HAMPs, studies have already revealed specific elements. For *Spodoptera exigua*, the volicitin found in its oral secretion is strongly linked to a PRR of the plasma membrane in maize plant cells (Truitt et al., 2004); the inceptin found in the oral secretion of *Spodoptera frugiperda* is a triggering factor for defense routes in cowpea and corn (Schmelz et al., 2006); the C-terminal region of a protein homologous to mucin found in the secretions of *Nilaparvata lugens* induces resistance in rice (Shangguan et al., 2018). Although specific receptors in plants have not yet been isolated and identified for these biomolecules, some putative receptors have been proposed, such as rice G-TYPE LECTIN RECEPTOR KINASES (LecRKs) for the secretion of *Nilaparvata lugens*, as well as for NAD(H) and ATP (Liu et al., 2015), and LEUCINE-RICH REPEAT RECEPTOR KINASE (LRR-RK) for the perception and signaling of defense against *Chilo suppressalis* compounds (Hu et al., 2018). Figure 2.7 shows a model of various DAMPs and their respective putative plasma membrane receptors.

Among the elements that participate in the perception and or signaling from the HAMPs by the cell is calcium, whether linked to calmodulin or not. There is a transient increase in the concentration of this nutrient in the cytoplasm, and consequently, a depolarization of the plasma membrane, which may affect the activity of voltage-dependent transmembrane transporters. Other mechanisms, if not sensory, but at least close to the reception of the signal, are the accumulation of reactive oxygen species (ROS) and the activity of MITOGEN-ACTIVATED PROTEIN KINASE (MAPK). The formation of ROS results from the action of NAD(P)H oxidases exposed at the injured site of the cells. MAPKs are important activators of the action of jasmonic acid and of gene transcription factors that participate in the synthesis of defense compounds. Although there is synthesis of jasmonic acid, its performance immediately after the signal is perceived in a matter of seconds, which, in this case, shows there is only cell stock activation at first.

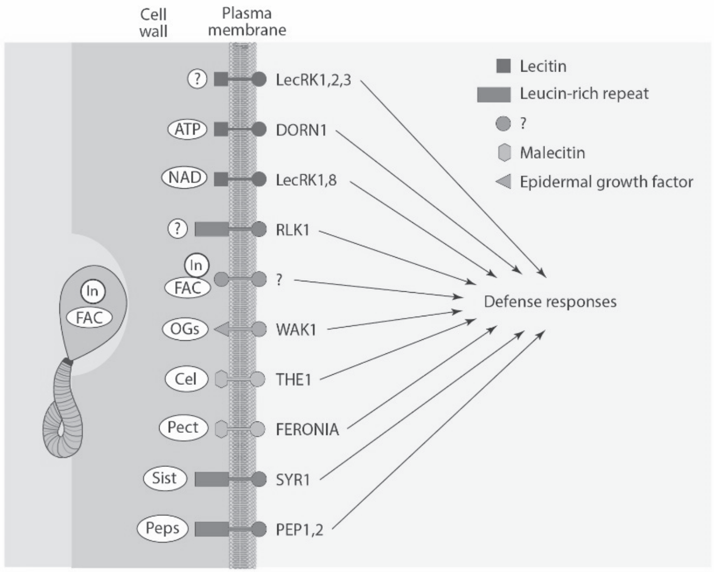

Figure 2.7. Model encompassing sensory elements of DAMPs (damage associated molecular pattern) from herbivory. In (inceptin) and FAC (fatty acid and their conjugated amino acids) are components of the oral secretions of herbivores. OGs: oligogalacturonides; Cel: cellulose; Pect: pectin; Sist: systemin; Peps: peptides, molecules that, together with NAD and ATP, make up the DAMPs from the oral secretion of herbivores, are present in cell exudates or from the breakdown of cell wall components, which act as DAMPs recognizable by the LecRK transmembrane proteins (Lecitin Receptor Kinase), DORN1 (DOES NOT RESPOND TO NUCLEOTIDES 1), RLK (RECEPTOR-LIKE KINASE), WAK1 (WALL-ASSOCIATED KINASE 1), THE1 (THESEUS1), FERONIA, SYR1 (SYSTEMIN RECEPTOR 1), PEP (Pep RECEPTORS). From the perception of DAMPs, the cells activate defense pathways. Modified from Erb and Reymond (2019).

Plants perceive herbivore eggs

Herbivores' eggs deposited on plant organs, usually leaves, are a potential damage factor since, when they hatch, herbivore larvae will begin to feed on the leaves. Plants, in turn, can predict this potential damage and develop strategies to destroy eggs. Naturally, for that to occur, it is necessary for plants to perceive them.

Many plant strategies to combat eggs have been reported, such as the release of volatile compounds that attract eggs or larvae parasitoids; separation or destruction of leaf eggs by the formation of necroses to isolate the eggs or tissue neoplasms capable of crushing the eggs; production of ovicidal chemicals (Figure 2.8).

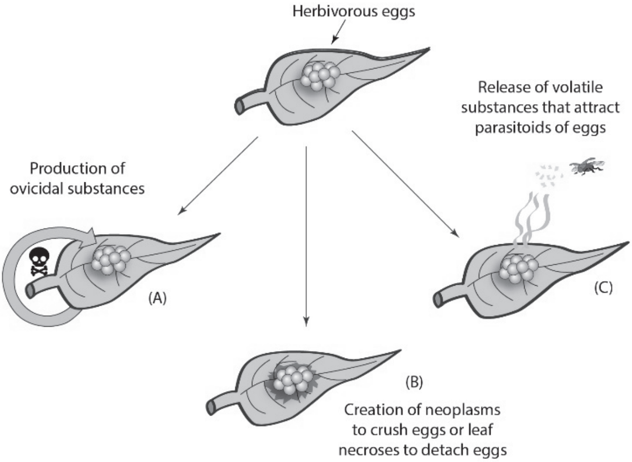

Figure 2.8. Plant defense mechanisms for herbivore eggs. Upon perceiving the eggs, the leaves can produce toxic ovicidal substances (A), create leaf necrosis for the purpose of detaching the eggs, produce new tissues (neoplasms) to crush the eggs (B), or even produce volatile substances that attract parasitoids of the eggs (C).

Plant responses to eggs can be nonspecific or specific (Hilker and Fatouros, 2016). Similarly to plants' combat against pathogens, after plants perceive the eggs, a non-specific response is the isolation of the oviposition site due to the death of tissues. To that end, many types of ROS are sent to the site to act on cell death. The isolation also helps the plant to maintain its immune potential to fight larvae, since it is known that the interaction between eggs and leaves weakens the plant's fighting arsenal when the eggs hatch, making the plant more defenseless from being fed on by the larvae. In turn, the eggs, by specific or nonspecific responses, induce changes in the gene expression of plants, as well as in the production of secondary metabolites that act in the plant's defense against foraging. Another strategy is to change the chemical composition of volatile compounds, phenolic compounds and glucosinolates they produce.

Plants recognize herbivore secretions

Secretions from herbivores may contain substances recognizable by plants, such as volicitin, an amino acid-conjugated fatty acid compound (FACs), inceptins, which are peptide fragments derived from plants, and caeliferins, which are sulfated fatty acids (Basu et al., 2018). These substances are recognized by plants as herbivore-associated molecular patterns (HAMPs), which, after being received, trigger the activity of metabolic immunization pathways. Some of these HAMPs can trigger the release of volatile compounds by plants that may influence ecological relationships that cause damage to herbivores, or even trigger the activity of the oxylipine pathway, through the activation of 12-oxo-phytoodienic acid (OPDA), precursor of jasmonic acid, whose importance in the defense against herbivory has already been described in the topic "Hormones and immunology". Other signs observed associated to herbivore secretions are an increase in hydrogen peroxide, a change in voltage potentials in the plasma membrane and an increase in cytosolic calcium, recurrent mechanisms in cellular defense against various damage agents.

The presence of biomolecules such as auxins, β-GLUCOSIDASE, GLUCOSE OXIDASE and APYRASE has been found in the oral secretions of herbivores and they can potentially modulate biotic interaction.

The different chemical components found in the secretions of herbivores, such as saliva, have been considered elicitors of disarming mechanisms of plant defenses. The microbes present in the secretions, identified as harmful by plants, have the power to change the mechanisms of plant defense against herbivores to mechanisms of defense against microbes. They can also trigger signals that cause plant metabolism to produce substances that scare competitors away. For example, it was observed that the foraging of maize leaves by *Spodoptera frugiperda* led the plant to activate an increase in phenylpropanoids in the roots that promoted increased root resistance to the attack of *Diabrotica virgifera* (Erb et al., 2015; Huang et al., 2017). The attack on the aerial part by *Spodoptera frugiperda* caused a decrease in the volatiles ethylene and β-caryophyllene, which act as clues for *Diabrotica virgifera* to locate its target.

Plants literally smell danger

In the biotic interactions of plants with other organisms, the other is usually the one reported as the one that smells the compounds eliminated by the plants. However, the reverse is also true.

Plant responses to volatile herbivore compounds appear to be induced, with no evidence of their being constitutive. There are several purposes for herbivores to release volatiles, such as sexual attraction, danger or defense and aggregation alarms. In an experiment carried out by Helms et al. (2013), the *Solidago altissima* plant was exposed to volatiles from male fruit flies, *Eurosta solidaginis*. Males tend to appear before females and release large amounts of volatiles, with a predominance of spiroacetals, which can attract females. However, plants exposed to the volatiles of males had a reduction of more than 70% in the incidence of oviposition of females of the same species, as well as a lower incidence and foraging by herbivorous insects, compared to unexposed plants, showing that the male volatile had induced higher plant tolerance. Although the reception sensors of the volatiles were not identified, it was noticed that there was a substantial increase in the content of jasmonic acid in the plants exposed to the volatiles after the beginning of foraging by herbivores, precisely the most important hormone to signal the plant's defense against herbivory. In a parallel experiment, it was observed that pheromones from other herbivores did not trigger tolerance responses in *S. altissima*.

Still related to the experiment mentioned in the previous paragraph, in a study by Helms et al. (2017), a compound from the set of volatiles emitted by *E. solidaginis* was identified, which is responsible for all the modifications suffered by *S. altissima*, the *E,S*-conophthorin, triggering effects on the plant in very low concentrations, the others being innocuous volatiles. However, the olfactory mechanism of plants remains unknown.

Plants hear the sound of danger

In an experiment developed by Appel and Crocoft (2014), using *Arabidopsis thaliana* and the *Pieris rapae* caterpillar, it was demonstrated that plants subjected to recorded acoustic vibrations from caterpillar chewing increased their chemical defenses of glucosinolates and anthocyanin, substances that have increased synthesis because of foraging by herbivores. It was also proven that this response is not derived from aleatory vibration since the plants did not respond to other acoustic vibrations produced by the wind or by the song of cicadas, although the patterns of these vibrations are different, because the wind has low frequencies and the singing of the cicadas, although it is high frequency, as is the case of vibrations emitted by chewing, it has a different temporal pattern than that exercised by the caterpillar. The leaves subjected to vibration had a 32% increase in aliphatic glucosinolates compared to the control, while the untreated leaves, but which received the stimulus signal in a systemic manner, had an increase of

24%, and the increase in the glucosinolates was proportional to the vibrational amplitude. Although the chewing vibrations did not cause an effect of direct anthocyanins increase, the previously vibrated tissues had a greater capacity to produce them after their exposure to the herbivore.

Taking glucosinolates as an example and knowing that the response of the plant is dependent on the received vibrational amplitude, this means that the stimulus will only reach neighboring plants if they not are very close, however it is a signal that can be transmitted systemically to the different organs of a same organism. In view of the observations, they are responses that depend on the vibrational frequency (wave oscillation speed), amplitude (energy) and exposure dynamics, such as frequency and stimulus time. Although we know several receptors for energy waves in plants, such as the photoreceptors for electromagnetic waves, specific receptors for mechanical waves of sound have not yet been identified, but we do already know many elements of mechanical perception, such as those covered in the section “Touch Perception” (Chap. 1). Since the mechanical waves are propagated quickly and systemically to the entire plant, it is suggested that there is participation of electrical signaling, the communication path to be explored in the next chapter, or the triggering of olfactory signals by volatile compounds, or even, that there is a combination of several communicative signals after the perception of the acoustic signal.

Immunology in response to the touch of herbivores

As already discussed in previous topics, foraging, oviposition and the presence of secretions from herbivores can trigger plant defense responses. However, simple physical contact can also trigger defense regulated by the jasmonic acid route.

In research developed by Peiffer et al. (2009) it was observed in tomato plants that the contact of herbivores triggered the breakdown of glandular trichomes, which contain *OPR3*, a key gene that participates in the synthesis of jasmonic acid (see the topic “Hormones and immunology”), and which also contain jasmonic acid. When broken, there was production of H_2O_2 in the trichome, a substance that induces the biosynthesis of this hormone. These observations not only reinforce the idea that trichomes have a mechanism to trigger defense responses regulated by jasmonic acid, but also prove that the defense genes known and regulated by jasmonic acid can be expressed in trichomes. It is already known that genes involved in the development of the trichomes themselves are positively regulated by jasmonic acid. In a parallel experiment, the researchers observed that the application of jasmonic acid in tomatoes induced an increase in the density of trichomes, as well as an improvement in the defense against herbivory.

Summary

Plants have a great sensory apparatus for the perception of herbivores. HAMPs, which are molecular patterns associated with herbivores, can come from secretions or oviposition of herbivores, recognizable by the plant cell. DAMPs, which are molecular patterns associated with damage, can come from the action of herbivores on the cell wall, giving rise to small fragments recognizable by the cells, and cell laceration can expose substances such as ATP, or reducing power such as NAD(P)(H), both recognizable as DAMPs by the plant cell. The specific recognition of HAMPs or DAMPs is made by many transmembrane proteins that act in cell defense signaling. The defense responses to herbivory signals are positively regulated by jasmonic acid, which acts immediately after damage, coming from the cell stocks, as well as having its biosynthesis regulated upwards. Specific responses of plants to the attack of specific herbivores have been reported, showing the existence of an immunology beyond the basal and constitutive one. Specificity is commonly observed by the production of specific volatile compounds. Production of substances harmful to herbivores has been induced in plants exposed to acoustic vibrations characteristic of chewing.

Plant-Plant Chemical Communication

Communication between plants can occur in various scenarios, ranging from the recognition of their hosts by parasites, to the recognition of competitors, or even to alerting their peers to the presence of a damage agent. There may be several changes observed in the behavior of plants after the receivers recognize the signals coming from the emitters. Although many aspects of plant-to-plant communication are covered in this section, it is a topic that will not be finalized here. Communication between plants can be so precise that they recognize not only co-specific individuals, but also their siblings. Aspects related to self-recognition or recognition of relatives will be addressed in the last chapter.

Host-parasite plant recognition

Approximately 1% of plants (4,500 species) are parasites and benefit from the resources of others. It is well known that they have preferred hosts and that requires recognition capabilities.

At first, the recognition of the host plant by the parasite occurs by chemical signaling, but this is a process that depends a lot on the physical proximity between the organisms. However, even though relatively far away, the parasite tends to grow towards the preferred host, which suggests that the chemical signal used for recognition has good mobility. In that regard, it has always been suspected that volatile compounds participate in this process of recognition between species, and finally, in a detailed experiment carried out by Runyon et al. (2006), using *Solanum lycopersicum* as a host and the parasite *Cuscuta pentagona*, it was proven that plants perceive and respond to specific odors. Of the volatiles isolated from tomato, α-pinene, β-myrcene, 2-carene, *p*-cymene, β-phellandrene, limonene, (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene and an unidentified monoterpene, only β-phellandrene and β-myrcene and, after a small increase in the sample size, α-pinene induced a chemotropic response of *C. pentagona*. In a parallel study, a negative chemotropic response to (*Z*)-3-hexenyl acetate, a wheat compound, was observed. Thus, the recognition between plants by means of specific volatile compounds was exemplified, although the perception sensors have not been identified. Illustrative image of *C. pentagona* can be seen in Figure 2.9.

Figure 2.9. Image of the parasite *Cuscuta pentagona*, which has a positive or negative chemotropic growth response, depending on the volatile compounds released by possible hosts. Credit: image by Curtis Clark from Wikimedia Commons. License to use at https://commons.wikimedia.org/wiki/File:Cuscuta_pentagona_stems_2003-06-02.jpg

Seeds of parasitic plants can wait years in the soil without germinating until a sign of proximity to a possible host appears. The main sign to be perceived by the parasite is strigolactones, which are exuded by the host's roots. The perception of strigolactones by the parasite occurs by the D14 receptor (DWARF14) in species of the Orobanchaceae, or D14-LIKE or HTL (HYPOSENSITIVE TO LIGHT) in other species. D14 itself is involved in the seed germination process of some species, even though they are not parasites. Several D14-LIKE homologues are found in different parasitic species, indicating the possibility of recognizing different strigolactones.

Before the formation of the haustoria, there seems to be a need for the parasite to recognize the host through the specific type of flavonoids or quinones exuded by the host, substances that act as inductive or repressive factors in the formation of the haustoria. This perception of the specific metabolite inhibits the penetration of haustoria in species unfavorable to the association or invasion of roots of the species itself. There is still no knowledge of how the haustoria inductive factors are perceived by the parasite, but there is evidence of the involvement of oxidation-reduction processes, from the exudation of reactive oxygen species by the parasite, which will act in the conversion of non-reactive forms of quinones to reactive forms, to be recognized (Saucet and Shirasu, 2016).

After some species of hosts notice the invasion, there are a series of reactions such as localized production of ROS, deposition of chemical substances such as calluses or occlusion of conductive elements of sap to cause the death of the parasitic organ. Although it is not clear how the perception is initiated, it is known that the invasion triggers the same sensory mechanisms of cellular receptors to the invasion of pathogens. There is an action on sensors that trigger immunity responses. In this case, PAMPs (also known as ParAMPs, specifically for parasitic plants) would be the patterns to be perceived by PRRs. Details of this mechanism can be accessed in the topic “Plant-pathogen recognition”.

In Figure 2.10, a schematic representation of the host-parasite plant communication can be seen.

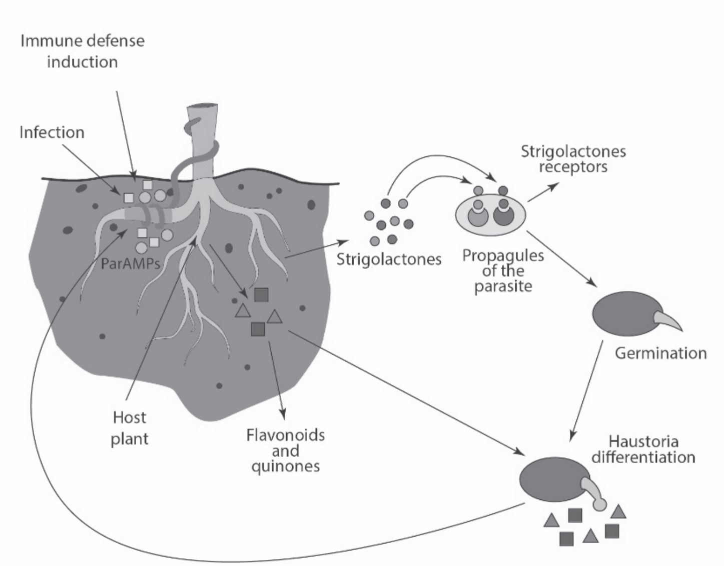

Figure 2.10. Communication between host and parasite plant. The propagules of the parasites are induced to germinate by strigolactones exuded by the host's roots. The reception of these substances is done by the D14 (DWARF14), D14-LIKE or HTL (HYPOSENSITIVE TO LIGHT) receptors. After germination, haustoria differentiation is modulated by flavonoids and quinones exudated by the host's roots. Once the haustoria is formed, infection of the host's roots will occur. This, in turn, when the host perceive the infection triggers defense mechanisms. Although there are points to be clarified, it is known that the recognition of infection by the host involves molecular patterns associated with parasitic plants (ParAMPs) and pattern recognition proteins (PRRs) from the host, triggering thereafter cellular mechanisms of immune defense.

Communication among competing plants

The balance in the community coexistence of plants is intermediated by allelopathy. Although this term may denote chemical substances from one organism that positively or negatively affect the development of another, this topic will only consider competitive plant-plant interaction.

For defense, allelopathic substances are called allelochemicals, and they are chemically diverse, including phenolic compounds (simple phenolics, flavonoids, coumarins and quinones), terpenoids (monoterpenes, sesquiterpenes, diterpenes, triterpenes and steroids), alkaloids and chemicals containing nitrogen (non-protein amino acids, benzoxazinoids, cyanogenic glycosides), among other substances, and some plant hormones (Kong et al., 2019). Plants can perceive them from aerial or underground organs. Unlike soil, which allows movement of substances in different physical states, for the substance to be received by aerial organs it must be in the form of volatile compounds.

There are several effects triggered by the presence of allelochemicals in the recipient plant, such as structural changes in cells, inhibition of cell division and growth, imbalance in the production of ROS and in the activity of antioxidant enzymes, changes in membrane permeability, hormonal imbalance, changes in synthesis of proteins and nucleic acids, and photosynthetic and respiratory changes (Chen and Chen, 2016).

Despite the vast knowledge about allelopathy and allelochemicals present in the literature, little is explored about how plants perceive allelopathic substances, although recognition is the initial factor that triggers responses. In the scope of recognition between plants, self-recognition and recognition of the neighbor are present (aspects to be better explored in the last chapter) since allelopathic interactions are intraspecific and interspecific. As in the plant-pathogen/herbivore interaction, the chemical defense of a plant against toxic substances released by a neighbor can be constitutive or induced.

Looking for a model for the action of allelochemicals, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and *Triticum aestivum* (wheat) were used in an experiment by Kong et al. (2018). The production of DIMBOA by wheat was dependent on the density of neighboring plants, whether co-specific or heterospecific, and its induction was promoted by more than one hundred species. Of the exudates of neighboring species, jasmonic acid, (-)-loliolide (in low concentrations) and salicylic acid (only in high concentrations) were the inducers of DIMBOA synthesis. In some cases, stimulation was also caused by luteolin. However, DIMBOA production was higher in heterospecific interactions, and even more so when the reference plant was exposed to its neighbors at a young age. It was also observed that the accumulation of DIMBOA was proportional to the content of (-)-loliolide, which did not occur due to the other inducers. The perception of exudates from neighboring plants by wheat occurred via chemical signaling through the soil and independent of mycorrhizae. Considering the lower concentration of jasmonic acid and (-)-loliolide needed to trigger DIMBOA synthesis, as well as their greater mobility in the soil in relation to the other substances, they are considered to be the main elicitors (Figure 2.11).

According to the research reported above, plants perceive competition since the increase in the allelochemical DIMBOA occurred due to the density of neighboring plants, as well as the content of (-)-loliolide. In this case, the (-)-loliolid acted as a chemical indicator of plant density and, consequently, of the need to compete for environmental resources. At least in this case, and it is expected that in others where there is auto-allelopathy, the release of allelochemicals by a plant depends on its perception of the degree of competition required in view of the environmental conditions.

The aforementioned research also reveals that wheat recognizes the identity of the neighboring plant since the production of DIMBOA was always lower when the neighbors were of the same species. An illustrative scheme of this interaction can be seen in Figure 2.11.

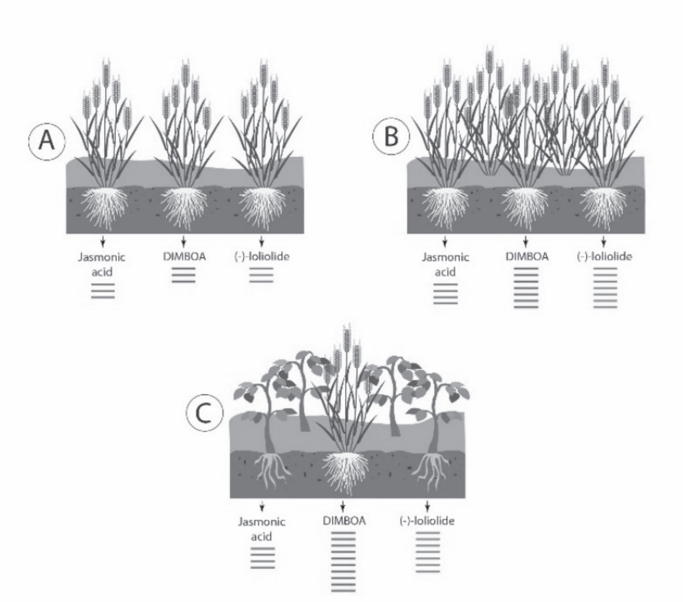

Figure 2.11. Production of allelochemicals according to the content of elicitors, density and identity of neighboring plants. The lower the density of neighboring plants in the environment of the reference plant (PR -central wheat-) is, the lower the soil content of substances exuded by the roots that induce the production of allelochemicals is. Jasmonic acid and (-)-loliolide are the main elicitors of the synthesis of the allelochemical DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) by PR (A). The increase in plant density increases the presence of elicitors in the soil, but (-)-loliolide is the one that proportionally induces the increase in production and release of DIMBOA by PR (B). When the increase in density of neighboring plants is of heterospecific plants, the production of DIMBOA by PR is greater than when it occurs by conspecific plants (C).

For jasmonic acid and salicylic acid, which can act as allelochemicals, it is expected that the receptors that recognize and trigger responses in plants will be the same as those already known in the field of plant development studies. Even though we are not yet aware of the other receptors for substances eliciting the biochemical routes triggered by allelochemicals, it is already a great advance to know the specific identity of the compounds as that greatly increases the possibilities of developing cultivation technologies, instead of just knowing the effect of an organ's macerated.

A high energy cost is assumed for the plant to produce allelochemicals, so much so that the production of DIMBOA in the reference experiment described above was very low when the introduction of neighboring plants was late; that is, when the reference plant evaluated was already well developed. From an energy point of view, it would be a waste to produce a large content of allelochemicals in a situation in which the plant would be little affected, since it would have the capacity to shade the competitors, inhibiting their growth.

It is intriguing that in dense grass there are rarely plants of other species, although there are many seeds of various species in the area. It is a clear sign of negative allelopathy being exerted by the grasses on the other species, although this does not exclude the existence of auto-allelopathy. To prove this effect, Ozaki and Kato-Noguchi (2016), realizing that right after the wheat harvest the rice germination percentage in a crop rotation was very low, analyzed the allelochemicals released by the wheat in the area and identified 6-methoxy-benzoxazolin2(3H)-one (MBOA) and benzoxazolin-2(3H)-one (BOA) from wheat plants as being allelochemicals that inhibited the action of gibberellin on the synthesis of α-amylase in rice caryopsis, a key enzyme in the germination process. In this case, it was not possible to know whether the allelochemicals caused damage to the reception or the signaling pathway triggered by gibberellin. However, it is yet another proven allelochemical action with precise identification of the perceived biologically active chemical component and one that signals competition between plants. From an ecological and energetic point of view, it would be a waste of resources for rice to germinate in an environment unfavorable for its growth, either due to the soil's chemical or resource conditions, or even when considering plant prediction (see last chapter) of the shade that was potentially occurring in the growth environment, promoted by the organisms that released those substances.

There is much technological potential for the use of allelochemicals, such as: interfering with seed germination; improving crop productivity; controlling weeds, pests and diseases, without environmental damage resulting from the introduction of substances strange to the environment; in the synthesis of new agrochemicals; in the reduction of nitrogen losses (ferulic acid, *p*-hydroxybenzoic acid and hydroxamic acid inhibit nitrification); and in genetic improvement for cultivars with greater allelopathic activity. Therefore, it is a promising area of knowledge that offers many technological benefits for plant cultivation, and certainly, the new elucidative studies on biological interactions, including cellular receptors, will contribute a lot to its progress.

Positive allelopathic interactions

As discussed in the previous topic, the constitution of a plant community has allelopathic substances as a regulatory factor. The fact that an allelochemical of a specific species acts as an inhibitor or repressor of the development of another species, can, at the same time, help in the establishment of a third species that would not be established with the presence of the repressed species; it means, "the enemy of my enemy is my friend". Another factor linked to the allelochemicals modifying the floristic composition can be perceived when the insertion and establishment of an exotic species insensitive to the allelochemicals of the native species, and due to its exotic allelochemicals, modify the composition of the community. Not only in plants, but in living organisms, the concern with the exotic is exactly the high probability that it is not sensitive to control factors inherent to the biota already there; a factor that shows that the control mechanisms between the species of an ecosystem develop over the course of evolution, considering the specificities of the organisms that cohabit a respective space.

An example related to ecological interactions involving allelochemicals is carvacrol, produced by thyme (*Thymus vulgaris*), which inhibits the development of a dominant grass, *Bromus erectus* (Ehlers et al., 2014). As the grass is suppressed by carvacrol, several other species, which until then did not exist in the area, are released from their repression imposed by the grass's allelochemicals and become part of the flora.

The composition of plant species that cohabit a space is also linked to their ability to tolerate toxic elements from neighbors. For example, there are species that can tolerate BOA, a highly toxic element (see previous topic) because they can inactivate it by glycosylation, while other species do not have this capacity. Another mechanism is the detoxification of various allelochemicals by glutathione, mediated by GLUTATHIONE TRANSFERASE, whose conjugate can be extruded to the apoplast by Mg^{2+}-ATPase specifically encoded by the gene *MULTIDRUG RESISTANCE-*

ASSOCIATED PROTEIN GENE (*MRP1*), as verified in *Arabidopsis thaliana* (Lu et al., 1997).

A beneficial form of tri-trophic interaction mediated by allelochemicals was observed by Glinwood et al. (2003). In their research the metabolites 5-hydroxy-indole3-acetic acid, DL-5-hydroxytryptophan, L-5-hydroxytryptophan hydrate and 6-hydroxy-1,2,3,4-tetrahydro-β-carboline-3-carboxylic (carboline) found in the root exudates of the *Elytrigia repens* grass induced greater tolerance of barley to the attack of the aphid *Rhopalosiphum padi*. However, the individual use of each substance as an aphid repellent was inefficient, which suggests that the combination of these substances stimulated the barley itself to produce differentiated volatile compounds that repel the aphid.

Summary

Plant-to-plant communication depends on the recognition of molecular patterns. In the host-parasite relationship, both the germination of the parasite's seeds and their growth directed to favorite hosts, as well as the formation of the haustoria depend on a recognition of chemical patterns coming from the hosts, whose patterns are different for each of these stages. The host plant is not passive, and soon after its invasion an increase in typical responses of plant recognition to pathogenic PAMPs by PRRs is observed, but in this case the molecular patterns of damage have been called ParAMPs.

Allelochemicals are substances with toxic potential exuded by plants that can inhibit or discourage the proliferation of new conspecific or heterospecific individuals in an area, respectively, depending on the density of plants compared to the amount of resources available in the environment and the chemical incompatibility between species. Due to the (in)sensitivity of a plant to the allelochemicals eliminated by its neighbors, there are drastic changes that are sometimes caused in the flora of an area due to the insertion of an exotic species, or it can even be the determining factor of the exotic's failure, because it has no tolerance or defense mechanisms for allelochemicals released by the natives. Although the sensory apparatus of plants for most allelochemicals is not yet known, many molecules that trigger responses have already been isolated and identified.

Acoustic Communication in Plants

Sounds in nature are abundant and reliable sources of information. However, the possibility of plants perceiving and responding to sound stimuli has only recently been considered. We already know that plants, even without eyes, perceive colors and even without noses, perceive smells. Therefore, the fact that they do not have sensory organs typical of big animals does not mean that they do not perceive the same stimuli. In previous situations we have already seen the influence of acoustic vibrations influencing plant responses. In the section “Drought and water perception” (Chap. 1), the recognition of the location of water by plants through acoustic vibrations was addressed, and in the topic “Plants hear the sound of danger” we saw plants’ detection of the danger of herbivory through acoustic vibrations. In this section, other interactions that involve acoustic vibrations will be described.

The response to sound is found even in unicellular beings, and even in organisms that have developed specialized organs for hearing, the factor that elicits sound perception is the vibration that the wave exerts on the tympanum or on other structures, such as the antennae of fruit flies.

The pressure exerted by sound vibrations on the plasma membrane can trigger a signaling cascade that culminates in phytoacoustic responses (Mishra et al., 2016). The way in which plants perceive this signal seems to be similar or very close to the way they perceive touch, which might be expected, since both are mechanical stimuli.

Acoustic vibration can be perceived by the transmembrane mechanosensitive channels, although in different studies carried out there was also a distinction between the touch and sound stimuli in the expression of the mechanosensitive channels themselves, but it is necessary to consider the pressure force exerted by both stimuli. Among these channels are MSL (MECHANO-SENSITIVE CHANNELS OF SMALL CONDUCTANCE) and MCA (MID1-COMPLEMENTING ACTIVITY), the latter being exclusively for calcium transport. The similarity of this mechanism triggered by sound to that triggered by touch, including the aforementioned mechanosensitive channels and other factors that will be approached in the sequence, can be seen in the section “Touch perception” (Chap. 1). Alterations have also been noticed in the reorganization of the actin filaments associated with the plasma membrane, which highlights them as potential mechanical wave receptors, consequently altering the tension of the plasma membrane and its responses downstream in cell signaling. In addition to the membrane, the cell wall is affected in the perception of the sound wave, since this stimulus causes an increase in cell wall remodeling proteins, such as xyloglucan endotransglucosidases (TCH4).

To verify the potential impact of sound on aspects of plant physiology, Ghosh et al. (2016) submitted arabidopsis to five defined frequencies, ranging from 250 to 3000 Hz, and an amplitude of 80 dB for an uninterrupted hour. The frequency of 500 Hz was the one that promoted the greatest differentiated expression of genes. Among them, those related to transferase action proteins, kinases, transcription factors, stress responses and different signal transduction pathways. Considering that sound is a mechanical wave and is expected to produce a similar effect to the touch stimulus or close to it, this idea was supported in transcriptomics, as many mechano-stimulation induction genes were up-regulated, such as *DIC2*, *TCH4*, *SZF1*, *MPK11*, *CRK41*, *MYB77*, *DREB26*, *MYB44*, *ERF2*, *RAV1*, *CYP81D8*, *EXO*, *NHL3*, *APD5* and *HSPRO2*. Corroborating the research by Rodrigo- Moreno et al. (2017), which proves the participation of calcium in phytoacoustic signaling, *CML38*, which encodes the calmodulin-type protein binding to Ca^{2+}, was also up-regulated. Oxidation-reduction homeostasis genes like *GRX* and *TRX*, genes involved in signal transduction through (de)phosphorylation such as *MPK11*, *WAKL2* and *CRK41*, genes involved in regulating gene transcription such as *DREB*, *ANAC*, *MYB*, *ERF*, *C3H*, *WRKY* and *RAV* were also differentially expressed according to sound vibrations.

Still in relation to the research by Gosh et al. (2016), there was an increase in the content of antioxidant enzymes such as APX, SOD, MDAR and CAT. These enzymes can be very important, because as we have seen in several situations discussed above, the increase in ROS is recurrent during signaling of stimuli, however we must not forget that many of them are free radicals, and therefore an important threshold signaling can be more harmful than beneficial. It was observed that acoustic vibration induced the up-regulation of FERREDOXIN $NADP^+$ REDUCTASE, which participates in the photochemical stage of photosynthesis. In addition, components of RUBISCO, a carboxylase-oxygenase protein from the Calvin-Benson cycle, which participates in the biochemical stage of photosynthesis, as well as proteins that participate in the glycolytic route and the cycle of tricarboxylic acids in cell respiration, were affected by both upward and downward regulation depending on the acoustic vibrations. In hormonal profile analysis, except for abscisic acid, the hormones auxins, gibberellins and jasmonic and salicylic acids were also up-regulated by sound, with salicylic acid being the most affected, which, for example, may induce greater tolerance of plants to some pathogens.

Although the study of phytoacoustics is still embryonic in plant biology, observing the potential impact of each, alone or in combination, of the many elements revealed by transcriptomics, proteomics and hormonal profile, it is evident that sound can modulate not only secondary metabolism but also the primary metabolism of plants in very diverse and unpredictable ways, requiring specific and systematic studies for the different objectives under analysis.

Plants have phonotropic responses

In addition to the study of roots growing towards the water source, previously discussed, another study with arabidopsis was developed by Rodrigo-Moreno et al. (2017) proving the roots have positive phonotropism to a 200 Hz sound source after two weeks of exposure to the stimulus, and that the sound, in a matter of minutes, triggered a rapid cytoplasmic influx of Ca^{2+}, with the participation of the MCA1 and MCA2 channels, from the apoplast and intracellular reserves. It was also observed that sound acted as an elicitor of ROS formation, mediated by NADPH OXIDASE, in addition to inducing K^+ efflux, mechanisms similar to touch responses. It is not known, however, whether the mechanical (or pressure) wave of the sound impacts the mechanosensitive transmembrane transporters (a strong impact could also affect the cytoskeleton structure, and, consequently, promote greater or lesser tension in the plasma membrane), which, in this case, would be the receptors, or if there is a receptor that precedes the activity of the transporters. If this is the case, the transporters would act only on the signaling path. In addition to phonotropism, the study also clarified that the roots of plants treated with acoustic vibrations became less sensitive to gravity and had less lateral growth, in addition to plants growing less, factors that may be associated with K^+ efflux. Similar responses of transmembrane transporters to sound are also known in animals.

There are many possibilities for sound to affect plant development. Studies have shown varied responses, such as: increased resistance to some pathogens and stress due to water deficiency; increase in the content of chemical components, such as ascorbic acid; increase in the percentage of seed germination; delay in fruit ripening and increase in agricultural productivity (Mishra et al., 2016; Khait et al., 2019).

In a simplified manner, Figure 2.12 shows the best-known elements in the reception of acoustic vibrations by the plant cell.

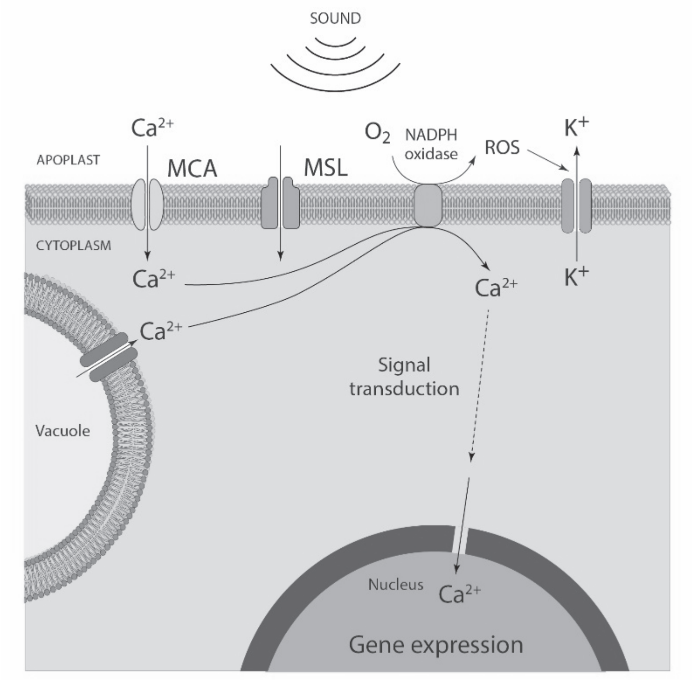

Figure 2.12. Reception and triggering of responses to sound stimuli by the plant cell. Acoustic vibrations with frequencies that sensitize the cell trigger the activity of mechanosensitive transmembrane channels, which are the most likely receptors, such as MSL (MECHANO-SENSITIVE CHANNELS OF SMALL CONDUCTANCE) and MCA (MID1- COMPLEMENTING ACTIVITY), this latter is exclusive for Ca^{2+}. The transient increase in cytosolic Ca^{2+}, either from the apoplast or from organelles such as the vacuole, triggers signaling pathways that culminate in the expression of genes responsive to mechanical impacts, as well as in the activation of NAD(P)H oxidases present in the membranes, whose activity may culminate in the production of ROS that trigger the activation of transmembrane channels for K^+ extrusion.

Acoustic plant-animal communication

As discussed in the section “Plant-herbivore communication”, the plant can perceive a sound that stimulates it to modify metabolic routes. In the case of caterpillar foraging, the metabolism changes to a greater production of defense metabolites according to the acoustic patterns perceived by the receiver. However, some sound-emitting animals are collaborators, so responses to non-defensive acoustic vibrations are expected.

In a study developed by Veits et al. (2019) with *Oenothera drummondii*, whose pollinators are bees and moths, it has been proven that flowers can act as a sound receiving device in plants. Using a laser vibrometer, the vibration of the petals in response to the sound vibrations of the pollinators, natural or artificial (reproduction by a sound device) was measured, as well as to silence, and the sugar content in the nectar was quantified according to the vibratory perception from pollinator. It was observed that the vibrations emitted by the wings of the bees, natural or recorded, promoted an increase of sugars in the nectar in just three minutes, unlike the vibrations of frequencies different from the natural/recorded or of silence; there was also vibration of the intact petals in a pattern similar to that of bees, which is expected to be due to the resonance frequency. Although it has not been evaluated, it is expected that the vibration of the petals will result in the activity of mechano-sensors, such as some transmembrane channels, activating metabolic pathways that culminate in different responses, such as greater production of nectar.

The plant's investment cost in elements that attract pollinators, such as pigments, volatile compounds and nectar, is expected to be high. When analyzing that the shape of the flowers adapts to the pollinators of interest, it is assumed that the plant wants to restrict the nectar to the specific pollinator. It is also widely observed that there is a rationing of the nectar so that the pollinator visits the flower several times, increasing the chance of pollination. In this context, the production of nectar by the sound perception of the specific pollinating agent not only brings an attractive benefit, but there is also an additional advantage to the rationing of the product, preventing other non-cooperative organisms, such as small insects, from taking advantage. It is a strategy that is added to the cyclic production of nectar, since the activity of pollinators is not constant as they have peak times of their activity.

Due to the specific vibration of the pollinator, by resonance, there is vibration of the anthers that have pores or slits and that only release pollen under the typical vibration promoted by their pollinator; it is called the buzz-pollination syndrome. This syndrome is presumed to occur in approximately twenty thousand species. Identifying elements of this vibration are frequency, amplitude and duration of the stimulus (De Luca and Vallejo-Marin, 2013).

Plants as sound reflectors

Figure 2.13. *Nepenthes hemsleyana*, an insectivorous plant whose trap shape acts on the acoustic reflection of the bat *Kerivoula hardwickii*, in a mutualistic interaction, in which the plant offers shelter in exchange for nutrients present in the bat feces. Credit: image by Bazile Vincent from Wikimedia Commons. License to use at https://commons.wikimedia.org/wiki/Nepenthes_hemsleyana#/media/File:Nepenthes_rafflesiana_var._elongata_lower_pitcher.jpg

Interestingly, plants are not only responsive to sound, but can use it as a means of communication to attract cooperative animals. This way of communication has been observed in echolocation over long distances through a specific sound pattern. As an example, a carnivorous species, *Nepenthes hemsleyana* (Figure 2.13), although it produces few attractions, such as nectar and volatile substances, has a vessel-shaped trap that facilitates a mutualistic relationship with the insectivorous bat *Kerivoula hardwickii* (the plant offers shelter in exchange for nutrients present in bat feces, especially nitrogen). With the use of sonar, it was observed that depending on the opening angle of the vessel cover, the concavity of the wall and the depth of the vessel, it starts to act on the reflection of ultrasound emitted by bats, and in this way, it helps them to find it by echolocation (Schöner et al, 2015). It is a highly efficient mechanism, as bats do not usually go to other neighboring shelters, even with very similar shapes. Other studies along the same line of observation have reported that leaves and flowers of some other species also have this ability to act as sound reflectors to attract bats.

Summary

Acoustic vibration, being a mechanical wave, is perceived by the plant cell, like other mechanical stimuli. The sensory apparatus of cellular perception of sound is similar to that for touch. Mechanosensitive transmembrane channels are the most likely receptors for sound vibration. Among them, calcium channels are considered the most important for triggering signal transduction pathways that culminate in the expression of genes sensitive to sound, and consequently, in morphophysiological effects. Specific responses require sound standardization, both in amplitude and frequency, as well as in the rhythm and time of exposure of the plant organ to the stimulus. Acoustic vibration has been observed as a very precise communication mechanism in plant-animal interactions, such as in the buzz-pollination syndrome, and even in mutual plant-bat interactions, in which the effectiveness of communication depends on plant organs reflecting specific acoustic patterns.

Electrical Communication in Plants

Electrical communication in plants has been widely explored, especially the signaling between cells, tissues or organs, aspects to be explored in the next chapter. Its importance in the interaction between plants and microorganisms has already been addressed in the section “Plant-microorganism communication”. However, its manifestation has also been observed in the plant-animal or plant-plant communication, which will be addressed in this section.

Plant-animal electrical communication

The studies of plant-animal electrical communication are limited to plant-pollinator interactions. Several aspects are known for a plant to attract a specific pollinator, using pigmentation, specific volatile compounds, texture, shape, humidity and impressions used for echolocation. However, an unusual aspect was reported by Clarke et al. (2013), which is the flower identification by pollinators through electric fields.

Some pollinators have positive electrical potential and flowers have negative electrical potential, which facilitates pollen adhesion over short distances. After pollination, the electrical potential of the flower is changed, which can be perceived by the pollinator as a flower with low potential for resources. It is notable in the study of pollination biology that multiple recognition factors increase pollinator visitation. In this case, the electric fields could act as an additional factor to the smell and or the color, however, in isolated experiments, specific recognition of the electric field in petunia has also been observed in the bumblebee *Bombus terrestris* (Clarke et al., 2013). Due to the opposite charges, the greater the proximity between the pollinator and the flower, the greater the charge acting in the attraction between them, reaching 5 kV/m at a distance of 2-3 cm (Clarke et al., 2017).

Electric fields in the flower are at the margin of the petals, stigmas, anthers and trichomes. However, the charges are differentiated, which raises the question of whether the electrical contrast is also decisive in the recognition by the pollinator. Electrical forces between pollen and pollinator are also important in moving pollen from anther to pollinator or from pollinator to stigma.

Plant-plant electrical communication

The fact that the soil can transmit electrical signals led Volkov et al. (2019) to develop a study in plant electrophysiology to verify the importance of this phenomenon to communication between plants. In isolated pots, connected through an electrical conductor, *Aloe vera*, tomato and cabbage plants were used to check if there was electrical stimulation between them through the soil. To that end, it was ensured that there was no communicative interference from mycorrhizae, volatile compounds or acoustic stimulation. It is important to note that the electrical conduction capacity of the soil is dependent on moisture, pH, chemical composition, texture, temperature and cation exchange capacity. It was discovered that there was intraspecific or interspecific wireless electrical communication (without direct contact between roots) after electrostimulation of the plants. Meanings were not explored in the study, but as has been and will continue to be addressed, there are several effects that electrical signals can trigger in plants, such as activation of membrane transporters, organ movement, enzymatic activation and changes in the pattern of gene expression. We hope that future research will discover how plants development can be affected by this form of communication.

Olfactory Communication in Plants

From among the more than 100,000 secondary plant metabolites, many of them, when volatile, are used as molecules of communication that serve to attract pollinators or natural enemies of damage-causing biological agents or even to induce greater tolerance in neighboring plants to factors of danger already detected by the emitting plants. The production and release of volatile compounds can be induced by various stressful conditions, whether biotic or abiotic, or simply be induced by the volatile chemical composition of the neighboring plant, even though it is in a non-stressful condition. Although there are many situations in which the influence of specific substances is proven, we still do not have clear studies regarding the receptors for the different chemical compounds.

Volatile compounds and defense communication

There is a specificity of volatile compounds emitted by different plant genotypes depending on the stressor, which means that it is possible to know which plant is under stress caused by a specific agent. Not only can plants identify different odors of volatile compounds, but herbivores can too, which is important for them to find their targets.

Depending on the herbivore, the plant modifies the composition of the volatile compounds emitted, and thus can attract natural enemies of the biological stressor that is harassing it. This behavior shows a coevolution of these organisms, whose learning for plant defense was established in their coexistence over time, and the learning has been passed through transgenerational memory (a theme to be explored in the penultimate chapter) to successive generations. In the same context, the insertion of an exotic organism in a system can result in the impossibility of recognizing the volatiles emitted by its components, since they were not evolutionary consorts in the same ecosystem.

A plant not infected by pathogens or infested by pests can increase its chemical defense when it comes into contact with specific volatile compounds from an infected or infested plant. This means that it recognizes “the smell of danger”, and as a consequence, it defends itself. Among the mechanisms triggered by this recognition is the expression of several genes that participate in metabolic defense pathways, which would be expressed in a condition in which the plants were effectively being affected by the damage agent. As a result of this differentiated gene expression, the production and release of other volatile repelling compounds may occur or it may trigger an increase in the mechanical barrier to invasion, such as greater deposition of callose on the cell wall or production of compounds toxic to the harmful agent. It is a defense that goes beyond the basal level of its constitutively produced immunological substances.

Recognition of the danger alert through volatile compounds in neighboring plants is more effective in those of the same species. It is not known whether this is because the different damage-causing agents have a different predilection, and therefore it makes more sense for the more susceptible genotype to protect itself, while the less susceptible genotype would not have to spend a lot of energy on defense in the face of low risk of damage, or because the less susceptible genotype does not have the same capacity to perceive the volatile signal emitted by the most susceptible genotype. In any case, it is considered that the volatile signal, after emitted, becomes public. There is a line of researchers who advocate that the plant produces it for itself, as a way of alerting its own organs not yet attacked by the damage agent. A discussion of public signs will be made in the topic "Plants as social organisms", Chap. 5.

There are many interactions whose communication is made by volatile compounds. As noted in Table 2.1, they are also important for tri-trophic relationships, such as in attracting natural enemies of agents that cause damage to plants. There are also the possibilities that: the presence of a plant species favors the neighbor simply by releasing constituent volatile compounds, which are harmful to the pests or pathogens of the receiver plant; the stage of development of the organs may favor the production of volatiles, which will act as an attractant for pollinators or dispersers; the plant's exposure to an abiotic stress condition can also favor the production of volatiles, which can favor the recovery of the plants from damage caused by stress (Table 2.1).

For a long time, the communicative potential of volatile compounds in defense relationships has been neglected in plant studies, although their importance for pollination and dispersion has long been known. Interest in the topic has grown in recent decades due to the perceived effectiveness in the closeness or distancing between pests-plants caused by different volatile compounds. Because of the technological potential of this knowledge for the cultivation of plants, today there is a substantial increase in studies, but many elements necessary for a broad understanding of its mechanisms are still lacking.

Volatile compounds action mechanisms

As an example of what occurs in plant physiology in biotic interactions mediated by volatile compounds, Arimura et al. (2000) and Yoneya et al.

(2014) observed in beans free from pests, but in the presence of volatiles from beans infested by mites, an increase in the expression of lipoxygenases (*LOX*), necessary for the synthesis of jasmonic acid, the central hormone in the regulation of plant defense against pests and some pathogens. They also observed an increase in the expression of the gene that encodes for PHENYLALANINE AMMONIA LYASE (PAL), a key enzyme in the pathway of phenylpropanoids, from where many secondary metabolites that act in the defense of plants originate. There was also an increase in the expression of the *FARNESYL PYROPHOSPHATE SYNTHASE* gene and PR-2 (BASIC PATHOGENESIS RELATED PROTEIN 2) and PR-3, important proteins in the field of plant defense. The isolated volatiles (*E*)-β-ocimene, (*E*)-4,8-dimethyl-1,3,7-nonatriene and (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene were the most effective in inducing the expression of the jasmonic acid synthesis genes. Several other studies have demonstrated the different volatiles influencing the expression of various other genes and it has been noted that in some situations it is the mixture of different volatiles that triggers the synthesis of secondary metabolites in the receiver plant. It is important to pay attention to the fact that even if it is the same chemical compound, its stereochemistry influences the induction of the response differently.

Although the exact mechanism of reception of volatiles is not known, it is known that even at the beginning of the signaling process, upstream of differentiated gene expressions, plasma membrane depolarization occurs by the transient increase in cytosolic Ca^{2+}, as well as the activity of (de)phosphorylation proteins, common events in signal transduction pathways in plant metabolism. There are reports that the distance between the emission and the reception of the volatile signal can vary from 40 cm to more than 10 m, but it is necessary to consider the response sensitivity of each species.

Since there is specificity of the plant-volatile response, it is assumed that there is a specific sensory system for the different elements. It is likely that the volatiles enter the plant body through stomata or through adsorption on the leaf surface, although the receptors are not known. It is likely that the effectiveness of some volatiles in communication depends on their (dis)conjugated forms, just as it does for plant tolerance to toxic metabolites, since some volatiles can be glycosylated or glutathionated, so that in this conjugated form they are not toxic to the cell, as occurs with (*Z*)-3-hexenyl vicianoside (Sugimoto et al., 2016).

The recent studies on aquaporins, in which these proteins have shown multiple roles, put them on agenda when we study the possible sensors for volatile compounds. It depends on the type of the aquaporin and its location in the plant. It is expected that very soon further studies bring us some more elucidative information about this subject.

Volatile compounds and pollination by sexual deception

In addition to volatile compounds being well known for attracting pollinators or dispersers, another facet of this communication is very intriguing. It is the so-called pollination by sexual deception, occurring among some plants, especially orchids, and their respective pollinators. The factor that elicits the production of the volatile seems to be only the stage of flower development, and it appears to be a purposeful action (intentional behavior in plants will be explored in the last chapter). This is what happens with the *Drakaea glyptodon* orchid (Figure 2.14A) which releases three alkylpyrazines and a hydroxymethylpyrazine, substances that act as pheromones to attract pollinating *Zaspilothynnus trilobatus* males. Not only that, but the plant also develops a petal imitating the physical shape of female wasps (Bohman et al., 2014). This tactic of sexual deception allows the plant to achieve pollination without a useful exchange with the pollinator since the flower does not even need to produce nectar. Another example of pollination by sexual deception is the action perpetrated by the orchid *Caladenia crebra* (Figure 2.14B), which attracts its only pollinator, the male wasps of *Campylothynnus flavopictus*, releasing (methylthio)-phenols identical to the female sex pheromones (Bohman et al., 2017). In Figure 1.14D there is yet another example, based on shape and smell, induced by the orchid *Ophrys bombyliflora* to attract its pollinators of the genus *Eucera*.

An example of pollination by sexual deception, involving an intricate relationship with more than two species, was observed by Oelschlägel et al. (2015), in which *Aristolochia rotunda* (Figure 1.14C) releases volatiles that mimic secretions of insects killed by spiders, a specific odor that attract a kleptoparasitic fly from the family Chloropidae, which is the pollinator of *A. rotunda*.

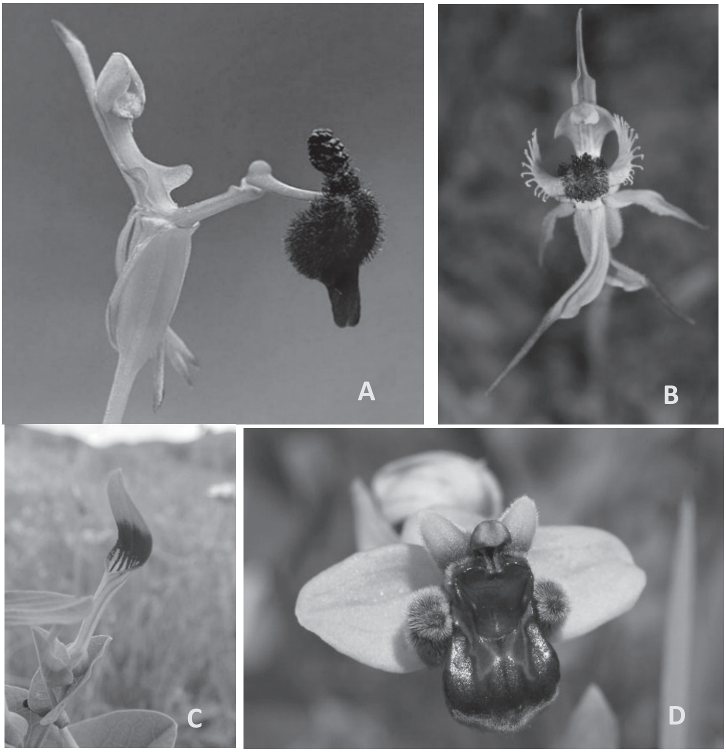

Figure 2.14. Plants that use volatile compounds that mimic pheromones to attract male pollinators and or that develop physical forms of female pollinators to attract males as a pollination tactic by sexual deception. 2.14A. *Drakaea glyptodon*, an orchid that uses a petal to imitate the physical shape of female wasps of *Zaspilothynnus trilobatus*, in addition to releasing volatile compounds that act as pheromones to attract male wasps. 2.14B. *Caladenia crebra*, an orchid that releases volatile compounds that attract the males of *Campylothynnus flavopictus*. 2.14C. *Aristolochia rotunda*, a plant that releases volatile compounds that mimic odors of insects killed by spiders to attract a kleptoparasitic fly, which is its pollinator. 2.14D. *Ophrys bombyliflora*, an orchid that releases volatile compounds that mimic pheromones of the pollinating females of the genus *Eucera*, in addition to taking the form of its pollinator. Credits: 2.14A Image by Brundrm from Wikimedia Commons. License to use at https://commons.wikimedia.org/wiki/File:Drakaea_glyptodon_2.jpg 2.14B Image by Terence Doust from Wikimedia Commons. License to use at https://commons.wikimedia.org/wiki/File: Caladenia_Crebra.jpg 2.14C Image by Krzysztof Ziarnek, Kenraiz from Wikimedia Commons. License to use at https://commons.wikimedia.org/wiki/File:Aristolochia _rotunda_kz08.jpg 2.14D Image by Hans Stieglitz from Wikimedia Commons. License to use at https://commons.wikimedia.org/wiki/File:Ophrys_bombyliflora-001.jpg

Various effects of communication by volatile compounds

The importance of volatile compounds, in addition to defence and pollination, as seen in the previous topic, extends to several other situations, which can be seen in Table 2.1 in which various factors stimulating their production are also shown.

There are countless possibilities for the technological use of this knowledge, which could be for purposes of defense against pathogens and pests, for reproduction, to produce substances for pharmacological use, for inducing stress tolerance, among other uses. Some techniques using aromatic plants have already been used in some plant cultivation systems, however, with the advent of the discovery of specific substances that elicit responses, which can be reproduced on a large scale in the laboratory, added to the research that is in progress on their mechanisms of action in plants, there is a visible prospect of a progressive increase in the use of volatile compounds, especially in agriculture.

Table 2.1. Agents that elicit the production and emission of volatile compounds, and their effects on plants.

Agent	Volatile induced	Plant species	Observed response	Reference
Neighboring plant *Artemesia tridentata*	1,8-cineole, (*E*)-ocimene and *p*-cymene	*Nicotiana attenuata*	More accelerated production of trypsin in *N. attenuata*, acting in the fight against the *Manduca sexta* caterpillar when there is presence of artemisia as a neighbor	Kessler et al., 2006
Rain and drought	α-pinene, camphene, β-pinene, limonene, β-caryophyllene, (*E, E*)-α-farnesene; (*E*) -2-hexenal, benzaldehyde and alcohol (*Z*) 3-hexen-1-ol	*Malus domestica*	Decrease in volatiles as rainfall increases, increasing compounds as water deficiency increases	Vallat et al., 2005
High temperature and intense light radiation	Isoprene, α-pinene, β-pinene, sabinene, 3-carene and limonene monoterpenes	*Helianthus annuus*	Accelerates recovery from stress, induces reduction of reactive oxygen species	Schuh et al., 1997
Salt	Isoprene	*Eucalyptus globulus*	Induces acceleration of stress recovery	Loreto and Delfine, 2000

Time, phenological stage	Phenylpropanoids	*Petunia hybrida*	Positive factor for expression of the *EMISSION OF BENZENOIDS II* gene, necessary to produce volatile attractive for pollinators	Spitzer-Rimon et al., 2010
Just touch (1 min/6 days)	Isoprene, S4-aldehyde, (*E*)-3 hexenal and terpenes	*Zea mays*	Increase in antioxidant proteins and terpenoids in the plants touched and in the neighboring plants, which made them more tolerant to aphids *Rhopalosiphum padi* and *Hordeum vulgare*	Markovic et al., 2019
Ozone and mite	(*E*)-4,8-dimethyl-1,3,7-nonatriene and (*E, E*)-4,8,12-trimethyl-1, 3,7,11-tridecatetraene and (*Z*)-3-acetate hexenyl	*Phaseolus lunatus*	Decrease in reactive oxygen species and increase in photosynthetic rate during recovery from both stresses	Vuorinen et al., 2004
Fungi *Sclerotium cepivorum*	Nerolidol and terpinolene	*Allium sativum*	Antifungal activity	Pontin et al., 2015
Tobacco Mosaic Virus - TMV	Methyl salicylate	*Nicotiana tabacum*	Increased resistance of the plant itself and of neighboring plants to the virus by the release of methyl salicylate by the infected plant	Shulaev et al., 1997

Bacterium *Pseudomonas syringae*	Nonanal and methyl salicylate	*Phaseolus lunatus*	Increased expression of the *PATHOGENESIS-RELATED PROTEIN2* (*PR-2*) gene, promoting greater tolerance to the bacterium	Yi et al., 2009
Insect *Diabrotica virgifera*	(*E*) -β- caryophyllene	*Zea mays*	Attracts the nematode *Heterorhabditis bacteriophora*, which fights the diabrotica itself	Hiltpold et al., 2010
Insect *Spodoptera littoralis*	Indole, linalool, (3*E*)-4.8 dimethyl-1,3,7 nonatriene and (*E*)-β-farnesene	*Zea mays*	Induction of genes positively regulated by abscisic acid; greater tolerance to caterpillar attack	Ton et al., 2007

Summary

The production and release of volatile compounds by plants, in addition to being constitutive, can be induced by different plant-environment interactions or plant interactions with other organisms that can induce the production of specific substances characteristic of the specific interaction. The release of volatile compounds can attract different organisms that participate in plant defense or pollination, chase away harmful organisms, or even contribute to the recovery of plants from different stresses. Although specific substances are known to trigger specific physiological responses, plant receptors are not yet known, but some elements of the signal transduction pathway that precede the expression of genes responsive to odors have already been proven. It is intriguing how plants, in addition to emitting volatile substances that imitate pheromones, also imitate physical forms of females of their pollinators to attract males, in pollination by sexual deception, widely observed in orchids.

Plant-Plant Communication via Mycorrhiza

The mycorrhizal interaction not only serves to increase the acquisition of nutrients and water by plants but implies plant-plant communication. The interconnection of plants by fungi allows the exchange of organic or inorganic nutritive substances, and even substances that signal danger, inducing greater defense capacity of the receiving plant.

Plant-to-plant nutrient transfer via mycorrhiza

The mycorrhizal interaction has long been known for increasing the acquisition of nutrients by the plants. However, in recent decades, another discovery has proved to be important in this interaction, which is the exchange of chemical compounds, via mycorrhizae, by plants interconnected by fungi, revealing a plant-plant communication occurring underground.

Recognition among symbionts was previously addressed in the topic “Plant-mycorrhizal fungal recognition”. Arbuscular mycorrhizal fungi can create a mycelial network of interconnection with various intraspecific or interspecific plants and thus act as a network capable of transmitting communication signals between them. Its role as an area amplifier for the absorption of mineral nutrients from the soil extends to the exchange of organic or inorganic nutrients between plants. The transfer of nitrogen from the donor plant to the receptor is more observed when the donor is a plant associated with atmospheric N_2 fixing bacteria. To prove this phenomenon, studies use different N isotopes to track the donor-receptor pathway (He et al., 2009).

Carbon isotopes have also been used to verify the transfer of organic carbon between plants. An example was found among seedlings of *Pseudotsuga menziesii* interconnected by the ectomycorrhizal fungus *Rhizopogon vinicolor* (Teste et al., 2010). It is expected that the receiving plant will benefit when it is difficult to produce photosynthates.

Allelochemicals plant-to-plant transfer via mycorrhizae

There is evidence that the mycelial network, in the interaction, plays an important role in plant-plant communication by allelopathic chemical signals. The possible transfer of a chemical signal between plants via mycorrhizae would be much more efficient than its release into the soil, because, in this case, it may not reach the neighboring organism given the possibility of losses or eventual modifications suffered in the environment, such as leaching or sorption by colloids.

The insertion of an exotic plant in an area can modify not only the floristic composition of the environment due to its allelochemicals not being tolerated by some species, as discussed in the section “Chemical plant-plant communication”, but also cause changes in the populations of mycorrhizal fungi. Although it has not been proven that the establishment of an exotic plant can cause the direct transfer of incompatible allelochemicals to native plants via mycorrhizae, in a study by Marler et al. (1999) it was observed that the exotic *Centaurea stoebe* caused a decrease in the mass of the native *Festuca idahoensis* only when there was the presence of mycorrhizal fungi, evidencing the possible transfer of allelochemicals via hyphae, or even organic carbon from the native to the invasive one, or even from any unidentified chemical (or electrical) signal that would inhibit the native's development. Nor is it ruled out that the native's loss of mass occurred due to the reduction of nutrients in the soil due to the greater absorption by exotic mycorrhizae, since it was not clear in the study whether there was a root-fungus-root interaction. In any case, this expands and instigates new studies on the potential of communication mediated by mycorrhizae.

Plant-to-plant defense signaling via mycorrhizae

The question raised in the last topic regarding the transfer of allelochemicals via mycorrhizae gains strength when other research proves the exchange of other substances in the mycorrhizal interactions between heterospecific plants. It was observed that the donor species *Pseudotsuga menziesii* attacked by *Choristoneura occidentalis* and interconnected with the receptor *Pinus ponderosae* by the ectomycorrhizal fungus *Wilcoxina rehmii* not only transfers organic carbon but also signs of the parasitic attack, stimulating the receptor to increase the production of defense substances, as well as antioxidant enzymes (Song et al., 2015). In a study conducted with *Pseudotsuga menziesii*, Gorzelak (2017) observed that when there is interconnection between relatives and non-relatives, the transfer of organic carbon or nitrogen is greater between relatives, and the transfer is increased in a situation of stress. This behavior also highlights the existence of recognition among interconnected relatives, although the flow of nutrients can also occur between non-relatives. The identity recognition mechanism has not been identified. Self-recognition and recognition of relatives is a subject of the last chapter.

Other evidence of root-fungus-root chemical communication was observed in tomatoes, in a study by Song et al. (2010). When plants were infected with *Alternaria solani*, there was an increase in chemical defense in the neighboring uninfected plant only when there was interconnection between them by the mycorrhizal fungus *Glomus mosseae*. Among the changes that occurred in the receptive plant, even isolated for aerial volatiles, there was an increase in putative defense molecules such as peroxidases, POLYPHENOL OXIDASE, CHITINASES, β-1,3-GLUCANASE, PHENYLALANINE AMMONIA LYASE (PAL) and LIPOXYGENASE. Another example following the increase in tolerance between plants, mediated by mycorrhizae, and with no other possibility of communication, was observed by Alaux et al. (2020) in potato plants. Healthy plants interconnected by the arbuscular mycorrhizal fungus *Rhizophagus irregularis* to plants infected with *Phytophthora infestans* had increased expression of genes involved in defense, such as those encoding for PHENYLALANINE AMMONIA LYASE (PAL), for PATHOGENESIS FACTORS (PR-1b), for RESPONSE FACTORS TO ETHYLENE (ERF3) and oxygenation of lipid precursors of jasmonic acid (LOX). Many other works showing this form of signal communication for defense have been published.

Figure 2.15 summarizes the phenomena involving movement of chemical plant-to-plant signals mediated by mycorrhizae.

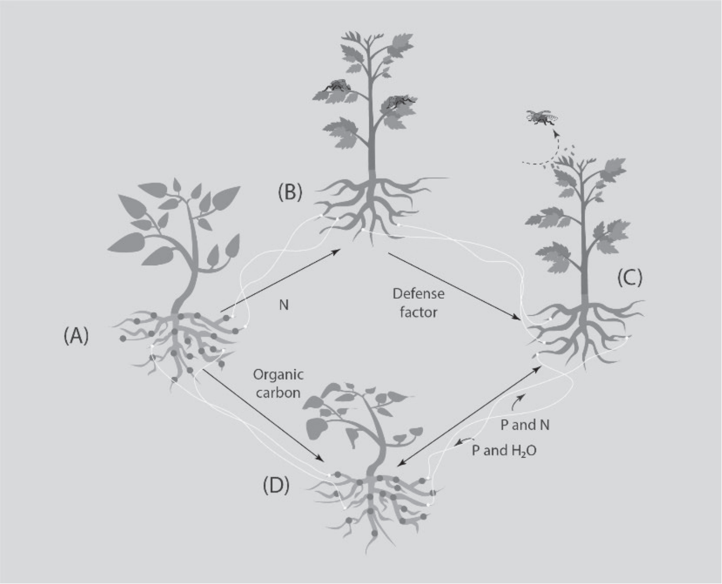

Figure 2.15. Communication between plants via mycorrhizae. Through mycorrhizal infections, plants can benefit from nutrients and water absorbed and channeled by fungi (C and D). Legumes in interaction with nitrogen-fixing bacteria (A) easily donate mineral or organic N, via mycorrhizal connection, to other interconnected plants, even if heterospecific (B), or can even donate organic carbon, especially to conspecifics with difficulties, such as those under stress (D). Interconnected plants can still transfer signals of defense among those affected by pests or pathogens (B) to those not yet affected (C), inducing them to produce repellant volatiles or substances toxic to the damage agents.

Plant-Plant Communication by Light

Light has previously been approached in different situations as a source of environmental information for plants. In this section, we will see that it can act as a plant-plant communication mode, triggering prediction responses of plants to shading.

How do plants predict shade?

Even though apparently there is no intention to communicate, the pattern of light reflected between two plants can serve as a source of information not only about the existence of a neighboring organism, but also its distance. A plant (A) can predict a future shade by the quantity and quality of the radiation that reaches it from the radiation filtered or reflected by a screen. If the screen is a neighboring plant (B), it absorbs a lot of the red (R), blue and UV-B radiation that reaches it, but transmits and reflects a lot in other spectral bands, such as the far-red (Fr) and the green. Thus, plant A, when perceiving the pattern of horizontal radiation reflected or transmitted by plant B, can recognize the presence and distance between it and the neighbor, since the R:Fr ratio, as well as the photon intensity in the blue and UV-B decreases as the distance between plants decreases. In this case, there is also a decrease in the Pfr:total phytochrome ratio, especially of photostable ones, as well as the activity of photostable blue photoreceptors, which can cause a shade avoidance response even before effective shading. For more details on light and photoreceptors, see the section "Light Perception" Chap. 1.

As already known from phototropism studies, the plant has the ability to perceive the direction of the light source. In the case of plants of unequal sizes, in addition to the direction, perceived through the angle of incidence, the quantitative and qualitative pattern of radiation intercepted by plant A indicates the probable size of a neighboring plant (B), which will soon be able to shade it during many hours of the day. In this case, even before there is effective shading, plant A can modify its stem growth projection to avoid shading, in addition to being able to use mechanisms of rapid growth and increased apical dominance to try to overcome the potential danger. When considering one plant lower than another, shading in the morning or afternoon is natural, depending on the distance between the plants and the position between them in relation to the cardinal points. In this context, the reference for a future shading will be the shading time before and after noon (moment of less inclination of the radiation incident on the leaves, having the zenith as a reference) to which plant A is exposed.

Although phyA and cry2 can be activated when receiving depleted red and blue radiation, respectively, phytochromes B to F, cryptochromes 1 and 3 and UVR8 tend to be less effective in the part of plant A that receives radiation filtered by plant B. These are the balances of specific phytochromes, cryptochromes and UVR8, activated or not activated, that act as indicators of the shade condition or potential shade danger. From the shade perception, the mechanisms for its avoidance are activated. There is evidence that, together with phyB and UVR8, phototropins also act in the repression of etiolation when the plant is discouraged from accelerated growth due to its exposure to full sun or to a condition with less danger of shading.

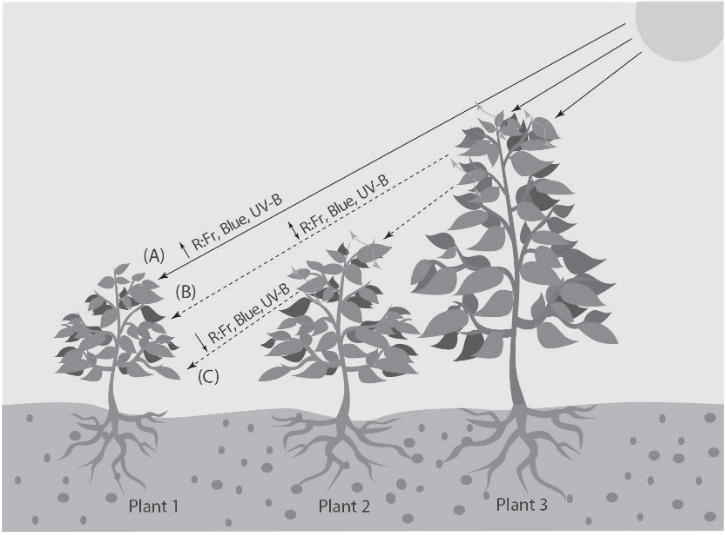

Figure 2.16. Perception of neighboring plants by the incident radiation pattern. Unfiltered radiation incident on leaf A of plant 1, characteristic of full sun, differs from the pattern incident on leaf of B and C, whose radiation was filtered by few or many leaves, respectively, of the taller neighboring plants. In this pattern, the angle of incidence of radiation is still embedded. Depending on the radiation incident on the different leaves of plant 1 (taking into account the number of leaves that act in the interception of the different radiation patterns and the time of incidence) changes will occur in the balances of active photoreceptors in different parts of the treetop, and so plant 1 can predict the distance and height of neighboring plants and may come to make decisions regarding the establishment of shade avoidance responses.

A quick response to the low R:Fr ratio condition is the expression of genes for phytochrome interaction factors, *PIFs 1,3,4,5* and *7*, which code for helix-loop-helix proteins that act as transcription factors of shade-responsive genes. In the shade condition, unfavorable for the action of phyB, there is release of its repressive control over the referred PIFs, which start to act positively for synthesis or for the action of hormones, such as auxins and gibberellins that regulate the accelerated growth of stem cells.

In studies with young plants, it is observed that in addition to the regulatory control of the PIFs, there is a circadian regulation of the elongation of the hypocotyl (Zou et al., 2020), which depends on the activity of the photoreceptors of the zeitlupe family, which, in turn, are involved in the activity of TOC1 and PRR5 in the dark condition, and in this way, preserve the natural oscillation of the growth of the hypocotyl. See details about the circadian clock in the section “Light Perception”, Chap. 1.

According to the information above, it is evident that all classes of known photoreceptors are involved in the perception of, and response to shade by plants.

An example of shade prevention was observed by Afifi and Swanton (2011) in maize, with ryegrass plants as neighbors. The low R:Fr ratio radiation reflected by the ryegrass plants promoted less development of the maize roots, as well as a high aerial part mass:root part mass ratio, showing the greater investment of the maize in aerial part growth to quickly overcome the risk of shading.

In tobacco and mustard, as the lateral distance between plants decreased, the R:Fr ratio was smaller, as well as the Pfr:total phytochrome ratio. A limit distance for a sudden change in response to the shade was around 30 cm, which indicates that the plant not only perceives the neighbor, but can distinguish the distance between them, and, when appropriate, trigger mechanisms for avoiding the shade, even before effective shading (Smith et al., 1990). Similar effect of response to patterns of light transmitted or reflected by neighboring plants was observed in other species by Ballaré et al. (1987) even before any effect of light on leaf photosynthesis was detected.

The study by Orr et al. (1996), once again highlights the role of light as a sign of communication between plants. Those authors observed that the growth of *Cuscuta planiflora*, under white light, was directed to regions with a low R:Fr ratio, indicator of shade by leaves, which, presumably, was the place where a possible host for this parasitic plant would be.

Summary

The radiation pattern received by the leaves acts as a sign of the presence and distance of screens. When the screen is leaves from other plants, the radiation arrives depleted in the R:Fr ratio, and in blue and UVB, which discourages the activity of photostable phytochrome and cryptochrome photoreceptors, as well as UVR8 in the receiver plant. The closer the screen is, the more severe the depletion of the aforementioned radiation is, as well as the greater the change in photoreceptor activity, which serves as a distance parameter from the neighboring organism. The angle of incidence of the radiation in its various patterns, as well as the time of permanence of the incidence of these patterns on the canopy or part of the canopy of a receiving plant, serve as signals to predict the risk of shading, which can induce the plant to change its growth behavior, either by increasing the

growth rate of the main branch, reducing the growth rate of lateral branches, or even changing the growth projection of the stem apex, in order to stand out or avoid a possible prolonged shading in the course of the day. In this sense, the radiation pattern, modified by the neighbors, is used by plants as a means of communication safe enough for them to make decisions that favor their development.

CHAPTER 3

INTRACORPOREAL COMMUNICATION IN PLANTS

It is fascinating that an organism, which can be enormous in stature, has a functional and interdependent harmony among its organs, even if they are quite spatially distant, without a central command and regulation organ, as is the case of the plants. Harmonic functionality would not be possible, not only among organs, but also among tissues and cells, without an effective and fine-tuned communication among the different structures.

Plant hormones have long been considered important substances in plant communication over long-distances. In the last decades, other substances, with hormonal potential or not, but that act as chemical communication signals have been discovered. In addition to chemical signaling, there have been advances in the discovery or understanding of the importance of other forms of communication, such as electrical and hydraulic ones.

The importance of communication is not restricted to the intercellular level, but considering the eukaryotic, and therefore compartmentalized condition of the plant cell, its homeostasis depends on a refined communication among its different compartments.

The most well-known mechanisms of intracellular and intercellular communication will be explored in this chapter. The putative mechanism of communication through neurotransmitters, object of study of plant neurobiology, will also be approached.

Intracellular Communication

A eukaryotic cell, such as a plant cell, is separated into compartments, so that incompatible reactions can occur at the same time in different locations. For that, it is essential that healthy biomembranes exist to promote the separation among compartments. On the other hand, the different environments cannot be completely isolated as they are interdependent, requiring exchange of information. Intracellular communication is fundamental to the plasticity of the metabolism, which is responsive to various factors of the environment, such as light, infections, mechanical impacts, temperature, herbivory, among other stimuli, which require adjustments in the interorganellar signal exchange.

The membranes that delimit cell compartments must, at the same time, retain what should not be mixed, so that the specific metabolism of each compartment is maintained, and allow the entry or exit of informational signals for metabolic readjustment, to maintain the homeostasis of the cell as a whole. Commonly, membrane permeability to chemical signals occurs through selective transmembrane transport proteins, which are key elements in the success of cellular communication. Because of this, it is not surprising that approximately 55% of biomembranes are of protein composition and that no less than 12% of the plant genome is dedicated to the encoding of transport proteins, and that the active transport of substances requires on average 36% of ATP from respiration.

In this section, the most well-known mechanisms for communication between different cellular compartments will be explored.

Chloroplast-mitochondria-nucleus communication

More than 3,000 plastid proteins are encoded in the nucleus, which requires an intense molecular transit between the two compartments, despite the chloroplast originating from an endosymbiosis, and therefore having its own DNA, which also contributes to the formation of elements of the photosynthetic apparatus. The transit of substances is fluctuating, and the variation in their behavior can serve as an information signal. Environmental conditions, such as stresses, light/dark, water and CO_2 availability affect the photosynthetic activity of the chloroplast, resulting in different nuclear demands. Therefore, there must be a communicator between these two organelles so that there is synchronized expression of their respective genes that encode for the proteins involved in the photosynthetic process. In this case, the regulation signal must go from the chloroplast towards the nucleus, what is called retrograde signaling. It is believed that the content of soluble carbohydrates in the cytoplasm acts as an information signal, which has a direct relationship with photosynthetic rates. For this, carbohydrates need to be perceived by different cell compartments. It is already known that high rates of cytoplasmic glucose suppress the expression of photosynthetic genes expressed in the nucleus, and that hexokinases are found not only in the cytoplasm, but can also enter the nucleus, in addition to acting in chloroplasts and mitochondria, which makes them strong candidates as interorganellar communicators (Häusler et al., 2014). Another support for this hypothesis is that mutants defective in hexokinases do not have photosynthesis affected by the accumulation of cytosolic carbohydrates, that means they are participating in the sensory apparatus.

Retrograde signaling from the mitochondria to the nucleus is also necessary for the expression of approximately 1,000 nuclear genes that encode for mitochondrial proteins, just as anterograde signaling (from the nucleus to the mitochondria) is necessary for interdependent functional balance. Since respiratory rates can be altered by different stimuli, it is essential that there is effective communication between these two organelles to synchronize the expression of their genes.

As a reference for communication studies in mitochondria, there is the activity of alternative oxidases (AOX), taken as a reference of what is happening in the respiratory process. Products resulting from or parallel to the activity of these enzymes are references for biomolecules that can cause changes in other organelles from the mitochondria. For example, the ABI4 transcription factor (ABSCISIC ACID-INSENSITIVE-4) keeps *AOX1* suppressed under normal environmental conditions. However, in the presence of abscisic acid, in a characteristic stressful situation, the repression of ABI4 is suppressed, and gene expression occurs. In addition to transcription factors, components involved in signal transduction such as KIN10 (catalytic subunit of a protein kinase) and CYCLIN DEPENDENT KINASE E;1 (CDKE;1) also participate in signaling. CDKE;1 is required in the expression of *AOX1*, just as it interacts with KIN10 in the nucleus, which interacts with ABI4 in the cytoplasm; that means it is a molecule that could perfectly participate in the coordinated interaction between the activity of these two compartments. And not only that, CDKE;1 is also required for the perfect coupling of chloroplasts' redox reactions. This time, it is a molecule that can integrate communication between three compartments with interdependent activities. In addition to this integration, KIN10 is known for regulating the metabolism of sugars and ABI4. Other evidence of the *AOX1* repressor ABI4's involvement in intracellular communication is that it is regulated by transcription factors that have transmembrane domains, and that by relocating from chloroplasts to the nucleus it can activate the expression of its genes. ABI4 is cell regulated by the action of the proteasome 26S and can therefore be destroyed when it is no longer needed. All these interactions reinforce the role of carbohydrates in signaling between different cell compartments, as previously mentioned not only due to the putative action of hexokinases, but also through several other biomolecules.

In Figure 3.1 there is an illustrative summary of the intricate participation of several biomolecules that integrate interorganellar communication considering carbohydrates as the chemical signaling agents.

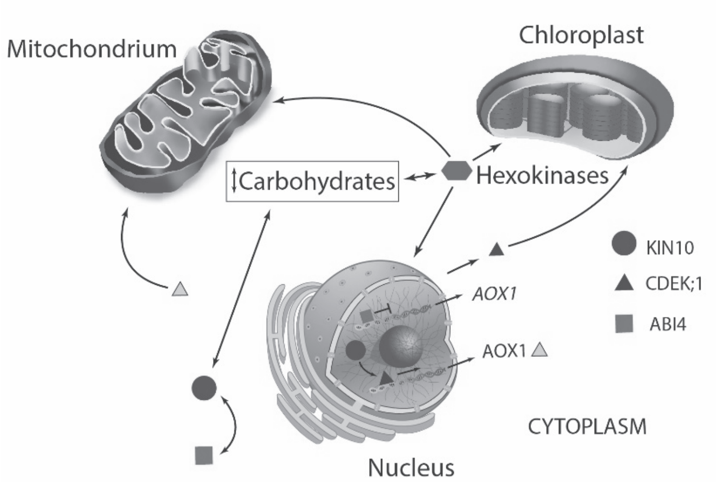

Figure 3.1. Molecules most likely to act as interorganellar signalers in carbohydrate-mediated communication. Hexokinases regulated by the content of cytoplasmic carbohydrates can influence activities of the chloroplasts, nucleus and mitochondria. Transcription factors, such as ABI4, can suppress the expression of nuclear genes that encode for the alternative oxidases (AOX1) of mitochondria. ABI4 also interacts with the signal transduction element KIN10, which is responsive to carbohydrate metabolism, which, in turn, also interacts with another signaler, CDEK;1, which acts as a positive transcription factor for *AOX1* and in couplings redox occurring in the chloroplast.

A by-product of sulfur assimilation, PAP (3'-phosphoadenosine 5'-phosphate), is another strong candidate for the chloroplast/mitochondria-nucleus communicating signal, as it acts as a retrograde signal from both organelles to the nucleus. It is also inferred that the mitochondria interfere in signaling of the chloroplast to the nucleus through it.

Linked to AOX activity is the redox state of oxidative phosphorylation, which may give rise to many ROS in the mitochondria. This can occur more frequently when there is a high $NADH/NAD^+$ ratio and when the ubiquinone pool is very reduced (poorly oxidized). As is already well known in several other situations, ROS themselves can act as secondary signals. Thus, they are candidates to be protagonists in mitochondria-nucleus communication, despite their short activity time. Although it is not known how, there is evidence that the nitric oxide generated in the mitochondria regulates mitochondrial transcripts in the nucleus. The same is true for H_2O_2, however, as this ROS is produced in several compartments, its organellar origin is unclear, but it seems to have greater ease of transit through the membrane than superoxide radicals, but this does not guarantee that it is the direct communicative signal at the nucleus.

The NADH/NAD$^+$ balance itself, as well as the ATP content, can act as indicators of the mitochondrial state that influences the expression of nuclear genes for transcripts that will act in the mitochondria. It is considered that many proteins that act in common in the nucleus and mitochondria, involved with gene expression or redox balance, such as some involved in the ascorbate-glutathione cycle, can participate in intracellular communication. Several other biomolecules, including plant hormones, in addition to the abscisic acid reported above, have been studied as potential interorganellar communicators, but still without secure information about their effectiveness (Schwarzländer and Finkemeier, 2013; Ng et al., 2014).

Many mitochondrial transcripts have circadian regulation, requiring a communication factor with the biological clock. The CHE transcription factors (CCA1 HIKING EXPEDITION) have been the strongest candidates for this interactive task with the oscillator TOC proteins, given the synchrony in the abundance of both transcripts. For details on the circadian clock see the section “Light perception”, Chap. 1.

Chloroplast-peroxisome communication

The greatest evidence of the communication between the chloroplast and the peroxisome comes from the increase in the metabolism of glycolate in the peroxisome from the increased oxidase reaction of the enzyme RUBISCO in the chloroplasts. In addition, a communication between these organelles is presumed to be related to the production and elimination of ROS, as well as to the synthesis of jasmonic acid. Using more modern techniques for detecting the position of organelles, such as probes for specific organellar elements, associated with fluorescence microscopy, a constitutive link between chloroplasts and peroxisomes has been perceived that would facilitate communication through MCS (Membrane Contact Sites) and they will also be covered in this section.

Endoplasmic reticulum-Golgi communication

The vesicular transport of many substances, such as glycoproteins, depends on the interaction between the endoplasmic reticulum and the Golgi complex since proteins are produced in the endoplasmic reticulum and then transported for processing in the Golgi complex for later vesicular transport. It has already been observed that both structures can associate and disassociate, and can remain connected even with cytoplasmic movement, which can facilitate communication. It is known that the Golgian protein CASP is necessary for the association between these two organelles.

Endoplasmic reticulum-peroxisome communication

While oily bodies are produced in the endoplasmic reticulum, their respiration depends on β-oxidation that occurs in special peroxisomes (glyoxysomes), which highlights the need for communication between the two organelles. The organellar extension of the peroxisome (*peroxules*, to be explored in this section) meeting the endoplasmic reticulum seems to be important from a communicative point of view. Although lipid bodies associated with peroxisomes are observed under a decrease in cellular carbohydrate, the presence of the endoplasmic reticulum in the interaction is still unclear.

Endoplasmic reticulum in intracellular communication

The distancing or proximity of organelles in the cell can hamper or contribute to communication among them. Membrane Contact Sites (MCS) are ubiquitous structures at the membrane interface between organelles that facilitate the exchange of signals for maintaining cellular homeostasis (Wang and Dehesh, 2018).

The membranes of the endoplasmic reticulum (ER) are functionally connected to the membranes of the other organelles, also participating in the transport by vesicles and the recycling of membranes by fusion. The contact of the ER with other organelles occurs in the MCS, which is an interaction different from the vesicular one (Figure 3.2). The MCS are not simple points of random contact between organelles, but are characterized by a transient membrane semi-fusion, with the contact site rich in lipids and or specific proteins necessary for the membrane junction or the transfer of interorganellar signals, in addition to functional change and or chemical composition in at least one of the adjacent membranes.

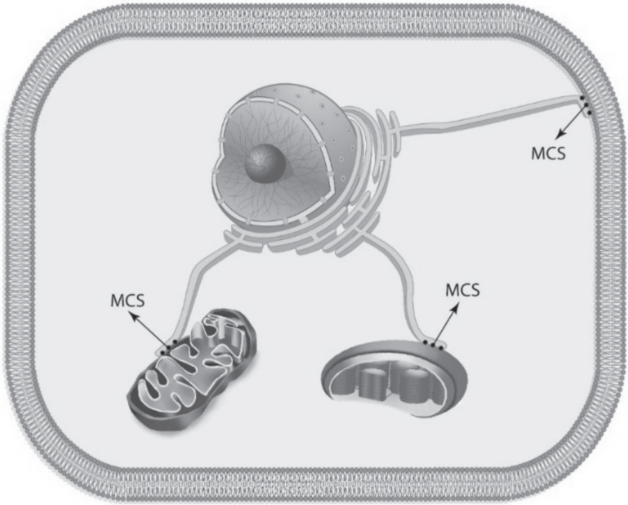

Figure 3.2. Representation of Membrane Contact Sites (MCS), which are special points of junction of the membrane of the endoplasmic reticulum with other membranes, such as plasma, chloroplasts and mitochondria ones.

A putative binding protein between ER and mitochondria in MCS is known as MELL1 (MITOCHONDRIA-ER-LOCALIZED LEA-RELATED LYSM DOMAIN PROTEIN 1). MCS between ER and plastids are known, although there are no proteins identified in the bond, but there is, nevertheless, a lipase, the acylhydrolase CLIP1 (CANDIDA LIPASE 1) that is considered a candidate for lipid transfer. At the contact points of the ER to the plasma membrane, MCS anchoring proteins are known, such as NET3C (NETWORKED 3C), which is part of a family of actin-binding proteins (NET), VAP27 (VESICLE-ASSOCIATED PROTEIN 27) and the actin microfilament and microtubule network itself.

It is known that fatty acids are synthesized in chloroplasts and subsequently transported to the ER where the synthesis of phospholipids and triacylglycerols occurs, an important process to produce material necessary for the formation of membranes in eukaryotic cells. Conversely, ER produces diacylglycerol, which is necessary for the formation of chloroplast membranes. In this case, it is essential that there should be channels of lipid flow between these organelles. Lipid traffic can occur via the vesicular route and also through the MCS, which is evidenced by the fact that the use of vesicular traffic blockers does not interfere with the exchange of lipids between organelles, as well as by the faster delivery of lipids to the plasma membrane from the ER than would occur by the vesicle traffic. Mitochondrial replication could not occur without lipid traffic, as they are unable to produce some lipids necessary for membrane formation.

MCS can also transfer calcium. It is known that ER can store calcium, and, via MCS, there can be a transfer of this ion from ER to mitochondria. The MCU uniport calcium transporter is of low affinity, but functional in the MCS when the ion is highly concentrated.

Calcium in intracellular communication

By exploring the cellular signaling mechanisms for the most different purposes studied so far, we can see that Ca^{2+} is involved in the vast majority. Its movement from the apoplast to the symplast is recurrent in signaling. The reason for the calcium input for the cytoplasm to be so expressive for the cell is because normally its concentration in the apoplast is 10,000 times higher than in the cytoplasm, and it is necessary to keep the cytoplasmic calcium content low for cellular homeostasis. In this case, the sudden increase of calcium in the cytoplasm is indicative that something different and important is taking place. However, the different signals do not trigger the same calcium pathway; there are differences in the transporters that are or are not activated, their cytoplasmic content, their intracellular interactions, the length of stay, as well as the frequency of their entry. Calcium is perceived by different receptor proteins, such as CaM (calmodulin), CML (CALMODULIN-LIKE PROTEINS) and CDPK (Ca^{2+}- DEPENDENT PROTEIN KINASE).

Both chloroplasts and mitochondria have a high capacity to sequester cytoplasmic calcium. The free calcium in the mitochondria is very low, equivalent to that of the cytoplasm, but under conditions of cold, oxidative stress and touch there is a sudden increase in mitochondrial calcium, coinciding with an increase in the respiratory rate. There are several calcium transporters found in the mitochondria. Some are from the MCF (Mitochondrial Carrier Family), exclusive to mitochondria, indicating different signaling pathways from other cellular compartments. Although specific calcium signaling mechanisms are not known in the mitochondria, it is known to act as a signaler since during hypoxia there is an efflux of calcium from the mitochondria, and when the environment is reoxygenated there is an influx of calcium in the mitochondria. Also there is a release of mitochondrial calcium when actin filament rupture occurs (Ng et al., 2014; Nomura and Shiina, 2014).

Chloroplast accumulates high concentrations of calcium, but not free; most of it is bound to proteins. Its role as a signal is evidenced when the dark condition promotes influx of calcium into the chloroplast, triggering nocturnal responses, as well as when biotic and abiotic stresses increase the content of free calcium in the plastid. As with mitochondria, it is unclear how calcium waves interfere with the physiology of the chloroplast.

Organelle movement and intracellular communication

The cytoplasmic flow apparently promotes a random movement of approach or distancing among organelles, although in some cells the movement patterns are well organized. The movement is admittedly dependent on the dynamics of the actin filaments, which includes their polymerization or depolymerization and slips. In addition to actin, the endoplasmic reticulum (ER) network appears to have a marked impact on the flow of cytoplasmic mass. As mentioned in the previous topic, there is interaction of the ER with the actin filaments. Perico and Sparkes (2018) showed that, in addition to the natural flow of cyclosis, small organelles, such as Golgi, mitochondria and peroxisomes, have autonomous movements, known as stop-go, with rapid acceleration, deceleration, pause and change of direction. Microtubules seem to be involved in the anchoring of the ER to the plasma membrane, and the points of their interaction with the actin filaments can act as a mechanical barrier forcing the pause of the movement of the organelles.

Motor proteins associated with the cytoskeleton, such as myosin, also participate in cytoplasmic movement. The N-terminus of myosin is attached to the actin filaments, while the C-terminus can attach to a structure to be pulled along its movement, such as organelles. Considering that the communication between the organelles depends on the MCS (Membrane Contact Sites) and that there may be autonomous movement of the organelles, their approach can occur through the cytoskeleton. It remains to be seen how organelles influence the movement of the motor proteins associated with them, or whether the movement occurs independently of the cytoskeleton. In addition to myosins, kinesins are also motor proteins and appear to be associated with microtubules participating in a more subtle and short distance movement of organelles. Some studies have also shown integration of both motor proteins in movement events.

The approaching movement between the nucleus and the chloroplast seems to be necessary, for example, to signaling mediated by ROS, since the increase in radiation that alters the levels of chloroplasts' ROS directly interferes in nuclear gene expression, as long as there is an MCS link between the two organelles.

Photoreceptors in intracellular communication

A classic light response mediated by photoreceptors in cells is the movement of chloroplasts, which are periclinally grouped to the cell wall directly exposed to the low radiation condition (Figure 3.3), in a clear sense of optimizing photon capture, which we know as an accumulation response. On the other hand, the avoidance of light by chloroplasts occurs when the high radiation exceeds a threshold, becoming harmful. In this case, the chloroplasts are grouped anticlinally to the walls directly exposed to radiation, parallel to the incidence of light (Figure 3.3), to avoid or reduce photo-oxidative damage.

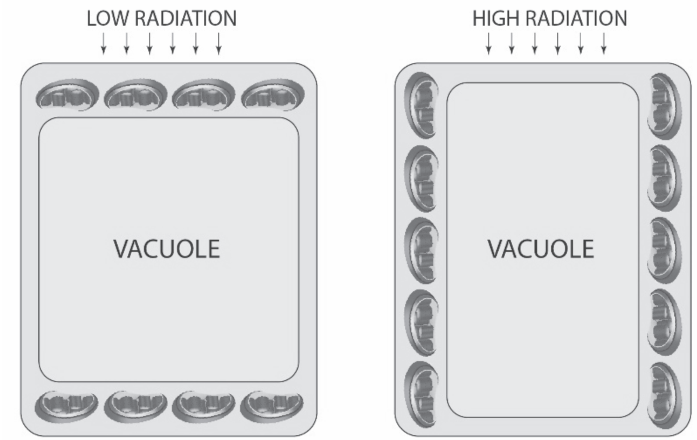

Figure 3.3. Front view diagram of the position of chloroplasts in the plant cell according to incident radiation. Under low radiation the chloroplasts are positioned periclinally to the cell wall directly exposed to radiation (figure on the left), to maximize the absorption of light. Under high radiation, chloroplasts position themselves anticlinally to the cell wall exposed directly to radiation (figure at right), in order to avoid photo-oxidative damage by excessive light absorption.

Phototropins are the photoreceptors that mediate the movement response of chloroplasts to light, which in this case is influenced by the light spectrum in the blue band. Using mutant arabidopsis, it was concluded that both the photoreceptors, phot1 and phot2, mediate the accumulation response, but phot2 is the mediator of the avoidance response. Under high radiation, phot1, which is photolabile, is destroyed (see topic “Plants perceive the condition of full sun and shade”, Chap. 1), leaving phot2 as the photoreceptor responsible for the avoidance response. Like chloroplasts, the avoidance movement of the nucleus at high intensity of blue light also occurs and is mediated by phot2.

In *Physcomitrella patens*, blue and red light (R), with the R effect being reversed by far-red (Fr), are responsible for the movement of chloroplasts. For the response to R radiation, the mediating photoreceptors are phytochromes, and for blue, they are phototropins. However, in mutants in phototropins there is no effect of R light, indicating that phytochromes can modulate the action of phototropins, but they do not replace them (Kasahara et al., 2004).

As discussed in the previous topic, organellar movement is linked to the cytoskeleton. However, since the photoreceptors perceive the light condition of the environment, there needs to be a regulation of the cytoskeleton mediated by the photoreceptors for the light-dependent movement (Figure 3.4).

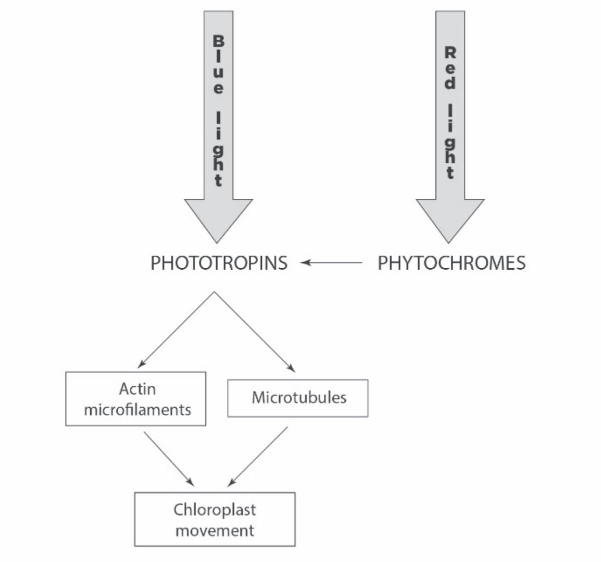

Figure 3.4. Influence of photoreceptors on the movement of chloroplasts. Blue light, mediated by phototropins, regulates the activity of the cytoskeleton, which in turn induces the movement of chloroplasts. Red light does not replace blue, but active phytochromes positively modulate the action of phototropins. Modified from Kasahara et al. (2004).

Retractable projections of organelles in intracellular communication

The classic image of stationary organelles from dead cells usually seen in didactic material does not give us the real picture of the living cell. With the advent of molecular probes, dyes and images obtained by fluorescence, we now have the possibility of monitoring the movement of organelles in living cells. Using images obtained by this technique, it was discovered that some organelles create transient projections, and, consequently, retractions of these projections, which implies a greater contact area as they are projected in the cytoplasm, which facilitates the communication processes dependent on the exchange of signals with the medium for maintaining cellular homeostasis.

The organellar extensions have a diameter of 0.3 to 0.8 μm and a length that can reach up to 70 μm (Mathur et al., 2012). The permanence of the extensions occurs according to the permanence of its elicitor factor, which can take hours or seconds. The elongation of the projections is linked to cytoskeletal organization since in the event of inhibition of actin there is also inhibition of the projections.

Sporadically, vesicles, vacuoles and nuclei can produce tubular extensions. However, projections are more common on other organelles. When they occur in chloroplasts, they are called *stromules*, when in peroxisomes, *peroxules*, and when in mitochondria, *matrixules*. Some of these projections can be seen in Figure 3.5. The formation of organellar extensions can facilitate a possible communication by MCS (Membrane Contact Sites) between two organelles that are moving at different speeds. Through *stromules* there can also be communication between different chloroplasts, which inevitably generates curiosity as to whether there is synchronized coordination of action between them due to different stimuli, although there is still no evidence for this.

Jaipargas et al. (2016) observed that the *peroxules* increase as there is an increase in the H_2O_2 content due to high radiation stress and that there is an increase in the interaction of these structures with the mitochondria when compared to the non-stress control treatment, which is an indication that *peroxules* act as oxidative stress relievers for mitochondria, because the characteristic of peroxisomes is having greater potential to combat these ROS.

The *stromules* have an increase in formation and duration in the presence of light, retracting in the dark condition. Their formation is expected to facilitate communication with peroxisomes and mitochondria.

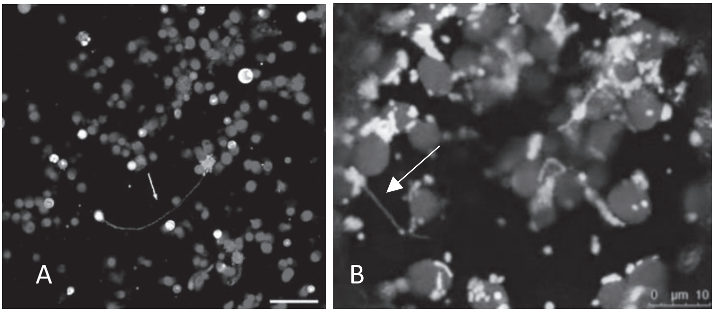

Figure 3.5. Retractable organellar extensions seen in living plant cells. 3.5A. *Stromules* present in *Nicotiniana bhentamian* chloroplasts, corresponding to 109 nm in length (indicated by the arrow). Bar corresponding to 30 µm. 3.5 B. *Peroxules* present in cotyledon cell peroxisomes. The arrow indicates the *peroxule* (varying in the 8-15 µm in the video), interacting with mitochondria. Big organelles are chloroplasts. Credits: 3.5A Image taken from a video by Ho J, Theg S from Wikimedia Commons. License to use at https://commons.wikimedia.org/wiki/File:The-Formation-of-Stromules-In-Vitro-from-Chloroplasts-Isolated-from-Nicotiana-benthamiana-pone.0146489. s001.ogv 3.5B Image taken from video by Jaipargas E, Mathur N, Bou Daher F, Wasteneys G, Mathur J from Wikimedia Commons. License to use at https://commons.wikimedia.org/wiki/File:High-Light-Intensity-Leads-to-Increased-Peroxule-Mitochondria-Interactions-in-Plants-Video5.ogv

Summary

Intracellular communication is necessary to maintain cellular homeostasis. Because the plant cell is eukaryotic, it maintains different incompatible metabolic events occurring simultaneously in different compartments separated by membranes. However, the different compartments are interdependent and require constant adjustments depending on the stimuli that reach the cell, thus requiring refined inter-organellar communication, without compromising the homeostasis of the cell as a whole. There are several chemical signals that can act to ensure harmonic synchrony in the metabolisms of the different compartments, such as hexokinases and gene transcription factors or elements of signal transduction pathways that act on carbohydrate metabolism. Products of sulfur metabolism, ROS or reducing power are also likely elements that act in the communication between the different compartments.

The different transient wave patterns of cytosolic Ca^{2+} are crucial to the metabolic adjustments that always occur. Photoreceptors in interaction with the cytoskeleton also participate in organellar movements according to stimuli. The contact between different organelles with the endoplasmic reticulum, through MCS (Membrane Contact Sites), which are specific and modified regions of biomembranes, facilitates interorganellar communication. In addition, retractable organellar projections enlarge the membranous surface for probable signal exchanges with the cytoplasmic environment or with membranes of other organelles.

Intercellular Communication

The communication among cells, tissues and organs is what guarantees the functional harmony of the organism as a whole. It is surprising how big plants, some of which, such as sequoias, reach 100m in height, can coordinate functional and interdependent synchrony among their organs, which may be meters apart, and do so without a central command organ.

For a long time the idea of communication was restricted to chemical signaling. Although it is of unquestionable relevance, it does not comprehend the totality of the communication apparatus of plants since the perception of some signals by plant organs goes far beyond the speed of movement of chemical substances. In the last decades, the discovery of electrical and hydraulic signaling has enabled the broadening of the understanding of plant communication, especially ultrafast and long-distance communication.

Throughout this section, the different mechanisms known in intercellular communication will be addressed. In addition to the mechanisms already elucidated and well known, another putative mechanism that has received attention from many scientists, that is, communication through neurotransmitters, will be discussed. The occurrence of canonical animal neurotransmitters in plants is intriguing, since in animals they are associated with nervous tissue, typical of communication, which does not occur in plants. Considering the hypothesis that they could be linked to plant communication, neurotransmitters will be approached, highlighting the response of plants to their presence, and then they will be explored as to their potential for action in the field of communication.

Neurotransmitters In Plants

Many classic neurotransmitters known in animals and microorganisms are also found in plants, which makes them apparently ubiquitous in living beings. Long lists of their occurrences in vegetables can be found in the work by Roshchina (2001).

Acetylcholine, dopamine, histamine, norepinephrine, adrenaline, serotonin, melatonin, glutamate and GABA are present in plant tissues (Figure 3.6). Hypothetically, they can be used by plants as informational signs, just as in other living organisms, however, until a few years ago, the report of their actions showed them more as growth regulators than properly as communication signals, even though regulators can have that function. The presence or content of neurotransmitters varies between species, organs, age and environmental conditions.

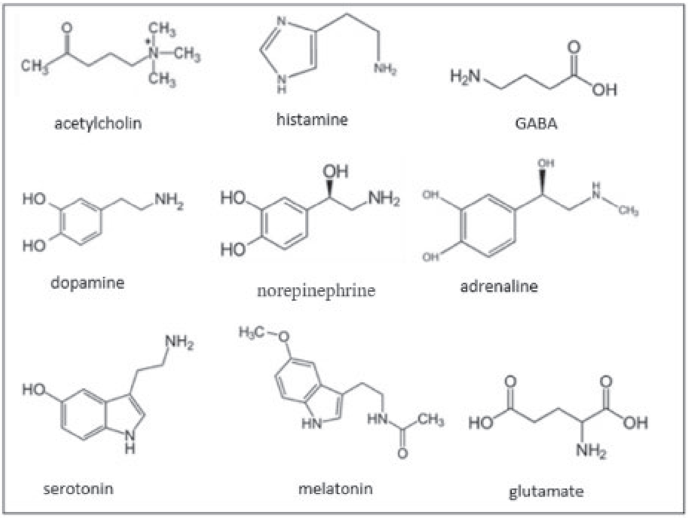

Figure 3.6 Animal neurotransmitters found in plants.

The action of these substances as plant neurotransmitters is considered a possibility, especially when they act on electrical potentials in biomembranes and when they trigger signals from their presence in the apoplast. Some other biomolecules, not considered to be neurotransmitters in animals, also play an important role in the studies of plant neurobiology, as will be discussed.

The location of some neurotransmitters in the plant cell was detected in a fluorescence study carried out by Roshchina et al. (2016). Catecholamines (dopamine, adrenaline and norepinephrine) were observed in the nucleus, chloroplasts and vacuoles, and histamine in the nucleus and chloroplasts. Neurotransmitter antagonists have also been observed, with d-tubocurarine (which binds to the acetylcholine receptor), yohimbine (which binds to the dopamine receptor) and inmecarb (which binds to the serotonin receptor) present on the surface of the plasma membranes, tonoplasts and membranes of nuclei and chloroplasts.

Acetylcholine

A classic effect of acetylcholine, present in nettle trichomes, is the triggering of pain, irritation and blistering when it contacts human skin. Derived from acetyl-CoA and choline, the synthesis of acetylcholine is regulated by phytochromes since it responds to the photoreversibility of R and Fr radiation. Studies that investigate the effects of acetylcholine and the influence of phytochromes together on plant responses are recurrent. Its stimulating action in plant development processes occurs in a synchronized manner with the effects of a high percentage of Pfr on the plant body. They are inactivated by the action of ACETYLCHOLINESTERASE.

The influence of acetylcholine on plant physiology is linked to its ability to alter the electrical potentials of biomembranes, causing rapid changes in the transmembrane flow of ions. Several studies have shown its role as a growth regulator, observed especially in seedling tissues. This effect can be proven using acetylcholine blockers, resulting in reduced seedling growth, which shows their participation in the remobilization of the seed reserve tissues. Among several effects, there is evidence of its participation in stimulating the growth of the pollen tube or acting in the regulation of plant-diazotrophic bacteria adhesion. Its production coincides with the peak of biological nitrogen fixation in the legume-bacteria interaction (Tretyn and Kendrick 1991; Bamel and Gupta, 2018; Akula et al., 2020).

In an experiment by Di Sansebastiano et al. (2014) it was observed that acetylcholine and auxin induce an increase in transcripts of the gene encoding expansin EXPA2 in tomatoes. The use of acetylcholine antagonists reduced its expression, showing its inductive role in cell expansion. However, its performance was more effective when combined with auxin than when isolated, which indicates a synergistic action between these two substances. Still, in fluorescence studies, it was observed in tomato cells that both the use of auxin and acetylcholine promoted the appearance of small vesicles at the base of the cells, and that the use of endocytosis inhibitors reduced the number of these vesicles to almost zero. This observation indicates a cell-to-cell transport, both of acetylcholine and auxin, mediated by endocytosis, a recurrent observation in several studies and a characteristic mechanism of chemical synapse in animal tissue, and therefore reinforces the role of acetylcholine as well as auxin as possible plant neurotransmitters.

Still on the synergism between acetylcholine and auxin, it is known that both participate as morphogens (substance that standardizes body plans, regulating tissue development patterns with the proper positions of each cell type during morphogenesis) as well as in the distribution of statoliths and gravity vector perception, as explored for auxin in the section "Perception of gravity", Chap. 1.

Catecholamines (dopamine, norepinephrine and adrenaline)

As monoamines (biogenic amines), dopamine, norepinephrine and adrenaline are derived from nitrogen metabolism, requiring decarboxylation or hydroxylation of specific amino acids such as tyrosine and glutamate, during their synthesis. Inactivation usually occurs when there is methylation. The 14-3-3 protein, which acts in many signal transduction processes in plants, is fundamental to the synthesis of catecholamines due to its binding to TYROSINE DECARBOXYLASE.

Effects of catecholamines on plants have also been observed as growth regulators, and are involved in cytoplasmic movement, as well as in the electric gradient of biomembranes and, consequently, in the transport of ions.

As in animal tissue, Skirycz et al. (2005) observed that catecholamines are associated with carbohydrate metabolism in plants, as whenever there is a decrease in starch and an increase in sucrose, fructose or glucose, a concomitant increase in the expression of TYROSINE DECARBOXYLASE genes and the HD1 dopamine receptor is observed (animal receptor), when present in transgenic potatoes. It has also been observed that gene expression decreases when there is a decrease in the activity of some enzymes such as SUCROSE SYNTHASE, PHOSPHOGLUCOMUTASE and ADP-GLUCOSE PYROPHOSPHORYLASE, the last two being part of the starch synthesis in the chloroplast.

The catecholamines present in vesicles, when they are released and perceived by the nuclear membrane receptors, act in the bioregulation of the division of the nucleus. They participate in intracellular signaling for plant growth and there is evidence of their participation as an electron donor in the chloroplast acyclic photophosphorylation chain. Acyclic photophosphorylation is also stimulated by low doses of dopamine and inhibited by its antagonist, yohimbine (Roshchina et al., 2016).

Dopamine is found in different plant organs and in latex, and like other catecholamines, its synthesis is usually increased after tissue injury and other stresses. Dopamine conjugates, as well as other amines, with hydroxycinnamic acid, increase greatly during the contact of pathogens with the plant cell, and as the location of the conjugate is the cell wall, a physical barrier effect against infection is suggested (Newman et al., 2001). Adrenaline conjugates, such as *p*-coumariladrenaline, have also been associated with resistance to pathogens.

At least in laboratory tests, dopamine has an antioxidant action with a potential similar to ascorbic acid and greater than some flavonoids. It also regulates the route of phenylpropanoids, modulating the activity of PHENYLALANINE AMMONIA LYASE, in addition to being reported as an inducer of SUPEROXIDE DISMUTASE activity, being effective for reducing ROS and lipid peroxidation. Both dopamine and adrenaline, in vitro, act as mediators of oxygen reduction in chloroplasts (Allen et al., 2003). More than 170 stress-associated genes are regulated by dopamine, including transcription factors for genes encoding ethylene response factors such as NAC and EFR, as well as calcium/calmodulin-dependent signaling genes and CYCLIC NUCLEOTIDE GATED CHANNELS (CNGC) channels.

Catecholamines, as well as acetylcholine, seem to positively modulate the action of auxins in stimulating cell growth, as well as acting in the inhibition of AIA-OXIDASE. They also appear as important suppressors of floral inhibitors.

In addition to stressful situations, norepinephrine is present in pulvinus motor cells, which respond to mechanical stimuli to close leaflets, although its importance in this process has not yet been elucidated. It has also been associated with oxidative processes in ripe fruits, a time of high accumulation of this substance (Roshchina, 2001; Kulma and Szopa, 2007; Akula et al., 2020).

Histamine

Histamine is a biogenic amine derived from the histidine decarboxylation process, and in addition to methylation it is also inhibited by acetylation. Like acetylcholine, it is well known for integrating the anti-herbivory irritants present in the nettle, causing pain and irritation in human skin. It is a causative agent of allergies when present in pollen, in which it participates in its germinative regulation. There is evidence of its performance as an allelochemical influencing seed germination (Roshchina and Yashin, 2014). The major concern with histamine in plant tissue, present in the scientific literature, has been regarding its potential for toxicity or to cause allergies in humans when it is present in food.

Serotonin and melatonin

Serotonin is derived from tryptophan, requiring actions of decarboxylases, which are modulated by light, temperature and stress. The role of serotonin on plant growth is still unclear, but it is known to be involved in the morphological regulation of the root apex. Although we do not know details, it is known that there is involvement of serotonin in cell signaling through ROS. Both serotonin and melatonin participate in long-distance chemical signaling in the plant, in addition to salicylic acid, jasmonic acid and nitric oxide, which are already well-known in signaling biotic stresses.

Melatonin is the most bioactive serotonin derivative. Among the substances involved so far in the study of plant neurobiology, phytomelatonin is the first to have a known receptor, CAND2/PMTR1 (CANDIDATE G-PROTEIN-COUPLED RECEPTOR2/PHYTOMELATONIN RECEPTOR) that participates in induction of stomatal closure by Ca^{2+} and H_2O_2 signaling (Wei et al., 2018). Melatonin stimulates the expression of genes encoding antioxidant, stress and defense proteins, such as those related to pathogenesis (PRs) and constitutive immunity (MAPK), as well as heat shock proteins, for ABA, salicylic acid, ethylene and receptors stress-responsive kinases, such as SOSs (Figure 1.16) and calcium signaling. It also stimulates the production of nitric oxide in plants infected by pathogens.

The ability of melatonin to improve plant tolerance to stress, specially salt stress, has promoted the maintenance of high photosynthetic rates and amino acid biosynthesis, even under oxidative stress. Physiological evidence suggests that there is a synergistic interaction between melatonin and cytokinin, such as inhibition of senescence, in addition to stimulating the expression of genes involved in the biosynthetic route of this hormone (Akula et al., 2020).

GABA

GABA (gamma-aminobutyric acid) comes from proline or from the degradation of polyamines. Its action modulates the cellular calcium content, acting, therefore, on signal transduction pathways. The most evident signaling occurs due to the carbon/nitrogen balance regulating the expression of several genes of both metabolisms. It also acts on the activation of malate-dependent aluminum efflux transporters and on membrane polarization, affecting the transport of nutrients. As we will see later, GABA is one of the most highly rated substances acting in the field of plant neurobiology.

Glutamate

Glutamate stimulates the expression of defense and stress genes in plants. It can act on the cell wall as an elicitor of signaling cascades in the cell. When it binds to calcium influx channels, it induces the depolarization of the plasma membrane and the depolymerization of microtubules, and in this way, it acts as an inhibitor of root growth and the formation of lateral roots. It has also been linked to increased tolerance of plants to pathogens. Its performance in the cell wall and in the depolymerization of components of the cytoskeleton makes it a molecule potentially central to the performance of plant neurobiology, just as it is in animal biology.

Summary and considerations

Although the occurrence and effects in plants of different classic neurotransmitters present in animals are known, their receptors or details of their mechanisms of action (with rare exceptions) are still unknown, despite evidence of their respective cellular locations and physiological effects. The use of the term neurotransmitters is due to what is already known from their actions in other organisms, but it does not necessarily mean that they have the same role in plant physiology. Their effects in the development of plants, in addition to the putative action on communication, have shown them to be substances of hormonal potential, involved in the regulation of biotic and abiotic stresses.

The importance of these substances as possible hormones and/or plant neurotransmitters means they need to be studied more. Although their occurrence in plants has been known for decades, only in recent years have they aroused greater interest among scientists of different areas, generating an expectation of advances in the knowledge of their roles in plant biology.

Putative Mechanism of Plant Neurotransmission

Although plant neurobiology is considered a contemporary theme, many of its bases are founded on ancient scientific investigations that are being revisited, using current methodological resources. Although its mechanisms are putative, built from elements of animal neurobiology, it is necessary to address them when we propose to study communication in plants.

Plant organs and tissues are composed of a network of cells interconnected not only by the cell wall, but also by plasmodesms. In addition to these connections, the cell's polarity is of great importance in the transmission of information, a characteristic already well known and explored in the polar auxin transport.

Studies using immunofluorescence of actin filaments have revealed that, through the cell walls, in the polar region of cells, where the intercellular apoplastic transport of auxin occurs, there is an enrichment of actin F and myosin VIII (Baluška et al., 2000). In this region, in addition to the wall being enriched with actin, it also has a high content of pectin, and this is where the plant synapses (stable adhesion point of information retransmission) must take place, where auxin and oligogalacturonides, molecules derived from pectin, act as transmitters in cell-cell communication. Both substances are known to promote rapid electrical responses in plants, triggering rapid calcium transients and influencing the polarization of the plasma membrane, as well as the activity of transmembrane transporters. Auxin and oligogalacturonides seem to act in an antagonistic way in terms of their physiological effects.

Some molecules known in the nervous system in animals are also found in plants and with potential involvement in communication, such as acetylcholine esterase; nitric oxide; ROS; GABA (gamma-aminobutyric acid), glutamate, glycine and endocannabinoid receptors.

In addition to hormones and morphogens, when present in the cell wall, auxin initiates electrical responses through the membrane ABP1 receptor, a response typically caused by a neurotransmitter (Bhalerao and Bennett, 2003). Its transport along the body of the plant is already well known to occur especially through phloem parenchymal cells, in which the entry through the upper pole of a cell occurs through the AUX transporters, and its exit through the basal pole, via PIN transporters. In the previous section, we have already discussed the transport of auxin through vesicles, which is a common form of neuronal communication in animals for other substances. Many research studies have observed that auxin transport inhibitors also inhibit recycling and trafficking of endosomal vesicles. Also, vesicle-defective mutants have auxin transport inhibited, leading to the belief that auxin efflux depends on PIN proteins and vesicular exocytosis (Baluška et al., 2005). Once having a possible size to be transported by plasmodesms but not being so, it is intriguing that its preferential transport occurs through the cell-to-cell apoplast, and since it causes an immediate electrical response when in direct contact with a wall, auxin would act not only on chemical signaling but could also act on the electrical synapse at the meeting points between two cells.

Auxin is known to be responsive to light and gravity stimuli. Its relationship with statoliths, as seen in the section "Perception of gravity" (Chap. 1), reveals its active face in the perception of the gravitational vector.

Therefore, a change in the inclination of the plant in relation to the gravitational vector would lead to a change in the synaptic domains (membrane-wall-membrane) of auxin, altering the site of vesicular exocytosis. The new location of exocytosis added to the new cell bottom where the statoliths are deposited then act as an indicator of the gravitational vector. Since the plasma membrane of the upper pole of the adjacent cell below has low tension, it is a favorable condition for it to receive material, by endocytosis, from the neighboring cell.

SNARE, SNAP (SOLUBLE N-ETHYLMALEIMIDE-SENSITIVE-FACTOR-ASSOCIATION PROTEIN) receptors, which are also sensitive to the gravitational vector, are plasma membrane proteins involved in the transport and fusion of vesicles, being involved in vesicular traffic in the synapse domains (immunological synapse) between the plant cell and the symbiotic or pathogenic microorganisms. An example of its role is as a traffic regulator of vesicles rich in hydrogen peroxide to harm a pathogen. ROS, such as H_2O_2, have already been extensively addressed as a signal in biotic and abiotic interactions of plants in previous sections, although it has not been clarified, so far, how they could act without causing damage or with reduced damage to the plant cell itself, when producing it.

Possible, plant synapses can be useful in explaining recognition processes between the roots of the plants themselves (a topic to be explored in the last chapter) and between plants and microorganisms, such as the entry of mycorrhizal fungi structures in the root cortex, apparently without there being any inhibitory chemical reaction by plant cells. It is hoped that new studies in plant neurobiology and associated areas can also clarify the occurrence of synaptotagmin genes in plants (Craxton, 2004), which are involved in synaptic vesicular exocytosis in animal neurotransmission processes.

Plant hormones and neurobiology

In addition to auxin, abscisic acid also induces an immediate electrical response when applied on the cells, which highlights its action as a possible neurotransmitter. It is even inferred that it has a role in the regulation of the animal nervous system, since it has already been found in its active form in the brain of pigs and rats (Le Page-Degivry et al., 1986). Salicylic acid is also known as an activator of MAPKs (MITOGEN-ACTIVATED PROTEIN KINASES) previously addressed in the topics of plant defense and immunology, which are activated by voltage pulses. Ethylene, on the other hand, is an anesthetic active in animal tissue (Campagna et al., 2003), and as such, it could induce the activation of mechanosensors in roots, and as it does, reduce root growth, just as other volatile anesthetics do in plants, such as menthol and camphor.

Plant electrophysiology and neurobiology

In the case of electrical synapses, in a study with *Mimosa pudica*, it was noticed that the flow of electrical current occurs in a bidirectional way between the flexor and extensor sides of the pulvinus, which is enabled by the plasmodesms connection (Section “Touch perception”, Chap. 1). The electrical coupling between vascular bundles and the tissues interconnected by the plasmodesms network allows the pulvinus to have properties similar to the electrical synapses of animal neurons (Volkov, 2017). For transmission of electrical signals, unlike animal nerve tissue, plants do it via phloem, especially through companion cells and sieved elements, for long-distance communication (as will be discussed in the next section), while the membranes of parenchymal cells interconnected by plasmodesms are important for transmission of signals over short distances.

The initial influx of Ca^{2+} mark the start of the transmission of the action potential throughout the vascular tissue of plants. While in animals there is transmembrane activity of Na^+/K^+ transporters in axons, in plants there is activity of Ca^{2+} and K^+ cation transport channels at synapse points during the transmission of the action potential.

Summary

There is evidence that auxin and acetylcholine are transported by vesicular chemical synapses between adjacent cell poles, a behavior similar to what occurs in chemical synapses in animal tissues. In addition, the presence of auxin, abscisic acid, oligogalacturonides, catecholamines, acetylcholine and glutamate, when present in the cell wall, trigger electrical responses in cells, a response typically induced by neurotransmitters. The movement of electrical signals in the tissues depends on points of electrical coupling, guaranteed by plasmodesms that interconnect the cell symplast between neighboring cells. Action potentials can diffuse over long-distances through sieved elements and phloem companion cells, whose signaling depends on transporters for Ca^{2+}, K^+ and Cl^-, unlike animal tissues that depend on Na^+/K^+ transporters.

Electrophysiology in Plant Communication

This section is dedicated to exploring the mechanisms through which electrical communication between tissues and organs in a plant occurs.

The electrical circuit in the plant connects the organ or the phytosensor tissue to the phytoactuator through the memristor. An electrical potential can be formed in cells of a certain organ, from various stimuli, and result in a flow of electrical current capable of reaching the phytoactuator, triggering a physiological effect, which will depend on the perception mechanisms, the electrical coupling between sensors and actuators, as well as the specific amplitude, duration and frequency of the electrical signal.

The action potential is active and propagates through interconnected membranes with constant amplitude, duration and speed. However, if the potential is passive, and therefore electrotonic, it loses amplitude exponentially with the distance covered. Potentials can propagate over short distances between parenchymal cells across plasma membranes interconnected by plasmodesms, and over long-distances through phloem, in which the plasma membranes of neighboring cells are also interconnected by plasmodesms.

Role of plasmodesms and phloem in the electric current

For many decades, electrical current transmission via plasmodesms has been known, and there is even a positive correlation between the number of plasmodesms and the electrical conductance between an injector point and a receiving point.

The phloem endings, either at the source or in the sink, are surrounded by parenchymal cells from the fundamental meristem, and it is expected that in most situations where there are electrical stimuli, these will start in the parenchymatic cells, so that only later do they reach the companion cells-sieved elements (CC-SE) (Figures 3.7C and 3.7D) of the phloem, mediated or not by the phloem parenchyma. For that, there needs to be an electrical coupling between the fundamental parenchyma and the phloem, which occurs through the plasmodesms that interconnect the cells. However, during the path of the electric current over a long-distance it is not interesting for the phloem cells to communicate with those of the fundamental parenchyma. In this case, the isolation of the vascular bundles usually occurs by layers of fibers in its surroundings (Figure 3.7A), important not only for mechanical protection of the tissue, but also as an electrical isolator, ensuring that the signal reaches different parts of the plant without modifications.

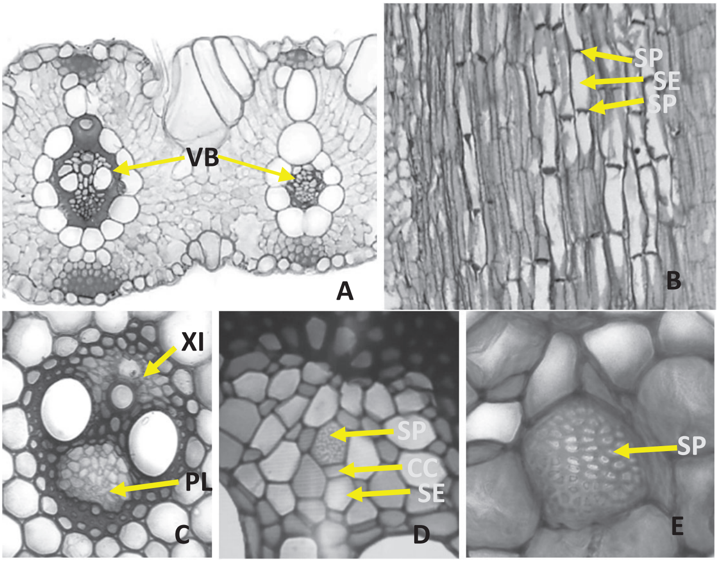

Figure 3.7. Plant cells and tissues involved in the transmission of electrical signals. 3.7A. Vascular bundles of smaller and larger caliber in cross section of *Echinolaena inflexa* leaves, showing the electrical isolation of vascular bundles (VB) surrounded by the fiber layer. 3.7B. Longitudinal stem section of *Cucurbita pepo* showing cells of the sieved element (SE), where the sieved plate (SP) is located at the poles, where the plasmodesms that interconnect the SE cells are concentrated. 3.7C. Cross section of the VB of the stem of *Zea mays* highlighting the location of the xylem (XI) and phloem (PL). 3.7D. Highlight of the phloem of *Zea mays*, in cross section, indicating the SP evidenced in one of the cells of the SE, and the companion cell (CC) adjacent to the SE, which have abundant plasmodesmatic connection between them. 3.7E. Highlight of the *Zea mays* SP in cross section. Material kindly provided by the Department of Botany of the Universidade Federal de Goiás.

In organs such as stem and petiole, there are few plasmodesmatic connections between the companion cell (CC) and the surrounding parenchymal cell (PC), just as there is rarely a plasmodesmatic connection between the sieved element (SE) and the PC, although there are many plasmodesmatic connections between CC-SE. In this case, CC-SE would be almost isolated components of a symplastic connection in the phloem of the stem, which is an ideal condition for the flow of an electric current, especially when there are plasmodesmatic connections between SE-SE (Figure 3.7B and 3.7E) at the poles of the cells, which allows a continuous path of low electrical resistance over a long-distance. In the CC-SE interaction, the CC membrane is slightly more electronegative, with a potential of approximately -149 mV, than the SE membranes, with an approximate potential of -141 mV. The number of proton pumps in the SE plasma membrane is less than the proton pumps present in the CC plasma membrane, and therefore this has a more important role in establishing the electrical membrane potentials. The electrical coupling between CC-SE is high exactly due to its plasmodesmatic connection. The propagation of electric waves over the CC-SE has been demonstrated by several research works (Rhodes et al., 1996; van Bel et al., 2008; Volkov and Shtessel, 2016).

Due to the considerable lateral electrical isolation of the CC/SE in the stem, electrical exchanges occur prominently at the CC-SE interface. Although the isolation is not complete, it is considered that the cells that communicate laterally with PC do not exceed the percentage of 15%. It is worth noting, however, that cell communication in the phloem loading at the source and unloading in other sinks does not necessarily follow the pattern of cell communication that occurs along the stem. This pattern of symplastic isolation of cells has long been observed between the guard cells and the subsidiary cells in the leaves, and this helps in the non-competition of lateral electrical stimuli, leaving the guard cell practically autonomous in its electrical regulation through transport of ions.

The common vascular symplastic isolation in stems is also observed in the *Mimosa pudica* petiole, whose closing of the leaflets by touch has already been covered previously in the section “Touch perception”, Chap. 1. Touch is an example of an external stimulus that can activate the transmembrane mechanosensors, which transform the environment stimulus into an electrical signal. The touch, even if only in a few leaflets, generates an electrical signal that propagates very quickly, culminating in the closing of all leaflets or even those present in the neighboring leaves. In the case of the mimosa petiole, the vascular isolation from the lateral parenchymal cells is made by a layer of sclerenchymatic cells, composed of fibers, which is positioned around it. After the arrival of the electrical signal in the pulvinus of the leaflets, the potential propagates through the electrical coupling of the cells of the vascular system via plasmodesmatic connections occurring with the cells of the collenchyma until it reaches the parenchymal cells of the pulvinus, where the H^+-ATPases and ion channels responsive to voltage will be activated, culminating in the rapid flow of ions and water from the flexor side towards the extensor side, and thus promoting the closing of the leaflets (Figure 1.8). The propagation of the action potential in mimosa has already been observed, in addition to phloem, in xylem parenchymal cells.

Using the case of mimosa as a reference, the phytosensors are in the leaflets touched, while the phytoactuator is in the pulvinus. In the interconnected membranes, from leaflets to pulvinus, are memristors, in which ion transporters participate, among them, at least some of the nine voltage-dependent K^+ channels known in plants. So much so that the use of proton channel blockers interrupts electrical signaling. In the same way that the touch stimulates the closing of the leaflets and the decumbence of the leaf, a discharge of 1.5V promotes a similar effect.

In addition to movement responses, previously seen in mimosa and venus flytrap (section "Touch perception", Chapter 1), electrostimulation also induces other responses, not necessarily involved in communication, such as the expression of the gene that encodes for the enzyme ACC SYNTHASE, essential in the biosynthetic route of ethylene, as well as in seed germination and plant growth.

The amplification of an electrical signal in plants can occur through the movement of K^+, Ca^{2+} and Cl^- by their respective membrane transporters (Figure 3.8). These ions are involved in the rapid depolarization and repolarization of membranes. Without initially depolarizing the membrane (due to the cytoplasmic influx of Ca^{2+}), there is no generation of wave potential (electrical). The potential responds to the molecular physical principle of "all or nothing" for its formation; that means it simply will not occur without reaching a minimum excitable charge, and that its propagation speed will not change if the amplitude occurs above the threshold of excitability. The transmission of the electrical signal through the transmitting channel requires a refractory period between one signal and another. This wave potential travels along paths with less resistance to its propagation with the same voltage, differently from the electrotonic potential, which loses strength quickly when passing through plasmodesms. In this sense, the electrotonic potential can act in short distance signaling, while the wave potential acts in the long-distance electrical signaling.

In addition to Ca^{2+}, for electrical signaling, K^+ transport is essential. Of the many voltage-dependent K^+ channels, AKT2/3 are the most abundant in phloem, and act for both influx and efflux of the ion, although there are other transporters exclusively for influx or efflux.

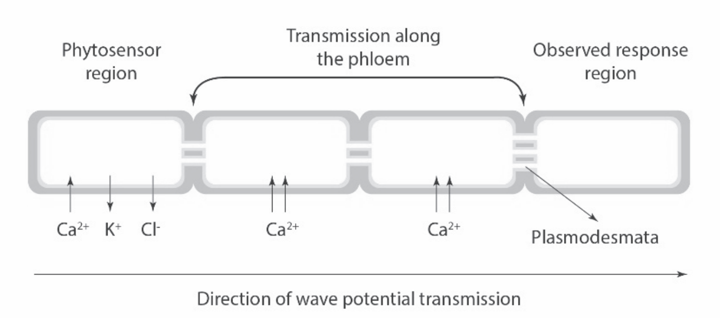

Figure 3.8. Representative scheme of the formation and transmission of the (electric) wave potential. For its formation, the movement of ions through the plasma membrane is essential, with Ca^{2+}, K^+ and Cl^- being the most important. There is no formation of the wave potential without membrane depolarization, which is promoted by the influx of Ca^{2+}. For its transmission, repolarization and depolarization are required, in which the K^+ and Cl^- flows also act. The potential travels along a low resistance pathway formed along the phloem by plasmodesmatic connections between the poles of the cells of the sieved elements or of the companion cells.

An example of lateral transport of chemical substances entering the CC-SE complex through plasmodesms, in fusion with the propagation of wave potentials, can be seen in the work by van Bel and Ehlers (2008). When electric shocks are applied to a specific part of a plant, which is seen as an injury signal, a wave is generated that spreads laterally towards the vascular bundle, and when it reaches it, the signal also propagates along the bundle. The electrical signal reaches the phloem cells in the loading or unloading region, via cells interconnected by plasmodesms, or even by the parenchymatic cells lateral to the bundle through the few existing plasmodesmatic connections, whose electrical signal still acts as an inducer of the transition from prosystemine to systemin (a polypeptide of hormonal potential formed in injured tissues and which activates the synthesis of proteinase inhibitors) still in parenchymatic cells. The systemin enters the CC-SE by symplastic via, causing a cytoplasmic increase in Ca^{2+}, which precedes the synthesis of the proteinase inhibitor, PI, a systemin-dependent defense protein. However, the electric shock causes the entry of calcium and induces the synthesis of PI in places too distant for the possibility of the systemin arriving by sap flow from the injury region, considering the speed of the flow. This indicates that electrical signaling by wave potential arrives much faster over long-distances than chemical signaling (Figure 3.9).

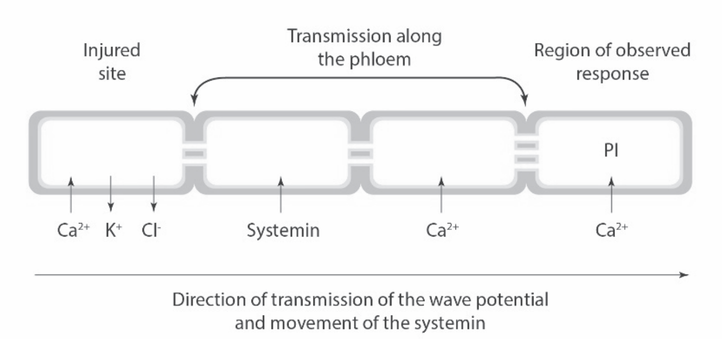

Figure 3.9. Representative scheme of the systemic propagation of electrical and chemical signals via phloem. Next to a damaged region of a plant, the formation of a wave potential and the production of a systemin, the biomolecule necessary for the formation of a defense protein, PI, occurs. The systemin enters the phloem close to the injured region, whose chemical signaling must propagate systemically and induce the production of PI even in regions distant from the injured area. It is noticed, however, that the influx of Ca^{2+} and the production of PI distant from the injured region occurs prior to the arrival of the systemin transported in the phloem sap flow, evidencing a response triggered by the propagation of the wave potential, whose speed is higher than the sap flow. Example based on the work of van Bel and Ehlers (2008).

The high speed of the propagation of the wave potential has already been measured in several situations. It has been verified that there is always a rapid depolarization of the CC-SE membrane from the triggering of an electrical stimulus, whose signal, through the phloem, reaches a certain destination without necessarily having a current of sap flow.

Corroborating the fundamentals of plant neurotransmission, seen in the topic “Putative mechanisms of plant neurotransmission”, from hormones and other substances that induce electrical signaling in plants, the synthesis of the PI defense protein can not only be formed in response to systemic signaling that reaches in several regions of the plant, initially induced by electric shock or injury from a single point, but can also occur by localized application of systemin, some oligosaccharides or methyl jasmonate.

Summary

There are several stimuli that can generate a potential wave (electric) in an organ or phytosensor tissue, a potential that must be propagated via memristors, which, in plants, are the cellular plasma membranes interconnected by plasmodesms, until reaching the phytoactuator region, triggering a physiological effect. Naturally, for the potential to propagate, it is necessary to have the proper electrical coupling between the different regions, which is guaranteed by the plasmodesmatic connection between the cells. For the propagation of the long-distance wave potential to be effective, a physical apparatus that acts as an electrical isolator is necessary, a characteristic found in the phloem, provided by its fibrous isolation, typical of the stem vascular bundles, in association with the symplastic isolation of the companion cell-sieved element (CC-SE) of the other adjacent cells, as in fact occurs. To create the potential wave, there is a need for membrane depolarization, which occurs through the cytoplasmic entry of Ca^{2+}, and for maintenance of the potential, subsequent repolarization and depolarization are required. For this, in addition to Ca^{2+}, other ions are important, such as K^+ and Cl^-. Unlike the electrotonic potential, which also occurs in plants, especially for electrical signaling over short distances, the potential wave maintains a constant amplitude throughout the transmission. Signaling speed is constant and much faster than chemical signals that move through the sap flow.

Hydraulic Signaling in Long-Distance Communication

The transport of water from the roots to the aerial part of the plants occurs via xylem, a tissue that depends on pressure to move water through its tracheal elements. The pressure condition can act as a long-distance communication signal, reaching all organs.

It is known that the tension in the xylem increases as water availability in the soil decreases, as well as being relieved by water replacement. The state of less or greater tension in the xylem, which is felt throughout the organism, can serve as a sign of specific environmental conditions to be perceived by the plant. The state of greatest tension promotes greater production of ABA, which in turn, as a hormone, has a pleiotropic and interaction effect with other hormones, starting to regulate various aspects of development depending on the water status of the organism.

Among the fastest effects observed with the advance of the lowering of the water potential (Ψ_w) is the loss of cellular turgor (Ψ_p) and the lowering of the osmotic (Ψ_π) and matric (Ψ_m) potential of the cell. Both Ψ_p and Ψ_m can trigger signaling pathways perceived by the same mechanosensors of the cell wall and plasma membrane already covered in the "Touch perception" section, Chap. 1. On the other hand, osmosensors can also trigger response pathways similar to those already explored in the section "Perception of drought and water", Chap. 1. The difference between the perception of touch (which is also a pressure stimulus) and the tension of the xylem is its extension, as touch can generate a localized response, while the tension of the xylem tends to be felt throughout the body (Christmann et al., 2013).

Hydraulic signaling is not always linked to the chemical effects triggered by ABA, whether it comes from the roots or the one whose synthesis is stimulated by the greater tension of the xylem felt by the aerial part. Gil et al. (2008) observed in avocado trees that signals from the lowering of the water potential (Ψ_w) of the soil, in a characteristic of drought state, as well as the return to irrigation, triggered leaf responses, such as stomatal conductance, at a much greater speed than that of the sap flow. It was found that it was electrical signaling generated by the fall of the Ψ_w and its increase at the time of the retorn of irrigation. Corroborating previous approaches, when they made a Malpighi ring at the base of the stem, there was an interruption of the electrical signal, indicating that the path of propagation was the phloem.

Long-Distance Chemical Communication

The synchronized development of the organs is necessary for the plants, and for that, effective long-distance communication is essential. Variations in the availability of water and soil nutrients perceived by the roots need to be informed to the aerial part so that it can adjust its development according to the availability of these resources, just as the root needs to be informed about the resources available in the aerial part, whose source is the soil, so that their acquisition is adjusted. For this, the transport of chemical substances, between root-aerial part, can act as communicating signals between organs.

Cellular perception via a chemical indicator can occur through substance presence, concentration, active or inactive form, ratio to other substances or speed of accumulation. Therefore, the xylem and phloem play a central role in this communication, since they integrate the vascular system of the plant; that means all organs are interconnected by the same tissue, so that any organ can communicate with another. Unlike other forms of communication, which tend to be fast, the limitation of chemical communication over long distances is its dependence on the speed of the sap flow, which fluctuates a lot. When considering the flow in the xylem we can have an amplitude from close to zero to 60 m/h and in the phloem from 0.3 to 2 m/h.

Long-distance communication by chemical signals can occur very quickly when considering the time between the release and reception of volatile compounds. However, in the case of compounds whose synthesis is induced by stimuli, it is necessary to consider the time needed between the stimulus and the synthesis. This type of communication by plant-to-plant volatiles has already been explored in the section “Olfactory communication in plants”, Chap. 2. Other forms of chemical long-distance communication will be covered in this section.

Peptides

In the section “Nutrients perception”, Chap. 1, two peptides, CLE (Clavata3/Embryo-Surrounding Region) and CEP (C-terminally Encoded Peptide), were presented as indicators of “satiety” and “hunger” for nitrogen (N), respectively. These are the peptides of greatest expression in long-distance communication in plants (Okamoto et al., 2016). Taking them as an example, they act as indicators of nutrient availability so that there is the so-called nutritional homeostasis among all organs, even because many nutrients, such as N, are absorbed in the soil and metabolized in the leaves, requiring a balance between both events. If there is a deficiency, there will be damage to the plant's development, and if there is an excess, the plant may suffer toxicity due to the accumulation of non-assimilated NH_4^+.

The peptides are recognized by membrane receptor kinases, triggering signaling involved in different aspects of development. The CLE acts as an upward sign from the roots to the aerial part, via xylem, whose increase in the sap discharged in the aerial part is an indicator of nitrate accumulation or success in biological nitrogen fixation in the symbiosis of root and diazotrophic bacteria. Its receptor is an LRR-RK (Leucine-Rich Repeat Receptor Kinase), HAR1 (HYPERNODULATION ABERRANT ROOT FORMATION 1) (Figure 3.10). Its binding to the receptor is an indicator of N satiety, culminating in the downward movement, via phloem, of secondary signals that act as negative regulators for the acquisition of N, including new symbiosis in the roots of legumes with rhizobia, thus avoiding unnecessary expenditure of photosynthates by plants, which would need to supply the bacteria with carbohydrates in exchange for N. Evidence suggests the participation of cytokinin produced in the aerial part as part of this downward signal.

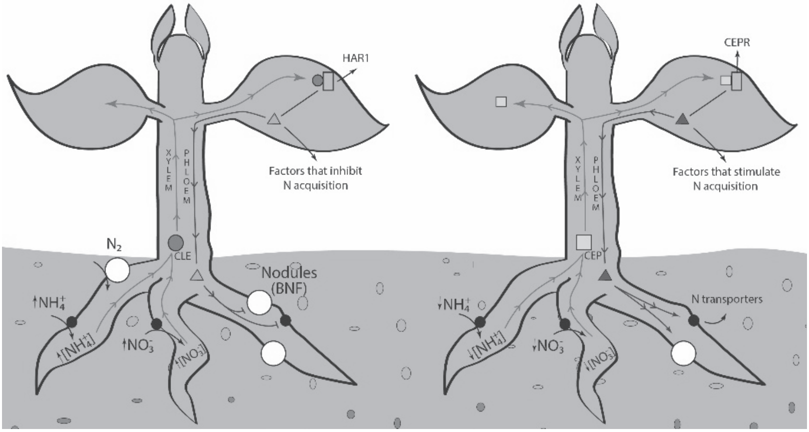

Figure 3.10. Peptide signaling in the root-aerial part communication indicating satiety or hunger of N. Under satiety, either by absorption of NO_3^- and NH_4^+ or by the accumulation of NH_4^+ or nitrogenous organic substances from the biological N fixation (BNF), the peptide CLE (Clavata3/ Embryo-Surrounding Region) is sent from the roots to the leaves via xylem. There, it is received by the HAR1 receptor (HYPERNODULATION ABERRANT ROOT FORMATION 1). Its leaf perception induces the synthesis of nodulation inhibitors and dawn-regulator of synthesis of N transporters, such as cytokinin, which are transported to the roots via phloem. In the inverse situation, of starvation of N, the CEP peptide (C-terminally Encoded Peptide) is sent from the roots to the leaves via xylem. There, it is received by CEPR (RECEPTOR KINASE CEP RECEPTOR). Its leaf perception induces the synthesis of stimulators of the synthesis of N transporters, which will be transported to the roots via phloem, and, in the case of legumes, they also stimulate the formation of new nodules in the roots.

In arabidopsis, no less than fifteen genes are found that encode for the CEP, whose transcripts increase many times as the availability of nitrogen decreases in the soil. Upon being transported by the xylem and reaching the leaves and being perceived by its CEPR1 receptor (RECEPTOR KINASE CEP RECEPTOR 1) there is a triggering of downward signaling, via phloem, to increase the expression of the genes that encode for nitrate transporters, such as NRT2.1, as well as inducing the formation of root nodules necessary for leguminous-rhizobia symbiosis, in order that the plant can redress its nitrogen deficiency.

Other peptides of the CLE family, such as Xylem Sap- Associated Peptide 4 (XAP4)/CLE32 have also been observed in flooded roots, showing their involvement with stress responses. CLE25, whose transcript is increased in roots in a dry condition, acts as a signal to produce more ABA in the leaves, which is more effective in closing the stomata than the ABA that reaches the leaves from the roots (McAdam et al., 2016). For this, CLE25 needs to reach the leaves via xylem, where it finds its receptor, BAM1 and BAM3 (BARELY ANY MERISTEM), whose BAM-CLE25 induces *NCED3* (*NINE-CIS-EPOXYCAROTENOID DIOXYGENASE3*) expression to increase leaf ABA, which will act on stomatal closure and on the regulation of genes related to stress due to water deficiency.

HY5

In addition to N availability, root-shoot regulation of the N/C balance (Nitrogen/Carbon) is also necessary, since without organic carbon available for assimilation of N, this could cause toxicity, if accumulated in the leaf. The root therefore needs a signal of carbon availability from the shoot to regulate nitrogen absorption. The transcription factor HY5 (Elongated Hypocotyl5) does this intermediation. The HY5 that reaches via phloem to the roots acts as a transcription factor for genes that encode for high-affinity nitrate transceptors, NRT2.1, the same that is positively regulated by the increase in sucrose (Figure 3.12).

ROS/Ca^{2+}

Abiotic stresses trigger the closure of stomata and promote a rapid increase in ROS/Ca^{2+} mediated by the action of ABA. According to Takahashi and Shinozaki (2019) these chemical signals can spread systemically throughout the plant and induce ABA action in other parts of the plant to promote stoma closure. ROS as metabolic signalers have already been observed in a wide range of responses previously discussed, but, in the case of free radicals, there is a need for better understand on their form of transport so that they do not cause major damage on the way. During the immunological synapse communication approach, we had evidence of this transport, in some situations, being carried out in vesicles. On the other hand, we know that Ca^{2+} does not have great mobility through the phloem, its long-distance movement being more restricted to the xylem flux.

Glutamate receptor

GLRs (Glutamate Receptor-Like) are strong candidates for synapse communication in the field of plant neurobiology. The presence of some of these receptors is detected in both phloem and xylem, and they are possibly involved in long-distance communication to signal injury and herbivory, triggering the synthesis of jasmonic acid, even far from the injured site. It is believed that some membrane transporters can bind and activate it, inducing an increase in cytoplasmatic calcium prior to defense responses.

An example of long-distance communication that shows an electrical signaling was observed in arabidopsis by Mousavi et al. (2013). When the plants were injured on a leaf, in addition to the membrane depolarization being observed, a typical effect of the beginning of electrical signaling, there was an increase in jasmonates in uninjured leaves and a consequent increase in the expression of defense genes, an effect that was reduced when glutamate receptor-defective mutants were used.

Proteins and RNA

The mobile protein FT (FLOWERING LOCUS T), if present in a leaf graft (scion) from a matrix plant already determined to flower, will induce the flowering of the rootstock, previously not induced. This classic effect represents a great example of long-distance protein communication via phloem (Figure 3.11). Like FT, other protein members of the PEPB family (Phosphatidyl Ethanolamine-Binding Protein) have been observed to move from grafted parts already determined to photo-dependent development events, such as tuberization, to rootstocks, previously not induced, breaking their dormancy (Thomas and Frank, 2019).

In addition to proteins, RNAs also participate in long-distance communication. The microRNA R2011, a repressor of the TOO MUCH LOVE transcription factor, which acts to inhibit root symbiosis with diazotrophic bacteria, has already been detected in movement from the aerial part to the roots, in addition to the CLE/CEP and HY5 signaling to balance the acquisition and availability of N between the aerial part and the root (Figure 3.12).

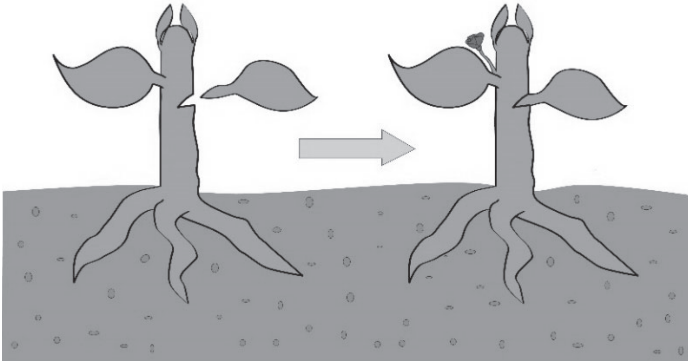

Figure 3.11. Representative scheme evidencing the movement of factors signaling floral induction. The graft of leaves from plants already determined to flower in plants that have not yet been induced is able to induce them after the transfer of an inducing signal, such as the FT protein (FLORESCENTE LOCUS T), from the graft (scion) to the rootstock, via phloem.

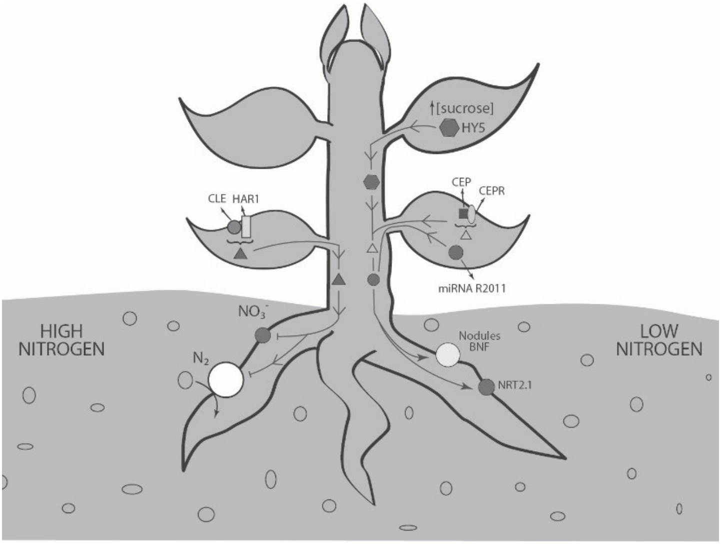

Figure 3.12. Chemical signals that act to balance the acquisition/availability of N or C/N between the aerial part and the root. Under high availability of N in the soil, leaf signals from the CLE peptide inhibit the formation of nodules and synthesis of N transporters in the roots. Under low availability of N in the soil, leaf signals from the CEP peptide, in addition to the miRNA R2011 and transcription factor HY5, stimulate the synthesis of NO_3^- transporters, such as NRT2.1, as well as, when possible, the formation of nodules for biological nitrogen fixation (BNF) in the roots.

MicroRNAs have also been observed in haustoria transit between arabidopsis and the parasite cuscuta. These miRNAs from cuscuta are another face of the virulence of parasitism (see section "Chemical plant-plant communication", Chap. 2), because they work causing the cleavage of host plant mRNAs, which are probably involved in plant defense, as well as promoting suppression of the host's mRNA accumulation and stimulating the production of secondary silencing RNAs (Shahid et al., 2018).

Hormones

Cytokinin, gibberellin, abscisic acid (ABA), strigolactones, jasmonates and salicylates are transported via the vascular system and act in long-distance signaling in plants, allowing harmonic development between distant, but interdependent organs. Auxin is transported in a non-symplastic polar form and with evidence of occurring by a chemical synapse mechanism through vesicular endocytosis-exocytosis with membrane recycling in phloem parenchymal cells, so it is not expected that there will be very fast chemical communication over the long-distance. Ethylene, being a gas, can easily diffuse through intercellular air spaces and can act more quickly than other hormones in communication. Brassinosteroids do not seem to play an important role in long-distance signaling, since their transport via sap flow is not common.

The transport of hormones across membranes occurs by specific transporters, with members of the ABC family (ATP-Binding Cassette Transporter) transporting ABA, strigolactone and cytokinin; those from the NPF family (Nitrate Transporter 1/Peptide Transporter) transport ABA, jasmonates and gibberellin; those in the MATE family (Multidrug and Toxic Compound Extrusion) transport ABA; while amino acid transporters transport aminocyclopropane, a precursor of ethylene (Lacombe and Achard, 2016).

It is important to note that not only is the presence or concentration of the hormone sufficient to trigger a signaling cascade, but also the availability of its receptors and hormone-sensitive proteins, which can act, for example, as transcription factors for genes responsive to the hormone.

Although it is expected that all hormones transported over a long-distance will have some signaling function in the target tissue/organ, the following examples of communicating signals are those best known to perform this role.

ABSCISIC ACID

Among the hormones, classically, ABA has a special prominence as a sign of abiotic stresses, known especially for having its content increasing in the roots as the water deficiency increases in the soil, reaching the leaves in greater concentration in the xylem sap, which is interpreted as a signal for stomatal closure in order for the plant to save water. However, as previously explored, CLE25 peptides from the roots under water deficiency ascend to the leaves via xylem and stimulate the synthesis of leaf ABA. The ABA synthesized in the leaves is more effective for stomatal closure than that originating from the roots. Not only that, much of the ABA accumulation in the roots after prolonged drought is due to its synthesis in the aerial part, which descended to the roots via phloem (Manzi et al., 2015). In this sense, it is important that new research be dedicated to a redesign on the importance and functionality of the higher ABA content in the sap flow during water stress. It is suggestive to verify the positive influence of ABA from roots and leaves on the already known expression of genes involved in the tolerance of plants to different stresses, as well as the importance of its conjugation with glucose-ester when transported in the xylem sap.

Not only is the presence and concentration of ABA important as a communication signal, but also its free or conjugated form, as well as the availability of its PYR1 (PYRABACTIN RESISTANCE1), PYL (PYR1-LIKE) and RCAR (REGULATORY COMPONENTS OF ABA RECEPTORS) receptors in plant tissue, without which it cannot be perceived and, consequently, trigger its effects.

CYTOKININS

Cytokinins are substances involved in long-distance communication for nutrient homeostasis. Nitrate stimulates the production of trans-zeatin in the roots, which ascends to the aerial part via xylem, where it will act in the regulation of development. On the other hand, isopentenyl-adenine descends to the roots from the aerial part, via phloem, acting in the regulation of the acquisition of nitrate and in the inhibition of the symbiosis of leguminous roots with rhizobia. The production of cytokinin in the aerial part, which will act on the roots as a suppressor of nodulation, is stimulated by the presence of CLE-HAR1, as already discussed in peptide-mediated long-distance communication.

The cellular perception of cytokinin depends on its receptors AHK2 (ARABIDOPSIS HISTIDINE KINASE2), AHK3 and CYTOKININ RESPONSE1/AHK4.

JASMONIC ACID

Jasmonic acid as a communicative signal over long distances is essential for greater tolerance of plants to attack by pests. As observed in several previous approaches, jasmonates are at the center of the regulation of defense against herbivory and necrotrophic pathogens, in addition to responses to mechanical damage. Details can be seen in the topic “Hormones and immunology”, Chap. 2.

After the damage caused to a specific organ of the plant, there is activity of the jasmonic acid pool of the injured site, as well as greater synthesis. From then on, the chemical signaling promoted by this hormone occurs in a systemic way via phloem, reaching non-attacked organs, where it induces the expression of several genes involved in defense (Figure 3.13), such as those that participate in the synthesis of toxic metabolites, pest repellant volatiles, volatiles that attract natural enemies of pests or even substances that are harmful to the functioning of the digestive tract of herbivores.

Jasmonic acid or its precursor, 12-oxo-phytodienic acid (OPDA), can be translocated in the sap of phloem and xylem. However, in addition to its direct induction of gene expression in the target tissue, many studies show that this target tissue begins to respond before the arrival of jasmonic acid at the site, indicative of its role in electrical signaling, the mechanism of which was previously discussed in the topic “Putative plant neurotransmission mechanism”.

Derivatives of jasmonic acid also have a signaling action on the plant. Methyl jasmonate can act as a volatile allelopathic substance, as well as trigger long-distance electrical signaling in the plant's body. On the other hand, jasmonoyl-isoleucine can be perceived by the plant cell by the SCF^{COI1}/JAZ co-receptor, triggering, from then on, defense responses.

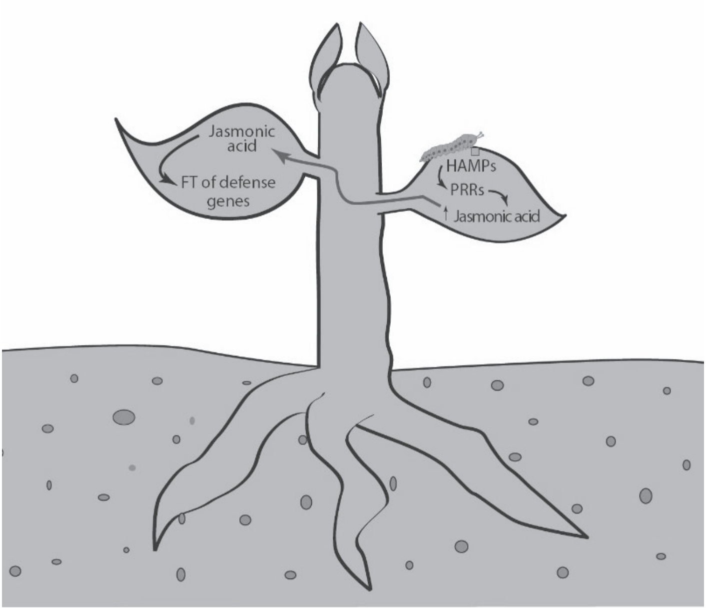

Figure 3.13. Long-distance systemic signaling promoted by jasmonic acid. Signs of damage, such as HAMPs (molecular patterns associated with herbivores) can be perceived by cells through PRRs (receptor and pattern recognition proteins), which will result in greater synthesis of jasmonic acid. After being loaded into the phloem, it will spread throughout the plant and induce greater activity or synthesis of FT (transcription factors) of genes related to plant defense, even in organs not yet effectively affected by the damage agents.

SALICYLIC ACID

A long-distance signaling for plant defense very similar to that triggered by jasmonic acid occurs by salicylic acid, which induces defense responses to biotrophic pathogens and herbivore-sucking in organs far from the affected site.

In infected or injured sites there is an increase in salicylic acid that acts as a signal to increase what is called systemic acquired resistance (SAR), which includes an increase in methyl salicylate, dehydroabietinal diterpenoid abietane (DA), azelaic acid (AzA) and glycerol-3-phosphate (G3P) (Shah and Zeier, 2013). These substances, and salicylic acid itself, are transported over long distances by the phloem. The transport of SARs often depends on the concomitant transport of the DIR1 protein (DEFECTIVE IN INDUCED

RESISTANCE 1). In target tissues, these SARs require other chemical compounds, such as salicylic acid itself or pipecolic acid, or patterns of environmental factors, such as light, to be effective. However, the salicylic acid that will interact with the SARs in target organs is not that derived from the infected organ, but the one synthesized in the uninfected organ. Its effectiveness is made by inducing the expression of genes related to plant defense, such as *PRs* (*PATHOGENESIS-RELATED*). The importance of salicylic acid to SARs production is confirmed by studies using mutants defective for this hormone. SARs participate as elements of transgenerational memory in plants, benefiting the offspring (a topic to be approached in the next chapter). Details on salicylic acid in plant pathogenesis can be seen in the topic "Hormones and immunology", Chap. 2.

The perception of salicylic acid by a plant cell occurs through NPR1 (NONEXPRESSOR OF PATHOGENESIS-RELATED GENE 1) and NPR3/NPR4 receptors.

Summary

The interdependence among the different organs of plants imposes the need for them to communicate with each other through the movement of chemical substances. For example, an abundance of leaf N beyond the plan's assimilation capacity, becomes toxic. In that case, it is necessary for the leaves send the roots chemical signals that inhibit nodulation for biological nitrogen fixation or for the synthesis of N absorption proteins. On the other hand, signs of N scarcity in the soil must reach the aerial part where they may even influence carbon assimilation as well as greater production of leaf chemical signals sent to the roots, which will act as stimulators of greater synthesis of transporters or of nodulation. For that balance to be established, there are many signaling elements, such as the CLE and CEP peptides, the HY5 transcription factor and the R2011 microRNA.

Other signals, such as ROSs, proteins and hormones, can move from an injured organ or one subjected to different stressful stimuli, and, via the vascular system, reach other non-injured organs, triggering defense responses in them even before they suffer effective biotic or abiotic stress. The same principle applies to the transfer of signals from different stimuli between graft scion and rootstock. Naturally, for the target organs to respond to chemical stimuli they must have a signal-sensory apparatus.

CHAPTER 4

PLANT MEMORY

Memory is a fundamental factor in the acquisition of a skill from a stimulus. The fact that a plant species acquires resistance (constitutive factor) due to its exposure to a pathogen over the generations evokes a skill built from a memory capable of being passed on from generation to generation. Likewise, a plant which in a single life cycle becomes more tolerant (non-constitutive factor) to stress after one or more exposures is another example of a situation that necessarily requires a memory to trigger specific signaling pathways. In this case, each time that the plant is exposed to the same stress situation, it will access the defense paths developed in previous exposure, a phenomenon that in plants is more common than hysteresis, that is, the maintenance of the active defense path even in the absence of the stimulus.

Much of what has already been covered in previous chapters necessarily requires the existence of a memory in plants, such as, for example, their recognition of specific symbiotic or pathogenic microorganisms, recognition of specific volatile compounds, as well as the signals used as factors of recognition of relatives.

As in animal biology, the memorization mechanism is not widely known; however some elements that interfere in the storage and access of the information have already been identified in plants. In addition to these elements, throughout this chapter, different types of plant memories will be described.

Memory Concepts

The concept of memory is very variable, according to the respective references, which can be the human or animal brain, a machine, a plant or simply a generic concept applicable to various organic or inorganic devices.

Some of the many concepts of memory, of an anthropocentric or generalist nature, are transcribed:

> *"It is the ability to acquire, store and retrieve (evoke) available information, either internally, in the brain (biological memory), or externally, in artificial*

> *devices (artificial memory). It is also the storage of information and facts obtained through experiences heard or lived”* (Wikipedia Encyclopedia, 2020)
>
> *“It is the ability to access past experiences, so that new responses incorporate relevant information from the past”* (Trewavas, 2003)
>
> *“In plants, the term memory applies to cases in which there is a storage of information from a stimulus, and, after some time, that information can be recovered, just as is the general concept for living organisms or machines”* (Verdus, et al., 2012)

Evidence of Memory in Plants

There is a lot of evidence of memory in plants, just as there are many types of memory, named according to the observed context. At first, general aspects related to memory will be addressed, such as the storage and retrieval of information, as well as explaining the importance of some factors for the effectiveness of those events. Then, the acquisition of information by priming and the importance of modulating factors for acquisition will be explored, and finally, the different types of memory observed in plants will be explored individually.

Below are some classic essays that demonstrate the storage and retrieval of information in plants. In these texts, it should be noted that there is a break from the concept that there is always the need for a linearity between a stimulus and a response since information can be stored for a while and then retrieved for a later response.

1. The first essay to be reported is by Thellier et al. (2000), and as the report of the experiment is being described, some conceptual inferences concerning the study of memory will be made. The experiment was carried out to assess the loss of symmetry in the growth of axillary buds of cotyledons in three-week-old *Bidens pilosa* seedlings (Figure 4.1A).
   a) After interrupting the apical dominance, by decapitating the stem apex, both buds developed symmetrically under optimal conditions of light and nutrients (Figure 4.1B).
   b) When the apex was decapitated at the beginning of the day, a set of seedlings received four needle punctures in cotyledon A, which promoted greater growth of the axillary bud of cotyledon B (Figure 4.1C).

c) Non-decapitated seedlings that received perforations in cotyledon A kept their axillary buds quiescent, but when decapitated between 2-14 days after perforation, the asymmetry evidenced by the greater growth of cotyledon bud B in relation to A was the same as that reported in topic 1b. The event that results in the retention of information, as in this case for the subsequent breaking of symmetry, is called storage (STO).

d) Maintaining the same treatments, but decapitating the seedlings at noon, instead of doing it at the beginning of the day, there was no asymmetry in the development of the lateral buds. However, when they were decapitated at noon and subjected to a heat treatment, the buds again grew asymmetrically. This requires some comment: 1. that there is an effect of the circadian clock linked to memory; 2. that there was storage of information, but some environmental conditions inhibited recall (the retrieval of information); 3. that changing environmental conditions can promote the retrieval of information that was stored. The retrieval of stored information is called recall (RCL).

e) The fact that the seedlings only retrieve the stored information from the symmetry break after the decapitation of the apex reveals the existence of a permissiveness factor for the information to be accessed, a factor known as permissitivity (PST). There are several factors that interfere in the RCL, including the quantity and quality of the stimulus, which can be reversed, while the STO is not erased or reversed by stimuli.

f) The application of macerated perforated cotyledons on unperforated cotyledons triggered the break in symmetry in the growth of axillary buds, which did not occur with the use of unperforated cotyledon macerate. In previous studies reporting this same symmetry break, Desbiez et al. (1994) and Desbiez et al. (1991) observed a similar breakdown response when applying dextran, fragments of oligosaccharides, D-glucose, D-galactose, D-fructose and myoinositol on cotyledons, as long as K^+ or Ca^{2+} were present in a satisfactory amounts, but not Na^+; other molecules such as D-arabinose, mannitol and DL-leucine had no effect on the breaking of symmetry, which reveals a chemical regulation in the establishment of STO.

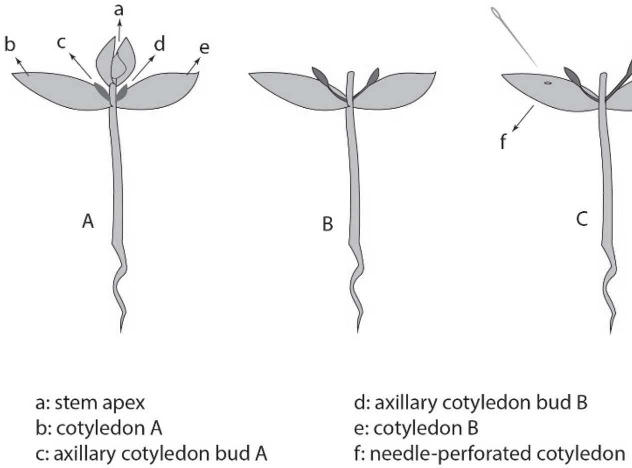

Figure 4.1. Representation of the experiment by Thellier et al. (2000). 4.1A. *Bidens pilosa* seedling intact, in which the axillary buds remain quiescent, while the apical stem is present; 4.1B. after breaking the apical dominance, by excision of the stem apex, the axillary buds grow symmetrically; 4.1C. when cotyledon A is pierced with a needle with concomitant excision of the stem apex, and between 2 to 14 days after piercing, the axillary bud of cotyledon B grows more than that of cotyledon A, and therefore, growth becomes asymmetrical.

2. The second essay to be reported was carried out with seedlings of *Linum usitatissimun* just a few days old, in an experiment developed by Verdus et al. (1997). During its description, inferences will be made on aspects of storage and retrieval of information previously explored during the description of essay 1. *L. usitatissimun* seedlings were subjected to a depletion in the supply of Ca^{2+}, showing a reduction in the lengthening of the hypocotyl and a formation of meristem in the epidermis of the hypocotyl (understood as the de-differentiation of cells, reassuming the meristematic function), and only of the hypocotyl, in regions neighboring the stomata. The depletion of K^+, Na^+ or Mg^{2+} also induced the appearance of meristems, but in a smaller number than that observed by the depletion of Ca^{2+}.
   a) Seedlings that were not transplanted between the germination and growth environments had a lower incidence in the appearance of meristems (up to 90% lower) when calcium depleted than those transplanted, which indicates that the transplanting acted as a stimulus for formation of meristems.

Transplanted plants had calcium depletion started at 4, 8 and 12 days after transplanting and all of them only started meristem formation after two days of calcium depletion. This means that the information generated in the transplantation to produce meristems (STO) was retrieved (RCL) two days after calcium depletion, an event that acted as a permissiveness factor (PST) for the retrieval of information. Calcium depletion still acted as a stimulus factor for the formation of meristems as non-transplanted seedlings also produced meristems after two days of calcium depletion, while no seedlings, regardless of their treatment, produced meristems unless they experienced calcium depletion.

b) The seedlings, transplanted or not, were submitted to wind and to water deficiency, and then submitted to 2-3 days of calcium depletion. The sum of stimuli, that means, wind, water deficiency and transplantation, after, and only after calcium depletion, promoted an increase in the number of meristems in transplanted plants compared to non-transplanted ones. That means the amount and intensity of the stimulus interferes in the storage of information (STO).

c) Exposure of seedlings to 2 days of calcium depletion, followed by exposure to a single stress (transplantation) or multiple stresses (transplantation, drought and wind) also induced greater meristem production in proportion to the accumulation of stimuli. In this case, when stimuli were applied 3 hours after the end of calcium depletion (hypocotyl still with low calcium content) the production of meristems was small, but when the stimuli were applied two days after the end of calcium depletion, the production of meristems increased a lot. This means that the perception of the signal and or the information storage (STO) depends on a high calcium content in the seedlings. On the other hand, as seen in topic 2a, the depletion of cellular Ca^{2+} acts as a permissiveness factor (PST) for information access (RCL), necessary for the continuity of the response (in this case, the formation of meristems), as observed in Figure 4.2. For the retrieval of information when the stimulus reappears or with the appearance of a new cumulative stimulus, there is a maximum calcium threshold for triggering the response. It is not known whether there is any factor that precedes calcium depletion that interferes in the triggering of the response.

d) A continuation of essay 2 was carried out by Verdus et al. (2012) with a focus on expanding the understanding of the importance of calcium in STO and RCL. It was observed that the activation of the RCL depends on a drastic depletion of calcium in the hypocotyl to less than 0.58 mM. When a second round of calcium depletion was imposed, a second wave of meristem production occurred, which means that memory can be accessed more than once. Calcium concentrations in the hypocotyl between 1.75 to 3.73 mM for the establishment of STO was excellent, but above or below this range it was unfavorable.

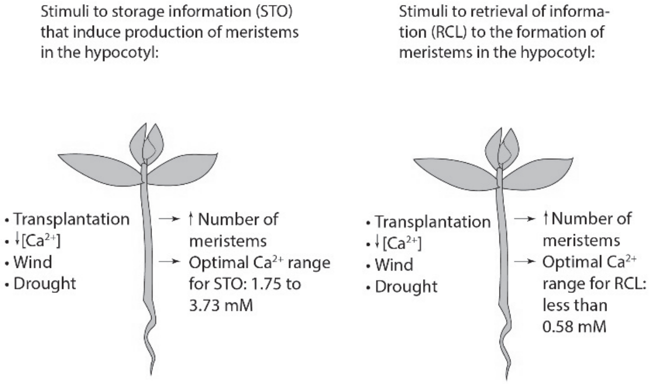

Figure 4.2. Calcium as a limiting factor for information storage (STO) and information retrieval (RCL) regarding stimuli to induce meristem formation in *Linum usitatissimun* hypocotyls, based on studies by Verdus et al. (1997) and Verdus et al. (2012).

3. The third essay to be reported concerns a mix of essays 1 and 2, an experiment carried out by Desbiez et al. (1987) with *Bidens pilosa* and another by Lefèvre et al. (1993) with tomato, obtaining the same results. Perforations performed in one or both seedling cotyledons growing in deionized water or very diluted saline caused a decrease in the growth rate of the hypocotyl, which did not occur when the seedlings grew in a nutrient solution suitable for their development. There were no variations in the growth of the hypocotyl of non-perforated seedlings depending on the culture medium. However, after the transfer of the perforated seedlings from the nutrient-rich medium to the deionized water (and therefore calcium-deficient), after one or two days the RCL of the perforate information occurred and the seedlings showed a decrease in the growth of the hypocotyl.

4. A fourth essay to be reported was carried out by Bourgeade et al. (1989), who conducted in vitro studies of callus from *Bryonia dioica* internodes. Still in the mother plant, the internodes that had suffered friction and developed with greater lignification and less growth were separated from those that had not suffered friction, which were taller and less lignified and represented the control group.
    a) In the first subculture both the callus of the control and those from rubbed internodes showed an increase of up to 5 times in the activity of peroxidases; specifically, GUAIACOL PEROXIDASE, which had less activity in the callus of the control group; in the course of the subcultures this difference was extinguished, but SYRINGALDAZINE PEROXIDASE was observed with greater activity in the callus from rubbed internodes in relation to the control until the fifth and last subculture. Cathodic isoperoxidases decreased their activities in calluses from successive subcultures, compared to internodes, but always with greater activity in calluses from rubbed internodes, while anodic isoperoxidases' activities increased in subcultures' calluses, compared to the original organ, but also maintaining greater activity in those from rubbed internodes.
    b) The activity of PHENYLALANINE AMMONIA LYASE (PAL) decreased in the callus in relation to the internodes of the control group but remained high in the callus of the rubbed internodes. The lignin content that was higher in the rubbed internodes was thus maintained until the fifth and last subculture.
    c) ACC SYNTHASE activity and ethylene content decreased from internode to callus but remained higher in calluses from rubbed internodes during subcultures.
    d) All of the evidence described in the topics above demonstrates the storage of previous information (STO) being transmitted by mitosis to the new cells, based on the condition experienced by the original organ.

Summary

The submission of plants to conditions that stimulate a response does not necessarily lead them to express the response, even though they are stimulated. Information inherent to the stimulus can be stored, and only after the plant is exposed to a permissiveness factor (PST), or its exposure to another stimulus that is cumulative to the first, is there a manifestation of the response from the previously stored information. Some factors, such as low availability of calcium, are necessary for the information retrieval (RCL) as well as high availability of calcium is necessary for the information storage (STO). Some chemical substances and the circadian clock modulate the plant memory. Access to information can occur repeatedly when the plant is resubmitted to the stimulus factor. Through the callus culture, where the organ is rubbed or non-rubbed internodes from the same plant, the activity of several enzymes was observed, being higher or lower in the successive subcultures depending on the treatment of the original material, which shows the maintenance of memory in the new cells produced during subcultures.

Evidence of Information Acquisition by Priming

The term priming is widely used to denote habituation, acclimation or conditioning. These are situations in which a stimulus factor usually perceived by the plant changes its signaling pathways. Many memorization events related to priming involve the information storage system (STO), as well as the recall system (RCL), much faster (minute scale) than the phenomena described in the previous section (day scale), giving the impression that there is no mandatory permissiveness factor (PST) for the retrieval of information. Let us examine the evidence.

1. As explored in the section “Temperature perception”, Chapter 1, a rapid decrease in cell temperature leads to a transient increase in cytosolic calcium. However, arabidopsis plants that previously had experienced prolonged periods under low temperatures, when exposed to cold shocks, strongly attenuated the transient increase in cytosolic calcium, but the increase was not attenuated when the shock promoted a cell cooling below the temperature at which the plants had been acclimated (Plieth et al., 1999). In other words, the condition of acclimatation of the plants to the cold desensitized them (a process associated with the non-influx or cellular efflux of calcium) to the cold shock. However, it was observed that the intensity of the stimulus changed the response. This first example shows a process commonly observed in animals, familiarization, in which the memory acquired in repeated previous experiences leads the organism to learn about the potential damage of a stimulus and, when appropriate, to ignore it when harmless.
2. Knowing that the kinetics and magnitude of the transient increase in cytosolic calcium differ among different stimuli, Knight et al. (1998) tested the concentration of cytosolic calcium in arabidopsis treated

with mannitol to mimic water stress (which triggers some signaling pathways common to osmotic stress) and compared the responses of plants previously exposed or not to oxidative or osmotic stress. It was observed that the history of the plant influenced its response to the stimulus, and those previously submitted (acclimated) to oxidative stress had a reduction in the transient increase in cytosolic calcium after their exposure to mannitol; those that had previously been exposed to osmotic stress had a transient increase in cytosolic calcium after exposure to mannitol. That behavior, in addition to showing the acquisition of a memory, also shows that a stress experienced by the plant can modulate the tolerance to other stresses that trigger common signal transduction pathways. In this second essay, we observe a process opposite to that of familiarization, mentioned in the first essay, which is also observed in animals, which is sensitization, in which the memory acquired in repeated previous experiences leads the organism to learn to react with more forcefulness to an unpleasant or harmful stimulus as was the case with mannitol-induced stress in plants previously exposed to osmotic stress.

3. In *Nicotiana plumbaginifolia* it was observed that when the plants came out of rest and were exposed to the wind, there was a rapid transient increase in cytosolic calcium; however, if the wind remained continuous, the calcium peaks would become progressively refractory in up to 1 minute (Knight et al., 1992). This response again demonstrates the desensitization effect already addressed in essay 1. Figure 4.3 demonstrates the effect of innocuous or harmful stimuli on the transient increase in cytosolic Ca^{2+}, showing the phenomena of familiarization and sensitization, respectively.

When comparing the tests that demonstrate the acquisition of information by priming, in which the RCL is very fast, with those addressed in the topic "Evidence of memory in plants", in which the events are slower, some possible advantages of the slower ones can be seen insofar as they are reversible. It is expected that the plant will only make efforts (resources) to prepare the response if the perception of the stimulus is relatively continuous or frequent, while in the faster responses, if the stimuli are unknown, or correlated with other harmful ones, responses tend to occur effusively, requiring energy expenditure without necessarily having a real need for defense, although adjustable over time. This does not mean that both speeds are not important, the slow event being important to avoid unnecessary energy expenditure, but applicable to morphogenesis responses, which are also slow, while the fast event can lead the plant to defend itself against a lethal agent, even if the threat proves to be not true.

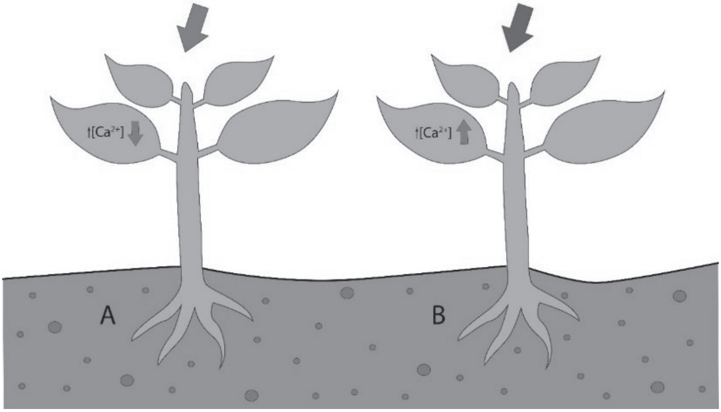

Figure 4.3. Sensitization and desensitization of plants due to harmful or non-harmful stimuli. A. Plants submitted to successive exposures to a non-harmful stimulus start to decrease the transient increase in cytosolic Ca^{2+} as the plant becomes familiar with the stimulus (desensitization). B. Plants submitted to successive exposures to a harmful stimulus start to increase the transient increase of cytosolic Ca^{2+} when they are resubmitted to the stimulus, a typical effect of sensitization. Both situations show the plant's memory related to the effect caused by the stimulus.

Summary

When young plants are exposed to some unknown stimuli, even though they are not harmful, there is a tendency to perceive them as possible causes of damage. After or during the perception of stimuli, there is a transient increase in cytosolic Ca^{2+}, which acts in signaling for defense responses. In this case, the plant is experimenting and getting used to the stimulus (priming). As successive exposures of the plant to the same stimulus factor occur, or even to associated stimuli, the transient increase in Ca^{2+} that occurs in the beginning can be altered downwards, due to the memory established in the plant because there were no harmful effects in previous exposures, thus avoiding an unnecessary expenditure of energy on defense. In this situation, there is a desensitization of the plant due to its familiarization with the stimulus. The opposite is also true; if there has been significant damage due to exposure to previous stimuli, there is an increase in the transient increase in cytosolic Ca^{2+} when the plant is again exposed to the stimulus. Unlike access to memories for events related to morphogenesis, apparently, access to memories necessary for rapid responses does not require permissiveness factors to access information, and they can manifest themselves effusively.

Importance of Calcium for Plant Memory

In addition to the importance of calcium for information storage (STO) and retrieval (RCL), discussed in the previous sections, we will see more details of its performance in the field of plant memory in this section.

Taking as an example the experiment of plants previously exposed to cold for memory evaluation in plants, seen in the previous section, it is known that there is an increase in the expression of genes related to calmodulin, as well as a transient increase in cytosolic calcium, which precedes events of phosphorylation and gene expression. In addition to this example, the participation of calcium in (de)phosphorylation and gene expression events is very common, stemming from the perception of various stimuli, as seen throughout the previous chapters. Naturally, calcium is an important part of the changes that occur related to temperature, but other changes are also observed, such as the fluidic decrease in membranes; activation of MAPK kinases (MITOGEN-ACTIVATED PROTEIN KINASES), which are very common in abiotic stress tolerance signaling pathways, and cytoskeleton reorganization.

A hypothesis for the importance of the sudden increase in cytosolic calcium, from different stimuli, is that it condenses around cellular electronegative structures such as nucleic acid and cytoskeleton, or simply decreases the typical electronegativity of the cytoplasm, so that this form of calcium condensation or state of lesser cytoplasmic electronegativity is a condition for typical cellular signaling processes, such as phosphorylation, protonation, methylation, acetylation of molecules and other (de)conjugations, conformational changes, compartmentalization of molecules, and other possible changes. Likewise, the calcium output to the apoplast or its intracellular compartmentalization acts like other metabolic signals. It is important to note that the magnitude of the content and the time the calcium remains in the cytoplasm is variable according to the stimulus, acting as a signal of recognition by the cell, and from these patterns there may be triggering of different transduction pathways of signals.

Throughout the examples that demonstrate memory, already explored, it became evident that the storage of certain information and its retrieval depend on an optimal range in the calcium content, although these ranges are different for STO and RCL. We have even seen that in some experiments, a low calcium content acts as a permissiveness factor (PST) to retrieve the stored information. If calcium is not directly the determining factor for STO, RCL and PST, it is at least some factor related to it because if there were no depletion in the culture medium and in the seedling, the response dependent on the stored information would not be effective, just as, if calcium depletion were delayed two days, the response would be delayed two days. Although the interference of its content and permanence in responses is known, it is not clear whether the calcium gradient itself between the apoplast and the symplast interferes in the response, as well as other related gradients, as, for example, the cytoplasm-vacuole or cytoplasm-endoplasmic reticulum ones.

The influence of Ca^{2+} on information retrieval

Attempts to establish a model for building memories or accessing information inevitably need to consider the role of calcium. Based on its effects related to memory for the formation of meristems, seen in the section "Evidence of memory in plants", Verdus et al. (2012), based on the work by Verdus et al. (1997), Tafforeau et al. (2002) and Henry-Vian et al. (1995), evoke some premises:

- Since the meristem formation response is increased with the accumulation of stimulus factors, it is believed that as the memory proteins are formed (STO) so also are the proteins for information retrieval (RCL), but the RCL-dependent response can be interrupted if the plant returns to high calcium concentration. In this case, calcium should stimulate phosphorylation/activation of memory protein inhibitors and/or inactivation of RCL proteins, such as, for example, triggering the activity of proteases of RCL kinases.
- Since successive calcium depletion results in successive retrieval of information, it is believed that there are successive activations of factors involved with the RCL, such as the decompression of chromatins related to it.
- A second wave of response can be more forceful than the first, and therefore, it is possible that this occurs because of the longer time available for greater production of proteins associated with memory (STO), which will be phosphorylated as soon as their kinases are expressed. This comes from the fact that the establishment of memory in some situations requires a certain time of accommodation (days), even because what is memorized is the signaling pathway

> from a specific stimulus, but there is no evidence that this includes memory of the exact moment of the stimulus manifestation, unlike what happens in animals.

Figure 4.4 is a summary of the factors involved in memory retrieved in plants, based on the putative assumptions of Verdus et al. (2012), concerning their involvement in the formation of meristems in the hypocotyls of *Linum usitatissimun* (experiment reported in essay 2 of the section "Evidence of memory in plants").

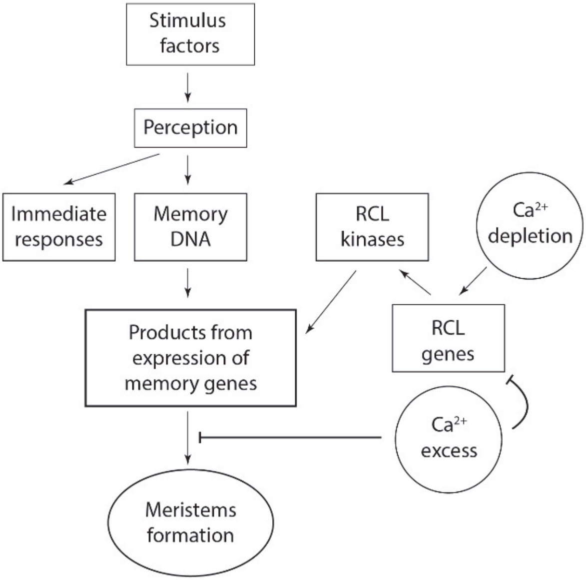

Figure 4.4. Factors involved in memory retrieval in plants. From the perception of repeated stimuli that lead the plant to retrieve the memory of previous exposures, there is an activation of DNAs (through, for example, decompression of chromatin and acetylation of histones) involved with the memory, resulting in the expression of response genes (in the present case, the formation of meristems). However, for there to be an effect, gene expression proteins depend on phosphorylation by kinases associated with information retrieval (RCL), which depend on the expression of genes associated with RCL, whose expression is dependent on Ca^{2+} depletion. The excess of Ca^{2+} inhibits the expression of genes that encode for RCL kinases and, consequently, the necessary phosphorylation of proteins that act in the signal transduction pathway for the formation of meristems.

Types of Memory in Plants

Several types of plant memory have been reported, which, although they have the same fundamentals of storage and recall, are named according to the context in which they are observed, as we will see in this section.

Transgenerational epigenetic memory

It is curious how a phenotypic change can occur in a plant, and from then on, the modified aspect will be transmitted to future generations. For this to occur, it is necessary that a memory triggered by the stimulus that caused the change be printed on the organism and that there is a mechanism for its transgenerational transmission. As we will see, advances have been made in acquiring knowledge of these mechanisms.

Epigenetics deals with stable changes in the genome that cannot be explained by changes in DNA sequences, but by other factors, such as changes in chromatin or DNA marks, as methylation. Epigenetic marks have a hereditary character, and therefore can be transmitted to generations through meiosis, as well as to new cells by mitosis. Although stable, there is evidence that, over time, these marks can be altered by the factors of the environment. Several environmental factors (such as abiotic stresses) or biotic factors (such as infection by pathogens) have been responsible for altering the expression of some genes, whose expression behavior is transmitted to future generations.

The mechanism by which epigenetic marks are transmitted to future generations is not widely known, but several studies have associated changes that have occurred with changes in DNA methylation, with small RNAs (sRNAs), with transposable elements and with the modification of histones, which are modifications that take place through methylation, acetylation, phosphorylation, ubiquitination, ADP-ribosylation, sumoylation and glycosylation (Annacondia and Martinez, 2019; Daxinger and Whitelaw, 2010; Quadrana and Colot, 2016).

For the occurrence of memory transmission, epialleles and the transmission of their marks are required. Epialleles are forms of the gene that have no change in its sequence but have a variation of epigenetic mark that alters their expression and the phenotypic variation of a hereditary character. The transgenerational inheritance of epigenetic marks requires the transmission to future generations of modification factors, such as DNA methylation.

Some roles of the elements involved in the transmission of memory are already known. Methylation is usually associated with gene silencing, but this does not always occur absolutely, as many genes continue to be expressed, although less, unlike when methylation occurs in transposons, in which silencing itself is most effective. The sRNAs act as markers that direct methylation. Both DNA methylation and histone modification are important for silencing transposable elements at different stages of plant life, but the methylation status can be regulated by the demethylation process if necessary.

As an example of an epigenetic phenomenon, Daxinger and Whitelaw (2010) reported a classic study with flowers of *Linaria vulgaris*, which assumed a radial symmetry from a bilateral one (Figure 4.5). This variation was attributed to the *Lcyc* loci, whose modification was due to methylation, but not to the DNA sequence. The researchers observed a phenotypic correspondence of the flower to the degree of DNA methylation, a mark that is transmitted to future generations. It has already been observed that methylations acquired in DNA are a mark transmitted by meiosis to many future generations even though there is a crossing with the unmarked genotype.

Figure 4.5. Example of an epigenetic phenomenon evidenced in the symmetry of *Linaria vulgaris* flowers. In the center, original image. On the left, enlarged detail of the flower design in bilateral symmetry. On the right, an enlarged detail of the flower design in radial symmetry, the result of methylation at the *Lcyc* loci. Credit: image by Pratt, Anne; Step, Edward (1905) from Wikimedia Commons. License to use at https://commons.wikimedia.org/wiki/File:The_flowering_plants,_grasses,_sedges,_and_ferns_of_Great_Britain_(Pl._160)_(8517612401).jpg

To observe the effect of the environment on DNA methylation and epigenetic events, a study with dandelions was carried out in which Verhoeven et al. (2010) compared apomictic seedlings (and therefore, clones) multi-stressed and not stressed. They observed that multiple stresses induced changes in DNA methylation and that those changes were transmitted to the next generation.

To study the effect of changes in histones and transposable elements interfering with epigenetic events, an experiment with arabidopsis exposed to very high temperatures or exposed to stress by UV-B radiation was developed by Lang-Mladek et al. (2010). They observed that transposable elements were activated and transmitted to two generations, and that the loss of silencing of a gene was correlated to the increase in histone acetylation, but without loss of DNA methylation. In general, methylation is more associated with the repression of gene expression and acetylation with activation or de-repression.

A CURIOSITY

It is well known that during the ripening of fruits, the hormone ethylene acts as a trigger for the expression of several genes involved in the process. However, it is also known that ethylene, even if applied exogenously, is not able to induce the ripening of green fruits. This is commonly associated with low cellular sensitivity to the hormone due to the low number and affinity of ethylene receptors at that stage. However, Zhong et al. (2013) observed an event that may also be associated with the low response of green fruits to ethylene: genes related to ripening were methylated while the fruits were green and their demethylation only occurred close to maturation; also, gene transcription factors came into action as demethylation progressed, and, furthermore, the use of methyltranferase-5-azacytidine inhibitors promoted early ripening.

As will be seen in Table 4.1, transgenerational epigenetic memory can be reset in some future generation; the importance of this “forgetfulness” will be discussed later in the topic “stress memory”. Evidence indicates that the loss of this memory is linked to the loss of methylation or the maintenance of methylation in genetic material, but with gain in acetylation.

Table 4.1. Examples of phenomena related to transgenerational epigenetic memory reported for different species and situations.

Species/ situation	Observed effects	Reference
Arabidopsis thaliana / pathogen	Biotic stress due to *Pseudomonas syringae* infection induced the expression of defense genes regulated by salicylic acid, whose acquired resistance was transmitted to a stress-free generation. It was observed that acetylated histones favored the expression of these genes in generations with or without infection.	Luna et al., 2012
Phaseolus vulgaris / pathogen	Offspring of parents infected with *Pseudomonas syringae* maintained high expression of the *PvPR1* gene, which is highly responsive to pathogenic priming. This characteristic was lost after two generations.	Ramírez-Carrasco et al., 2017
Arabidopsis thaliana / cold	Mutant plants defective in DICER-LIKE PROTEINS (DCL2 and DCL3), important to sRNAs production, did not transmit epigenetic characters of cold stress tolerance to the subsequent generation, unlike the wild genotype, showing the importance of these proteins for the establishment of epigenetic memory.	Migicovsky and Kovalchuk, 2015
Arabidopsis thaliana and *Solanum lycopersicum* / chemical damage and herbivory	The offspring of plants treated with methyl jasmonate were submitted to caterpillar herbivory and mechanical damage as defense primings. In both species the primings promoted an increase in defense capacity. For two subsequent generations, 50% less caterpillars were found in arabidopsis. Mutant arabidopsis defective in the synthesis of sRNAs or jasmonic acid did not inherit the defense characters.	Rasmann et al., 2012

Geranium sylvaticum / mass of seeds and mycorrhizae	Seed mass was positively correlated to the total degree of DNA methylation. Seeds from hermaphroditic plants had higher DNA methylation when the parents interacted with mycorrhizal fungi.	Varga and Soulsbury, 2017
Arabidopsis thaliana / salinity	After two generations suffered hyperosmotic stress, tolerance characteristics were transmitted to immediate offspring, but were lost in successive offspring in the absence of stress. It was observed that the acquired tolerance depends on DNA demethylation and reprogramming of transposable elements.	Wibowo et al., 2016
Arabidopsis thaliana / heat	Stressed plants transmitted epigenetic marks to their immediate offspring. During stress, there was a decrease in methylation and an increase in the expression of transposons. The increase in gene expression in offspring was related to epigenetic marks in histones.	Migicovsky et al., 2014
Trifolium repens / Water deficiency in different clones	The epigenetic marks of some genes, a character transmissible to several generations was observed to be common but variable among different clones of the same species (clones of different species varieties).	González et al., 2018
Arachis hypogaea / water deficiency in different genotypes	The formation of epigenetic marks induced by water stress depends on the genotype in question, with variation among the five genotypes (varieties) tested.	Racette et al., 2019

According to the studies listed in Table 4.1, when exploring knowledge inherent to transgenerational epigenetic memory, information emerged with potential for use in the technological field of plant cultivation, since DNA methylation influenced factors of importance to agricultural production, such as the seed mass. In addition, with immediate possibility of use, we saw that the symbiotic interaction between plant and microorganisms affects the quality of seeds, in addition to the fact that seeds from stressed plants have a greater potential to originate plants less susceptible to the respective stress. On the other hand, it is important to pay attention to the possibility that the greater natural investment in defense will disadvantage the plant's investment in growth and production of reproductive organs, even if they are grown in a stress-free environment, but, if the cultivation occurs under a stress condition, the plant tends to increase the production in relation to the others that did not come from stressed predecessors.

Circadian memory

In the topic “Can plants add time cycles?”, Chap. 1, we saw the intriguing case of a bamboo clone that blooms at the same time regardless of geographic location, despite variations in edaphoclimatic conditions. Obviously, we cannot dissociate this fact from a memory, which, in this case, is intrinsic to the circadian clock. This fact is an indicator that after the establishment of memory (STO) for flowering according to time, it will not be erased over the years (in some genotypes flowering will only happen after more than one hundred years), and even if the environmental conditions do not favor uniformity in other aspects of development, they will not be able to induce the resynchronization of the clock oscillator for the floral response. The lack or low influence of environmental factors on clock-dependent responses is typical of circadian regulation. This type of regulation of memory is not common since environmental conditions tend to interfere on the retrieval of information (RCL). However, for the case of synchronized flowering of bamboo, there is evidence that the memory was unchanged, and, after the closing of the circuit or the sum of temporal circuits, the phenomenon manifested itself. More information on the biological clock and circadian response control can be accessed in the topic “How are plants synchronized with the time of day?”, Chap. 1.

Circadian control over responses in plants is linked to an environmental condition, based on a memory built especially by the time (A) of sunrise and sunset, and therefore dependent on the activity of photoreceptors. When changing the start time of the light period (day) or dark (night) of the 24h from A to B, the previously established memory remains active determining the clock oscillator to work at the rhythm of A for a few days, until, by repetition, the new time of the beginning of the light or dark period B is printed in the memory of the clock, that is, it will be resynchronized for the new time of its responses. The participation of the biological clock in the scope of stimuli-responses inherent to memory can be seen in Figure 4.6.

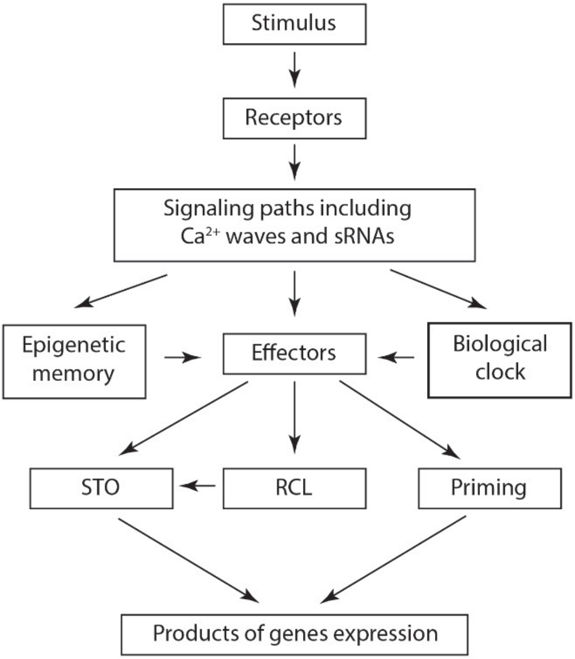

Figure 4.6. Proposed model of interactions between stimuli and responses inherent to memory. After the stimulus is perceived, the signaling pathways are activated, directly influencing the production of effector molecules, or indirectly, through memory epigenetics and the performance of the biological clock (circadian). The effectors activate the memory established by priming, which allows the source stimulus to directly trigger the expression of related genes. Effectors also stimulate the expression of memory genes related to information storage (STO) and information retrieval (RCL). The influence of previously stored information (STO) on gene expression depends on the effective action of the RCL. Model modified from the proposal by Torres et al. (2019).

In essay 1 on breaking symmetry in the growth of the axillary buds of the perforated cotyledons, dependent on the decapitation of the seedling apex (section "Evidence of memory in plants"), there was only a response to stimulating the perforations of the cotyledons if the decapitation of the apex was done at the beginning of the day, but not at noon. In essay 2 of the same section, which is about the formation of meristems in the hypocotyl due to calcium depletion, it was observed that this effect was more expressive between the months of April to June than in the other months of the year. Both examples denote a regulation of the biological clock over the STO/RCL, and in the case of the first essay, a circadian control of the response is also evident. Although the evidence for memory-clock interaction exists, the mechanism itself has not yet been elucidated.

Interactions between photoreceptors and aspects related to plant memory are beginning to be unveiled (see more information on photoreceptors-biological clock throughout the first chapter), which is an important step so that in the future we can understand the circadian influence on memory. For example, when working with transgenerational epigenetic memory, we saw the importance of processes of methylation/acetylation or alteration of histones influencing the responses inherent to memory. In the work of Jang et al. (2011) it was observed that phyA activation precedes the acetylation of histone H3, lysine 9/14 (H3K9/14) and H3K27, as well as the trimethylation of H3K4 in arabidopsis, demonstrating, therefore, that these are sites regulated by phyA. In fact, H3K27 starts to be methylated, with concomitant loss of acetylation, when phyA is inactivated.

In addition to phyA, other photoreceptors are also involved in regulating factors that can potentially participate both in circadian memory, due to their influence on the clock, and in epigenetics. PhyB and cry2 are known photoreceptors in the regulation of chromatin condensation levels, as well as mediating changes in the methylation and acetylation status of histones, as suggested by Torres et al. (2019) due to their influence on the synthesis of DEACETYLASE6 (HDA6) and METHYLTRANFERASE1 (MET1).

One factor that shows the circadian influence on plant memory is that histone acetylation is necessary for the expression of the clock's main oscillator genes which are expressed during the day, the *CCA1* and the *LHY* (Figure 1.5). The expression of *TOC1*, a nocturnal expression gene, is also dependent on histone acetylation. HY5, on the other hand, a well-known regulator of the signaling pathway in photomorphogenesis, has also been observed to promote inhibition of H3K27 methylation.

In view of the evidence presented above, it is clear that photoreceptors and other biomolecules that act in the regulation of clock-dependent events are involved in the processes of methylation or acetylation known in the context of epigenetic memory, but there are still no mechanisms to explain the interaction between the biological clock and the transfer of memory to future generations. However, there is evidence of this interaction, as in a study conducted with arabidopsis by Yerushalmi et al. (2011), crossing mutants with alterations in circadian rhythms; the F3 generation still had great differences in rhythms, which were related to the performance of the *PRR5*, *PRR7* and *PRR9* genes, which participate in the clock oscillator (Figure 1.5).

An experiment carried out with electrical discharges made evident a circadian memory, and therefore, related to specific times of the day, regarding the sensitivity or resistance of the plants to the discharges made. When electrical discharges in *Clivia miniata* were carried out at night, the kinetics of the discharge were significantly slower than for the daytime discharge (Volkov et al., 2012), which means that the capacitor's resistance to discharge increases at night. When analyzing the resistance at different times of the day, it was concluded that it started to increase at 4:00 pm reaching the maximum at 7:00 pm, even if the plants were kept under artificial light, which shows that the circadian memory of the evening period directly interferes with the electrical resistance of the plant. Throughout the work, the hypothesis was raised that this interference may be associated with a greater closure of the gates of the transmembrane channels, which participate in the sensitivity of the tissues to electrical signals.

In general, circadian phenomena are manifested by predicting a habitual environmental condition at a certain time, regardless of whether this condition will actually be established on a certain day. For that, it is essential that there should be a memory about the usual changes in the environment that occur over the 24h period.

Electrical memory

In the section “Touch Perception”, Chap. 1, aspects related to the closing of the trap of the venus flytrap were explored, a behavior that, in addition to touch (which generates an action potential), can also occur by electrical stimulation of 1.5 V applied for 1 s, without the need for any mechanical stimulation. To trigger the closure of the trap, a minimum electrical charge is required from the midrib (positive potential) reaching the modified leaf lobe (negative potential). However, if the charges are below the necessary voltage, with a short time between them (less than 50 s), the charges will be added by the plant and when the minimum limit is reached the effect will occur (Volkov et al., 2008), which is evidence, therefore, of the existence of a short-term electrical memory, of mechanism yet to be elucidated.

Spatial and temporal memory

A spatial memory can be observed in the phototropic curvature of *Zea mays* (Nick and Schäfer, 1988). The experiment developed was based on the changes in the radial distribution of auxin in the stem apex from the phototropic stimulus, which causes changes that culminate in the asymmetric-radial difference in the growth of cells, leading to the curvature of the organ. With a pulse of blue light, the phototropic curvature of coleoptiles was stimulated towards the light source, which occurred after 1.5 h of the stimulus. It was also observed that if a second stimulus was projected to another lateral point of the coleoptile before 65 min, from the first stimulus, the phototropic curvature would occur in the direction of the second stimulus, and if the second stimulus occurred between 65-90 min there was no curvature (Figure 4.7). This type of response reveals the existence of a spatial memory, time dependent on a scale of minutes. After the stimulus, it takes time for the organ to determine the response, which can be canceled or reversed by another stimulus before the organ is determined to respond.

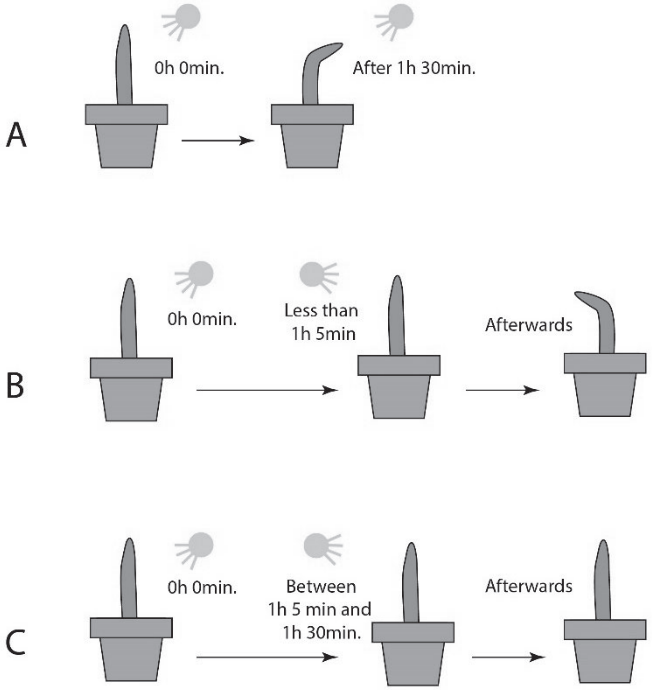

Figure 4.7. Phototropic curvature in maize coleoptiles showing the existence of a spatial memory in the organ, based on the experiment by Nick and Schäfer (1988). A. The lateral incidence of blue light induces phototropic curvature after 1.5 h of its incidence. B. If before 65 min of the first stimulus, the light is projected to another side of the coleoptile, the curvature will occur in the direction of the second stimulus. C. If the second stimulus occurs between 65 min and 90 min, from the first, no phototropic curvature will occur.

The responses observed in Figure 4.7 exemplify that the plant is not simply an organism that only responds to stimuli, but that it can compute different stimuli before responding; an important factor, as it avoids spending unnecessary energy in response to an unreliable stimulus. Naturally, there are mechanisms for this calculation, including chemical and electrical references, as discussed in the topic “Plants perceive the direction of light”, Chap. 1. Reliability of the perceived stimulus and the ability to analyze multiple signals by the plant will be addressed again in the next chapter.

The memory linked to the reversibility of stimuli, although not necessarily spatial, as in the example above, is classic in photoreversibility studies, typical of events mediated by phytochromes. Let us consider the hypothetical condition of a plant whose only factor necessary for floral induction is its exposure to a peak of radiation R. When exposed to that radiation there will be the triggering of metabolic events that will lead to the floral determination supposedly in 2 hours. After 2 hours from the start of the stimulus (radiation R), it will no longer be possible to reverse the effect of R by exposing the plant to an inhibiting factor (Fr radiation). However, if, for example, after 15 min of the plant's exposure to R radiation it is exposed to Fr radiation, the effect will be reversed and the plant will not be determined to flower.

In a simplified way, what is expected from reversible events is that after a specific stimulus, accumulation/activation of A molecules and inactivation/destruction of B molecules occurs (in the case of phototropism, in different regions of the same organ), as well as the effect otherwise triggered by the opposite stimulus. When a certain A/B balance is reached that serves as a threshold to trigger a response, the time limit in which it could be reversed by another stimulus is finished. In that case, the activity of the signaling pathways that hang to the A or B side of the balance reveals a mechanism triggered by the last stimulus, and in the case of photoreversibility, of the last radiation to which the plant was exposed. It is important to note that, although the radiation is the same perceived by the plant since its seedling phase, its effect on flowering will only be effective after the organism has passed the juvenile phase and reached the adult stage, which indicates the need for the establishment of a condition, probably related to chemical balances, of permissiveness (PST) for information inherent to reproduction to be retrieved (RCL) from a stimulus.

Acoustic memory

In the topic "Plants hear the sound of danger", Chap.2, we saw an experiment in which *Arabidopsis thaliana* increased their glucosinolates against a caterpillar only when subjected to recorded acoustic vibrations from the caterpillar chewing, but not when subjected to others acoustic patterns. The same behavior was observed in the section "Acoustic communication in plants", Chap. 2, when greater gene expression took place under specific pattern of sound, namely, 500 Hz of frequency and amplitude of 80 dB for one hour uninterrupted, differently of other frequencies. In the same chapter, in the topic "Plants have phonotropic responses" the same behavior was observed in relation to the positive phonotropic curvature of the roots to acoustic stimulus of 200 Hz during two weeks of plant exposure. Based on these observations we conclude that plants only respond to sounds of specific patterns (amplitude, frequency, time and dynamic of exposure), although they could be subjected to a wide variety of sound stimuli, what necessarily requires a memory of a specific sound pattern for triggering a specific response. Although in the section about the acoustic communication in plants we could see a broad range of genes responsive to the acoustic vibration, still we do not know which are related to the acoustic memory. It is expected that soon, new studies will broaden our knowledge about the acoustic memory circuit, just as we already have knowledge of many genes related to the stress memory circuit.

Winter memory

Many species need to be subjected to many hours of cold as a stimulus to floral induction. However, when the formation of reproductive organs still depends on other factors, such as photoperiod, after reaching the minimum number of cold hours required for induction, the plant can stay months without flowering due to not being exposed to the necessary photoperiod, but as soon as it is subjected to the inducing photoperiodic condition, it will flower. That is, the memory of floral induction built during the cold can be stored for months.

Prolonged cold is well known as a factor of repression of the *FLC* gene (*FLOWERING LOCUS C*), being a strong inhibitor of floral morphogenesis, and even after vernalization remains repressed. During vernalization, there is an increase in the methylation of histones H3K9 and H3K27 linked to the *FLC* (Sung and Amasino, 2006), which, as seen in previous sections, is indicative of repression. As previously explored in the topic "Plants can add hours of cold", Chap. 1, and since the induction to flowering by cold can be interrupted until the plant reaches the minimum required hours limit, it is assumed that memory for maintenance of the repression of the *FLC* genes after the cold period is established late, close to the minimum limit of hours necessary for floral induction.

The maintenance of floral induction memory is also well known when leaves of plants already induced to flowering are used as grafts on non-induced plants (rootstocks). The memory present in the graft is sufficient to reprogram the non-induced rootstock to flower (Figure 3.11).

Immunological memory

What is called immunological memory concerns the defense of the plant by becoming more robust after the recognition of a pathogen that previously infected it. In the topic on transgenerational epigenetic memory, it has already been seen that the acquisition of greater defense of a plant due to its exposure to a dangerous condition can be transmitted to future generations by genetic heritability, but the capacity for increased defense induced in a single life cycle by repeated exposure to a pathogen has not yet been explored.

A memory constructed by priming can be established both by exposing the plant to a pathogen and by substances that activate pathogenic signaling pathways, such as salicylic acid, azelaic acid, pipecolic acid, methyl jasmonate and xenobiotics. As an example, in a study with arabidopsis, Beckers et al. (2009) observed that infection by *Pseudomonas syringae* or the use of benzo-(1-2-3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH), salicylic acid, 3-hydroxybenzoic acid (i.e. substances that mimic the infection), used as primings in three to four leaves, triggered an increase in mRNA and MITOGEN-ACTIVATED PROTEIN KINASES proteins (MPK3/6), which are involved in defense signaling. It was also observed that MPK3/6 are kept inactive in cells submitted to priming but are activated quickly in the case of a new pathogenic infection, in addition to further increasing their synthesis when comparing plants submitted to priming with those not submitted. When using mutants defective for these two MPKs, there was no improvement in subsequent tolerance, indicating the importance of these molecules to the formation of memory.

Greater accumulation of inactive proteins, such as MPKs, in periods of non-stress, in addition to the increase in receptors for some substances (such as hormones linked to stress) and changes in chromatin status, are factors observed after the submission of a plant to a priming and are characters that are associated with the greater tolerance of the plant when it is resubmitted to the stressor. Although we use the term immunological memory, observations are made not only of pathogenic infection, but they also extend to herbivory and various abiotic stresses.

An example of immune memory triggered by herbivory was observed by Ruuhola et al. (2007) in which *Betula pubescens* foraged for five years by *Epirrita autumnata* had a more forceful defense response than plants without a history of herbivory. The pupae present in the conditioned plants had less weight and the larval period was more prolonged. It was observed that the quercetin:kaempferol ratio was higher in conditioned plants, which probably caused greater damage to the pest's digestive tract.

Stress memory

Evidence of memories stored from abiotic stresses has been mentioned in the topic of transgenerational epigenetic memory. Here, the memory established in plants subjected to successive stresses in a single life cycle will be specifically addressed. This memory, being involved in the defense of the plant, can also be considered as an immunological memory, although the term stress memory is common.

It is desirable that perennial plants acquire tolerance to frequent stresses, but it is questionable whether the acquisition of long-term tolerance would be beneficial to infrequent stresses, as it is common for the plant to decrease its growth and reproduction capacity when under stress. As noted in the topic of transgenerational epigenetic memory, the acquisition of a defense or tolerance skill can be erased in subsequent generations depending on the environmental condition, especially when there is no more exposure to stress; factors that imply processes of (de)methylation and acetylation of genetic material. The present question is whether perennial plants have the same capacity to “forget” the signaling pathways to respond to stress, in the post-stress period, due to disuse, and thus start to invest more resources in growth and reproduction at the expense of defense. Also included in this calculation is the cost of maintaining some of the defense elements produced during stress, such as MPKs, on stand-by. In Figure 4.8 there is a representation of what is expected from the production of plant reproductive organs due to the construction of, and access to the stress memory.

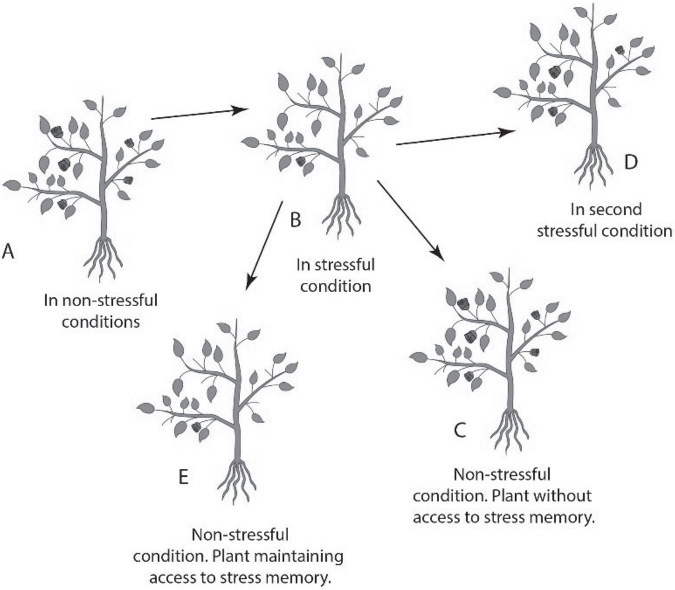

Figure 4.8. Expectation of the production of plant reproductive organs due to the construction and access of the stress memory. A. Non-stressed plants tend to invest heavily in the production of reproductive organs. B. Under the first stress, the plant tends to invest a lot in defense and to invest less in growth and reproduction. C. After the plant returns to a non-stress condition, it tends not to access the stress memory and thus does not invest unnecessarily in defense, returning to invest a lot in reproduction. D. In the condition of second stress, the plant tends to access the memory built during the first stress, facilitating the construction or activation of the defense arsenal, which requires less investment than in the previous stress, and thus, more resources remain to invest in reproduction, compared to the first stress. E. The continuous access to stress memory could induces the plant to maintain high investment in defense at the expense of reproduction, even under non-stressful conditions.

In the post-stress period, it is expected that many of the stress response genes will be repressed, and that other genes repressed during stress will be released from repression. The maintenance of the memory established during stress may, however, act in maintaining the expression of some of the defense genes, even in the post-stress period. It is questionable whether this cost of investment in greater defense is compensatory to the plant due to the frequency of its exposure or the risk inherent to stress. To maintain the defense metabolism, it is necessary, however, that there be an effective action of permissiveness factors to access stored information (PST). There is a possibility that the defense information is stored, but the cellular metabolic condition in the non-stress period does not favor its access (RCL), a condition more common than the maintenance of the active defense pathways.

Although most signs of stress disappear in the post-stress period, some can remain active for some time. In a water stress study with *Medicago truncatula*, it was observed that 24 hours after stress 10% of genes active involved in the water deficiency response were still active (Zhang et al., 2014). In the same line of investigation, it was observed in arabidopsis, malnourished for phosphorus for 21 days, that 31 days after stress there were still 80 genes differentially expressed because of stress (Secco et al., 2015). In those studies, gene silencing was once again associated with chromatin condensation and the hypermethylation of DNA and transposable elements.

A little-known factor, but with the potential to participate in the construction of stress memory, is the proportion of different RNAs during and after periods of stress. The content of RNA-binding proteins, such as Rpb4 and Rpb7, also fluctuates greatly due to stress and non-stress and facilitates rapid transitions in the abundance of mRNAs in the cell. It is expected that the balance between different transcripts and the drop in the abundance of some specifics will be considered in the construction of cellular memory. Another piece of evidence is that the DNA polymerases IV and V transcribe genes that act in triggering and mediating the methylation of specific DNA sequences via sRNAs (Crisp et al., 2016).

In the sequence, some experiments will be reported that resulted in important information concerning plant memory inherent to different stresses.

HEAT

The proteins LARP1 (LA RELATED PROTEIN 1A) and XRN4 (EXORIBONUCLEASE 4), associated with high temperature stress, promote a drop of approximately 4500 mRNA, an event necessary to establish heat tolerance. XRN4 is also associated with regulation of the levels of HSFA2 (HEAT SHOCK TRANSCRIPTION FACTOR A2) transcription factors, since *xrn4*-defective arabidopsis mutants maintain high levels of HSFA2 after stress release longer than non-mutants (Merret et al., 2013; Nguyen et al., 2015); that means, the presence of XRN4 inhibits the access to stress memory in periods of non-stress.

In another experiment, it was observed that the presence of sRNA was essential for plants that had undergone high temperature stress to have greater tolerance to a second stress, in addition to the fact that the presence of miRNA156 was fundamental to the transition of the performance of transcription factors between the period of stress and the period of non-stress, this miRNA being constitutive and induced by high temperature (Stief et al., 2014). This experiment highlights the importance of RNAs both for the transition from the period of stress to non-stress as well as for access to the memory of past stress.

COLD

It was observed that the conditioning of seven-day-old arabidopsis seedlings to cold did not result in greater tolerance to stress when they were resubmitted to it; greater tolerance only occurred when priming was imposed on plants already with true leaves (14-21 days) (Leuendorf et al., 2020), which indicates the importance of the physiological state of the plant to the storage of information (STO).

In another experiment Griebel et al. (2020) observed that the conditioning of arabidopsis for only 24 h in cold conditions of 4ºC, not only induced greater tolerance to future exposure to cold but also promoted greater tolerance to infection by *Pseudomonas seryngae*, even after 5 days of priming, this effect being dependent on the activity of chloroplast ascorbate peroxidase.

DROUGHT STRESS

In an experiment developed by Melo et al. (2019), solutions of 0 to 200 μM of ABA sprayed once a week on rubber tree leaves for twelve weeks were used, followed by suspension of irrigation for 13 days. During the irrigation suspension period, the plants that had received the highest doses of ABA maintained greater tolerance to drought, evidenced by the prolongation in days of high rates of net photosynthesis and potential photochemical efficiency of photosystem II (Fv/Fm). There was a natural abscission of the leaves of all plants during or after stress period. After growing, and 120 days after the first stress, the plants were resubmitted to another 13 days of irrigation suspension, this time without leaf treatment of ABA. All plants, including the control plants, which had never been treated with ABA, had an increase in drought tolerance during the second stress, prolonging the high photosynthetic rates and the Fv/Fm ratio in relation to the first stress by up to six days. This result shows that during the second stress the plants were able to retrieve information (RCL) of drought tolerance stored (STO) during the first stress, and the phenomenon is independent of whether previously stressed leaves are maintained or not. Another study carried out with coffee trees, Guedes et al. (2018) pointed dozens of genes probably related to water stress memory, many of them that also participate in the ABA signaling pathways.

In another experiment, which was carried out with wheat, Wang et al. (2014) exposed the plants to one or two periods of water stress before anthesis (soil with 35-40% water). Fifteen days after anthesis, the plants were submitted to another period of stress (soil with 20-25% water). Plants treated with one or two stresses prior to anthesis had higher grain yield than unconditioned plants. In addition to production, conditioned plants also had higher photosynthetic rates and peroxidase activity, as well as lower malondialdehyde content (a marker of oxidative stress) during post-anthesis stress.

SALINITY

Tomato seeds conditioned by their submission to a 6 M NaCl solution gave rise to plants with less dry mass of roots and shoots, but with a higher content of soluble sugars and organic acids in the leaves, compared to plants from unconditioned seeds (Cayuela et al., 1996). In *Trigonella foenum-graecum* the osmotic conditioning of seeds also provided a better performance of the plant to stress using as evaluation parameters, the growth, pigment content and increased CATALASE and GLUTATHIONE PEROXIDASE activity (Mahmoudi et al., 2019).

In rice, Amaral et al. (2020) observed alteration in the expression of many genes involved in salt stress signaling pathways and some of them apparently are important to the salt stress memory, which encode to the Zinc Finger Family Protein (ZFP), AP2 DOMAIN CONTAINING PROTEINS and CYTOCHROME 450.

Summary

All memory is based on information stored and retrieved after a time. What differentiates the different types of memories mentioned in plants is the context in which the information is stored and accessed. Transgenerational epigenetic memory alludes to a memory built under a given circumstance, which is transmitted to future generations, so that there are no changes in the DNA sequences, but in markings, such as the methylation of genetic material. This is a memory that can be forgotten in future generations not exposed to the same circumstance as the generation previously affected.

Circadian memory is that linked to the oscillator of the biological clock, whose responses anticipate effective environmental changes, especially in light, which is closely linked to the action of photoreceptors. Even if eventually the change does not occur in some day the response dependent on circadian memory occurs. There are cases in which the time for manifesting a stored memory is counted in years, which shows a memory built at the beginning of the plant's life cycle and which is maintained for decades until the closing of a time circuit that triggers the synchronized manifestation of inherent phenomena such as death or flowering of some bamboo genotypes.

The electrical, spatial and temporal memories are dependent on the temporal or spatial frequency of stimuli. Electric memory is known as short-term one, since cumulative electrical stimuli (charge) can be added, and from then on, trigger an effect, such as the closing of the insectivorous plant trap, which only occurs if the frequency of stimuli to be added does not exceed a few dozen seconds. Spatial memory, on the other hand, can be considered medium-term, because if a phototropism-inducing light stimulus shines on side A of the stem apex followed by another stimulus on side B of the same apex, distant in a time scale of some dozens of minutes, phototropic curvature can be maintained according to first stimulus, changed to the B side, or simply be canceled.

Acoustic memory is related to response of a plant to a specific pattern of sound, depending on its amplitude, frequency, time and dynamic of plant exposure. It occurs, as example, in plants that improves their defense mechanisms when subjected to a specific sound of a caterpillar chewing recorded by a sound device.

Winter memory is one in which a developmental event, such as flowering, depends on vernalization, with a minimum limit of hours of cold as well as photoperiodic induction. Competent flowering plants, when vernalized, can maintain the memory of the cold stimulus for months until they are exposed to the inducing photoperiod to flower. In this situation, it is a long-term memory.

Immune and stress memory is that involved with defense and tolerance, whether to biotic or abiotic stressors. The memory acquired in the first exposures of the plant to an adverse condition favors its tolerance or defense to subsequent exposures.

CHAPTER 5

INTELLIGENCE IN PLANTS

Plants are organisms continually exposed to situations that challenge their development. Different stresses, such as thermal, water, hypoxic and nutritional ones, in addition to the attack of herbivores, pests and infection by pathogens, are situations, isolated or combined, commonly experienced by plants in their natural habitat. To overcome these obstacles, it is necessary that they not only develop mechanisms of tolerance to recidivist adverse situations in a single life cycle, but also, throughout evolution, that they acquire constitutive resistance against the most frequent dangerous factors occurring in their ecosystem. The fact that a specimen or genotype molds itself to different situations in the environment to maintain or improve its development is a sign of intelligence.

Studies related to “plant intelligence” started approximately two decades ago, although many of its foundations have been known for a long time. Dozens of concepts are found in the literature for “intelligence”, some of which are anthropocentric and others of a generalist nature. Considering the semantic aspects applicable to plants, the objective of this chapter is to explore the capabilities of plants in the thematic scope of intelligence.

Some cognitive abilities, essential to the understanding of plant behavior, have already been addressed in previous chapters, such as the perception of stimuli by the plant, its ability to communicate intracorporeally or with the environment stimulus, as well as its ability to memorize. Other topics that are part of the study of intelligence, not yet discussed, will be explored in this chapter, namely, (self)recognition, learning, behavior (in a *stricto sensu* perspective), prediction, decision-making, cognition, attention, awareness and sociability. For the purposes of familiarizing and facilitating understanding, when possible, experiments and subjects already explored in previous chapters will be revisited and put under the perspective of the current discussions.

Intelligence Concepts

The different existing concepts for "intelligence" have instigated many debates about their applicability to plant behavior (Struik et al., 2008; Baluška and Mancuso, 2009; Cvrčková et al., 2016; Adams, 2018; Second-Ortin and Calvo, 2019; Taiz et al., 2019; Calvo et al., 2020; Calvo and Trewavas, 2020; Parise et al., 2020; Robinson et al., 2020). However, the discussion can become sterile when the aim is to compare skills dependent on different instruments inherent to different organisms. It is not convenient, for example, to compare intelligent behaviors dependent on human brain activity with those manifested by plants, a no-brain organism. On the other hand, the discussion can be fruitful when respecting the limitations of each organism and considering the possibility of intelligent behavior independent of brain activity.

There are more than 70 definitions found in the literature for "intelligence", which vary immensely, according to the focus of the study. Wide variation of concepts is also found for "behavior" and "cognition". While some concepts are limited to human capacities, others are comprehensive, applicable to other forms of life, or even to inanimate forms, the object of artificial intelligence. In the sequence, some concepts for intelligence will be transcribed, whether of an anthropocentric or general nature.

> "*a very general mental capacity that, among other things, involves the ability to reason, plan, solve problems, think abstractly, understand complex ideas, learn fast and learn from experience. It is not a mere literary learning, a strictly academic skill or a talent for doing well in tests. Rather, the concept refers to a broader and deeper ability to understand the world around you – "catching up", "catching" the meaning of things or "perceiving" something*" (Wikipedia, 2020)
>
> "*intelligent behavior is an aspect of complex adaptive behavior that provides a capacity for problem solving*" (Trewavas, 2005)
>
> "*an intrinsic ability to process information from abiotic and biotic stimuli that allows for optimal decision-making about future activities in a certain environment*" (Brenner et al., 2006)
>
> "*adaptively variable behavior during the individual's life*" (Stenhouse, 1974)

According to most of the concepts transcribed above, plants are organisms that correspond to the premises that define a being as intelligent. However, for that, a detailed analysis of its capabilities is required in different aspects that encompass this conceptual domain. Undoubtedly, the perception capacity is essential to the intelligent organism, since it is from there that there can be a response to stimuli. Communication, in turn, is fundamental to cellular homeostasis and harmony between distant and interdependent organs, as well as interactions with external stimuli. Adaptation, or learning, would not be possible without the ability to memorize. Therefore, the three themes explored in the previous chapters are part of the study of intelligence in plants. Matters not yet approached, but inherent to the thematic, will be the focus of the sections that follow.

Identity Recognition

In this section, the ability of plants to self-recognize and to recognize their neighbors as conspecific or heterospecific will be explored, as well as the degree of kinship of the conspecific neighbor. Of the mechanisms of action for the recognition of identity, the most elucidated are those for inhibiting self-pollination. However, other organs, in addition to reproductive ones, also participate in identity recognition.

Self-recognition

From an evolutionary point of view, it is proposed that the reproductive self-incompatibility that occurs in at least 40% of phanerogams is an important factor in the preservation of species, in the same way that self-fertilization can lead to a greater risk of extinction (Igic et al., 2008). Although there are many strategies to avoid self-pollination, in some it is necessary for the plant to recognize its own pollen and thus trigger mechanisms so that self-fertilization does not occur. As suggested by Fujii et al. (2016), it is expected that the mechanisms developed for self-incompatibility, based on self-recognition, be the result of learning throughout evolution, a subject to be explored in this chapter.

According to Takayama et al. (2000), the recognition of “self” or “non-self” in Brassicaceae depends on two polymorphic genes (when more than one allele occupies the gene's locus in at least 1% of a population). The determining factor of male recognition is known as S-Locus Protein 11 (SP11) or S-Locus Cysteine-Rich protein (SCR). The determining factor for female recognition is the S-Locus Receptor Kinase (SRK), located on the plasma membrane of the stigma papillary cells (Figure 5.1). The molecular interaction between SP11 and SRK of the same haplotype (combination of a group of adjacent loci alleles, which are part of the same chromosome) leads to self-recognition and induces incompatibility, culminating in the rejection of pollen by stigma. Downstream of the SP11-SRK complex, pollen rejection is signaled. Although the sequence of events is not known, some steps are known, such as the interaction of SRK with M-Locus Protein Kinase (MLPK), also present in the papilla membrane; the action of a ubiquitin ligase (ARC1) that signals the degradation of pollen acceptance factors, such as Exo70A1 and the influx of Ca^{2+} through channels linked to glutamate receptors.

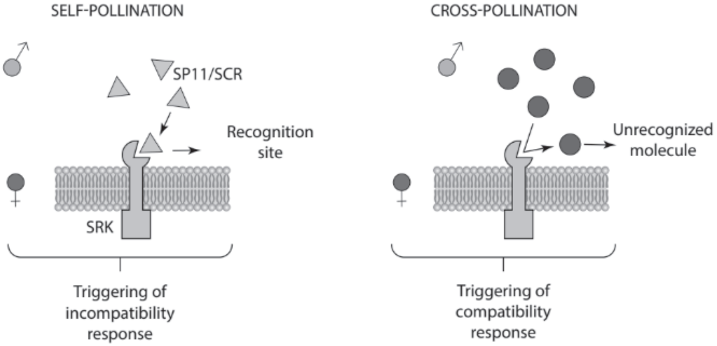

Figure 5.1 Mechanism of recognition of “self” and “non-self” during self-pollination or cross-pollination in Brassicaceae. S-Locus Protein 11 (SP11) or S-Locus Cysteine-Rich protein (SCR), male recognition factors, when coupled to the S-Locus Receptor Kinase (SRK), female recognition factor present in the plasma membrane of the papillary cells of the stigma, indicates self-pollination. Since it is unwanted, an incompatibility response is triggered. In case there is no male factor coupling in the SRK, it is indicative of the desired cross-pollination, thus triggering a compatibility response.

Another known mechanism of recognition between the “self” and the “non-self” occurs in Solanaceae. According to Sijacic et al. (2004) the female determining factor of recognition is the S-RNase glycoprotein, which has a ribonuclease-cytotoxic effect, inhibiting the growth of the pollen tube in the stylet, where it is abundant. The pollen, in turn, has an *S-Locus F-box* (*SLF*) gene that binds to S-RNase, which encodes a protein, SLF, which is part of the ubiquitin ligase recognition system SCF (Skp1 – Cullin1 –F-box). It is likely that SLF acts in the recognition of strange RNase by ubiquitin ligase, which after ubiquitination is degraded by proteasome 26S, allowing the continuation of the growth of the pollen tube in the stylet (Figure 5.2). However, there is no copy of SLF for RNase of the same haplotype, and, because of that there is no destruction of it, then the RNase persists and there is no growth of the pollen tube. On the other hand, when recognizing other haplotypes, copies of SLF promote the degradation of toxic S-RNase, allowing the growth of the pollen tube and facilitating cross-fertilization.

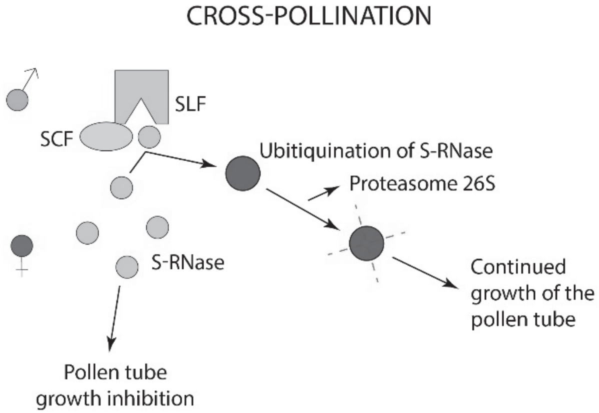

Figure 5.2. Mechanism of recognition of the "non-self" that occurs in Solaneceae. S-RNase is the female recognition factor, abundant in the stylet, but with an inhibitory effect on the growth of the pollen tube. S-Locus F-box (SLF) is the male recognition factor, which is part of the ubiquitin ligase SCF. In the absence of recognition of S-RNase by SLF, it is ubiquitinized by the SLF-SCF, which serves as a mark to be further degraded by the proteasome 26S. After its degradation, the pollen tube continues to grow in the stylet. However, there is no copy of SLF for RNase of the same haplotype, and therefore, there is no recognition of it and it is not ubiquitinized. The S-RNase persists and so the pollen tube does not grow in the presence of pollen from self-pollination, and thus, there is inhibition of self-fertilization.

In Papaveraceae, the mechanism of self-recognition and self-incompatibility is known for the presence of polymorphic proteins in both the male and female reproductive apparatus (Wheeler et al., 2009). The papillae of the stigma secrete the PrsS proteins (Papaver rhoeas style S), which, when linked to the pollen of the same haplotype, probably received by a protein called PrpS, results in rejection, triggering mechanisms that culminate in its cell death (Figure 5.3). In the signal transduction pathways for cell death, there is an increase in cytosolic calcium, phosphorylation of pyrophosphatase, depolymerization of actin and microtubule filaments, increase in ROS, cytosolic acidification, protease activity and DNA fragmentation.

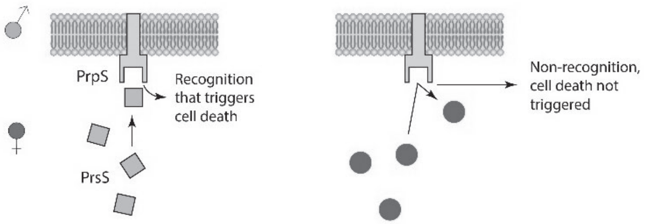

Figure 5.3. Mechanism of (self)recognition during self-pollination and cross-pollination in Papaveraceae. The female recognition factor, PrsS (Papaver rhoeas style S), which are proteins secreted by the stigma, upon being recognized by the male recognition factor, the PrpS proteins, there is a rejection response that leads to cell death. In the absence of recognition, there is no triggering of cell death.

When analyzing the three mechanisms of molecular recognition of "self" and "non-self" in three families, and each having a particular mechanism, it is assumed that there are several others to be known in other taxa.

Recognition of neighbors

There is abundant evidence of identity recognition among neighboring plants, necessarily implying self-perception and the perception of the other. Unlike the previous topic, in which the focus of the approach was trained on reproductive structures, in this one, the focus will be on recognition involving other structures. Some studies that show this ability were selected and listed in Table 5.1.

Table 5.1. Evidence of self-recognition and recognition of conspecific or heterospecific neighbors by vegetative organs.

Species	Observed effects	Reference
Ambrosia dumosa and *Larrea tridentata*	In a homogeneous environment in water and nutritive availability, roots of *A. dumosa* that self-touched continued to elongate normally, but when they touched *L. tridentata*, the elongation decreased; *L. tridentata* reduced the elongation of the roots by the presence of any neighbor, conspecific or heterospecific, without need physical contact.	Mahall and Callaway, 1991
Trifolium repens	Clones interconnected by stolons had reduced growth and root mass. However, there was a greater development of roots after being disconnected, showing a loss in the capacity of intraclonal recognition.	Falik et al., 2006
Kalanchoë daigremontiana	Conspecific plants were grown interacting with clones and non-clones. In the non-clonal interaction, there were more roots and fewer seedlings formed.	Yamawo et al., 2017
Arabidopsis thaliana, siblings and strangers	Plants were treated with their own exudates, from the siblings or from conspecific strangers. Only exudates from strangers induced greater formation of lateral roots, a suppressed response when using secretion blockers. Exudates from siblings or strangers, but not itself, inhibited the increase in the length of the roots. In short, the ways of recognition between itself, the relative and the stranger differ; exudates are necessary for recognition.	Biedrzycki et al, 2010

Centaurea maculosa and *Festuca idahoensis*	*C. maculosa*, treated with methyl jasmonate, when in conspecific interaction produced more phenolic defense compounds than when in interaction with *F. idahoensis*. Only in the heterospecific interaction *C. maculosa* invested more biomass in growth.	Broz et al., 2010
Artemisia tridentata	It was observed that volatile compounds from damaged stems induced increased resistance to herbivory only in clones, but not in conspecific non-clone neighbors.	Karban and Shiojiri, 2009
Momordica charantia and *Passiflora caerulea*	In both species, tendrils curled preferentially in the neighboring plant than in itself. In *M. charantia*, the tendrils were curled faster among different cultivars than among plants of the same cultivars.	Sato et al., 2018
Pinus contorta	Pines infected by the fungus *Grosmannia clavigera* released volatile compounds that were perceived only by related chemotypes, inducing an increase in defense mechanisms, a response that evidences the recognition of a substance among conspecifics.	Hussain et al., 2019
Cultivars “alva” and “kara” of *Hordeum vulgare*	The cv. “kara” in presence of volatile compounds released from “alva” has accumulated more biomass in roots than in presence of its own volatiles or absence of volatiles.	Ninkovic, 2003
Brassica nigra and three heterospecifics	The allelochemical sinigrine released by *B. nigra* inhibited the development of heterospecifics in a relationship dependent on physical contact between roots but did not affect the root development of conspecific neighbors.	Lankau, 2008

Although there is evidence of identity recognition by roots, the mechanisms are still elusive. There are many aspects to be isolated to be sure of the source of the stimulus. The soil is complex, differing in water availability, nutrition, microbiota, content and composition of allelochemicals, among other factors that could interfere in the identity recognition responses. In addition, there are different pathways for recognition involving roots, as in some species there is a need for physical contact for a response, while in others it does not. There is also variation in responses depending on the identity of the other. After recognition, some species project their roots to the opposite side of the neighbor, while others project towards the neighbor, and others tend to contain their roots in the space they already occupied (Figure 5.11), just deepening their roots, be it the neighbor conspecific or heterospecific. There are still cases of species that simply seem to ignore their neighbors.

As expected, the presence of chemical substances plays an important role in the recognition and responsiveness of roots to the presence of neighbors. This evidence can be seen in the behavior of *A. thaliana* (Table 5.1) according to exudates to which the plants were exposed.

Not only is the presence of chemical substances the factor involved in the recognition of identity among neighboring plants, but also the oscillation of their release has been considered an important information for the recognition of the other, even if they are of the same genotype, but in distinct physiological states. In Table 5.1, it was observed that while *T. repens* clones were connected by stolons there was a typical self-recognition response, however, apparently the recognition was decreased or lost by disconnection. In this case, because they are clones, there is not expected to be a chemical difference, at least qualitative, among neighbors sufficiently capable of loss of identity recognition. Although in an elusive way, for this type of situation the possibility has been raised that the recognition among neighbors is affected by hormonal oscillations inherent to the different physiological states among plants. There is a difficulty in understanding how these signals could be perceived by neighboring organisms, especially if they are not very close. The hypothesis is that, in this situation, communication occurs through electrical signals established by the oscillation and quantity of exudates, causing changes in electrical fields to be perceived by the mechanisms covered in previous chapters, as in the section “Perception of electric fields”, Chap. 1, and “Electrical communication in plants”, Chap. 2. The recognition of the electrical signal will depend on its resonant amplification by the receiving organism.

The identity recognition evidenced by the behavior of *M. charantia* tendrils (Table 5.1) is probably associated with differentiated volatile chemical compounds released by plants of different cultivars. Rapid curling in the other cultivar can be an adaptive advantage due to the greater chance of the plant gaining more space and light. When the tendrils are curled in the neighboring plant of the same genotype, but not in the plant itself, as observed in *M. charantia* and *Passiflora caerulea*, it is believed that the perception of the other depends on the different physiological states of the neighbors, as discussed in the previous paragraph. There are studies carried out with other genotypes without identifying aspects of identity recognition evidenced by the tendrils' behavior.

In Table 5.1 we can find a recurrent response in plants of different genotypes, which is an increase in mass, either root or of aerial part organs, as occurred in *T. repens*, *C. maculosa*, *K. daigremontiana* and *H. vulgare* in the presence of neighbors, especially heterospecific, conspecific non-siblings or non-clones, which results in increased competitive capacity. This behavior reveals that plants alone invest a lot in reproductive organs instead of vegetative organs. However, when there are neighbors that instigate greater competitive need, they decrease investment in reproductive organs and increase in vegetative organs. Figure 5.4 illustrates this behavior.

Figure 5.4. Growth behavior of aerial part and root, as well as the production of reproductive organs due to the presence of neighbors and identity recognition. In situation A there is a representation of plants growing in isolation, a situation in which there is greater investment in reproductive organs and less investment in vegetative organs. In situation B there is a representation of plants growing in the presence of neighbors, in which there is greater investment in vegetative organs to increase the capacity for competition, with less investment in reproductive organs. Greater lateral growth of the roots is also observed, especially towards the heterospecific neighbor.

It is important to highlight a fact observed in the experiment with *C. maculosa* (Table 5.1). During heterospecific interaction, plants produced more maleic acid, fumaric acid, succinic acid, fructose, some amino acids, phosphoric acid, ethanolamine, glycerol, glycerophosphate, linoleic acid, hexacosanol, octacosanol and hexacosanoic acid. On the other hand, galactose, inositol, chlorogenic acid and quinic acid were observed in greater content in conspecific interaction, which reveals that due to the recognition of the neighbor's identity, there is a wide alteration of chemical substances produced by plants.

Recognition of relatives

The responsiveness of plants to the neighbor is not always due to the availability of resources in the environment, although in many cases the scarcer the resources, the more forceful are the competitive responses. The competition among plants is differentiated according to the competitor's identity. Many studies show greater cooperation among relatives than among non-relatives. The degree of kinship is not restricted to species or varieties, but also to plants of the same genotype that derive from the same mother or from different mothers, that means, conspecific siblings or strangers, respectively. Recognition responses have also been observed in relation to the familiarity of plants as to whether they belong to the same population or not.

In a social and evolutionary context, the recognition of kinship strengthens the inclusive aptitude of both neighboring relatives and the consequent perpetuation of the genotype in a certain area. However, it is observed that just as there are genotypes that are sensitive to the presence and identity of neighbors, there are those that are indifferent (Schenk et al., 1999). A selection of studies that demonstrate parental recognition among neighbors is listed in Table 5.2.

Table 5.2. Evidence of the recognition of relatives by plants.

Species	Observed effects	Reference
Cakile edentula	Neighboring conspecific plants originating from seeds of different mothers had a greater accumulation of root biomass, and therefore greater competition power, compared to those from the same mother.	Dudley and File, 2007
Lupinus angustifolius	When grown among brothers and genotypes of the same population, the plants had less biomass production than when grown among plants of other populations.	Milla et al., 2009
Impatiens pallida	Competition signs were observed whether the neighbors were relatives or strangers. When strangers, *I. pallida* invested a lot in the root biomass, and when relatives, invested a lot in growth of the aerial part.	Murphy and Dudley, 2009
Genotypes of *Oryza sativa*	In some genotypes, such as the cultivars Huagan-3 and Lingyou-6173, there was recognition of relatives, with the non-relative neighbor causing greater accumulation of biomass in the roots and an increase in the formation of lateral roots, which also occurred when the neighbors were of different cultivars. In the cultivation among relatives there was a higher grain yield.	Yang et al., 2018
Pisum sativum	Even without direct physical contact between roots, when the neighbor was not relative, in comparison to the relative, the plants produced 24% more lateral roots, 17% more in length and root diameter and 71% more biomass.	Falik et al., 2003
Arabidopsis thaliana	When neighboring rows were relatives, there was less projection of leaves towards neighbors than when neighbors were not relative.	Crepy and Casal, 2015

The mechanisms of action used by plants for parental recognition still need a lot of research to be elucidated, but there are already some known elements, which will be addressed.

In addition to the exudates, seen in the previous topic, mediating the recognition of relatives in *A. thaliana* (Table 5.1), it was observed that the transporters of secondary metabolites of the ABC family are involved in identity recognition. The use of inhibitors of the expression of the *AtPGP1*, *AtATH10* and *AtATH1* genes led to less recognition of relatives or strangers (Biedrzycki and Bais, 2011), which implies their importance to the sensory recognition apparatus.

Other genes, some also encoding for transporter proteins, have been reported to mediate kin recognition responses in rice. In Table 5.2 we see that even domesticated cultivars of *O. sativa* still maintain the capacity for kin recognition. When non-relatives were recognized as neighbors, the plants exuded more allantoin than normal and this induced greater growth of their own roots, perhaps due to the substance's nitrogen, but reduced the allocation of photosynthates to produce caryopses. It was found that the genotypes that produced more allantoin had greater expression of the *OsYUCCA* genes, involved in auxin biosynthesis, as well as the *OsAUX1*, *OsPIN1* and *OsPIN2* genes, involved in polar auxin transport, and *OsARF12*, involved in the sensitivity of cells to auxin. In this case, auxin appears as a substance to be considered in studies involving recognition in plants.

In addition to chemical compounds, volatile or not, the recognition of kinship among neighbors can occur by different light patterns. The behavior observed in *A. thaliana* (Table 5.2) occurs due to the difference in the quality of light reflected horizontally between neighboring plants. The quality of the reflected light (or transmitted laterally by the neighbor, depending on the position of the sun) is altered by the different architectures of the different genotypes. With the use of selective filters for R and blue radiation, as well as photoreceptor-defective mutants, it was concluded that phyB, cry1, cry2, phot1 and phot2 participate in the neighbor's identity recognition. In addition, the use of auxin-defective mutants also resulted in phenotypes that are not responsive to neighbors, which indicates that this hormone is necessary for leaf repositioning, just as it is important for stem repositioning in the phenomenon of phototropism. The possibility of architectural change of plants mediated by photoreceptors, although not related to relatives, was previously approached in the section “Plant-plant communication by light”, Chap. 2.

Summary and considerations

Self-recognition in plants is a phenomenon that has been known for a long time, and with a wealth of details of its mechanism of action when it comes to molecular recognition in reproductive structures. This ability allows plants to avoid unwanted self-pollination.

The recognition of identity is not limited to the “self” and the “non-self” but extends to the recognition of the conspecific or heterospecific neighbor, and even more refined than that, whether the conspecific is a stranger or a relative (siblings). Although not all species investigated so far have exhibited the ability to recognize, most have. When the neighbor is conspecific and a relative, the plants tend to exhibit a less competitive behavior, which is revealed in greater investment in reproduction and less investment in growth. When the neighbor is not a relative or conspecific, plants tend to invest more in growth and less in reproduction, which translates into greater competitive capacity.

Chemical substances, volatile or not, and light are the elements most likely to mediate the recognition of identity among plants, but the possibility of the existence of other signs, such as electrical patterns, is not ruled out. Using mutant plants or chemical expression inhibitors, some genes fundamental to the sensory apparatus necessary for identity recognition have been identified.

The recognition capacity, in addition to being a phenomenon observed among plants, is also known in the specificities of plant-microorganism interactions, so that the plant attracts and facilitates its own infection by mycorrhizal fungi and diazotrophic bacteria of specific taxa, as well as hindering the infection by pathogenic organisms or those that do not bring benefits (see section “Plant-microorganism communication”, Chap. 2). In addition, parasitic plant interactions (see section “Chemical plant-plant communication”, Chap. 2) can be very specific and, therefore, dependent on recognition.

Knowledge about the ability to recognize the identity and kinship of plants can be easily used for technological purposes of cultivation, since interactions that result in greater competitiveness require a high investment of the plant in growth to increase its competitive performance. On the other hand, less competitive interactions commonly result in greater investment in reproductive organs.

Learning in Plants

The ability to learn is fundamental to the adaptation of an organism to the variations that occur in the environment, central focus of most concepts of intelligence. An indispensable requirement for learning is the ability to memorize. Although the plant does not have a central memory device at the level of organs or tissues, unlike the brain in big animals, it has the ability to memorize, as discussed throughout Chap. 4. In this sense, it is expected that the memory in plants will be stored and accessed at the cellular unit level, a capacity apparently innate to any living cell. The ability of a cell to memorize and learn is no longer new in the field of animal cell biology, as it deals with some specific cells of the nervous and immune system, but it is a little explored knowledge in the field of plant biology, despite the abundant evidence of this capacity. We know that a single plant cell has the superpower to give rise to an entire plant, with all variations of tissue shape and function, of organs and general architecture, because of its totipotentiality, a capacity that has been known for a long time. It means that is, in some way, the organism's memory is embedded in every somatic cell, regardless of its type; after all their genetic loads are the same. Considering the approaches presented in the previous chapter, it is necessary to know which are the endogenous or exogenous elements that act as factors of permissiveness and retrieval for this cellular memory.

Learning has been defined as a process derived from acquired memories, through which adaptive changes in the behavior of organisms arise because of experiences (Okano et al., 2000). A simple way of verifying an organism's ability to learn is to subject it to habituation, which can lead it to an adaptive process, allowing it to focus (which requires attention) on the most important information in the environment, while filtering stimuli or events that, over time, are repeatedly irrelevant to their development (Rankin et al., 2009).

Habituation learning

Based on the conceptual premises of learning and habituation, Gagliano et al. (2014) carried out an experiment with *Mimosa pudica* to investigate the plant's ability to learn. The evaluations focused on the opening and closing of the leaflets due to the mechanical impacts resulting from the controlled fall of the mimosa vessels from a height of 15 cm. Habituation training consisted of seven series of 60 consecutive falls throughout the day. It was observed throughout the experiment that:

- The leaflets that closed for long periods in response to the first falls at the beginning of the training, with time started to open from the fourth to the sixth fall of a series, and with the progress of the training they started to no longer close due to the falls, which means that the plant perceived the importance of that impact for its development, changing its behavior according to the lived experience.

- Six hours after habituation training promoted by falls (first stimulus), the plants were subjected to a second stimulus, this time, controlled agitation (which could serve to dishabituate the first stimulus). The first stimulus did not interfere with the plant's responses to the second stimulus. Ten minutes after being submitted to the agitation stimulus, the plants were exposed again to the first stimulus, and the memory of the first habituation was retrieved, as was the response of the control plants (which were not submitted to the second stimulus).
- Six days after habituation, the plants were resubmitted to the same sequence of falls and it was verified that the memory of the habituation remained operative. Some individuals did not get to close the leaflets completely, while others started to open them after the second or third fall.
- 28 days after habituation, when resubmitted to the stimulation of falls, the plants still maintained the memory of the training operative, and with a greater degree of opening of the leaflets and uniformity of response than that observed during the initial period of the experiment.

A simplified scheme of the experiment reported above can be seen in Figure 5.5.

The responses observed in the experiment reported above explicit the plants' ability to learn by habituation. This ability was already implicit in previously explored situations. In the section “Evidence of information acquisition by priming”, Chap. 4, we had two examples in line with habituation learning. The first was of arabidopsis subjected to successive cold shocks; once they were accustomed, there was a significant attenuation of the transient increase in cytosolic calcium (an event typically occurring when the plant was exposed to stress), however, when exposed to a colder temperature than usual, there was a cytosolic increase in calcium at the level of unaccustomed plants, which reveals learning about the importance of the stimulus. It is important to note that learning did not make the plant more susceptible to the same stimulus if it happens to be stronger. In the second example, it was observed that when the plants were exposed to an osmotic stress condition and resubmitted to the same condition, there was a transient increase in cytosolic calcium in plants previously stressed, which denotes that the plant learnt about the high potential for damage caused by that stimulus, enhancing the defense signaling.

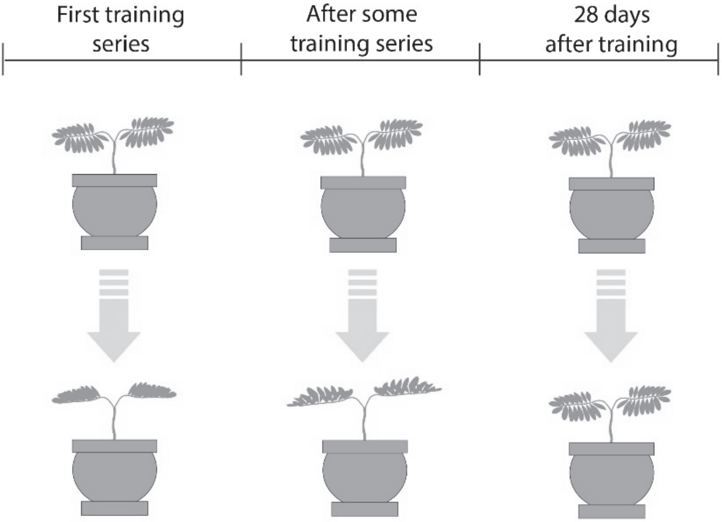

Figure 5.5. Plant learning by habituation training, based on the experiment by Gagliano et al. (2014). *Mimosa pudica* vases were subjected to frequent series of falls from a height of 15 cm. In the first training series, plants closed their leaflets completely. As training progressed, when they realized that the mechanical impact of the fall was not relevant to their development, the plants started not to close the leaflets, although there was no uniformity in the response of 100% of the plants or in the opening angle of the leaflets. 28 days after training, when exposed to falls again, it was observed that the memory was not only still operative, but the uniformity of response and the opening angle of the leaflets was greater in relation to the initial period of habituation training.

As in other different organisms, it is not clear or simple to understand how learning takes place. In Chap. 4, we saw that Ca^{2+} plays a central role, acting as a necessary factor for the construction of memory (STO), as well as permissiveness (PST) for information retrieval (RCL), which also occurs in other organisms. Since repeated submissions to a certain environmental condition can cause a behavioral change in plants due to changes in gene expression mediated by acetylation or methylation of genetic material (topic “Transgenerational epigenetic memory”, Chap. 4), this is probably another component involved in the learning mechanism. In general, due to the close link between learning and memory, it is conceivable that the same factors that influence memory also influence learning.

It is interesting to note that, as in humans the solid establishment of memory requires a period of sleep after exposure to new information, plants also seem to require a certain period of accommodation for the solid establishment of some memories and possibly linked to some specific condition of the organism. That was observable in the mimosa experiment, reported above, in which the largest angle of opening of the leaflets and the greater uniformity of the response were observed 28 days after the initial training period of plant habituation, although there was already a (less uniform) response during the training phase. Similar behavior occurred with *Bidens pilosa* after having a cotyledon perforated; the most striking response triggered by the damage, the asymmetric growth of the cotyledon buds, only occurred if the decapitation of the stem apex was carried out two days after the perforations (see section "Evidence acquisition of information by priming", Chap. 4). Apparently, the requirement for a time frame for memory consolidation, and consequent learning, is linked to the production of chemical substances related to the phenomenon. It is important to note, however, that for some responses that demonstrate the manifestation of learning, there is no requirement for this period on a scale of days, but of minutes. That is the case with plants exposed to the wind, in which, through familiarity/desensitization, the frequency of transient cytosolic calcium peaks decreases in a matter of minutes after the plant is exposed to the stimulus (see topic "How do plants perceive the wind?", Chap. 1).

Although the use of stressful stimuli, as previously seen in relation to falling, agitation, perforation, cold and osmoregulators, is instructive for the study of habituation learning, it is important to note that it can occur in plants in a non-stress situation. A remarkable phenomenon to be mentioned in this context, which is not necessarily stressful, is the resynchronization of the biological clock. Throughout the topic "How are plants synchronized at the time of day?", Chap. 1, it was reported that the most important synchronization references for the biological clock are sunrise and sunset, but if we subject the plant to a change of photoperiod/nictoperiod, the circadian-dependent events, for a few days, will maintain the same time rhythm as they did prior to the change. The repetition of the new start time of the light/dark period for a few consecutive days will induce the clock to resynchronize, and, consequently, the events regulated by it will adjust to the new photoperiodic condition. In other words, resynchronization corresponds to the time necessary to learn about the new condition of the environment. In this context, the time demanded on consecutive days is important for the plant to be sure about the perceived change, and thus adjust its metabolism to the new condition.

Metabolic plasticity depending on the conditions of the environment is essential so that the development of the plant is not perpetually harmed by occasional adverse situations. At several moments this book has mentioned a recurrent behavior observed in plants, namely, a loss of reproductive performance and growth due to greater investment in defense. In that context, we can infer that the learning established in a life cycle is transmitted to future generations through transgenerational epigenetic memory (Chap. 4). Let us consider that the condition of the environment experienced by a generation can be different from that experienced by its ancestors. Couldn't the manifestation of behavior inherent in the learning of predecessor generations be harmless or even harmful? For example, wouldn't the high investment of a plant to the defense against a pathogen no longer exist, would it not induce it to invest many resources in defense, unnecessarily, in detriment of growth and reproduction? According to information contained in Table 4.1, we can see that the manifestation of memory/learning is not mandatory. Access to memory may require a condition of permissiveness, which is probably established in the situation of need, so that specific information stored is accessed, in addition to the possibility of the plant forgetting the information when it no longer needs it, which occurs through (de)methylation or acetylation of genetic material. That would not impose the obligation of future generations to maintain the activation of non-useful metabolic pathways. This same principle of plasticity applies to sporadic adverse situations experienced by a plant in a single life cycle. As seen in the topic "Stress memory", Chap. 4, even though there is a memory and a defense learning due to a stressful experience, after the stress period, the cellular metabolism tends to readjust to the non-stress conditions, and therefore, the plant starts to invest less resources in defense, although the organism has acquired a greater defensive skill in dealing with the eventual stressful situation in the future. This plastic behavior in plants is much more common than hysteresis, that is, to preserve the defense apparatus, even in the absence of the threat that induced its formation.

Learning by association

Learning by association implies making choices that satisfy needs, using prediction or expectation of what will be better. To verify this ability in vegetables, Gagliano et al. (2016) studied young plants of *Pisum sativum* growing in Y-shaped tubes in a dark environment, receiving two treatments: blue light input (non-conditioning stimulus) and ventilation (conditioning stimulus), with the stimuli entering from opposite sides of the bifurcation (T1) or both stimuli entered from the same side of the bifurcation (T2) (Figure 5.6). T1 corresponds to a negative association between light and wind and T2 corresponds to a positive association between both stimuli. Let's see the sequence:

- For three consecutive days, at T1, wind and light entered from opposite sides of the bifurcation, but in order not to condition the side of the bifurcation through which the light or wind entered, it was alternated at each training session. In T2, wind and light entered simultaneously from the same side of the bifurcation. The wind preceded the light by 1h and was applied simultaneously with light for another 1/2h. Similarly to T1, the sides through which the stimuli entered were alternated at each session. Three daily sessions of 1 hour of light were performed. Between the stimulus sessions, there was 1 hour of rest (darkness).
- On the fourth day, the plants were separated into 2 groups: a test group, in which the seedlings were treated only with wind, and a control group, in which the plants received no treatment (neither wind nor light). In the test group of plants from T1, the wind was placed on the same side as the last exposure to light, and in the test group of plants from T2, the wind was placed on the side opposite to the last exposure to light.
- On the fifth day, the phototropic curvature was evaluated. In the control group, all plants had phototropic curvature directed to the side that had received light in the last training session, regardless of whether the plants were from T1 or T2. In the test group, it was observed that 69% plants from T1 had phototropic curvature directed to the opposite side of the wind-treated bifurcation on the fourth day, although the wind-treated side was the same as that which received light in the last training session, while the group of plants from T2 had 62% of them directed to the bifurcation treated with wind on the fourth day, although this was the opposite side to the one that received light in the last training session.
- Because the difference found between the control group and the plants in the test group from T1 and T2, it is evident that the wind acted as a conditioning for the seedlings to predict the most likely side on which they would find light. Even though T1 plants received the last light treatment on the same side of the wind on the fourth day, 69% predicted that the light would come from the opposite side to the wind, due to the conditioning treatment, in which the stimuli came from opposite sides. Even with 100% of the plants of the control treatment bending towards the last side treated with light, plants of the test group originating from T2, because they were conditioned to the positive association between light and wind, 62% grew in the direction of the bifurcation treated with wind on the fourth day, even though this side is the opposite of the one that received light during the last training session.

Based on the treatment and responses observed from the above experiment, it is evident that plants can learn by association, whose nature is more complex than simple habituation. Figure 5.6 displays an illustrative scheme of the experiment.

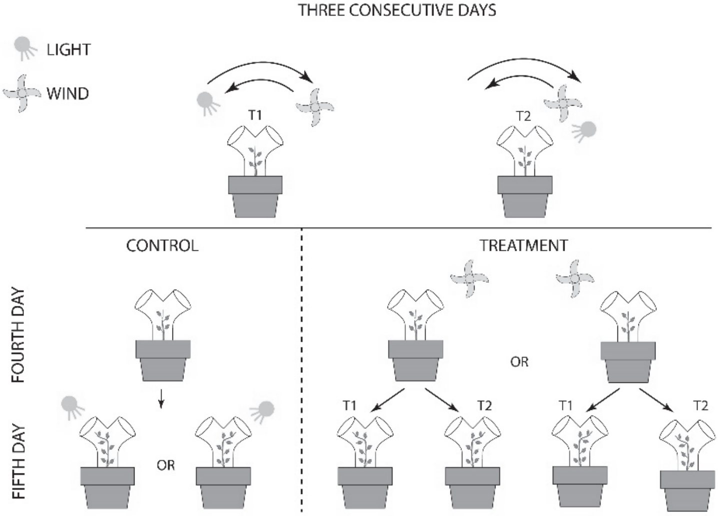

Figure 5.6. Representation of plant learning by association, based on the experiment by Gagliano et al. (2016). For three consecutive days, seedlings of *Pisum sativum* were treated with three daily series of blue light (non-conditioning stimulus) and wind (conditioning stimulus), whose stimuli reached the seedlings on opposite sides of a bifurcated tube (T1) or both stimuli arrived concurrently from the same side of the bifurcation (T2). At each daily session, the sides through which the stimuli entered, whether in T1 or T2, were alternated to prevent the position from becoming a conditioning factor. On the fourth day, one group of plants received no treatment (control), while another group was submitted to ventilation only (test/treatment group). Plants in the test group from T1 received wind from the same side of the bifurcation that received light in the last training session, while plants from the test group from T2 received wind from the side of the bifurcation opposite to the last training session. On the fifth day, the phototropic curvature of the plants was evaluated. It was found that 100% of the plants in the control group had phototropic curvature directed to the bifurcation that received light in the last training session, whether coming from T1 or T2. 69% of the plants in the test group from T1 had phototropic curvature directed to the opposite side of the wind exposure on the fourth day, while 62% of plants in the test group from T2 had phototropic curvature directed to the same side of the bifurcation that had been exposed to the wind on the fourth day. These results prove the conditioning effect of the wind and the ability of the plant to learn by association.

To know that plants are able to learn by habituation and or association is fundamental to our understanding of how some plant behaviors, discussed in previous chapters, were established. For example, this helps us to understand how plants learned to recognize the organisms with which they interacted during evolution. We know that recognition can be extremely refined, to the point where a plant recognizes its siblings among conspecific neighbors. Similarly, they learn to recognize beneficial or harmful microorganisms.

As seen in the section "Plant-microorganism communication", Chap. 2, plants can recognize harmful microorganisms through the mechanism of recognition of pathogen-associated molecular patterns (PAMPs) via receptor proteins and recognition of these patterns (PRRs) and recognize the beneficial ones through chemicals inherent in the taxon. Taking the plant-pathogen interaction as an example, it is likely that plants have learned to recognize them associating molecular and or electrical patterns characteristic of these organisms to the damage they have always suffered because of their presence. In other words, learning was established from both the association of events and habituation. On the other hand, if we take as an example the plant-fungus relationship, in which there is a specificity between the species, the plant must, at the same time, facilitate its infection to those fungi with which they form a symbiotic interaction, such as mycorrhizal, and make it difficult to those with whom they would form a parasitic interaction, such as pathogens, although they are all in the same environment. It is expected that the learning of the plant that led it to recognize a specific beneficial fungus is the result of the presence of specific substances, such as specific residues in the (lipo)-chito-oligosaccharides, differentially present in these microorganisms, associated with the benefits obtained when they are present. Like plants, the microorganism recognizes the specific host by the substances of attraction it releases, such as flavonoids, cutin monomers or strigolactones. A similar relationship applies when we think about how specific recognitions between plants and bacteria occur, between neighboring plants, or how a parasitic plant recognizes its host.

Some recognition relationships are more complex, like the tri-trophic ones. Take as an example the relationship in which the maize roots are attacked by the pest *Diabrotica virgifera*, and, from then on, these roots release the volatile compound (*E*)-β-caryophylene, a substance that attracts the nematode *Heterorhabditis bacteriophora*, a natural enemy of the *D. virgifera* (Table 2.1). On the one hand, it is easy to associate the recognition between the plant and the pest through learning established during the coevolution of these organisms in the same ecosystem, but on the other hand, it is questionable whether the release of caryophylene by the plant would be purposeful (and, therefore, conscious) to attract the enemy nematode of *D. virgifera*. This example does not give us enough basis to make that extrapolation, because the plant may have learned to simply associate the absence of the damage caused by the pest to the release of caryophylene, unaware of what the release of this chemical would imply. However, in the same way that the tri-trophic relationship addressed does not allow us to affirm that the plant has learned to attract the nematode consciously, it also does not allow us to affirm the opposite. This is because, other interactions, some even more complex, highlight likely intentional behaviors of plants and will be explored in the section "Consciousness in plants".

Following the same principle of the previous examples, but in the opposite sense, there are organisms that did not coevolve in the same ecosystem, and therefore, that did not learn to live together, either approaching, when compatible, avoiding or protecting themselves from harmful effects, when incompatible. This sometimes leads to drastic changes in a system when an exotic organism is inserted, as discussed in the section "Chemical plant-plant communication", Chap. 2.

Summary

Learning is seen as a central capacity of the intelligent being. It means that based on lived experiences, the organism's behaviors are modified to better adapt to new situations. Therefore, the ability to memorize is indispensable, since a change does not necessarily mean a perpetual change from a stimulus, but to respond in a different way when it comes to possible future stimulation. Through specific learning research methodologies, it has been concluded that plants can learn by habituation, that is, from repetitive stimuli; they can readapt their response according to the impact of the stimulus on their development, becoming more or less sensitive to the stimulus factor, when it recurs. More complex than simple habituation learning, is when two or more stimulus factors need to be associated positively or negatively to establish learning, a process called learning by association. In an experiment conducted with two stimulus factors (one conditioning and one non-conditioning) for phototropic curvature, it has been proved that plants are able to learn by association.

Prediction in Plants

Predicting events is a skill that has favored the adaptive success of living beings throughout evolution; it allows organisms to prepare their defense against an imminent threatening situation and therefore be less affected if it actually does occur.

When analyzing the experiment described in the previous section (Figure 5.6), it can be seen that when the plant took on a side for its phototropic curvature, it did so by predicting a luminous condition that would favor it. This prediction is the result of what it learned during its exposure to treatments. Therefore, in this case, learning itself is the central mechanism for prediction.

In addition to the learning established in a single life cycle being useful for prediction, those established throughout evolution are also important to the predictive ability of plants. For example, when young plants bend to avoid shading even before they are actually shaded (topic “How plants predict shade”, Chap. 2) it is a situation that shows prediction; the behavior is not the result of previous individual experience, but of learning inherited from their ancestors. Another case of prediction, in the same context of heritability, was previously explored in the topic “How plants perceive water”, Chap. 1: in an experiment carried out with inverted Y tubes the roots grew not only in the direction of environments with greater availability of water, but also in the absence of minimum availability, they predicted the location of a water source in function of the acoustic vibrations emitted by the moving water, towards which they directed their growth (Figure 1.13). Naturally prior learning was required for this prediction to have occurred in young plants, in this case, inherited from their ancestors, which could recognize vibrations emitted by water. That means they learned to recognize by association between a specific perceived vibration and the presence of a specific resource.

A detail of the research on the growth of the roots towards or not towards the moving water source draws attention: the plants only directed their roots to the water source in a situation of soil Ψ_w below an already very negative threshold. This encourages us to question whether the plant has different degrees of confidence about its ability to identify signs, as the roots did not grow towards a water source even in the face of an increasing decrease in water availability on one side of the tube. That which reveals either a low confidence in its mechanism of identifying the location of the water source through acoustic vibrations, or the plant was avoiding (and therefore, predicting) a hypoxia condition in the water source environment. Although it is not possible, from the experiment in question, to reach a more solid conclusive idea on this issue, the reliability of the perceived stimulus is a factor considered by the plant when making a decision. We can see this in different situations, such as: the need for more than one touch to trigger the trap of the venus flytrap (section “Perception to touch”, Chap. 1); *Acacia drepanolobium* only decreases the greater production of nectaries and bulbous thorn when in conditions of more than two years free from herbivory (to be seen); the cell needs to take into account several signs in order to distinguish between a possible beneficial or harmful microorganism, for further facilitation or hinder of the infection (Figure 5.7); when the perception of the light signal by the plant occurs in different positions of the stem apex, the stimulus to phototropism can be canceled (Figure 4.7).

When considering the question of reliability for the manifestation of a plant response, we must ask whether different species have equal sensitivity of perception, or whether there is a hereditary influence of ancestors who experienced peculiar situations regarding the situation to be faced. This is because we have already seen several situations (Chap. 4) in which a stimulus is neglected or even overvalued by the plant depending on its learning as to the degree of importance of this stimulus to its development. It is questionable whether the learning that leads to desensitization can affect the perceptual sensitivity of the stimulus. This context encourages us to be curious to discover if plants whose ancestors were subjected to severe water deficiency would be more sensitive to recognize vibrations from a moving water source.

A complex situation mentioned above, and involving reliability, is the plant cell’s need to predict in order to choose between triggering a pathway of resistance and defense signaling or facilitating infection in a situation of anchoring microorganisms, such as bacteria, on the cell wall. This is because, many pathogenic bacteria, in addition to having molecular patterns common to beneficial bacteria, to get past the first immunological layers of the cell, may emit signals that serve as beneficial bacteria recognition factors for the plant cell. In a similar context, secretions of herbivores can deceive plant cells, inhibiting their herbivore-specific defense, as they contain bacteria of pathogenic potential to plants, inducing them to invest specifically in pathogenic defense, which we previously called fake news (see section “Plants have a sense of danger”, Chap. 2). The cell, therefore, needs to learn to associate various signals of recognition to predict what type of organism it is, and thus, to direct, relax or intensify its defense strategies. Figure 5.7 illustrates the prediction of plant cells as to the pathogenic potential of bacteria, based on multiple signals to be perceived.

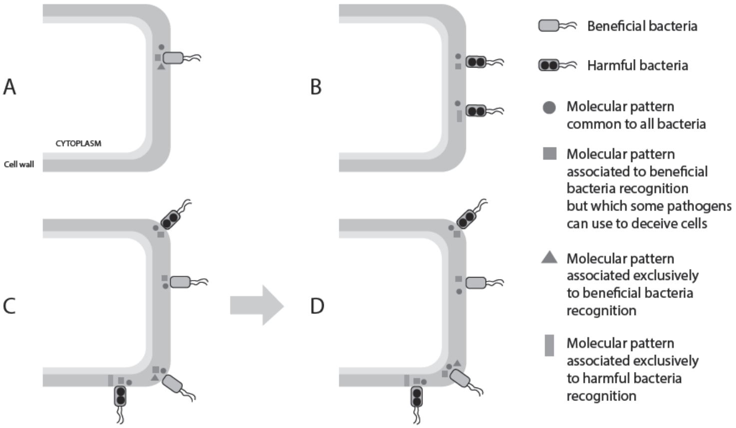

Figure 5.7. Cell prediction of microorganisms as beneficial (diazotrophic bacteria) or malefic (pathogenic bacteria) by association of molecular patterns. Some patterns are common to all bacteria, others are common only to harmful or to beneficial ones. In situation A, the cell predicts that the organism anchored in the cell wall is beneficial due to the presence of two patterns associated with beneficial bacteria, thus facilitating infection. In situation B, the cell prevents the infection of harmful bacteria anchored to the wall by the presence of an exclusive pattern of harmful bacteria or by a pattern that does not signal a pathogenic bacterium, but which is not fully reliable. When analyzing infection from situation C to D, the non-recognition whether the bacterium is beneficial or harmful due to the presence of only one distinguishing factor, can lead to the cell stopping both, as a means of prevention (or perhaps, in a situation of drastic absence of nitrogen, the cell taking risks, and favoring infection by both). The recognition of multiple molecular patterns by the plant cell favors the prediction if which is the harmful or beneficial bacteria, favoring infection only by the beneficial microorganism.

A well-known prediction system in plants is the circadian clock itself, because it anticipates the manifestation of environmental phenomena. Daytime circadian events anticipate the effective dawn, just as nighttime events anticipate the effective dusk. The plant predicts the occurrence of phenomena due to the activity of the clock oscillator, which acts as the predictive mechanism. This is important, for example, so that on a cloudy day, circadian-dependent diurnal responses occur normally, regardless of the availability of light ideal for triggering certain metabolisms. If this were not the case, an entire complex machinery would cease to function due to the unavailability or insufficient availability of a single resource. In this context, predicting the dawn is a success factor in the evolutionary adaptation. An example of a circadian predictive event is the movement of young sunflower flowers to the east at the end of the day, from where the sun is expected to emerge in the coming dawn (see illustrative image of the synchronized movement of the sunflower in Figure 1.4) The description of the mechanism used by this predictive apparatus is present in the topic “How are plants synchronized with the time of day?”, Chap. 1. Circadian-dependent responses only occur prior the effective change in environment because the plant has learned the changes that usually occur in the environment during the 24 h cycle.

Another phenomenon occurring in plants that can be associated with prediction is photoblastism. Positive photoblastic seeds are those that do not germinate in an environment rich in Fr radiation, which stimulates plant organs to keep most phytochromes in the biologically inactive form, Pr (details on the light-photoreceptor interaction can be seen throughout the section “Light perception”, Chap. 1). An environment with a low R:Fr ratio is typical of shade, the screen being leaves. If the seed germinates under this condition, most likely there will not be enough light for the development of the new plant, and thus the seed's potential will be lost. However, when the environment is high in the R:Fr ratio, most photostable phytochromes assume the biologically active form, Pfr, and germination occurs. This is a phenomenon that favors seeds of plants adapted to full sunlight or seeds with very small reserve tissue. In this sense, the Pfr/Pr ratio in the seed serves as a predictive apparatus for the ambient light condition. On the other hand, a poor R:Fr ratio may favor the germination of negative photoblastic seeds, from shade or half-shade plants, since their germination is induced by the low Pfr:Pr ratio of photostable phytochromes (Melo, 2020). The influence of ambient light patterns on the germination of positive photoblastic seeds can be seen in Figure 5.8.

Photoblastism does not necessarily suggest a conscious prediction of the seed, however, it is the result of an evolutionary adaptation, built from plant learning, resulting in the structuring of an organ whose germination should preferably occur under conditions that meet the different specificities of light required for the development of plants of different taxa.

The physiological plasticity of seeds in terms of photoblastism is an amazing phenomenon. If an ideal light condition for germination is never established, theoretically, the seeds would not germinate, and thus, they would have their potential lost. However, photoblastism is not an absolute determinant fact influencing germination, with rare exceptions, because there is a certain percentage of seeds germinating, even in unfavorable light conditions, while most seeds, as a precaution, remain waiting for a more favorable condition to germinate. On the other hand, the germination percentage increases in any light condition as the seeds get older; that means "in view of the risk of death, it is worth trying". This means that the prediction of a favorable light environment for the development of a new plant exists, but its influence on germination is relative, as is the influence of prediction on the behavior adopted by other organisms with this cognitive capacity in different situations.

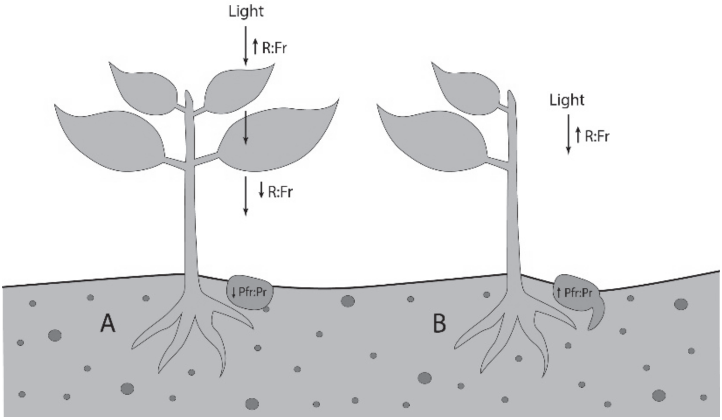

Figure 5.8. Photoblastism as a predictive mechanism of the environmental condition of light favorable to the development of a new plant. In situation A, a positive photoblastic seed is shaded by leaves, which, by absorbing a large part of the R (red) radiation from the environment, establish a shadow rich in Fr (Far-red), a radiation condition that induces the establishment of a low Pfr:Pr ratio in the seed, inhibiting its germination in a light environment that would be unfavorable to the development of the new plant. In situation B, the absence of leaf shading, and therefore, with radiation rich in R, there is an induction of the establishment of a high Pfr:Pr ratio in the seed, triggering its germination in a light environment favorable to the development of the new plant.

Summary

The ability of plants to predict is a direct consequence of their ability to learn by association. When analyzing the experiment in which *Pisum sativum* establishes phototropic growth in the opposite direction to the last exposure to light because of the negative association that this makes with the wind, naturally there is evidence of the ability of plants not only to learn but also to predict the side of growth that would best favor them. Despite this evidence coming from more recent studies in the field of plant intelligence, the manifestation of prediction was already known, at least implicitly, in events regulated by the circadian clock, which do not necessarily need effective exposure to a certain environmental stimulus to manifest themselves. The anticipation of the stimulus occurs due to a prediction of the plant regarding the usual changes in the environment due to the time of day, which, consequently, makes the circadian clock oscillator a predictive mechanism.

Aware of the ability of plants to predict by different mechanisms, we can associate this ability to the difficulty or facilitation interposed by the plant cell to infection by microorganisms anchored to its wall. If the power of the cell were only to recognize, but not to predict the agent's damage potential, it would be a useless skill, because then there would be no reason for triggering the resistance or defense mechanisms in anticipation of infection. Prediction also makes it easier to understand aspects of plant behavior due to an environmental condition that favors its development or not, such as the germination of photoblastic seeds.

Predictive instruments or mechanisms such as the circadian clock, photoblastism or molecular pattern recognition proteins that allow the cell to identify microorganisms or decoys perpetrated by maleficent organisms, are very useful tools for the adaptive success of plants.

Decision-Making

Intelligence denotes capacity for discerning choice. For a choice to be made it is necessary to decide. Based on previous studies of memory and learning in plants, these decisions are made based on past experiences. In the experiment on learning by association (see topic "Learning by association"), in which there was a negative association between light and wind, most plants in the test treatment assumed a phototropic curvature based on the experience. It is important to note that this curvature is not just a response to a stimulus, because while 69% bent to one side, 31% bent to the other, which shows a choice, since all plants were under the same conditions of stimulus.

Another situation that also denotes choice and decision is when the parasitic plant *Cuscuta europaea* shows signs of acceptance or rejection to the host *Crataegus monogyna* depending on its nutritional status (Kelly, 1992). Acceptance is evidenced by the curling in the host, and the denial by the non-curling and continued lateral growth. It was observed that hosts treated with nitrogenous nutrients had a 20-30% greater acceptance than untreated hosts. This shows both a choice and a prediction of what would be best for the parasite. It is assumed that the host's nutritional status is perceived by the parasite because of the quality or quantity of volatile compounds released depending on the nutritional status.

The dilemma faced by plants as to stomatal closure or opening is yet another situation that highlights the need for choice and decision making. On the one hand, the closure contributes to water savings, which is especially important in an environment with water scarcity, and, on the other hand, openness is fundamental to the acquisition of CO_2, both of which are essential resources for plant development. It is questioned whether, in a condition of water stress, the closing or opening of stomata occurs spontaneously from the establishment of chemical balances that favor one response or another, or if it is an event that involves a more complex decision making by plants, based on the prediction of future consequences. Throughout evolution, plants have developed different ways of dealing with this dilemma. C4 plants reduced the need for a large stomatal opening to acquire sufficient CO_2 to their demand, and, as a consequence, they are more efficient in the use of water compared to C3 plants; resurgent plants avoided the dilemma by entering a state of quiescence in the drought season; CAMs (Crassulaceae Acid Mechanism) started to open their stomata at night, and therefore have less water loss due to the lower vapor pressure gradient between the leaf and the atmosphere; most C3s, compared to C4 and CAM ones however, are the ones that most need to fine-tune their decision making regarding the opening or closing of stomata to reduce errors that lead to avoidable losses in growth performance.

The fact that different plants have assumed different mechanisms to deal with the water/CO_2 dilemma indicates a discomfort suffered by C3 plants throughout evolution, since the evolutionary adaptations C4 and CAM are derived from C3. In this sense, stomatal closure and opening does not seem to be just a spontaneous event because of chemical balances, about which the plant does not need to “worry”, that means, they needed to focus attention (subject to be addressed later) on this phenomenon in the course of evolution.

When considering a momentary condition for decision making regarding the opening or closing of stomata, a chemical signal that seems to be an important reference throughout the day in C3 and C4 plants is an ABA/DAP balance (abscisic acid and 1,3-diaminopropane (DAP) acetylated), which results in the formation of electrical gradients in the membrane of the guard-cells and the consequent activation of transmembrane ion transporters that are important, respectively, to the closing and opening of the stomata (Jammes et al., 2014; Tian et al., 2015). However, as we will see later, stomata sensitivity to ABA is not homogeneous, even considering a single leaf, which implies the existence of other elements that interfere in the control of stomatal closure.

A plant receives many signals at the same time. Based on the various aspects explored above, it must be acknowledged that a plant's decision to make changes induced by them cannot be a simple matter.

Studies in natural computation have found functional behaviors in plant cells or even in some molecules similar to those of computational decision-making (Kawano et al., 2012). In this sense, it seems that the artificial intelligence developed from the observation of biological behavior can assist us in understanding how broad a functional mechanism among different living organisms can be.

Summary

The plant's ability to predict a condition that favors it, such as the phototropic curvature (not homogeneous, even under the same stimulus condition) observed in *Pisum sativum* induced by the association of two stimuli, shows that it is an organism with the ability to make a careful decision based on a set of signals. Another evidence of this capacity is the fact that parasitic plants choose, among neighboring hosts, those best nourished, that means, the criteria for choice and decision making considers the organism with the greatest potential to favor its own development.

The ability of plants to make decisions, even if implicitly, has always been involved in discussions about the dilemma of closure and stomatal opening in situations of water deficiency, since closure promotes water savings, but makes it difficult to acquire CO_2. It is likely that stomata movement is not just a spontaneous response to a momentary stimulus. It is presumably a problem for the plant, since adaptations have emerged throughout evolution, such as resurgent plants, C4 and CAM, which have different mechanisms that help to mitigate the problem of excessive water loss associated to stomatal opening in a water deficient condition. These observations show a more complex scenario for decision making than a simple response based on a momentary chemical balance.

Plant Behavior

Behavior is defined as a response to an event or environmental change during an individual's life (Silvertown, 1998). In this sense, it is a term of similar meaning to what is known in biology as phenotypic plasticity. In a simpler and broader sense, Trewavas (2009) defines plant behavior as "what plants do". The approach to the theme in this section will be directed to factors to be observed by plants for behavioral changes.

As a classic example of morphophysiological plasticity characteristic of some *stricto sensu* concepts of behavior in plants, there is the photoreversible behavior of responses mediated by phytochromes (addressed in the section "Perception of light" Chap. 1), because while the period lasts induction, such as floral induction promoted by R light, can be reversed by Fr light and, depending on the photoreversibility, a flower may or may not appear, although the plasticity ends as soon as the plant determines flowering (Figure 5.9).

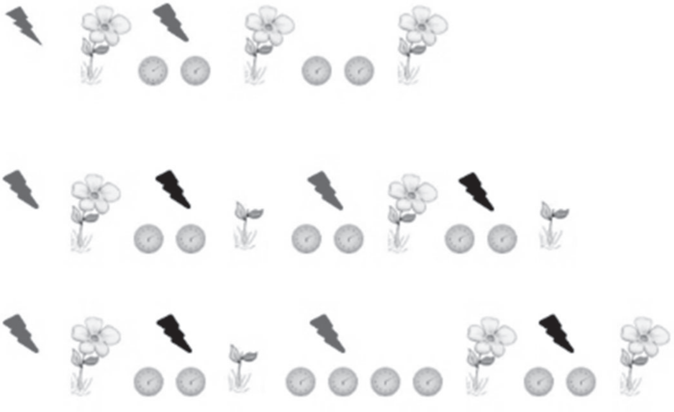

Figure 5.9. Flowering induction of some plants as an example of reversible behavior. In the first line, when the plant is induced to flower by exposure to red light (lighter ray) its flowering will occur regardless of whether it will be maintained or not under the stimulus condition. In the second line, the induction to flowering promoted by red light can be reversed by exposing the plant to far-red light (darker ray) if the plant has not yet determined to flower, and thus, the reversal will occur successively in relation to the last quality of light to which the plant is exposed. In the third line, it can be noted that if the plant's exposure to the inducing stimulus exceeds the time threshold necessary for floral determination, the response cannot be reversed. Plant with flower indicates a plant induced to flowering and plant without flower indicates a non-induced plant to flowering.

Another example of plastic and reversible behavior is the movement of the young sunflower flowers depending on the position of the sun in relation to the plant. However, plasticity does not always require reversion to the previous state, such as changes in the positioning of organs like stems or roots due to the availability of light and nutrients fixed in one point, respectively; alteration in the proportion of male:female flowers under stressful conditions experienced by monoecious plants; higher rate of abortion in unfavorable environmental conditions for fruit development; anticipation or postponement of flowering due to environmental changes; and activation of specific cellular defense pathways induced by the recognition of permanent harmful agents.

Changes in plant behavior have a cost which requires “prudence” from plants in determining a change. Investment in increasing the defense arsenal against harmful insects can lead to a decrease in resources for investment in defense against pathogens, as observed by Bostoch (2005), just as investment in shade avoidance can decrease the investment to defend against herbivores (Izaguirre et al., 2006). However, there are situations in which the investment in a specific defense or tolerance response can favor the organism against other threats when there are points of synergistic crossings or convergence in the metabolic pathways involved. In this context, the damage potential of one or more perceived stimuli, as well as the compatibility of responses to them, need to be observed by plants before they determine changes.

An example of a situation in which the potential for damage or danger is considered by the plant is seen in the tritrophic relationship that occurs with *Acacia drepanolobium* (Figure 5.10), which produces 25% more nectaries and bulbous thorns to attract ants that defend it from herbivores, only when the risk of herbivory in the environment is high (Huntzinger et al., 2004). It is questionable whether the increase in mechanisms involved in defense against herbivory would occur, at least at the same level, if the risk of herbivory was elevated concomitantly with a pathogenic infection. Pathways positively regulated by jasmonic acid against herbivory, at least in its beginning, have some antagonistic crossings with those positively regulated by salicylic acid against pathogenic infection, as explored in the section “Plant-herbivore communication”, Chap. 2. In this sense, the determination to change behavior due to the perception of a stimulus by the plant is not expected to occur in isolation, independent of the perception of other stimuli and that requires the plant's already explored ability to predict as well as to calculate risks that justify an investment or behavioral change. The risk balance is evidenced, for example, in the relaxation of the greater investment in nectaries and bulbous thorns by *A. drepanolobium*, since this behavior only occurred in the conditions in which the plants were exposed to non-herbivory for more than two years; that means, there had to be a guarantee greater than one year of experience for the plant to gain confidence in behavioral change.

Figure 5.10. *Acacia drepanolobium*, a species that, in a situation of high risk of herbivory, increases the production of nectarines and bulbous thorns (which serve as shelter) by 25% to attract defending ants against herbivores. Credits: first image by Dick Culbert / Gibsons from Wikimedia Commons. License to use at https://commons.wikimedia.org/w/ index.php?curid=50052162 Second image by Sohmen from Wikimedia Commons. License to use at https://commons.wikimedia.org/wiki/ File:Figure-1-Acacia-drepanolobium-with-its-long-swollen-thorns-which-are-occupied-by-ants--Crematogaster-species-when-young.jpg

The natural environment of plants is a place of multiple stresses which require concurrent decision-making due to multiple signals. Even if a plant is isolated in a controlled laboratory condition, subjected to a single stress, will still be a need to calculate the risk before triggering a defense response due to the potential damage of the stimulus, even if there is a simple reference, such as reaching certain thresholds from substances, such as ROS, which would justify the change in behavior. This avoids a lot of investment of resources in defense without the risk of damage being really important to plant development thereby avoiding an unnecessary loss of resources that would be necessary for the growth or production of reproductive organs. Niinemets (2010) highlights this issue of plants' observation of thresholds and contexts when addressing the magnitude of the release of volatile defense compounds linked to the plant's tolerance to stress, as well as its duration and severity. Therefore, there is a context to be observed regarding the triggering of a response to a stimulus.

In addition to the influence of chemical balance thresholds or electrical gradients on the triggering of responses, other factors, such as cellular sensitivity to hormones, can interfere with the behavioral changes of plants in relation to stimuli. An example of cellular sensitivity was reported by Raschke (1988) in plants of *Commelina communis*, in which certain concentrations of ABA induced the closure of some stomata, but, even if multiplied by six, the concentration was not able to induce the closure of others, although it was observed that as the concentration increased, the number of closed stomata also increased. This observation again shows that the behavior of the plant, at least on some occasions, is not defined solely by the stimulus-response, indicating that there is a factor of permissiveness to the response. It is not clear, however, what the level of complexity of this factor is. It is not known whether it involves the age of guard cells, whether there is a control of leaf tissues or even of interaction among organs.

When considering that the plant knows how to identify the danger situation to increase or decrease the production of nectaries and bulbous thorns, as seen in the study discussed above (even more when it is not about effective herbivory, but rather its risk due to the history of the plant, that means, it is not a response to a simple momentary chemical change) its capacity for computation, and, therefore, for analysis, is evidenced. In this context, we arrive at what Trewavas (2009) calls intelligent behavior, which is a level of capacity more subtle and complex than learning and memorization *per se*, since it culminates in adaptive responses for problem solving. It is important to emphasize, however, that the intelligent behavior of the plant is inherent to the plant's capacity, respecting its limitations, and it is not convenient to anthropophize its meaning.

Summary

In a simple and broad way, Trewavas conceptualizes plant behavior as "what the plant does". The morphological or physiological plasticity of the plant, depending on the different conditions of the environment, can lead it to an adaptation that favors its development or to the resolution of a problem. Therefore, it is an intelligent behavior. In this sense, the plant is not only an organism responsive to stimuli because there is not always an automatic response. There are situations in which the potential damage resulting from the presence of a stimulus needs to be computed, considering the degree of resistance of the organism, the duration of the threat or the frequency of its manifestation, as well as its severity, in addition to its relationship to the other stimuli. Evidence of this analytical ability in plants has been reported such as the increase or decrease in the production of bulbous thorns and nectaries, attractive to defending ants, due to the danger of herbivory, as well as the quantum released of volatile compounds depending on the potential for damage from stress and the resistance of the plant. The ability of plants to analyze the context to determine whether to change their behavior can result in savings of important resources for their growth and reproduction.

Cognition in Plants

Many of the aspects that have been covered so far, such as perception, communication, memory, learning, (self)recognition, prediction, decision-making and intelligent behavior are cognitive abilities. Just as the debate about intelligence in plants becomes useless when based exclusively on a comparison with brain skills, it would also be useless to refer the discussion about the cognition of sessile organisms, such as plants, to sensory-motor aspects, as it is classically explored in regard to humans. The sessile condition of the plants was once a reason to exclude them from the list of sexual organisms, because, at the time, they did not meet the established criteria for the characterization of the sexual being, among which was movement in search of the partner. That mistake is worth remembering, because it is not the skill that has to fit the concept, but the opposite.

Like intelligence and behavior, which are terms that have dozens of concepts, cognition is another term with a wide semantic field. Ulrich Neisser (1967) defines cognition as "all the processes by which the sensory input is transformed, reduced, elaborated, stored, retrieved and used". Given this definition and the plant abilities already explored in previous sections and chapters, the manifestation of cognitive abilities in plants becomes evident.

Karban (2008) considers central three plant behaviors associated with cognition:

- plants can anticipate environmental changes that have not yet occurred;
- plants can change their behavior after experiencing new situations;
- plants can change behavior through communication signals.

Many examples covered in the sections of prediction, learning, as well as the communication and memory in plants chapters have the characteristics considered by Karban. Plants are able to perceive the environment, and as a result, respond, adjusting their metabolism in a conciliatory way to the conditions of the environment to maintain (and improve) their development. Knowing this ability of plants reinforces and exemplifies the idea of biologist Bateson (1979), who considered that cognition is an act of knowledge implicit in all forms of life.

There is a lot of evidence not only of cognition but of manifestations of extended cognition (Parise et al., 2020) in plants, whereby the organism projects itself to the environment in a way capable of manipulating it; the organism alters the environment for its own benefit. Considering the premises of the concept, an example of extended cognition capacity in plants is observed in pollination by sexual deception, in which flowers take the shape of the female of their pollinator and may also release volatile substances that act as attractive pheromones for males. That means the plant manipulates the environment in its favor. In fact, it is deceiving the pollinator, benefiting itself from pollination, and, in fact, in no way favoring the pollinator, because in so behaving, the plant exempts itself even from the production of nectar. Previously, in the topic “Volatile compounds and pollination by sexual deception”, Chap. 2, examples of this phenomenon were cited for *Drakaea glyptodon*, *Aristolochia rotunda*, *Ophrys bombyliflora* (see Figure 2.14). It is not convincing that these complex, specific and recurring phenomena arose solely as a result of evolutionary coincidences. There is great expectation that geneticists and evolutionists will be able to shed a little light on how this phenomenon arose and enhance our understanding of it.

Other events that denote the involvement of extended cognition can be observed in plants with roots that exude specific substances to attract mycorrhizal fungi or diazotrophic bacteria, inducing them to infect their roots to increase the acquisition of nutrients (see section “Plant-microorganism communication”, Chap. 2). This action results in the modification of the environment for its own benefit, although the plant needs to reward the symbiotic organisms with photosynthates produced by it. If it were not enough to modify aspects of the rhizosphere to attract microorganisms, the plant decreases the attractiveness or even breaks the trophic connection with some nodules when there is an increase in the availability of nutrients in the soil. That means it occurs when the plants no longer need the microorganism for their sufficient acquisition of nutrients. We should also not forget that plants modify the environment for their own benefit by exhaling volatile substances attractive to pollinators and dispersers. Our awareness that plants’ behavior in releasing volatile attracters for pollinators or dispersers has been learnt and established throughout evolution does not disprove their manipulation of the environment.

Summary

Learning, prediction and plasticity capable of making the plant modify its (morpho)physiology and behavior to adapt to changes in the environment are premises of an organism's cognitive manifestations, and all of them have already been observed in plants. Therefore, it is evident that cognitive manifestations are not exclusive to brain activity, albeit acknowledging their limits of coverage. The cognitive abilities of plants can make them modify the environment in their favor, which is known as extended cognition. This is evident, for example, when plants modify the rhizosphere by releasing substances in the soil that attract symbiotic microorganisms to infect their roots, and thus increase their acquisition of nutrients, as well as in the flowers that take the form of the female pollinator, in addition to exhaling volatile substances that mimic pheromones attractive to male pollinators, and thus guarantee a pollination, even without providing an effective reward to the pollinator. In addition to pollination by sexual deception, the release of volatile compounds to attract pollinators and dispersers, even with an effective reward, is still a behavior that changes the environment for its own benefit, and, therefore, is another example of the manifestation of extended cognition.

Attention in Plants

A little explored aspect, even in studies of intelligence in plants, is regarding attention. Marder (2013) argues that although memory is a fundamental element of intelligence, it has the past as a reference, with attention being an important component to the intelligent conduct of the present. In that light, the momentary perception of the self or the environment requires a state of alertness, and therefore of attention.

Take the phenomenon of differentiation of meristematic cells from a stem or root apex as a reference for discussion about the importance of attention in plants. These cells have no differentiated shape or function, added to the fact that they are somatic, and therefore have the same genetic apparatus. In this context, it is intriguing how the positioning of cells in the meristem defines their future differentiation, which suggests the presence of a coordination and vigilance factor. Vigilance is a state intrinsic to attention. It is known that hormonal balances are not the same in every position of the meristem (hence, a coordination factor is necessary), resulting in differentiated expression of genes and the consequent cellular differentiation for the formation of different tissues. Some substances such as acetylcholine and auxin are considered morphogens (section “Intercellular communication”, Chap. 3), which, theoretically, have the function of regulating cell differentiation patterns. In this case, it would be the substances that would act in the vigilance and coordination of the adequate difference in hormonal balances and consequent differentiated expression of genes in the meristem cells due to their respective positioning in the meristematic tissue. Therefore, we can infer that the morphogens act as a mechanism of attention.

In the item “Plants perceive the location of nutrients” (Chap.1) we saw that the growth of the roots is directed to the places where the availability of nutrients is greater when the spatial distribution of the latter is irregular. This is a typical event of a behavior based on the condition of now and that reveals the plant as a being continuously attentive to the possibilities of well-being and improvements.

The change in the spatial distribution of roots in the soil according to perception and even the recognition of the neighbor's identity (see topic “Recognition of neighbors”), ensures the plant a greater adaptive advantage. The root distribution in response to the neighbor (which does not have the same pattern among different species) depends on an active state of attention of the plant, associated with a spatial awareness (perception) (Figure 5.11), which in attention studies is linked to the margin concept. Although changes in root distribution in response to the neighbor is a common behavior observed in most plants studied for this purpose, it was not identified in some genotypes. This time, it is questionable whether the different genotypes have the same ability to project the focus of attention to the presence of neighbors or whether the projection of the focus is stimulated by the significance of the stimulus, that means, it depends on the momentary context. Anyway, it is a situation that exposes the manifestation of three important elements in the study of attention: focus, context and margin. Figure 5.11 illustrates different responses found in plants that make spatial awareness explicit, as well as the expression of focus and context implicit.

In the approach carried out on habituation learning, we could see that the plant familiarized with stimuli that are not important to its development becomes less responsive when resubmitted to them. The opposite effect is observed when plants learn that a stimulus factor is harmful to their development. This means that, depending on the context, the focus of attention is changed, resulting in a change in behavior regarding the responsiveness of the plant to the stimulus.

When considering aspects of intracellular communication, attention is expected to act at the cellular unit level, so that the eukaryotic cell is in constantly alert to the need for communication among compartments to ensure its homeostasis. This same consideration applies to intercellular communication, so that the organism is aware of the need for communication among tissues and interdependent organs, guaranteeing a harmonious growth and functioning. If there is a disturbance due to some factor, such as stress due to herbivory, the focus of attention is now more focused on mitigating the problem, which generates greater investment in defense, which may result in less growth or production of reproductive organs. When the threat is of a competitive nature, the focus of attention is projected on the increase in the size or distribution of organs that guarantee greater power of competition.

Figure 5.11. Behavioral representation of the root distribution among neighboring plants denoting the elements margin, focus and context in the scope of attention. In situation A, either the species is insensitive to the presence of neighbors or there is not enough reason (context) for the plant to direct the focus of attention to the neighbor, and, therefore, there is no change in the distribution of the roots due to the neighbor, observed behavior in *Ambrosia dumosa* by Mahall and Callaway (1991). In situation B, there is reason for the plants to focus their attention on the presence of neighbors, changing the projection of the roots to increase their capacity for competition in the same volume of soil, a behavior observed in *Pisum sativum* by Falik et al. (2003). Situation C is also inserted in a context that demands focus on attention to neighbors, but it is noted that the spatial perception (margin) allows the growth projection of the roots of plants that do not have neighbors on one side to a volume of soil in which there is no competitive pressure, behavior observed in *Larrea tridentata* by Brisson and Reynolds (1994).

Although memory and learning are linked to past events, they play an important role in the focus of attention, which is momentary, even because there is a learning about what is more or less important, and therefore about what worth focusing attention on. There are situations in which memory and attention need to act concurrently. In the topic "Rapid responses to touch", Chap. 1, the role of electrical memory on the closure of the venus flytrap (Figure 1.20), which is an event that requires the manifestation of an alert state, was approached. From the first touch of the fly on a trichome, an "expectation" (greater centralization of the focus of attention) of a second touch in less than 30 seconds (electrical memory) is generated in the organ, so that the plant has greater reliability in the perceived signal, and so, immediately trigger the trap. In the absence of a second touch within the timed timeframe, the action potential required for the action is not reached and the subpotential generated by the first touch is dissipated. This is a strategy ingeniously developed by the plant to increase the degree of confidence of the action to be performed and, thus, to reduce the risk of high energy expenditure without reward.

It is expected that the cell be aware of different signals, as it responds in different ways to different stimuli. Some physical structures of the plant seem to serve especially as instruments of attention, such as the aforementioned sensory trichomes of the venus flytrap, responsible for triggering a drastic movement of an entire organ. Another example of physical structure that acts as a mechanism of attention are the PRR proteins, constitutive immune components with act as instrument of constant state of alert, and therefore of attention, to the presence of PAMPs, molecular patterns characteristic of pathogenic organisms (see topic "Plant-pathogen recognition", Chap. 2).

When we refer to PRRs, the sensory trichome, the perception of the need for intracellular or intercellular communication, the perception of the self or the neighbor, as well as the different factors of environmental stimuli within the scope of attention in plants, we perceive an intricate relationship between attention and perception, which naturally involves the plant's sensory ability. They really are attributes that cannot be unlinked, since they are part of the same functional scope, because there is no reason for attention without the ability to perceive.

In line with the above, it is important be aware to what Chamovitz (2012) emphasizes about attention in plants, whose manifestation occurs as an instrument of action, with no known elements that evidence contemplative attention.

Summary

The memory of past events is essential for the plant to learn to direct the focus of attention to the stimuli that really make a difference to its development. However, the continuous adjustments that allow improvements and well-being to the plant depend on a focused attention to the conditions of the present. Naturally, the plant needs to perceive the conditions of the now so that the focus of attention is directed to the most important stimuli. This means that attention responds to context. By using the fact that the roots of one species of plant are indifferent to the presence of neighbors, and that the growth of roots of another species is projected to the opposite side or even to the neighbor's root system, there are three fundamental elements of attention: the perception of space (margin), a direction of focus and a context. If the presence of the neighbor is a factor that affects its development (context), there will be a focus of attention (focus) on this obstacle, and thus the roots will respond by increasing the power of competition for space or seeking a less disputed space.

When a plant is repeatedly subjected to a stimulus, its responsiveness can increase or decrease depending on how important it was to its development. This means that learning can change the focus of attention. On the other hand, there are situations in which there is a constant focus of attention, as is the case of PRRs proteins, receptors of molecular patterns of pathogens, constantly alert for the presence of PAMPs, molecular patterns associated with pathogens, a situation that is typical of what it expects a state of alertness, and therefore of attention, which is inseparable from the stimulus' perception capacity.

Consciousness in Plants

Etymologically, “conscience” derives from the Latin term *conscientia*, which means “to be aware”, “to perceive”. An anthropocentric appropriation made the meaning of this term almost an exclusivity inherent to the manifestations of the human mentality. In plants, although physical structures of the perceptual apparatus were discovered more than six decades ago, such as phytochromes, there are still clashes over the appropriateness of the use of the term “consciousness” associated with plant behavior. Unless, inappropriately, the discussion is based on references unrelated to plants, such as brain activity, questioning consciousness in plants is, in the *stricto sensu*, questioning its perception capacity. It is impossible for phototropism, thermotropism, chemotropism, among many other phenomena observed in plants, to occur without the organism noticing the stimuli. The first chapter of this book was especially devoted to exploring what is known about the apparatus and the perceptual mechanism of plants.

It is important to note that the plant is not only aware of the stimulus but acts to adapt itself to different situations to maintain or improve its development in line with the changes that occur in the environment, that means, it is aware of the importance of the perceived stimulus to its development.

Perhaps the most intriguing factor in the discussion of plant consciousness is the relationship we make between consciousness (perception) and conscious action (purposeful, intentional). To explore this issue, let us consider different behaviors. Let us take as a first example the communication that occurs from the leaves to the roots regarding the need to increase or decrease the acquisition of nitrogen (section “Perception of nutrients”, Chap. 1; section “Chemical communication at long distance”, Chap. 3). It is questionable whether there is an intention for the leaves to communicate to the roots about nitrogen deficiency or excess, sending biomolecules signaling “hunger” or “satiety” so that the roots increase or decrease the absorption of N, or if the movement of these chemical signals results only from their spontaneous accumulation according to the state of leaf N metabolism. Whether or not there is an intention to communicate between the organs, the biomolecules involved will not cease to be part of a communication mechanism, without which there would be no necessary changes in the behavior of the roots in terms of nitrogen acquisition according to the needs of the leaves. What is under discussion, however, is the intentionality of communication. In this specific example, it is not possible to reach a conclusion. However, in many of the behaviors observed in plants, several already explored, there is evidence of intentional action:

- When *Aristolochia rotunda* (topic "Communication by volatile compounds in pollination", Chap. 2; Figure 2.14C) releases volatile compounds that mimic secretions of insects killed by spiders to attract a specific cleptoparasitic fly that pollinates it, there is an indication that the plant knows the eating habit of its pollinator, and thus, purposely, produces and releases very specific substances that attract it. It is not convincing that an extremely complex and specific behavior like this arose only from evolutionary coincidences, especially when it is a recurring behavior in several other species. The evidence is that there was learning during evolution, whose bases and mechanisms involved in its emergence have not yet been elucidated. This same comment applies to the next evidence of conscious action in plants.
- *Drakaea glyptodon* (Figure 2.14A) develops a petal mimicking the physical shape of female *Zaspilothynnus trilobatus* wasps and also produces and releases chemicals that act as specific pheromones to attract male pollinating *Z. trilobatus* wasps. This behavior evidences that *D. glyptodon* is conscious about the shape and sexual behavior of its pollinator. It is suggestive that it is a structure developed specifically for the plant to deceive and take advantage of its pollinator since it does not even need to produce nectar as a counterpart for "pollination by sexual deception". Similar behavior is observed in *Ophrys bombyliflora* (Figure 2.14D), *Ophrys insectifera*, *Ophrys speculum* and *Caladenia tentaculata* (Figure 5.12), and in some cases, in addition to the shape and smell, even the pollinator's color is mimicked.

Figure 5.12. *Ophrys insectifera* (left), *Ophrys speculum* (central) and *Caladenia tentaculata* (right), plants that mimic the shape, smell and/or color of female pollinators to attract male pollinators (Borg-Karlson et al., 1993; Paulus and Gack, 1990; Peakall and Beattie, 1996). Credits: image to the left by Ian Capper from Geograph. License to use at https://www.geograph.org.uk/photo/6483166 Central image by Lumbar ~ commonswiki from Wikimedia Commons. License to use at https://commons.wikimedia.org/wiki/File:Ophrys_speculum_d.JPG Image to the right by Reiner Richter from Wikimedia Commons. License to use at https://commons.wikimedia.org/wiki/File:Caladenia_tentaculata_flower.jpg

- The recognition between conspecific neighbors, relatives or non-relatives (siblings or non-siblings), (Table 5.2), makes the plant trigger an apparently purposeful behavior in manifesting or not morphophysiological mechanisms that increase the competitive capacity due to kinship. And not only that, but this manifestation is also modulated by the availability of resources in the environment, that means, the competition response is accentuated as the scarcity of resources increases. The same occurs with clones connected or disconnected by stolons, in which competitive behavior is only observed after being disconnected (Figure 5.13). It is not advisable, therefore, to simplify these changes just to a question of stimulus-response due to chemical changes in the environment, since they are conspecific and even clones, where expressive chemical variations are not expected.

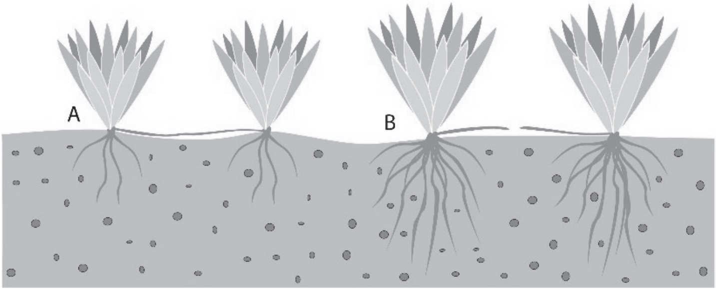

Figure 5.13 Competitive behavior between clones dependent on identity recognition. A. Clones connected by stolons have a typical growth of close relatives, whereby they invest less resources for aerial and root growth and invest more in reproduction. B. When there is a disconnection between neighboring clones due to the shutdown of the stolon, apparently there is a loss of a factor necessary for recognition, resulting in a behavior typical of strange neighbors, with greater growth of vegetative organs, which ensures greater competitive power. Based on the studies by Falik et al. (2006) and Yamawo et al. (2017).

- Based on the plant behaviors discussed in the section “Plant-microorganism communication”, Chap. 2, facilitating or hindering root infection by beneficial or harmful fungi and bacteria, is evidence not only of the plant's recognition of the microorganism present but also its influence on its development, triggering, from then on, a purposeful action that culminates in the strengthening or relaxation of immunological barriers that influence the infection. Even when considering that this behavior is the result of learning built over evolution, it does not detract from the conscious action of plants in the construction of the recognition mechanism to facilitate/hinder infection during learning.
- When *Acacia drepanolobium* (Figure 5.10) at risk of herbivory, and only in this condition, produces 25% more nectaries and bulbous thorns to attract ants that defend them from herbivores, it is a behavior that demonstrates a conscious action by the plant. We could simply link this behavior of increasing the production of ant attractions to chemical changes that occurred due to herbivory, such as changes in the hormonal balance, if it were not a phenomenon that occurred due to the history of plant herbivory, without which the plant must necessarily come under effective attack by herbivores.
- When the phloem sieve elements suffer damage that leads to sap spill and consequent loss of positive pressure, such as perforation by the

> mouthpiece of an aphid, it is common for protein P, abundant in the liquid cell matrix, to agglomerate in the injured region to prevent the overflow from continuing. In this case, protein P is a mechanism used purposefully by the cell when it becomes aware of an undesirable situation. Here, it is important to note that we are making an explicit inference of consciousness at the level of the cellular unit.

The situations explored above show that the plant is not a passive and unconscious organism before the environment. However, it is important to be aware that this must be the consciousness of the plant, which, it is presumed to be quite distinct from that of animal or human. According to Calvo (2017), in his article “What is it like to be a plant?”, he points out that the plant's awareness must not only differ from that of the animal or human, but must also differ among the different plant species, as well as differs among animal species. In this context, it would not be prudent to state, for example, that because there is evidence that *Aristolochia rotunda* is aware of the food habit of its pollinator, *Drakaea glyptodon* is also aware of it. The evidence we have of *D. glyptodon* is of its awareness of the sexual behavior of its pollinator. If we take as an example the awareness of the venus flytrap as regards the capture of insects, we will see that it is a peculiarity of the species, not applicable to all plants. The same can be observed for *Mimosa pudica*'s sensitivity regarding the closing of the leaflets. Already when considering the inherent awareness of a sieve element cell as to the importance of protein P, although it is an expected manifestation of occurrence in all plants, given that it is an omnipresent structure and that performs the same function, even so, there may be different degrees of responsiveness of action corresponding to this protein when cell damage occurs among different species. Therefore, dealing with conscious or intelligent behavior in plants is not an issue that necessarily implies homogeneity of the skills evidenced for the different plant species.

It is important to note that, just as for attention, the evidence of consciousness that we know today in plants only allows us to conceive it as a mechanism of action, and not of contemplation.

We do not know any structure in plants with functional similarity to the brain of animals, and, therefore, that acts as an influential command center on the behavior of the organism. Although we have evidence of the autonomy of conscious and intelligent behavior of the cell unit, as in the case reported above about the action of protein P in an injured cell, it is also expected that there are factors regulating cellular behavior at the supracellular level. An example of this evidence was approached in the section “Attention in plants” regarding the organized differentiation of the meristematic cells of the stem or root apex, whose regulation was attributed to morphogens. Plant neurobiology studies have raised the possibility of there being small command centers in young regions of the roots or of points of convergence of information, as in meristems. Anyway, it is plausible to think that the manifestations of intelligent behavior, or behavior that shows awareness, are inherent to the individualized capacities of the plant cell, even if they can be modulated by factors of the environment. In this sense, unlike what is so far studied in animal cell biology, in which memory and learning are attributes of specific cells of the nervous or immune system, the evidence we have in plants is that cognitive abilities are attributes of any living cell.

The potential of a single cell is surprising. Depending on the stimuli of the environment, a stem cell (meristematic) can differentiate into any cell of the most varied tissues. In addition to meristems, meristematic potentiality is also present in cells of other tissues. There are situations in which already differentiated cells are dedifferentiated; that means they reassume the meristematic role, or they may even be re-differentiated, changing form and function, as occurs in the transition of some parenchymal cells to sclerenchymatous cells.

Any somatic cell with meristematic potentiality is totipotent, that is, it has the capacity to give rise to an entire organism, genetically identical to its mother. In plants, this extraordinary cellular capacity is tangible and relatively simple to put into practice in a tissue culture laboratory. Therefore, linking overly complex events to the existence of organs or command centers does not always seem to be an indispensable condition when analyzing individual cellular capacities. What could be more complex from a morphophysiological point of view than structuring an entire organism from one or a few somatic cells in response to some initial stimuli? After all, the principle involved is very similar to that of embryogenesis.

Summary

The use of the term “consciousness” in plants may still sound strange due to the association we make of it with our own mental capacity, however, consciousness means to be aware, to perceive. In the first chapter, we already explored sensory apparatus and mechanisms for many stimuli. The plant is not only able to perceive, but also change itself morphologically and physiologically in line with changes in the environment, seeking improvements for its development; that means it is aware of the importance of what it perceives for itself. However, the evidence we have access to is that the plant has consciousness as an instrument or mechanism of action, but not of contemplation.

As already seen in the extended cognition approach, the plant can manipulate the environment to its advantage, indicating intentional behavior, as we observed especially in some orchids, which mimic shapes, colors and smells of female pollinators to attract males, and in this way, being pollinated without even providing nectar to the pollinator, a phenomenon we know as “pollination by sexual deception”. This behavior shows the manifestation of a purposeful action of the plant to manipulate the environment, and, for that, it needs consciousness of the form and sexual behavior of its pollinator.

The manifestation of consciousness in plants is not attributed to the capacity of an organ or other specific structure. There is evidence of autonomous consciousness in cell units and there is also evidence of supracellular regulation of cell behavior. The meristematic potentiality, associated with the totipotentiality of unspecific vegetable somatic cells, reveals the potential of cellular independence from a command center, even if it can be modulated by the environment, as well as revealing its autonomy regarding the manifestations of cognitive abilities.

Plants as Social Organisms

It has long been known that plants form complex relationships among themselves and with other organisms. Studies in the field of plant intelligence encourage us to rethink the establishment of these relationships, putting the plant as an active element in the process, especially considering its potential to modify the environment in its surroundings, which is the focus of extended cognition studies.

Some situations, previously explored, such as communication by volatiles among plants, differentiated growth according to the degree of kinship of neighbors and relationships that involve allelopathy, encourage us to question the scope of the potential of sociability of the plants.

There has been great progress in the contemporary perception that we have of plants, when compared to 1983 (not so long ago), when David Rhoades was harassed by a scandalized scientific world for expounding the idea of an aerial signal emitted by a plant that triggered greater tolerance of the conspecific neighbors to a pest attack. It was a situation that demanded knowledge and acceptance of what we now know as extended cognition. At the time, it was unacceptable that the plant, a passive being, with only the ability to respond to stimuli, could manipulate the environment around it. However, even to this day, some remnants of the ideas common to that time are perpetuated, such as the inference we make that volatile compounds that induce tolerance and defense in neighboring plants are merely public signals generated by the emitting plant in order to communicate and induce defense in organs of the same plant not yet affected by the damage agent, but, because it is public, the signal can be used by commonly conspecific neighboring plants that have access to it, as long as they have receptors for such compounds. In adopting this idea, we may be trying to deny the intentionality of communication among plants.

It is suggestive that the idea of emitting public signals to communicate itself is rethink, as it ignores ecology studies that are dedicated to exploring the investment and return of behavioral changes in plants. It is assumed that there is a high cost for the synthesis of volatile compounds. So much so that many are produced only when there is a need, and others, which are constitutive, have an increase in production only when there is greater demand. In this sense, why would the plant that emits volatiles take on such an expensive form and of dubious efficiency for inducing the defense of its own organs? A volatile as a public signal is not a guarantee that the information will reach the unaffected organs, as it is easily lost and can be dragged by the wind out of the system. Meanwhile, several other safe and highly efficient internal communication possibilities could lose investment, such as the internal chemical signaling by jasmonic acid, salicylic acid and ABA, widely observed, depending on the stressor. In addition, there are highly efficient electrical signaling for fast communication. When looking at the issue from this angle, the production and release of volatile compounds by a plant as an information signal, which will become public, to communicate to itself, does not seem interesting, not even for the energy aspect (of resources) or that of efficiency, which strengthens the idea that this mechanism is intentionally used for communication among plants.

What would be the need to “worry” about the neighboring plant? This does not necessarily make the emitting plant an “altruistic” being, because, when allogamous, the healthier its conspecifics are, the greater the chance of reproductive success, therefore, protecting its equals is an investment in its own reproduction. On the other hand, the larger the population of healthy conspecifics is, the less chance there is of territorial invasion by species that are not compatible or that do not bring any benefit, albeit avoiding a population density threshold that would generate unwanted intraspecific competition for the available resources in the environment, whose control can be carried out by allelochemicals.

The release of allelochemicals as a way of restricting the development or proliferation of incompatible plants, or seed germination even of conspecifics, as a way of reducing competitive pressure also exemplifies social behavior.

In the section on the recognition of neighbors, relatives and non-relatives, many examples were explored that show changes in behavior due to the degree of kinship. A recurrent behavior was observed: when the neighbor was a stranger, the plants increased the investment of resources in the production and growth of lateral roots, as well as in the growth of the aerial part, resulting in plants with greater competitive power. On the other hand, they were plants that produced fewer reproductive organs. This is typical behavior of territorial defense. On the other hand, when the neighbor was a conspecific, a relative or a clone, the tendency was for plants to slow down the growth of vegetative organs and invest more in reproduction, which is a sign of less competition among equals. For most cases, the closer the degree of kinship, the less evidence there is of competitive behavior. Once again, there is a sociability behavior with the potential to modify the environment, and, therefore, consistent with the premises of extended cognition and conscious action.

Summary

Approaching plants as social beings raises evidence of intentional behaviors in the relationships among them that result in the modification of the environment. In previous chapters, allelopathic interactions had already been explored, in which a plant can directly interfere in the development and establishment of others. However, the matter had not yet been discussed from the perspective of purposeful interference. The plant's ability to modify the environment to its advantage, the focus of studies on extended cognition, reveals an intelligent behavior in which the plant is able to create favorable conditions for its development. From the point of view of sociability, this capacity can be exemplified in the communication among plants by volatile compounds, in which a plant induces greater protection and defense of its conspecifics in a system. Although this capacity can be exploited simply within the context of public signals, there is no ecological evidence to support this hypothesis. The friendly social relations of plants towards their clones and siblings and the competitive one towards strangers, or even non-relative conspecifics, is yet another example that shows a social relationship based on conscious behaviors.

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