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Electrical signals and their physiological significance in plants

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ABSTRACT

Electrical excitability and signalling, frequently associated with rapid responses to environmental stimuli, are well known in some algae and higher plants. The presence of electrical signals, such as action potentials (AP), in both animal and plant cells suggested that plant cells, too, make use of ion channels to transmit information over long distances. In the light of rapid progress in plant biology during the past decade, the assumption that electrical signals do not only trigger rapid leaf movements in 'sensitive' plants such as *Mimosa pudica* or *Dionaea muscipula*, but also physiological processes in ordinary plants proved to be correct. Summarizing recent progress in the field of electrical signalling in plants, the present review will focus on the generation and propagation of various electrical signals, their ways of transmission within the plant body and various physiological effects.

Key-words: action potential, aphid technique, chlorophyll fluorescence, gas exchange, phloem, signal transmission, variation potential.

Abbreviations: AP, action potential; $\Delta F/F'_m$, photosystem II (PSII) electron quantum yield; g_{H2O}, stomatal conductance; J_{CO2}, assimilation rate; pS, picosiemens; VP, variation potential.

INTRODUCTION

Initiated by a correspondence with Charles Darwin which included some carnivorous venus flytrap plants, Burdon-Sanderson (1873) was the first to discover APs in plants following stimulation of a *Dionaea* leaf. Hence, electrical signals do not belong exclusively to animal kingdom. In 1926, Bose used isolated vascular bundles of a fern to show that excitation was transmitted as an electric disturbance that appeared to be controlled by similar physiological events as in animal nerves. In 1930, APs were recorded with inserted microelectrodes in *Nitella* cells (Umrath 1930), earlier than the first intracellular recording of an AP in animal cells (Nastuk & Hodgkin 1950; Tasaki 1952). In the 1950s, Sibaoka was also able to demonstrate that the propagation of electrical signals in *Mimosa pudica* showed characteristics similar to APs in nerves. Comprehensive reviews on APs in plants have then been published by Sibaoka (1966, 1969) and Pickard (1973), indicating that all higher plants may be utilizing electrical signals to regulate a variety of physiological functions. In 1984, ion channels, the basis for APs, were discovered in plants, too (Schroeder, Hedrich & Ferandez 1984). Since then, one of the important questions has been whether excitability in animals and plants is based on a similar set of ion channels. Recently, most of the chemistry of the neuromotoric system of animals has been found in plants, for example, neurotransmitters such as acetylcholine, and cellular messengers and cellular motors such as calmodulin and actin, respectively (Baluska *et al*. 2006; Murch 2006). Although this nerve-like cellular equipment never develops the same degree of complexity as in animal nerves, a simple neural network is formed, especially within phloem cells, which is responsible for the communication over long distances. The reason why plants have developed pathways for electrical signal transmission is most probably the necessity to respond rapidly to external stimuli, for example, environmental stress factors. It has been shown recently that different environmental stimuli evoke specific responses in living cells that are capable of transmitting an electrical signal to the responding region (Lautner *et al*. 2005). In contrast to chemical signals such as hormones, electrical signals are able to rapidly transmit information over long distances. Because numerous physiological effects of electrical signalling have been discovered in the past two decades (Fromm 2006; Fromm & Lautner 2006; Trebacz, Dziubinska & Krol 2006), this kind of rapid transmission between living cells may also prove to be essential to plants.

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TECHNIQUES FOR MEASURING ELECTRICAL SIGNALS IN PLANTS

In general, two different methods are being used to measure electric potentials in plants, viz. extracellular and intracellular recording. Extracellular potential measurements on the surface of higher plants have been widely performed in the past, and offer the advantage of being able to detect electrical potential differences over long periods of time (several days). By contrast, intracellular measurements with penetrating glass microelectrodes are only effective for short periods of time such as 1–2 h, because some of the electrolyte within the electrode usually diffuses into the cell to be measured and changes its original bioelectric condition. However, intracellular recording has the

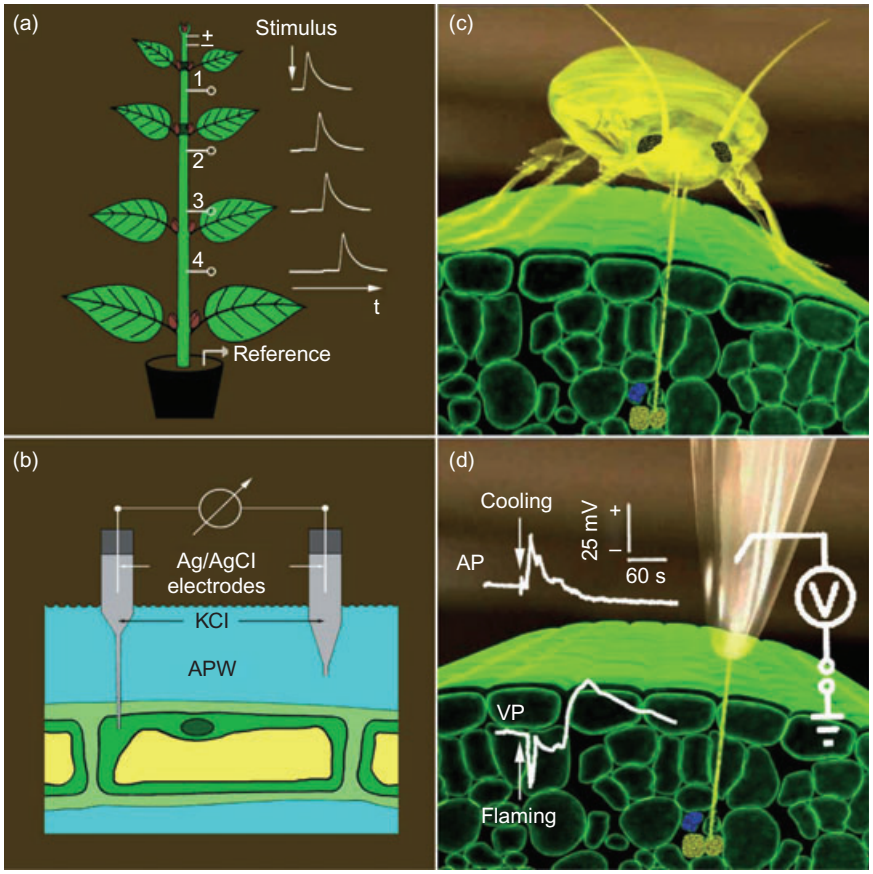

Figure 1. Techniques for measuring electrical signals in plants. (a) Extracellular recording with four channels and a reference electrode inserted in the soil. ±, electrical stimulation. An AP (right) generated by electrical stimulation appeared successively at electrodes 1, 2, 3 and 4. (b) Intracellular measurement of the membrane potential with a microelectrode inserted into the cytoplasm of an algal cell while the reference electrode is in contact with the artificial pond water (APW) outside the cell. Both electrodes are filled with KCl, clamped in Ag/AgCl pellet holders and connected to an electrometer. (c) Phloem potential measurements; an aphid in feeding position with its stylet inserted into a sieve element on the upper side of a leaf. (d) After the aphid is separated from its stylet by a laser pulse, the stylet stump exuded sieve tube sap to which the tip of a microelectrode was attached. Cooling the shoot evoked an AP transmitted acropetally within the phloem, while flaming of a leaf generated a VP with different form and of long duration. t, time.

advantage of being more precise because membrane potentials and electrical signals may be deduced from specific cells.

Extracellular recording

Extracellular measurements are well known from animal electrophysiology and are based upon measurements of the sum total of bioelectrical activity in large groups of cells. For example, electrocardiograms (ECG) and electroencephalograms (EEG) are widely used in medical practice. In higher plants, two types of measurements of extracellular potentials can be performed, surface recordings and measurements using inserted metal electrodes. The latter method causes wound reactions when inserting the electrode, and therefore the electrodes have to consist of thin metal wires (e.g. Ag/AgCl-wires 0.4–1.0 mm in diameter). Upon insertion into the shoot or leaf veins, the electrodes come in contact with tissue embodying larger groups of cells. For instance, such recordings are made in the cambial region of various tree species – showing daily and yearly rhythms as well as 5 min oscillations related to cambial growth (Fensom 1963), or in soybean plants that transmit electrochemical signals induced by blue light (Volkov 2006). On the other hand, surface measurements appear better suited as they are non-invasive and physically stable; they may also be performed simultaneously with other physiological methods such as gas exchange recordings (Fromm & Fei 1998). Such electrodes usually consist of Ag/AgCl wire, moistened with 0.1% (w/v) KCl in agar and wrapped in cotton to provide the appropriate contact with the plant surface (Fromm & Spanswick 1993), or of Ag/AgCl pelleted electrodes that can be connected to the plant surface by means of a conductive aqueous gel of the type commonly used in ECG (Mancuso 1999). At different positions of a plant surface, electrodes can be connected by screened cables to a high-input impedance electrometer with many channels. An identical electrode can either be placed on the distal region of a plant or in the soil to serve as a reference electrode (Fig. 1a). When all channels show stabilized potentials, the plant can be stimulated electrically at the apex (e.g. 3 V for 2 s) or by other stimuli (flaming, cold shock) applied to a leaf. Usually, the electrical responses to an apical stimulus can be shown by all electrodes, from top to bottom of the plant (Fig. 1a), indicating that the transmission of an electrical signal is occurring throughout the plant. For example, a similar experimental set-up has been used in sunflower to analyse the characteristics of APs and VPs (Stankovic *et al*. 1998).

Intracellular recording

The intracellular measurement of membrane potentials and electrical signals is usually carried out with glass

microelectrodes with tip diameters of less than 1 μm. These electrodes are filled with KCl, clamped in Ag/AgCl pellet holders and connected to a high-input impedance amplifier. After the amplifier has been zeroed with both electrodes outside the cell, one microelectrode is carefully inserted into the cytoplasm (or the vacuole) of a cell using micromanipulators, while the reference electrode is in contact with the solution surrounding the cell (Fig. 1b). When the microelectrode pops through the cell membrane, the amplifier reports the negative change of the recorded potential. This is the resting membrane potential, usually with values between –80 and –200 mV in plant cells.

Because phloem cells facilitate long-distance electrical signalling due to relatively low resistance connections (sieve pores), intracellular recordings of intact sieve elements are important to the detection of signals with high velocities. However, the phloem is located inside the plant body, which makes it difficult to insert microelectrodes. Recordings in combination with dyes injected into the cell after obtaining electrical signals is a time-consuming technique because often, the microelectrode tip is not properly inserted into the phloem, as revealed by microscopic checks after the experiment. However, making use of the 'aphid technique' allows the detection the membrane potential of sieve tubes and its changes after stimulating the plant (Fromm & Bauer 1994). To describe this method briefly: aphids are transferred to a mature leaf and allowed to settle overnight (Fig. 1c). On the following day, an aphid is severed from its stylet by laser pulse. The stylet stump exudes sieve tube sap to which the tip of a microelectrode is attached (Fig. 1d; Wright & Fisher 1981; Fromm & Eschrich 1988b). The successful use of stylets to detect electrical signals within the phloem depends on their function as an effective salt bridge between the cytoplasm and the microelectrode. Sieve tube exudates typically contain high K^+ levels; measurements on barley leaves gave values ranging from 50 to 110 mM (Fromm & Eschrich 1989). The dimensions of the stylet's food canal can be used to roughly calculate its electrical resistance. Taking an average area of 6 μm^2 and supposing the canal to be filled with 100 mM KCl, its resistance would be about 2.6×10.0^9 Ω (Wright & Fisher 1981). Although this value is about three times greater than the typical resistance of a microelectrode, it is still within the capacities of the electrometer used (input impedance > 10^{12} Ω). In addition, the stylet is embedded in hardened saliva, which provides electrical insulation. For example, in poplar, the microelectrode tip was brought into contact with the stylet stump at a leaf, attached to the shoot with its cut end sealed into saline solution to which the reference electrode was connected (Lautner *et al*. 2005). After successful connection of stylet and microelectrode tip, a resting potential of –140 mV was established. Cooling the lower part of the shoot with ice water evoked a rapidly moving AP transmitted acropetally within the phloem at a speed of 4–8 mm s^{-1} (Fig. 1d). By contrast, flaming evoked a more slowly moving signal (1–2 mm s^{-1}), also called VP, which is different in form and of long duration.

GENERATION OF ELECTRICAL SIGNALS

Perception of environmental stimuli

Environmental stimuli such as spontaneous changes in temperature, light, touch or wounding can induce electrical signals at any site of the symplastic continuum. With regard to APs, the plasma membrane is depolarized first, for example, by mechanical stimulation as observed in *Chara* (Kishimoto 1968). This process is known as formation of the receptor potential, which is an electrical replica of the stimulus lasting for the period of time that the stimulus is present. When the stimulus is sufficiently great to depolarize the membrane to below a certain threshold, an AP is generated. On the other hand, light stimuli, too, may induce APs. When the liverwort *Conocephalum conicum* is shaded, its thallus cells hyperpolarize, whereas they depolarize upon reillumination in a dose-dependent manner (Trebacz *et al*. 2006). Light-induced generator potentials, when strong enough, lead to the exceeding of a threshold and the generation of APs (Trebacz & Zawadzki 1985). Following perception, APs can be transmitted via plasmodesmata to other cells of the symplast.

With regard to wounding or flaming, generally so-called VPs are induced by a hydraulic wave transmitted through the xylem. The mechanism by which the hydraulic wave is able to cause a local electrical response in the neighbouring cells is not clear. Probably, the large influx of water into living cells can lead to a stretching of membranes and may therefore affect mechanosensitive ion channels (Cosgrove & Hedrich 1991) or, if a chemical is being transported, perhaps via ligand-activated channels (Malone 1996).

TYPES OF ELECTRICAL SIGNALS

AP

APs are rapidly propagated electrical messages that are well known in animals. They speed along the axons of the nervous system and over the surface of some muscle and glandular cells. In axons they are brief (in ms range), travel at constant velocity and maintain a constant amplitude (Hille 1992). They usually have an all-or-nothing character, that is, after a stimulus reaches a certain threshold (which leads to membrane depolarization), further increases in stimulus strength do not change its amplitude and shape. The response is an all-or-none depolarization that spreads passively from the excited region of membrane to the neighbouring non-excited region. Thus, APs propagate electrically, with depolarization being the stimulus for passive propagation. While the ionic mechanism of APs in animal axons depends on inward-flowing Na^+ (depolarization) and outward-flowing K^+ ions (repolarization), excitation of plant cells depends on Ca^{2+}, Cl^- and K^+ ions. The best studied plant AP is in the giant internodal cells of the green algae *Chara* and *Nitella*. Such cells have vigorous protoplasmic streaming that is arrested by APs propagating at a speed of 10–20 mm s^{-1}. Homann & Thiel (1994) found a 40 pS K^+ channel in *Chara* that may play a role in

conducting an outward current in the repolarization phase. Because voltage-dependent anion channels in plants require elevated cytoplasmic Ca^{2+} levels, the activation of Ca^{2+}-permeable channels is assumed to present an initial step within an AP (Hedrich & Becker 1994). Indeed, the increase in intracellular calcium has been demonstrated in response to various stimuli (Knight *et al*. 1991). In the Characeae internodal cells, Ca^{2+} entry activates a Cl^- channel and arrests cytoplasmic streaming (Lunevsky *et al*. 1983). Ca^{2+}-dependent anion channels were characterized in the *Chara* plasmalemma, showing unitary conductance of 9 (Okihara *et al*. 1991), 17 and 38 pS (Homann & Thiel 1994). Thorough investigations using the manganese quench technique revealed that Ca^{2+} is predominantly released from internal stores (possibly the endoplasmic reticulum) during an AP in *Chara* (Plieth *et al*. 1999). In combination, Ca^{2+}, Cl^- and K^+ channels allow plant cells to respond to changing environmental conditions by changes in their electrical activity. These ion shifts during an AP are confirmed in trees by energy-dispersive X-ray analysis and inhibitors of ion channels (Fromm & Spanswick 1993). Using a vibrating probe in combination with a microelectrode, an apparent efflux of anions and cations of 200–700 pmol cm^{-2} per AP was calculated in willow (Fromm, Meyer & Weisenseel 1997). The information within the transmitted signal may be encoded by the shape of a single AP determined by the relative contribution of the ionic conductances at rest or by the frequency of numerous signals (firing).

A classic example of the conductance of APs in higher plants is the sensitive plant, *M. pudica*, where the propagated folding of leaflets is associated with the transmission of an AP. Rapid cooling of the apical end of a leaf pinna evokes a rapidly moving AP with a duration of 5 s transmitted basipetally within the rhachis at a rate of up to 20–30 mm s^{-1} (Fig. 2a). This is similar to the speed of an AP in the nerves of *Anodonta* (45 mm s^{-1}), but much slower than in *Octopus* (3 m s^{-1}) and mammalian nerves (up to 100 m s^{-1}, Lüttge, Kluge & Bauer 2005). The *Mimosa* AP becomes immediately evident as the bending tertiary pulvini cause impressive movements of the paired leaflets. When an AP reaches a pulvinus, it is transmitted laterally via plasmodesmata into the cells of the motor cortex. They respond to the signal by ion efflux associated with water efflux, which leads to leaf movements (Fromm & Eschrich 1988b,c; Fromm 1991). In addition, in *Mimosa*, the depolarizing phase is accompanied by a membrane conductance increase and the appearance of Cl^- ions in the extracellular medium, and the signal height decreases when Cl^- ions are added to the medium (Samejima & Sibaoka 1982). Interestingly, the transmission of an AP induced by touch or cold shock stops at the base of the single pinna and no further transmission occurs, leaving leaflets from neighbouring pinna unfolded. Concerning refractory periods, that is, the period following an AP when the cells are not excitable, they were much longer in plants than in animal systems. Absolute refractory periods last 0.0005 s in mammalian nerves, but 4–40 s in the Characeae and 2–4 min in *Conocephalum*, while relative refractory periods are 0.001–0.01 s in mammalian nerves, 60–150 s in the Characeae and 6–8 min in *Conocephalum* (Dziubinska *et al*. 1983, Lüttge *et al*. 2005).

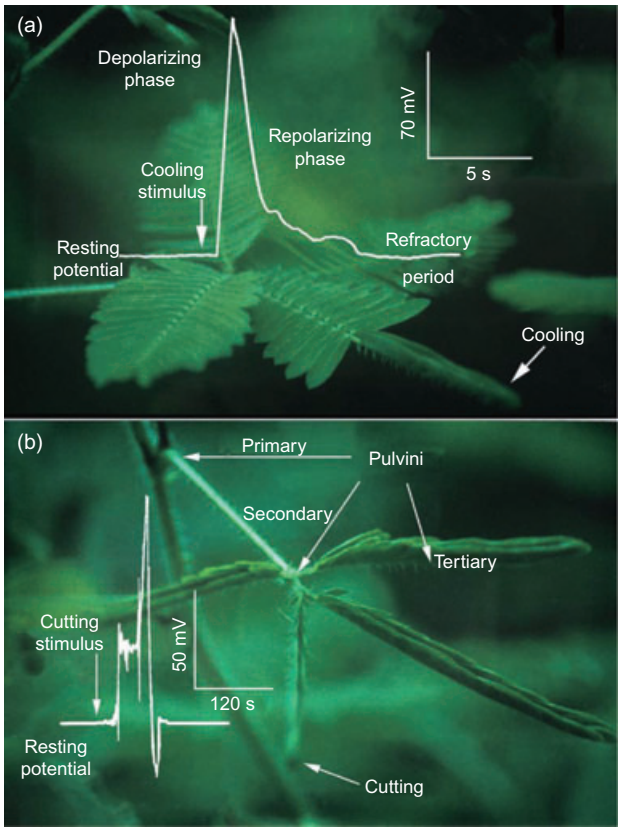

Figure 2. Electrical signalling in *Mimosa pudica*. (a) When the tip of a leaf pinna is stimulated by spontaneous cooling with ice water or mechanically by touch, an AP is evoked and transmitted basipetally within the rhachis with a speed of 20–30 mm s^{-1}. The tertiary pulvini at the base of the leaflets respond to the AP, causing ion and water fluxes that lead to leaf movements. This type of signal stops at the base of the pinna, and no further transmission occurrs. (b) When the leaf is stimulated by cutting, a basipetally moving VP is generated in the rhachis, irregular in shape and long in duration. Its speed is slower (5–6 mm s^{-1}) than that of the AP; however, it is able to pass through secondary pulvini at the base of the pinna and causes leaflet movements of neighbouring pinna, and also bending of the primary pulvinus at the base of the petiolus.

VP

VPs or slow wave potentials are propagating electrical signals which also consist of a transient change in membrane potential (depolarization and subsequent repolarization). The main difference to APs lies in longer, delayed repolarizations and a large range of variation. This signal varies with the intensity of the stimulus, is non-self-perpetuating and appears to be a local change to either a hydraulic pressure wave or chemicals transmitted in the dead xylem. It can be generated by wounding, organ

excision or flaming, and was studied in numerous plant species such as cucumber and pea seedlings (Stahlberg & Cosgrove 1992, 1994), or in woody species, for example, *Vitis vinifera* (Mancuso 1999). The VP is characterized by amplitudes and speeds that decrease with increasing distance from the injured site, by its ability to pass dead regions of tissue, and its dependence on xylem tension. At saturating humidity, when xylem tension becomes negligible, VPs will not be generated. VPs are also called slow wave potentials by some authors (Stahlberg, Cleland & Van Volkenburgh 2006) because of their slow repolarization phase. Their ionic mechanism differs from that underlying APs; it is thought to involve a transient shutdown of a P-type H^+ ATPase in the plasma membrane (Stahlberg *et al*. 2006). In *M. pudica*, cutting the tip of a leaf pinna generated a VP in the rhachis, which was of irregular form and longer duration than an AP (Fig. 2b). In contrast to the AP, the VP passes through the secondary pulvinus at the base of a pinna and moves into the three neighbouring pinna to cause the folding of all leaflet pairs. Simultaneously, it propagates basipetally through the petiolus, causes a bending movement of the primary pulvinus and of pulvini from distant leaves (Fig. 2b). To conclude, in *Mimosa*, a VP can pass through secondary and primary pulvini to cause leaf movements in the entire plant, whereas the transmission of an AP is restricted to a short distance as in a single leaf pinna (Fig. 2a).

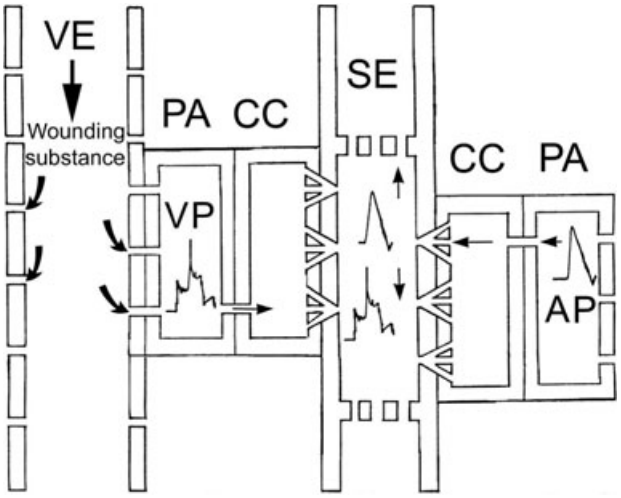

Figure 3. Electrical communication over long distances. An AP (right) can propagate over short distances through plasmodesmata, and after it has reached the sieve element/companion cell (SE/CC) complex, it can travel over long distances along the SE plasma membrane in both directions. In contrast, a VP is generated at the plasma membrane of parenchyma cells (PAs) adjacent to xylem vessels (VEs) by an hydraulic wave or a wounding substance. Because VPs were measured in SEs (Lautner *et al*. 2005), it is suggested that they also can pass through the plasmodesmal network and can reach the phloem pathway. However, in contrast to APs, their amplitude will be reduced with increasing distance from the site of generation.

MEANS OF SIGNAL TRANSMISSION

After an electrical signal has been generated in the symplast, it can be transmitted via plasmodesmata to all the other symplasmic cells (Van Bel & Ehlers 2004). Evidence of electrical coupling of cells was demonstrated as early as 1967 by Spanswick and Costerton who injected a current into a *Nitella* cell and managed to trace it several cells away from the injected cell. In addition, in *Elodea* leaves and *Avena* coleoptiles, electrical coupling was shown (Spanswick 1972), indicating that plasmodesmata are relays in a signalling network at the local level. If the information has to be transmitted to distant parts of the plant, electrical signalling via the phloem appears to be used (Fig. 3, sieve element). The phloem extends continuously throughout the entire plant, and sieve elements may be considered low-resistance pathways for AP transmission. Because of the relatively large, unoccluded sieve plate pores and the continuity of the plasma membrane and endoplasmic reticulum, sieve elements appear suitable for electrical signalling over long distances. Moreover, in numerous species, the vascular bundles are surrounded by a sclerenchyma sheath in order to restrict electrical signalling to the phloem. For example, in the petiolus of *Mimosa*, the phloem is surrounded by such a barrier (Fromm 2006). The low degree of electrical coupling in lateral direction caused by only few plasmodesmata at the interface between companion cells (CC) and phloem parenchyma cells (PAs) (Kempers, Ammerlaan & van Bel 1998) also facilitates long-distance signalling. However, the plasmodesmata may open up following stimulation and may make way for APs to propagate laterally from neighbouring cells into the SEs/CCs (Fig. 3, right). The transmission of electrical signals along sieve tubes is achieved by ion channels in their plasma membranes, and some channels have been identified, mainly K^+ channels. The membrane potential of the sieve tubes has been shown to be dominated by K^+ conductance (Ache *et al*. 2001), while corresponding AKT2/3-like channels expressed in the phloem have been identified in *Arabidopsis*, maize and broad bean (Marten *et al*. 1999; Bauer *et al*. 2000; Deeken *et al*. 2000; Lacombe *et al*. 2000). In addition, Ca^{2+} channels have been localized in the phloem of leaf veins from *Nicotiana* and *Pistia* (Volk & Franceschi 2000), indicating that sieve elements are enriched with ion channels which may be involved in long-distance electrical signalling. By using severed aphid stylets, electrical signals have been measured in sieve tubes of *M. pudica* (Fromm & Eschrich 1988b), *Zea mays* (Fromm & Bauer 1994) and *Populus trichocarpa* (Lautner *et al*. 2005) over distances of up to 20 cm.

The large propagating depolarizations of VPs are generated by a rapid loss of tension in the xylem vessels (VEs) after wounding. This hydraulic wave is transduced into local changes in ion flux through mechanosensory channels in the adjacent living cells (Stankovic *et al*. 1998; Davies & Stankovic 2006). After being generated in these cells, the VP can move laterally, via plasmodesmata, into the sieve elements from where it can be transmitted over long distances

(Fig. 3). Alternatively, some wounding substance can also be transported in the xylem via the hydraulic shift and can evoke a VP via ligand-activated channels (Fig. 3, left). To summarize, the transmission of electrical signals within the plant depends on the electrical conductance of plasmodesmata in lateral direction and on the high degree of electrical coupling via the sieve pores in longitudinal direction.

PHYSIOLOGICAL EFFECTS OF ELECTRICAL SIGNALS

Apart from the different induction mechanisms, both AP and VP are capable of informing distant cells about local stimuli, causing them to act appropriately. Numerous functions of electrical signalling exist in plants (Table 1); insectivorous plants are well-known examples of the occurrence of short-distance signalling. For instance, *Drosera* and *Dionaea*, which live in nitrogen-depleted areas, use APs within specialized leaf traps to catch insects in order to secure their nitrogen supply (Sibaoka 1969; Williams & Pickard 1972a,b). In *Dionaea*, catching starts with the release of calcium into the cytosol of the sensor cells (Hodick & Sievers 1988), induced by mechanical pressure of one of the trigger hairs. Subsequently, an AP is generated without any response of the trap. If any of the trigger hairs is bent no later than 40 s after the first, a second fast AP ($20\ cm\ s^{-1}$) is evoked to close the trap. After closure, enzymes are exuded to digest the prey. The process of two APs serves to protect the plant against accidental stimulation, but it remains an open question why the trap, having a kind of memory, only responds to the second AP. Another well-documented implication of short-distance electrical signalling is an increase in respiration following cutting as well as electrical stimulation of the thallus of *C. conicum* (Dziubinska, Trebacz & Zawadzki 1989; Trebacz *et al*. 2006). Changes in the respiration rate concomitant with the transmission of electrical signals are also reported for *Vicia faba* seedlings (Filek & Koscielniak 1997) and after the pollination of flowers (Sinyukhin & Britikov 1967, Fromm, Hajirezaei & Wilke 1995). In *Hibiscus* flowers, self-pollination as well as cross pollination induce a series of 10–15 APs (firing) which cause a transient increase in the ovarian respiration rate, indicating that its metabolism is prepared for fertilization. By contrast, cold shock of the stigma evokes a single AP of different duration, whereas wounding causes a strong depolarization of irregular form. Both treatments cause a spontaneous decrease in the ovarian respiration rate and reduce metabolite concentrations (Fromm *et al*. 1995).

Apart from short-distance signalling, long-distance transmission via the phloem pathway is a well-known mechanism in many plants. In maize, APs generated by re-watering plants in drying soil cause increases in the CO_2 and H_2O gas exchange of the leaves (Fromm & Fei 1998), whereas APs triggered by cold shock of leaf tips cause a reduction in phloem transport of distant leaf parts (Fromm & Bauer 1994). In *Mimosa*, both AP and VP cause the leaflets to fold together (Fig. 2), making the leaf look dead and unappealing to a would-be herbivore. Apart from the role of electrical signals in the regulation of leaf movements, it has been demonstrated that electrical signals affect photosynthesis in *Mimosa* (Koziolek *et al*. 2004). Flaming of a leaf pinna evokes a VP that travels rapidly into the neighbouring pinna to eliminate the net CO_2 uptake rate and reduce the quantum yield of electron transport through photosystem II (PSII). In coincidence with *Mimosa*, further

Table 1. Well-documented physiological effects of electrical signals in plants

Stimulus	Signal	Plant	Physiological effect	Reference(s)
Mechanical	AP	*Dionaea*	Trap closure Release of digestive enzymes	Sibaoka 1969
Mechanical	AP	*Drosera*	Tentacle movement to wrap around the insect	Williams & Pickard 1972a,b
Cold shock, mechanical	AP	*Mimosa*	Regulation of leaf movement	Fromm & Eschrich 1988a,b,c; Sibaoka 1966, 1969
Electrical	AP	*Chara*	Cessation of cytoplasmic streaming	Hayama, Shimmen & Tazawa 1979
Electrical	AP	*Conocephalum*	Increase in respiration	Dziubinska *et al*. 1989
Pollination	AP	*Incarvilea, Hibiscus*	Increase in respiration	Sinyukhin & Britikov 1967; Fromm Hajirezaei & Wilke 1995
Re-irrigation	AP	*Zea*	Increase in gas exchange	Fromm & Fei 1998
Cold shock	AP	*Zea*	Reduction in phloem transport	Fromm & Bauer 1994
Electrical, cooling	AP	*Luffa*	Decrease of elongation growth of the stem	Shiina & Tazawa 1986
Electrical	AP	*Lycopersicon*	Induction of *pin2* gene expression	Stankovic & Davies 1996
Heating	VP			
Heating	VP	*Vicia*	Increase in respiration	Filek & Koscielniak 1997
Heating	VP	*Solanum*	Induction of jasmonic acid biosynthesis and *pin2* gene expression	Fisahn *et al*. 2004
Wounding	VP	*Pisum*	Inhibition of protein synthesis, formation of polysomes	Davies, Ramaiah & Abe 1986; Davies & Stankovic 2006
Heating	VP	*Mimosa, Populus*	Transient reduction of photosynthesis	Koziolek *et al*. 2004; Lautner *et al*. 2005

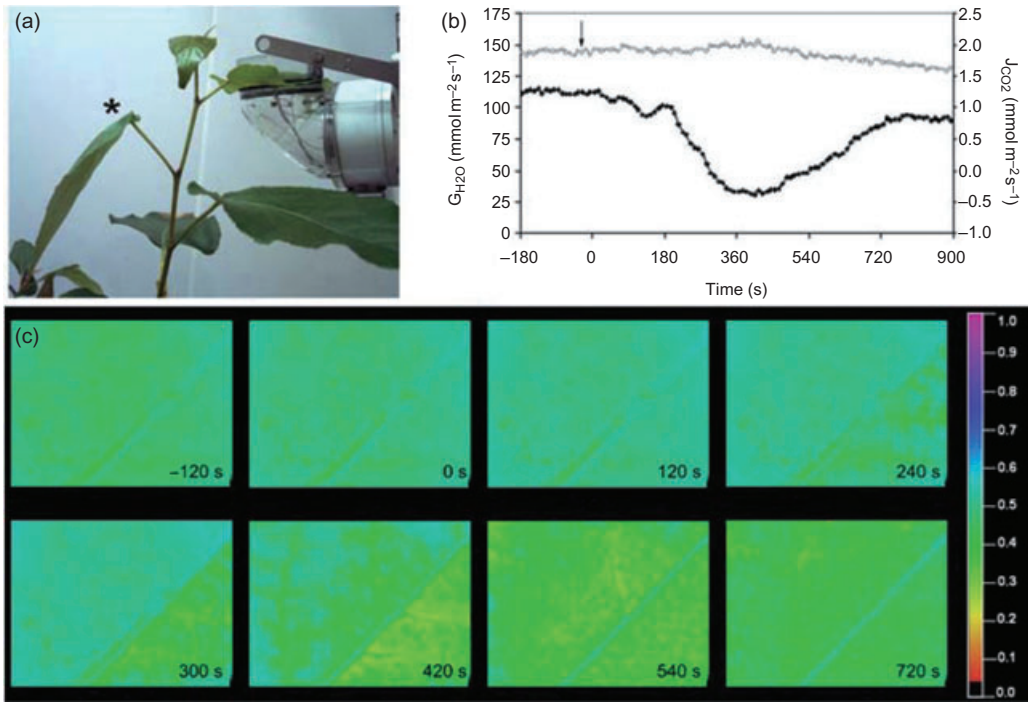

Figure 4. Photosynthetic response of electrical signalling in poplar. (a) Experimental arrangement of gas exchange recordings. The plant was heat stimulated for 3 s by the flame of a lighter at the base of a mature leaf (*) to evoke a VP (see Fig. 1d). (b) Typical response of J_{CO2} and g_{H2O} of the opposite, right leaf at a distance of 15 cm upon flaming of the left leaf. The arrow denotes the instant of injury. At 180 s after stimulation, the net CO_2 uptake rate (black graph) decreased immediately and then recovered almost completely after 900 s, while the g_{H2O} (grey graph) remained stable. (c) Spatio-temporal changes of $\Delta F/F'_m$ assessed by chlorophyll fluorescence imaging. The image area (length, 22 mm; width, 17 mm) covers the center of a leaf while the opposite leaf was stimulated by flaming (*) at a distance of 15 cm similar to the set-up shown in (a). Times are given in relation to the instant of injury (at time 0). Changes in $\Delta F/F'_m$ took 240 s to become apparent. A false colour shift from blue to yellow in the intervein area, equivalent to a reduction of $\Delta F/F'_m$ from 0.6 to about 0.2, indicates the decrease of photosynthesis. The bar translates the false colour code into values of $\Delta F/F'_m$.

research on poplar trees shows that the flaming of a leaf also evokes electrical signals that travel across the shoot to adjacent leaves where the net CO_2 uptake rate as well as the quantum yield of electron transport through PSII are temporarily reduced (Fig. 4, Lautner *et al*. 2005). Recently, evidence has been found in *Chara corallina* that the electrical signals arising at the plasmalemma are transmitted to the thylakoid membranes and that fluorescence changes derive from the increase in pH gradient at the thylakoid membrane (Bulychev & Kamzolkina 2006). Further research needs to be done on the responsiveness of molecules involved in electron transport and CO_2 uptake during electrical signalling. Clearly, the involved ion fluxes or/and the amplitude and duration of the electrical signal play a key role in the generation of the photosynthetic response. For example, upon flaming, calcium-deficient plants show reduced electrical signal amplitudes and no response in leaf gas exchange (Lautner *et al*. 2005).

Furthermore, numerous other physiological consequences of plant excitation have been reported by various groups (Table 1). In *Luffa cylindrica*, elongation growth of the stem decreases after the generation of a single AP (Shiina & Tazawa 1986). In *Arabidopsis*, changes in gene expression are seen within minutes after touching, and recent research is revealing the types of genes up-regulated by touch and implicate Ca^{2+} signalling, cell wall modification and disease resistance as potential downstream responses (McCormack *et al*. 2006). Special interest has been paid to the regulation of the proteinase inhibitor gene (*pin2*) expression in tomato which responds to wounding by induction of proteinase inhibitor activity in distant parts of the plant. Wildon *et al*. (1992) provided first evidence that the systemic wound signal is a transmitted electrical signal, while Stankovic & Davies (1996) showed that both, AP as well as VP, induce *pin2* gene expression. In addition to *pin2* gene expression, Fisahn *et al*. (2004) demonstrated that jasmonic acid biosynthesis, too, is induced by electrical signals in potato plants.

CONCLUSIONS AND OUTLOOK

To introduce the reader to the characteristics and physiological consequences of electrical signals in plants, we chose two examples for signals, AP and VP. Their features were discussed with respect to possible functions in plant physiology. Overall, the knowledge of electrical signalling in plants will help to unravel the nature of information exchange within plant cells and organs, and will give rise to new and fascinating questions. Future studies will be directed towards a better understanding of the electrical

signalling control mechanism, the interlink between ion fluxes and physiological responses, and the molecular identity of different channel types that participate in electrical signals.

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