12 Long-Distance Electrical Signaling and Physiological Functions in Higher Plants

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12.1 Introduction

Plants possess most of the chemistry of the neuromotoric system in animals, i.e. neurotransmitter such as acetylcholine, cellular messengers like calmodulin, cellular motors, e.g. actin and myosin, voltage-gated ion channels and sensors for touch, light, gravity and temperature. Although this nerve-like cellular equipment has not reached the same great complexity as is the case in nerves, a simple neural network has been formed within the phloem, enabling it to communicate successfully over long distances. The reason why plants have developed pathways for electrical signal transmission most probably lies in the necessity to respond rapidly to environmental stress factors. Different environmental stimuli evoke specific responses in living cells which have the capacity to transmit a signal to the responding region. In contrast to chemical signals such as hormones, electrical signals are able to rapidly transmit information over long distances. Most of the plant action potentials studied so far have a velocity in the range of 0.01–0.2 m s^{-1}. However, in soybean, action potentials reached conduction rates of up to 30 m s^{-1}, similar to the speed of action potentials in nerves (Volkov et al. 2000).

As regards the origin of the neuronal system in plants, it appears unlikely that it was adopted from animals. In our search for the common evolutionary roots of action potentials in plants and animals, we need to look at unicellular ancestors which do not need to transmit signals over long distances. The function of electrical transmission has most probably evolved at a later evolutionary stage. The assumption is that in the course of evolution the development of plants and animals branched off into different directions. Since cellular excitability was found to exist in primitive organisms, it is obvious that both plants and animals inherited their basic neuronal capabilities from their bacterial ancestors (Simons 1992). Szmelcman and Adler (1976) observed changes in membrane potential during bacterial chemotaxis. Even the sensitivity to mechanical touch is known to be an early evolutionary achievement. Martinac et al. (1987) detected pressure-sensitive ion channels

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in *Escherichia coli*, suggesting that these channels have an osmotic function. For the early evolution of action potentials, an osmotic function can also be assumed in unicellular alga such as *Acetabularia* (Mummert and Gradmann 1976). A mechanosensitive ion channel was also found in the yeast plasma membrane (Gustin et al. 1988), providing convincing evidence that plants inherited mechanical sensitivity from bacterial ancestors in the course of millions of years of evolution. The characean algae, which include *Chara* and *Nitella*, are also known to be the ancestors of higher plants. Action potentials were observed in the internodal cells of *Nitella* in 1898 by Hörmann, who used extracellular electrodes long before they were observed in isolated nerve cells by Adrian and Bronk (1928). Characean internodal cells respond to electrical stimulation in a manner similar to the contraction response displayed by skeletal muscles following electrical stimulation by nerve cells. In characean cells, electrical stimulation causes the cessation of protoplasmic streaming which is incited by the same interactions between actin and myosin that cause contraction in muscles (Hörmann 1898). In the course of evolution, once plants had gained and settled on dry land, their excitability and neuronal capability were used to develop numerous survival tactics. For instance, one important step was the development of fast-moving stomatal guard cells in response to environmental changes, while another was the electrical communication system which uses the phloem to transmit information over long distances within the plant body (Fromm and Lautner 2005).

12.2 Perception of electrical signals

Electrical signals can be generated at any site of the symplastic continuum by environmental stimuli such as changes in temperature, touch or wounding. Recently, it was found that acid rain also induces action potentials (Shvetsova et al. 2002), as well as irradiation at various wavelengths which induces action potentials in soybean with duration times and amplitudes of approximately 0.3 ms and 60 mV, respectively (Volkov et al. 2004). Upon perception, electrical signals can be propagated via plasmodesmata to other cells of the symplast (van Bel and Ehlers 2005). As a first step, the plasma membrane is being depolarized, a process known as formation of the receptor potential, e.g. by mechanical stimulation as observed in *Chara* (Kishimoto 1968). The receptor potential is an electrical replica of the stimulus lasting for the period of time that the stimulus is present. An action potential is evoked when the stimulus is great enough to depolarize the membrane to below a certain threshold. Subsequently, the action potential characterized by a large transient depolarization allows the rapid transmission of information via plasmodesmata (Fig. 12.1). An action potential usually has an all-or-nothing and self-amplifying character, and it travels with constant velocity and magnitude (Zawadzki et al. 1991). Electrical coupling via plasmodesmata was demonstrated in a variety of species such as *Nitella*

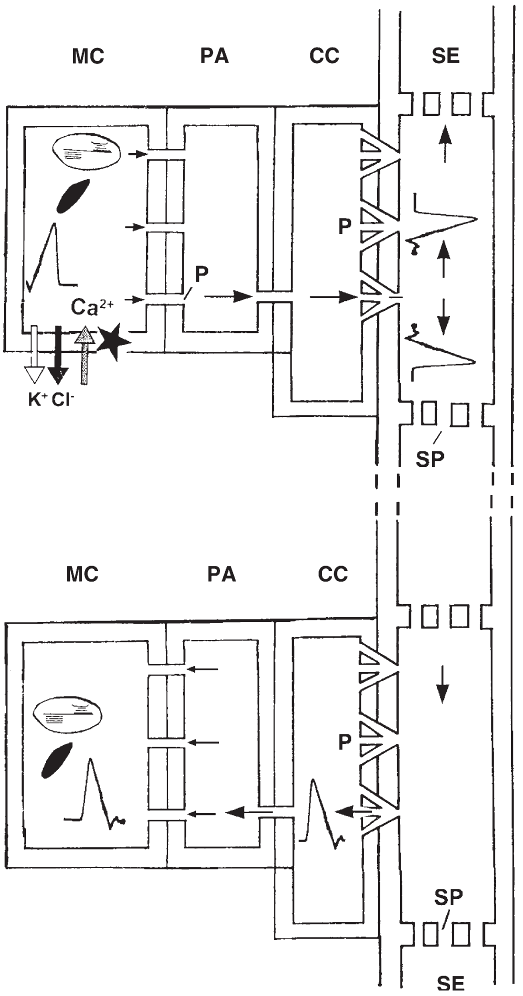

Fig. 12.1. Electrical signaling in higher plants. Stimulation by cold-shock or touch (*star*) induces calcium influx into a living cell, e.g. a mesophyll cell (*MC, above*). After the membrane potential is depolarized below a certain threshold level, an action potential is elicited by chloride and potassium efflux. The signal is propagated over short distances through plasmodesmal (*P*) networks and, after it passed the few plasmodesmata between sieve element/companion cells (*SE/CCs*) and phloem parenchyma cells (*PA*), will enter the SE/CC-complex to be transmitted over long distances. Sieve pores (*SP*) with their large diameters present low-resistance corridors for a rapid propagation of electrical signals along the SE plasma membrane. Such signals can leave the phloem at any site via plasmodesmata (*below*) to affect certain physiological processes in the neighbouring tissue

(Spanswick and Costerton 1967), *Elodea* and *Avena* (Spanswick 1972) and *Lupinus* (van Bel and van Rijen 1994), indicating that plasmodesmata are relays in the signaling network between cells. However, long distances between different organs can be bridged rapidly only via low resistance connections, which extend continuously throughout the whole plant. The sieve tube system seems to fulfill these conditions, because the structure of the sieve tube members is unique and appears to be suitable for the transmission of electrical signals due to the relatively large, unoccluded sieve plate pores, continuity of the plasma membrane and ER (Evert et al. 1973), as well as lack of vacuoles. Moreover, the low degree of electrical coupling in lateral direction caused by only few plasmodesmata at the interface between companion cells and phloem parenchyma cells (Kempers et al. 1998) facilitates long distance signaling. However, the plasmodesmata may open up, making it possible for lateral electrical signaling from neighboring cells to be transmitted to the sieve elements/companion cells (SE/CC, Fig. 12.1). In summary, signal transmission within the plant depends on the electrical conductance of plasmodesmata in lateral direction as well as on the high degree of electrical coupling via the sieve pores in longitudinal direction.

12.3 Aphid technique as a tool for measuring electrical signals in the phloem

Since the phloem is located inside the plant body several cell layers distant from the plant surface, experiments on electrical signaling via the phloem of intact plants are difficult to perform. Microelectrode measurements in combination with dye solutions injected into the cell to be measured after obtaining electrophysiological results is a time-consuming technique because the measured cell type can only be roughly estimated at the beginning and very often the microelectrode tip was not properly inserted in the phloem as revealed by microscopic checks after the experiment. Microelectrodes brought into contact with sieve tube exudates that appear at the cut end of an aphid stylet (Wright and Fisher 1981; Fromm and Eschrich 1988b), enabled us to monitor the membrane potential of sieve tubes and its changes after plant stimulation (Fig. 12.2A, B). The successful use of aphid stylets to measure electrical signals within the sieve tubes depends on their functioning as an effective salt bridge between the sieve tube cytoplasm and the microelectrode. Sieve tube exudates typically contain high K^+ concentrations; measurements on barley leaves gave values ranging from 50 to 110 mM (Fromm and Eschrich 1989). The stylet's food canal dimensions can be used to roughly calculate its electrical resistance. Using an average area of 6 μm^2 and assuming the canal to be filled with 100 mM KCl, its resistance would be about $2.6 \times 10^9\ \Omega$ (Wright and Fisher 1981). Although this value is about 3 times greater than the typical resistance of a glass microelectrode, it is still within the measuring capacities of the electrometer used (input impedance $>10^{12}\ \Omega$). The stylets are embedded in hardened saliva, which insulates electrically. For

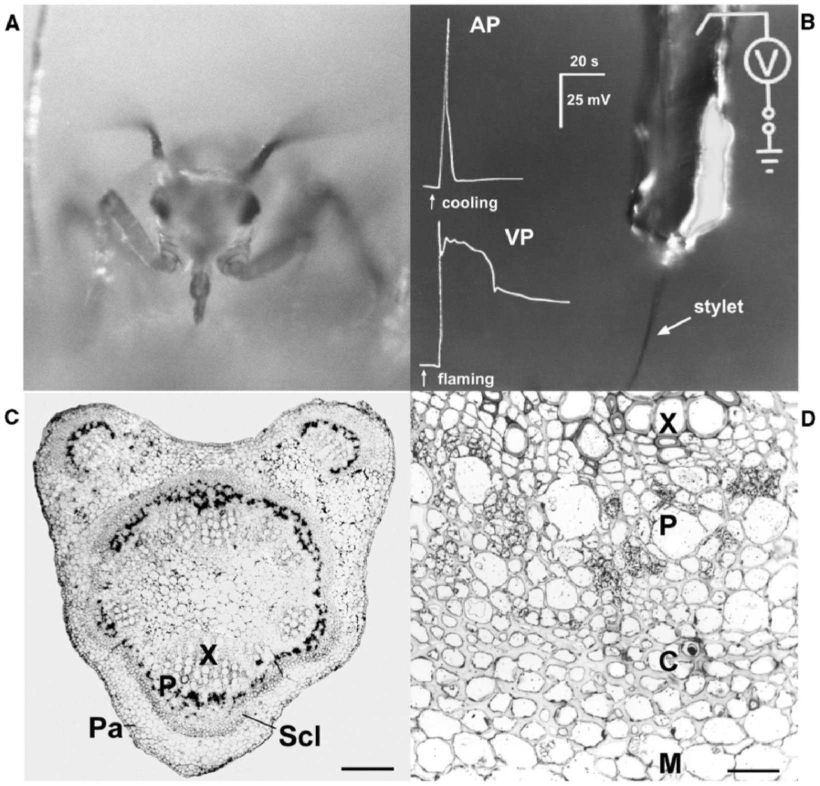

Fig. 12.2. Transmission of action potentials (*AP*) and variation potentials (*VP*) in sieve tubes of *Mimosa*. **A** Front-view of *Rhopalosiphum padi* sucking at the base of a petiolus with its stylet inserted into a sieve element (×32). **B** After the aphid separated from its stylet by a laser pulse, the stylet stump exuded sieve tube sap to which the tip of a microelectrode was attached (×400). Cooling the apical end of the petilous evoked an action potential (*AP*) while flaming triggered a variation potential (*VP*) transmitted basipetally within the sieve tubes. **C** Microautoradiography of the petiolus. ^{14}C-labeled photoassimilates from the leaves accumulated in the phloem (P) which is surrounded by a sclerenchyma sheath (*Scl*) in order to restrict electrical signaling to the phloem. *Bar*, 150 µm. **D** Microautoradiograph of a cross section of the primary pulvinus at the base of the petiolus. Labeled photoassimilates are restricted to the phloem strands (*P*). Since sclerenchyma tissue is absent, electrical signals can be transmitted laterally from the phloem via living collenchyma cells (*C*) to the motor cells (*M*) which cause the leaf movements by either losing or gaining turgor. *X* xylem; *Pa* parenchyma. *Bar*, 30 µm

instance in *Mimosa pudica*, a classic example for the conductance of rapid excitation in higher plants, the microelectrode tip was brought into contact with the stylet stump at the petiolus with its cut end sealed into saline solution to which the Ag/AgCl reference electrode was connected. After successful connection of stylet and mirocelectrode tip a resting potential of −160 mV was established, well in line with values found in other species and by other methods (Eschrich et al. 1988; van Bel and van Rijen 1994). Cooling the apical end of the petiolus evoked a rapidly moving action potential transmitted basipetally within the sieve tubes at the rate of up to 3–5 cm s^{-1} (AP, Fig. 12.2B). By contrast, wounding by flaming induced a more slowly moving signal, also called variation potential, with irregular form and of long duration (VP, Fig. 12.2B). The variation potential varies with the degree of stimulus, is non-self-perpetuating and appears to be a local change to either a hydraulic surge or chemicals transmitted in the dead xylem (Davies 2004). In *Mimosa*, both action and variation potentials become immediately evident as the bending pulvini cause impressive movements of the paired leaflets. Microautoradiography of the petiolus showed the localization of the phloem by ^{14}C-labeled photoassimilates from the leaves exposed to $^{14}CO_2$ (Fig. 12.2C). The vascular bundles are surrounded by a sclerenchyma sheath in order to restrict electrical signaling to the phloem. When a phloem-transmitted action potential reaches the pulvinus which has no sclerenchyma (Fig. 12.2D; Fromm and Eschrich 1988a), it is transmitted laterally via plasmodesmata into the cells of the motor cortex. The latter possess voltage-gated ion channels which respond to the signal, causing ion efflux associated with water efflux, which leads to leaf movements (Fromm and Eschrich 1988c).

12.4 Electrical properties of the phloem and characteristics of phloem-transmitted signals

The phloem presents a network for assimilate allocation as well as chemical and electrical communication within the plant. Concerning assimilate transport, osmolytes like sucrose generate the hydrostatic pressure which drives nutrient and water flow between the source and the sink phloem. Proton-coupled sucrose symporters, such as ZmSUT1, localized to the sieve tube and companion cell plasma membrane are capable of mediating both, the sucrose uptake into the phloem in mature leaves and the desorption of sugar from the phloem into sink tissues. The use of patch-clamp techniques revealed that the ZmSUT1-mediated sucrose-coupled proton current depended on the direction of the sucrose and pH gradient as well as on the membrane potential across the transporter (Carpaneto et al. 2005). Concerning the membrane potential, it has been shown that a sink-source-regulated and sugar-inducible K^+ channel (VFK1) dominates the electrical properties of the sieve tube plasma membrane (Ache et al. 2001). The source site of phloem cells is characterized by K^+ concentrations of about 100 mM within the cytoplasm and 10 mM in the apoplast (Mühling and Sattelmacher 1997), resulting in an equilibrium potential for potassium ions (E_K) of around –60 mV. Since the membrane potential of the SE/CC is between –130 and –200 mV (van Bel 1993; Ache et al. 2001) and thus more negative than E_K, VFK1 loads K^+ into the phloem. Furthermore,

K^+ channels of the AKT2/3 family have been identified as photosynthate-induced phloem channels. From studies of an AKT2/3 loss-of-function mutant, it was shown that this mutant exhibited reduced K^+ dependence of the phloem potential and that AKT2/3 regulates sucrose/H^+ symporters via the membrane potential (Deeken et al. 2002). Furthermore, there is an electrogenic component of the sieve tube membrane potential, the magnitude of which is substantially greater than that predicted for E_K (Wright and Fisher 1981). With regard to calcium, (DHP)-type Ca^{2+} channels were localized in the phloem of leaf veins from *Nicotiana* and *Pistia* by immunolabeling techniques (Volk and Franceschi 2000), indicating that Ca^{2+} channels are also abundant in sieve elements. Most likely, these channels are involved in the generation of electrical signals, making them the subject of further studies.

The ion transport processes which create the conditions necessary for the generation of an action potential were investigated intensively in members of the green algal family Characaea (Tazawa et al. 1987). One of the ion transport mechanisms responsible for depolarization is based on chloride the efflux of which increases upon membrane stimulation (Gaffey and Mullins 1958; Oda 1976). Another ion involved in plasma membrane excitation is calcium where studies showed that both the peak of the action potential (Hope 1961) and the inward current (Findlay 1961, 1962) are dependent on the calcium concentration outside the cell. Some workers suggest that both chloride and calcium are involved in the formation of an individual action potential (Beilby and Coster 1979; Lunevsky et al. 1983). In addition to these ions, it was found that potassium efflux from the cell increases upon stimulation of the membrane (Spyropoulos et al. 1961; Oda 1976).

These ion shifts during an action potential were confirmed in trees by a method which uses inhibitors of ionic channels as well as energy-dispersive X-ray microanalysis (Fromm and Spanswick 1993). Results indicate that calcium influx as well as potassium and chloride efflux are involved in the generation of action potentials. When action potentials were induced by electrical stimulation in willow, it became clear that the required stimulus depends on both, its intensity and duration (Fromm and Spanswick 1993). An increase in stimulus strength does not produce any change in the amplitude nor in the form of the action potential once it has been induced, showing that it conforms to the all-or-nothing law. Concerning refractory periods, they were found to be much longer in plants than in animal systems, with durations between 50 s (Fromm and Bauer 1994) up to 5 h (Zawadzki et al. 1991).

12.5 Electrical signaling via the phloem and its effect on phloem transport

Strong evidence has accumulated that electrical transmission in sieve elements also occurs in species that do not perform rapid leaf movements as, e.g. in Mimosa. In zucchini plants, electrical signal transmission via sieve tubes between a growing fruit and the petiole of a mature leaf reached maximum velocities of 10 cm s^{-1} (Eschrich et al. 1988). This is in the same velocity range as the movement of the action potential in sieve tubes of *Mimosa pudica* (Fromm and Eschrich 1988b). It is obvious that no chemical substance is capable of moving so fast in the assimilate flow. By contrast, hydraulic signals might transmit stimulations, but they would not be able to carry encoded plus- or minus-signals for hyperpolarization or depolarization, respectively, as shown in poplar sieve tubes (Lautner et al. 2005). It has not yet been shown whether hydraulic signals occur in the turgescent sieve tube system. In the wounded tomato plant, the pathway for systemic electrical signal transmission is also associated with the phloem (Rhodes et al. 1996), indicating that it regulates the induction of proteinase inhibitor activity in parts of the shoot distant from the wound (Wildon et al. 1992). As regards the function of the phloem, it has been shown that action potentials propagating in sieve tubes of *Mimosa* trigger phloem unloading in the pulvini (Fromm and Eschrich 1990; Fromm 1991).

In maize leaves, both electrical stimulation as well as cold-stimulation induce action potentials with amplitudes higher than 50 mV that are propagated basipetally in sieve tubes at speeds of 3–5 cm s^{-1} (Fromm and Bauer 1994). Stimulation with ice water has been reported to induce action potentials in a number of plant species, including *Biophytum* (Sibaoka 1973) as well as pumpkin and tomato (van Sambeek and Pickard 1976). The fact that Woodley et al. (1976) observed that localized chilling temporarily stops or reduces translocation of ^{14}C in sunflowers for 10–15 min and that this reduction in translocation corresponds closely to electrical changes measured along the stem gave rise to the idea of a possible relationship between action potentials and the cold-shock-induced inhibition of phloem transport. In addition, Minchin and Thorpe (1983) showed that rapid temperature drops of only 2.5 °C caused a brief abeyance of phloem transport in *Ipomea purpurea*, *Phaseolus vulgaris* and *Nymphoides geminata*, a phenomenon not observed when the temperature was reduced at a slower rate. In maize leaves, rapid cold-shock treatments cause sieve elements to trigger action potentials while phloem transport in distant leaf parts is strongly reduced, as shown by autoradiography at a distance of over 15 cm from the site of cold-stimulation (Fromm and Bauer 1994). When a maize leaf was stimulated electrically (10 V) via surface electrodes action potentials were induced and phloem transport was interrupted at the site of stimulation. Evidence of a link between electrical signaling and the reduction of phloem transport was found based on the decrease in symplastic K^+ and Cl^- concentration. In *Luffa cylindrica* action potentials affected elongation growth of the stem, most likely by K^+ and Cl^- efflux which reduced cell turgor and caused growth retardation (Shiina and Tazawa 1986). Since the concentrations of either ion are also reduced in the sieve element cytoplasm after stimulation (Fromm and Bauer 1994), decreased cell turgor may have caused the reduction in phloem translocation since the latter requires the intracellular movement of water as a transport medium. However, the reduction in phloem translocation may have also been caused by a closure of sieve pores or a reduction of phloem loading because the latter depends on the membrane potential as well as on the K^+ concentration in sieve tubes, both of which changed during stimulation. To obtain a better insight into the electrical controlling points in the phloem transport system, further work is required.

12.6 Role of electrical signals in root-to-shoot communication of water-stressed plants

Non-hydraulic signaling between roots and shoots of plants growing in drying soil has evoked considerable interest in recent years. Since plants growing in drying soil showed stomatal closure and leaf growth inhibition before the reductions in leaf turgor were measured, non-hydraulic signals from roots may serve as a sensitive link between soil water changes and shoot responses (Davies and Zhang 1991). Therefore, stomata appear to be able to receive information on the soil water status independent of the leaf water potential. Evidence that the nature of this information is chemical was obtained by analyzing the xylem sap from unwatered plants, indicating the involvement of ion content, pH, amino acids and hormones (Schurr and Gollan 1990). Since the velocity of a chemical substance in the phloem is relatively slow and typically proved to be 50–100 cm h^{-1} (Canny 1975), the open question was how is the leaf capable of responding rapidly to the changing water status of the soil. Evidence of electrical root-to-shoot signaling was obtained by both, extra- and intracellular potential measurements on 80 cm tall maize plants. They were subjected to a drying cycle of 5 days showing a decrease in CO_2 uptake and transpiration rate while the electrical potential difference between two surface points showed a daily rhythm which seemed to be correlated with the soil water status (Fromm and Fei 1998). After soil drying the plants were watered and increases in CO_2 and H_2O exchange were demonstrated to follow the arrival of an action potential in the leaves. Experiments with dye solution showed that the increase in gas exchange could not be triggered by water ascent. In addition, the use of aphid stylets as “bioelectrodes” showed that sieve tubes served as a pathway for electrical signaling. The membrane potential of the sieve tubes responded rapidly upon watering the dried plants as well as after inducing spontaneous water stress to the roots by polyethylene glycol (Fromm and Fei 1998). Results therefore suggest that electrical root to shoot communication plays an essential role in the co-ordination of processes between roots and leaves, especially via long pathways.

12.7 Role of electrical signaling during fertilization

Strong evidence also exists that electrical signals evoke specific responses of the ovary during the processes of pollination and fertilization. As regards pollination, two different kinds of electric potential changes were measured in the style of flowers. First, Sinyukhin and Britikov (1967) recorded an action potential in the style of *Lilium martagon* and *Incarvillea grandiflora* a few minutes after placing pollen on the stigma lobes. Furthermore, an action potential was detected after mere mechanical irritation of the *Incarvillea* lobe, causing closure of the stigma lobes without further transmission. In both species the pollen-induced action potentials propagated towards the ovary to stimulate the oxygen consumption by 5–11%, 60–90 s after arrival of the action potential. At this moment, most likely post-pollination effects begin, such as the induction of ovary enlargement and wilting of the corolla, which occur long before fertilization. Second, electrical potential changes were measured in the style of *Lilium longiflorum* flowers 5–6 h after pollination (Spanjers 1981). No signals were detectable when applying killed pollen or pollen of other species. In *Hibiscus rosa-sinensis*, different stimuli applied to the stigma of flowers evoke specific electrical signals that propagate toward the ovary at speeds of 1.3–3.5 cm s^{-1} (Fromm et al. 1995). To investigate the first reactions of the ovarian metabolism, various metabolites were analysed 10 min after stimulating the stigma by pollen, wounding or cold-shock. Self- as well as cross-pollination hyperpolarized the resting potential of style cells 50–100 s later, followed by a series of 10–15 action potentials. At 3–5 min after pollination, the ovarian respiration rate increased transiently by 12%, with the levels of ATP, ADP and starch rising significantly (Fromm et al. 1995). By contrast, cold-shock of the stigma caused a single action potential, whereas wounding generated a strong depolarization of the membrane potential with an irregular form and at a lower transmission rate. Either treatment caused a spontaneous decrease in the ovarian respiration rate, as well as reduced metabolite concentrations in the ovary. Since there was no evidence that a chemical substance had been transported within 10 min over a distance of 8–10 cm from the stigma to the ovary, the metabolism must have responded to the electrical signals (Fromm et al. 1995). In the light of these results, the question arises how does an electrical signal cause the biochemical response. Most likely the latter may be achieved through subcellular changes of K^+, Cl^-, and Ca^{2+} ions which are responsible for the generation of action potentials. According to Davies (1987) local changes in ion concentration can lead to modified activities of enzymes in the cell wall, the plasmalemma, and the cytoplasm. This kind of mechanism may also be involved in the fluctuation of the starch level of the ovary after stigma stimulation. The biochemical regulation of starch synthesis is centered almost exclusively on ADP-Glc-pyrophosphorylase (Preiss et al. 1985). The characteristics of this enzyme in ovaries will therefore be analyzed in future to gain a better understanding of the biochemical role of electrical signaling during fertilization.

12.8 Long-distance electrical signaling in woody plants

In trees in particular, communication over long distances may be achieved through phloem-transmitted electrical signals. Bridging long distances between different organs, these rapid signals possess the ability to coordinate physiological activities. Due to environmental changes, different electrical signals can be evoked in the symplast and transmitted to distant organs, with concomitant specific effects on various physiological processes.

12.8.1 Membrane potential, electrical signals and growth of willow roots

Since willow roots were shown to respond to hormones with propagating action potentials (Fromm and Eschrich 1993), it was an important challenge to measure the magnitude of the current that flows during action potentials. With the use of the vibrating probe technique it was possible to quantify the current, the sensitivity of the probe being in the range of $\mu A\ cm^{-2}$, i.e. sufficiently sensitive to measure ion fluxes of $pmol\ cm^{-2}\ s^{-1}$ (Fromm et al. 1997). Therefore, microelectrode recordings and vibrating probe measurements were used in tandem to correlate changes in membrane potential with changes in endogenous current. Transient depolarizations were elicited in root cortex cells by spermine, while abscisic acid caused a transient hyperpolarization. For the latter we assume that K^+ leaves the cortex cells, similar to the K^+ efflux measured in guard cells (Mansfield et al. 1990). All changes in membrane potential were accompanied by transient responses of the endogenous current. These responses suggested that first anions and then cations leave the root during spermine-induced depolarizations. From the changes in the endogenous current an apparent efflux of anions (presumably Cl^-) and cations (presumably K^+) of 200–700 $pmol\ cm^{-2}$ per signal was calculated (Fromm et al. 1997). Furthermore, it was possible to demonstrate the effect of the growth regulators spermine and abscisic acid on root growth. The mean growth rate of roots increased by up to 30% after application of spermine, while it almost came to a standstill after treatment with abscisic acid.

12.8.2 Electrical properties of wood-producing cells

In the course of the evolutionary process, plants found it necessary to develop wood in order to increase their mechanical strength so as to be able to reach tree heights of 100 m and more. Extensive literature exists that addresses wood anatomy, chemistry and physical properties. However, we have only just begun to form an understanding of the molecular and electrophysiological mechanisms of cambial activity and wood formation, a field now considered a main research area in tree physiology. One of the main model tree species for basic wood research is poplar. Because of its suitability for genetic transformation and its ease of vegetative propagation, poplar has become the commonly used model tree species in Europe and the United States. To give a description of the electrophysiological processes in wood formation biophysical and molecular techniques have been used to analyze K^+ transporters of poplar. K^+ transporters homologous to those of known function in *Arabidopsis* phloem and xylem physiology were isolated from a poplar wood EST library and the expression profile of three distinct K^+ channel types was analysed by quantitative RT-PCR (Langer et al. 2002). Thus, it was found that the *P. tremula* outward rectifying K^+ channel (PTORK) and the *P. tremula* K^+ channel 2 (PTK2) correlated with the seasonal wood production. Both K^+ channel genes are expressed in young poplar twigs, and while PTK2 was predominantly found in the phloem fraction, PTORK was detected in both phloem and xylem fractions. Following the heterologous expression in *Xenopus* oocytes the biophysical properties of the different channels were determined. PTORK, upon membrane depolarization mediates potassium release, while PTK2 is almost voltage-independent, carrying inward K^+ flux at hyperpolarized potential and K^+ release upon depolarization (Langer et al. 2002). In addition, in-vivo patch-clamp studies were performed on isolated protoplasts from PTORK and PTK2 expressing suspension cultures. Poplar branches were therefore induced to build callus and the resulting meristematic tissues were used to generate suspension cultures. Protoplasts were isolated and the plasma membrane potassium conductances were compared with the electrical properties of *Xenopus* oocytes expressing PTORK and PTK2 individually. Concerning PTORK, it was shown that the properties of this channel are similar in both experimental systems and also to other plant depolarization-activated K^+ release channels (Gaymard et al. 1998; Ache et al. 2000; Langer et al. 2002). In coincidence with the activity of the K^+ channels a plasma membrane H^+-ATPase, generating the necessary H^+ gradient (protonmotive force) for the uptake of K^+ into xylem cells, was localized in the poplar stem using specific antibodies (Arend et al. 2002, 2004). Since potassium is the most abundant cation in plants, playing a central role in many aspects of plant physiology, we conclude that K^+ channels are involved in the regulation of K^+-dependent wood formation. Since seasonal changes in cambial potassium content correlate strongly with the osmotic potential of the cambial zone (Wind et al. 2004), potassium may well play a key role in the regulation of wood formation due to its strong impact on osmoregulation in expanding cambial cells. On the other hand, since PTORK appears in the plasma membrane of sieve elements of the phloem as well as in xylem rays, this channel may play a role in the generation of electrical signals within the poplar phloem and xylem. However, the potential role of xylem ray cells in radial transmission of electrical signals within the tree stem will have to be proved in the future.

12.8.3 Role of electrical signaling in the regulation of photosynthesis

Most of the work on functions for electrical signals in plants focused on responses evoked by heating and evidence exists of their role in transcription, translation and respiration (Stankovic and Davies 1997; Davies 2004). Recently, evidence was found of a link between electrical signaling and photosynthetic response in *Mimosa* (Koziolek et al. 2004). Flaming of a leaf pinna evoked a variation potential that travels at a speed of 4–8 mm s^{-1} into the neighboring pinna of the leaf to transiently reduce the net CO_2 uptake rate. Simultaneously, the PSII quantum yield of electron transport is reduced. Two-dimensional imaging analysis of the chlorophyll fluorescence signal showed that the yield reduction spreads acropetally through the pinna and via the veins through the leaflets. The results provide evidence of the role of electrical signals in the regulation of photosynthesis because the high speed of the signals rules out the involvement of a slow-moving chemical signal. In addition to the photosynthetic response, it was shown that wounding causes lateral chloroplast movement within 10 min after wounding in *Elodea canadensis* (Gamalei et al. 1994). The time course of chloroplast movement coincides with rapid changes in the membrane potential with low amplitudes (humming, 4–7 mV), recorded by microelectrodes impaled into the midrib of the attached leaf.

With regard to trees, hormone-induced action potentials in the roots were shown to propagate throughout willow plants at velocities of 2–5 cm s^{-1} in order to affect the gas exchange of the leaves (Fromm and Eschrich 1993). To gain a deeper understanding of the role of electrical signaling in the photosynthesis of trees, poplar shoots were stimulated by flaming. In this species, depolarizing signals travel over long distances across the stem from heat-wounded leaves to adjacent leaves where the net CO_2 uptake rate is temporarily depressed towards compensation (Lautner et al. 2005). Surprisingly, signals induced by cold-shock did not affect photosynthesis. In coincidence with the results on *Mimosa,* electrical signaling also significantly reduced the quantum yield of electron transport through PSII in poplar. Cold-blocking of the stem proved that the electrical signal transmission via the phloem becomes disrupted, causing the leaf gas exchange to remain unaffected. Furthermore, calcium-deficient trees showed a marked contrast inasmuch as the amplitude of the electrical signal was distinctly reduced, concomitant with the absence of a significant response in leaf gas exchange upon flame-wounding (Lautner et al. 2005). Further research has to be done on the responsiveness of the various types of molecules that are involved in electron transport as well as on enzymes involved in the uptake of CO_2 during electrical signaling.

12.9 Conclusions

So far, we have seen glimpses of a complex electrical long-distance signaling system in plants and obtained evidence of the role played by electrical signals in the daily processes of plant life. Obviously, plants have developed a simple neural network which responds to a variety of environmental stimuli which may be both, abiotic as well as biotic. Due to the impulses generated by environmental changes action and variation potentials serve as information carriers. The primary step in signal perception may be the opening of plasmalemmal calcium or chloride channels, leading to ion fluxes which generate action or variation potentials. Astounding similarities exist between action potentials in plants and animals. The generation of action potentials in plants follows the all-or-nothing law too (Shiina and Tazawa 1986; Fromm and Spanswick 1993), and plant action potentials also show refractory periods. Furthermore, the use of new methods provides opportunities for detection of fast action potentials which reach speeds up to 40 m s^{-1} (Volkov and Mwesigwa 2000), i.e. similar to the velocities of action potentials in nerves. In higher plants, electrical signals are transmitted from cell to cell via plasmodesmata over short distances, while propagation over long-distances along the plasma membrane of sieve tubes occurs through the successive opening and closure of ion channels (Fig. 12.1). Calcium, chloride and potassium channels are involved in the generation of action potentials and several ion channels postulated to be involved in electrical transmission were identified in the phloem and xylem (Ache et al. 2001; Langer et al. 2002).

Concerning the physiological functions of electrical signals, numerous examples exist. Apart from the role of action and variation potentials in carnivorous plants and *Mimosa*, a concrete relationship between electrical stimulation and the increased production of proteinase inhibitors was found to exist in tomato (Stankovic and Davies 1997). Other work showed that action potentials regulate respiration (Dziubinska et al. 1989), phloem transport (Fromm and Bauer 1994), fertilization (Sinyukhin and Britikov 1967; Fromm et al. 1995) and photosynthesis (Koziolek et al. 2004; Lautner et al. 2005). It is to be expected that future improvements in investigation methods will reveal more aspects of the signaling complexity and its physiological responses that are as yet not fully understood.

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