

# Electrical Long-Distance Signaling in Plants

Matthias R. Zimmermann and Axel Mithöfer

**Abstract** In higher plants at least three different types of electrical long-distance signaling exist: action potential (AP), variation potential (VP), and system potential (SP), all of which have their own characteristics concerning their generation, duration, amplitude, velocity, and propagation. Whereas both AP and VP are due to a transient depolarization of the plasma membrane, the SP is based on hyperpolarization. For more than 100 years the AP is known and described for some specialized plants such as the Venus flytrap. Meanwhile, all three types of electrical signaling have been shown for many common plants, monocots as well as dicots, indicating that the capability to generate long-distance electrical signals is not the exception but a general physiological feature of plants. In spite of this, positive proofs for the involvement of these kinds of electrical signaling in the induction of many different plant responses to (a)biotic stresses or in developmental processes still wait to be established.

**Keywords** Action potential • Variation potential • System potential • Downstream signaling • DIR1

## 1 Introduction

### 1.1 *Electrical Long-Distance Signals: Brief History and Presence*

The unequal separation of charged molecules and ions across the plasma membrane to generate a membrane potential is usually due to the activity of plasma

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M.R. Zimmermann  
Botanisches Institut I, Justus-Liebig-Universität, Gießen, Germany

A. Mithöfer (✉)  
Bioorganische Chemie, Max-Planck-Institut für Chemische Ökologie, Hans-Knöll-Str 8,  
Jena, Germany  
e-mail: [amithoefer@ice.mpg.de](mailto:amithoefer@ice.mpg.de)

membrane-located channels and pumps. The presence of a membrane potential is an essential feature for all kinds of living cells and necessary for the uptake of nutrients and minerals as well as to establish and regulate intracellular physiological processes. In addition, the interaction between different cells or different tissue and organs via an electrical signaling for communication over a long distance is a well-established phenomenon for animals and known for more than 200 years. Even for plants fast electrical signals were described about 140 years ago: In 1873, Burdon-Sanderson studied the leaf closing mechanism in the carnivorous Venus flytrap, *Dionaea muscipula*. In addition, Darwin (1875) investigated tentacle movements of sundew plants, *Drosera spp.* Some years later, Kunkel (1898) and Bose (1907) showed that in *Mimosa pudica* the fast leaf(let) movements depended on electrical signals. All those plants represent remarkable and very specialized species, which, thus, were highly attractive to the scientists. General studies on long-distance electrical signaling in other common plants were not very popular among physiologists for many decades although some studies suggested that upon wounding electrical signals may travel through phloem and/or xylem elements (Pickard 1973; Davies 1987; Rhodes et al. 1996). Fortunately, in recent years an increasing number of studies are published dealing with the phenomenon of electrical long-distance signaling in higher plants.

## 2 Electrical Signals in Plants

In animals only one genuine electrical signal is known, the action potential. In contrast, three different signal types have been identified in plants—(1) action potential, (2) variation potential, and (3) system potential; all of which will be described in the following.

### 2.1 Action Potential

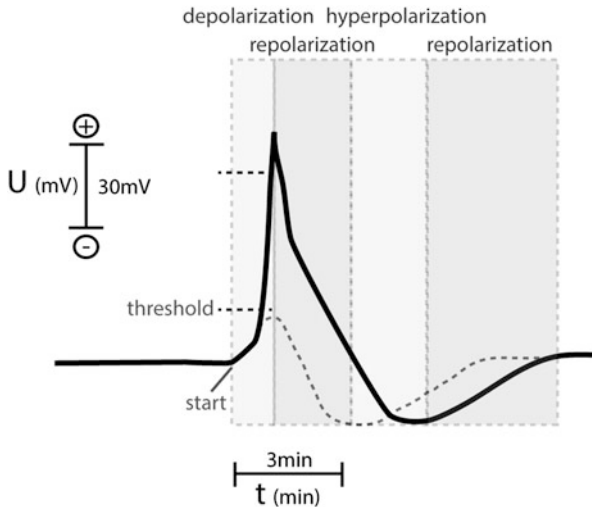
The action potential (AP) is a transient depolarization of the plasma membrane with a very typical voltage signature that is interestingly found in animals as well as in plants. Plant- and animal-originated APs share fundamental characteristics: (1) the distinctive voltage signature, (2) the all-or-nothing law, (3) the ability of self-propagation, and (4) a refractory period, but they differ in (1) time frame and (2) molecular compounds involved (Pickard 1973; Beilby 2007; Pyatygin et al. 2008).

Most details about the complex and fine-adjusted molecular mechanism of APs were found for lower plants, e.g., *Acetabularia spec.* (Gradmann 1976), *Chara spec.* (Hope and Findlay 1964; Beilby 2007), *Nitella spec.* (Blatt 1974; Kikuyama 1987), and *Conocephalum conicum* (Dziubinska et al. 1983; Trebacz et al. 1989, 1994, 1997; Trebacz 1992). In higher plants, APs have been mainly examined in plants with motor activity, i.e., *Mimosa pudica*, *Drosera spp.*, or *Dionaea*

**Table 1** Action potential characteristics and various examples for higher plants

Species	Stimulus	Amplitude (mV)	Time (s)	Velocity (cm min <sup>-1</sup> )	Setup	Location	Reference
<i>Zea mays</i>	Electrical/cold-shock	+50–80	30–120	180–300	Intra	Sieve tubes/leaf	Fromm and Bauer (1994)
<i>Z. mays</i>	Electrical/cold-shock			12–120	Intra	Mesophyll cells/leaf	Fromm and Bauer (1994)
<i>Salix viminalis</i>	Electrical	+30–50	10	120	Intra	Stem, cortex	Fromm and Spanswick (1993)
<i>Vicia faba</i>	Heat	+100–110			Intra	Sieve tubes/leaf	Furch et al. (2007)
<i>Lycopersicon esculentum</i>	Heat	+79			Intra		Rhodes et al. (1996)
<i>Cucurbita pepo</i>	Sucrose (100 mM)	+90	60		Intra	Sieve tubes/petiole	Eschrich et al. (1988)
<i>C. pepo</i>	Heat	+60	20–30		Intra	Sieve tubes/fruit	Eschrich et al. (1988)
<i>L. esculentum</i>	Heat	+50	120		Intra	Main vein	Herde et al. (1998)
<i>Lupinus angustifolius</i>	Electrical	–60	180	3.6–5.6	Extra	Stem	Paszewski and Zawadzki (1976)
<i>Mimosa pudica</i>	Heat	–74–60			Extra	Stem	Roblin (1985)
<i>Salix viminalis</i>	Electrical	–50		120	Extra	Stem, cortex	Fromm and Spanswick (1993)
<i>Helianthus annuus</i>	Electrical	–50	20–30	11	Extra	Stem, leaf	Dziubinska et al. (2001)
<i>Arabidopsis thaliana</i>	KCl (1 M) + prick	–43–8	60	0.78–9	Extra	Midrib and petiole/leaf	Favre et al. (2001)
<i>Vicia faba</i>	Heat	–76–56		6.3–12.1	Extra	Stem, leaf	Dziubinska et al. (2003)
<i>C. pepo</i>	Cooling	–120	120–600		Extra	Hypocotyl	Opritov et al. (2005)
<i>Hordeum vulgare</i>	KCl, CaCl <sub>2</sub> , glutamic acid	–80–70	900	20–30	Extra	Substomatal cavity/leaf	Felle and Zimmermann (2007)

*Note.* Voltage changes of intracellular recordings are positive going (=depolarization) and all kinds of extracellular measurements show a negative shift of voltage (=“hyperpolarization”). Prefix indicates direction of voltage change. Extracellular (extra) recordings were executed with surface, blindly pierced, or substomatal-placed electrodes. Intracellular (intra) measurements were achieved with severed stylets of aphids or impaled microelectrodes. Velocities are converted to cm min<sup>-1</sup>. Data are arranged to the category setup



**Fig. 1** Illustration of an intracellular recorded action potential (AP) in higher plants. An AP is separated into three steps: depolarization, repolarization, and hyperpolarization. The depolarization is again separated in three different steps. First, a minor slope is observed within the initiate phase of the depolarization. Passing the threshold, the typical rapid and steep depolarization is observed and persists merely a few seconds (app. 10 s). At the end of the depolarization, the kinetics is getting slower again (*dashed line*). The subsequent repolarization is divided into two steps separated by a distinct edge and fading to hyperpolarization.  $U$  = voltage

*muscipula* (Houwink 1935; Sibaoka 1969; Hodick and Sievers 1988; Volkov et al. 2010a, b). In addition, numerous studies with higher plant species without motor activities confirmed the general occurrence and characterized APs in plants (Table 1).

Up to now, diverse stimuli (touch, heat, cold, KCl, CaCl<sub>2</sub>, glutamic acid, electrical current) have been identified to initiate the orchestrated molecular cascade of an AP (Fig. 1) via three different mechanisms: (1) a depolarization (KCl, electrical current), (2) an increase of apoplastic Ca<sup>2+</sup> (CaCl<sub>2</sub>), or (3) a ligand-binding receptor (glutamic acid; Felle and Zimmermann 2007). In each case, the consequence is a Ca<sup>2+</sup> influx from the apoplast into the cytoplasm. This increase of cytosolic Ca<sup>2+</sup> causes the opening of anion channels in the plasma membrane and, subsequently, an efflux of anions (mainly Cl<sup>-</sup>) along the electrochemical gradient from the cytoplasm into the apoplast (Bradley and Williams 1966; Tarr et al. 1970; Lunevsky et al. 1983; Tsutsui et al. 1986; Felle and Zimmermann 2007). Interestingly, Mg<sup>2+</sup> is not able to replace Ca<sup>2+</sup> confirming the specific role of Ca<sup>2+</sup> during an AP (Tarr et al. 1970). Only if a certain threshold is passed all anion channels will open and the characteristic rapid and strong depolarization (“break-through”) can be observed (Fig. 1). The massive loss of negative charges affects the membrane potential and the Nernstian potential of potassium. This results in a passively driven K<sup>+</sup> efflux from the cytoplasm into the apoplast (Tsutsui et al. 1986; Felle and Zimmermann 2007). At the end of the depolarization phase when anion- and K<sup>+</sup>-

efflux is balanced and the  $H^+$ -ATPases start working, the peak of the depolarization is reached (Fig. 1). To some extent, the single phases are reflected in the voltage pattern of the depolarization phase with its typical kinetics (Fig. 1). The repolarization is based upon an active symport of  $H^+/Cl^-$  and a passively driven  $K^+$ -influx from the apoplast into the cytoplasm (Felle and Zimmermann 2007).

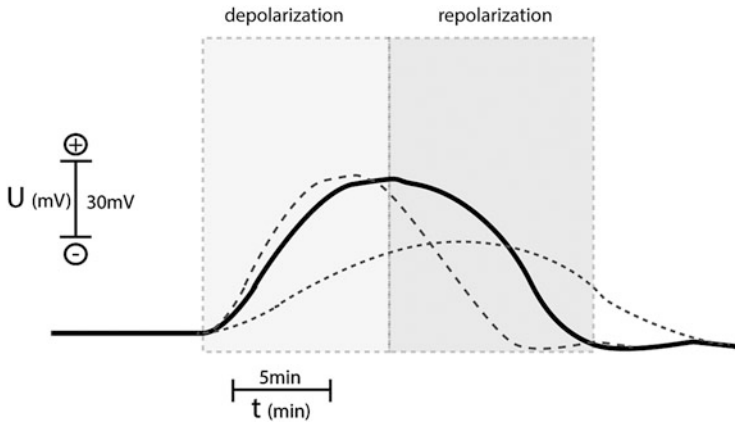
The reported rate of transmission (velocity) ranges from 0.78 to 300  $cm\ min^{-1}$  (Table 1) suggesting differences in the electrical coupling within diverse plant species. In general, the phloem is supposed to be the main responsible cellular translocation pathway for an AP within a plant (Eschrich et al. 1988; Fromm and Bauer 1994). Sieve elements and companion cells exhibit a relative high electrical coupling via abundant cell-to-cell contacts (plasmodesmata; van Bel and Ehlers 2005). Direct local and systemic measurements of APs within sieve elements supported the thesis (Eschrich et al. 1988). But also the apoplast was suggested to have a crucial role in long-distance transmission of APs (Herde et al. 1998; Felle and Zimmermann 2007). Without doubt, plants have to cope with the problem of a missing electrical shield around the phloem (vascular system). The consequence is a leakage current and, thus, a longitudinal loss of voltage (see also cable theory; Adam et al. 2009). In mammalian, a myelin sheath acts as an electrical shield. That handicap might be compensated in plants—at least in part—by an intensive interplay of the phloem and the corresponding apoplast.

Finally, any AP-originated disturbance of the membrane potential has to be reset to the original conditions. During this time frame, called (absolute/relative) refractory period, cells are restricted for a next excitation. The refractory period is characteristic of APs. In the literature, a time range from 50 s (*Mimosa pudica*) to 2 h is reported indicating the refractory period to be plant specific (Paszewski and Zawadzki 1976; Fromm and Spanswick 1993; Fromm and Bauer 1994).

## 2.2 Variation Potential

The variation potentials (VPs), also known as slow wave potentials (SWPs), are transient depolarizations of the plasma membrane with variable shape, amplitude, and time fame (Fig. 2; Table 2) (Houwink 1935; Sibaoka 1953; Roblin and Bonnemain 1985; Stahlberg and Cosgrove 1996). VPs have been reported for a wide range of herbaceous plants (Table 2) and have been detected solely or in combination with APs (Roblin 1985; Furch et al. 2007). Diverse mechanical/physical stimuli such as heat, wounding (cutting, excision), and pressure as well as chemical factors (Ricca's factor, cyanide, aziden 2,4-dinitrophenol, sodium cholate) have been identified as external stimuli (Table 2) (Ricca 1916; van Sambeek and Pickard 1976b; Stahlberg and Cosgrove 1997a; Stanković et al. 1997). The observed inconsistent but characteristic voltage pattern is a result of the underlying molecular mechanism.

The depolarization of VPs results primarily from an inhibition of the plasma membrane  $H^+$ -ATPases. It was shown that during a VP, no changes in cell input



**Fig. 2** Illustration of an intracellular recorded variation potential (VP) in higher plants. VPs reflect a wave-like, transient depolarization with variable shape and duration displayed here with three different voltage patterns (*continuous, dashed, and dotted lines*). Amplitude and duration depend on the distance between stimulus and recording site. A subsequent hyperpolarization can be observed to some extent.  $U$  = voltage

resistance occurred, which actually is typical for APs, and a decreased activity of proton pumps was concluded (Julien et al. 1991; Stahlberg and Cosgrove 1992). Congruously, a study with diverse proton pump inhibitors showed voltage patterns (Stahlberg and Cosgrove 1996) that are comparable with those evoked by mechanical stimuli. In addition, heat, cutting, or exogenous pressure application induces an alteration of the hydraulic pressure conditions within the xylem/apoplast via an import of energy (heat, pressure) or an opening of the vascular system (cutting). Indeed, changes of turgor pressure could be measured locally and systemically following application of heat or exogenous pressure (Malone and Stanković 1991; Malone 1992). Moreover, a combined determination of stem length and leaf thickness via transducers as a sign of hydraulic pressure alteration and electrical recordings showed a temporal correlation (Malone 1992; Stanković et al. 1997). The change of hydraulic pressure propagates in terms of a diminished wave along the xylem vessels and affects the surrounding cells by an inhibition of the proton pumps in the plasma membrane causing a transient depolarization. An essential turgor sensitivity of proton pumps was reported earlier (ref. see Stahlberg and Cosgrove 1992, 1996). The decline of proton pump activity results in reduced  $H^+$ -extrusion from the cytoplasm into the xylem/apoplast. There, the consequent alkalization could be determined with a pH-sensitive fluorescent dye, for example, in *Pisum sativum* from pH 4.5 to 6.0 (Stahlberg and Cosgrove 1996); this is additionally supported by the observed pH dependence of VPs (Julien et al. 1991). So VPs reflect the local electrical consequence of a hydraulic pressure wave running along xylem/apoplast (Malone 1992; Stahlberg et al. 2005).

The variable strength (amplitude and duration, time frame) of a VP is positively correlated with the force of the locally affecting hydraulic pressure wave. It could

**Table 2** Variation potential characteristics and various examples for higher plants

Species	Stimulus	Amplitude (mV)	Time (min)	Velocity (cm min <sup>-1</sup> )	Setup	Location	Reference
<i>Pisum sativum</i>	Wound	+40–45	5–8	6	Intra	Epidermal cells	Stahlberg and Cosgrove (1992)
<i>Cucumis sativus</i>	Pressure	-100–40	20–30	0.5–3	Intra	Epidermal cells	Stahlberg and Cosgrove (1997b)
<i>Vicia faba</i>	Heat	+90	20		Intra	Sieve tube/leaf	Furch et al. (2007)
<i>Cucurbita maxima</i>	Heat	+50–75	20		Intra	Sieve Tube/leaf	Furch et al. (2010)
<i>Lycopersicon esculentum</i>	Heat	-40–30	3–8		Extra	Stem, leaflet	van Sambeek and Pickard (1976a)
<i>L. esculentum</i>	Crude extract + wound	-35			Extra	Rhachis	van Sambeek and Pickard (1976b)
<i>L. esculentum</i>	Mannitol (1 M) + wound	-12			Extra		van Sambeek and Pickard (1976b)
<i>L. esculentum</i>	KCl (1 M) + wound	-22			Extra		van Sambeek and Pickard (1976b)
<i>L. esculentum</i>	KCl (0.1 M) + wound	-7			Extra		van Sambeek and Pickard (1976b)
<i>Mimosa pudica</i>	Heat	-82–52			Extra	Stem	Roblin (1985)
<i>Vicia faba</i>	Heat	-59–17		9	Extra	Stem	Roblin (1985)
<i>L. esculentum</i>	Heat	-54–33			Extra	Stem	Roblin (1985)
<i>Triticum durum</i>	Heat	-20–10	10–15		Extra	Leaf	Malone and Stanković (1991)
<i>P. sativum</i>	Wound	-35	5–8	6	Extra	Epidermal cells	Stahlberg and Cosgrove (1992)
<i>Triticum aestivum</i>	Heat	20		600	Extra	Leaf	Malone (1992)
<i>P. sativum</i>	Pressure/heat	-80–60	5–10		Extra	Epicotyl	Stahlberg and Cosgrove 1996
<i>L. esculentum</i>	Heat	-23			Extra		Rhodes et al. (1996)
<i>Helianthus annuus</i>	Pressure + wound	-50–10		5–100	Extra	Stem	Stanković et al. (1997)
<i>P. sativum</i>	Pressure	-80–40	5	2–3	Extra	Stem, hypocotyl	Stahlberg and Cosgrove (1997b)
<i>Helianthus annuus</i>	Heat	-100	8–12	5–25	Extra	Stem, leaf	Dziubinska et al. (2001)
<i>Arabidopsis thaliana</i>	KCl (1 M) + pricks			1.8	Extra	Midrib and petiole/leaf	Favre et al. (2001)
<i>Vicia faba</i>	Heat	-100–20		5.1	Extra	Stem, leaf	Dziubinska et al. (2003)

(continued)

Table 2 (continued)

Species	Stimulus	Amplitude (mV)	Time (min)	Velocity (cm min <sup>-1</sup> )	Setup	Location	Reference
<i>Nicotiana tabacum</i>	Heat	-25–10	30–60	90–120	Extra	Leaf	Hlaváčková et al. (2006)
<i>C. maxima</i>	Heat	-115–66	60	0.6	Extra	Vein, leaf	Furch et al. (2010)
<i>C. maxima</i>	Wound	-52–38	60–180	240–480	Extra	Vein/petiolum	Zimmermann et al. (2013)
<i>C. maxima</i>	Wound	-28–16	60–240	240–480	Extra	Vein/petiolum	Zimmermann et al. (2013)

Note. Voltage changes of intracellular recordings are positive going (=depolarization) and all kinds of extracellular measurements show a negative shift of voltage (=“hyperpolarization”). Prefix indicates direction of voltage change. Extracellular (extra) recordings were executed with surface, blindly pierced, or substomatal-placed electrodes. Intracellular (intra) measurements were achieved with severed stylets of aphids or impaled microelectrodes. Velocities are converted to cm min<sup>-1</sup>. Wound includes damage, excision and cutting. Data are arranged to the category setup



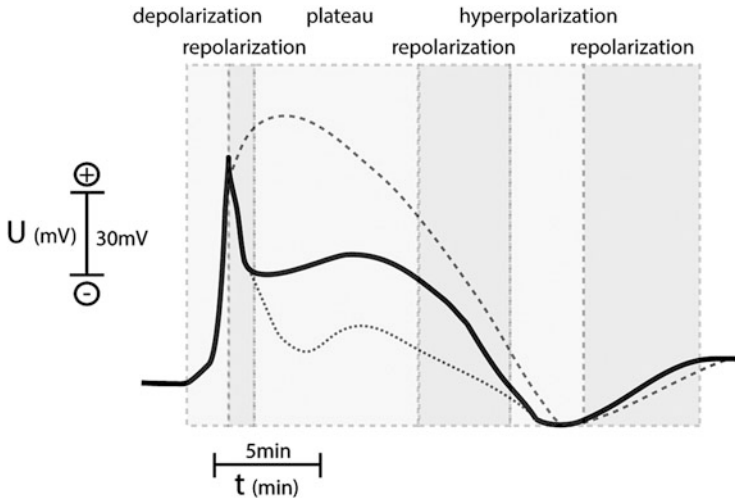
be observed that an increasing exogenous pressure application (30–100 kPa) at the root of *Pisum sativum* provoked stronger shapes of VPs, and simultaneously, a negative regression analysis displayed a decline of VPs with an increase of distance to the given continuous stimulus (50 kPa). The related radial leak of xylem pressure was calculated to be approximately  $4 \text{ kPa cm}^{-1}$  and is in direct context to the strength of a VP (Stahlberg and Cosgrove 1997a).

Between the occurrence of a hydraulic pressure wave and the VP, a lag time was observed depending on the strength of hydraulic pressure (Malone and Stanković 1991; Malone 1992; Stanković et al. 1997; Stahlberg et al. 2005). A faster stimulation of a VP was measured with stronger hydraulic pressure explaining why a wide range of measured velocities can be found in the literature (Table 2) due to the fact that the rate of propagation depends on the distance between stimulus and recording site and declined with an increasing distance (Stanković et al. 1997; Hlaváčková et al. 2006). Here, it has to be noticed that in contrast to APs, VPs are not able to self-propagate excluding them to be a genuine long-distance signal (Stahlberg and Cosgrove 1997a). That aspect is additionally supported by the results that chemically induced VPs via proton pump inhibitors could be merely measured at the site of application with the exception of Ricca's factor and sodium cholate (Ricca 1916; Stahlberg and Cosgrove 1997a).

Concerning physical stimuli, it is likely that at least mechanosensitive channels are also influenced during a VP. Depolarization with various proton pump inhibitors did not evoke APs, thus indicating a participation of mechanosensitive  $\text{Ca}^{2+}$  channels during VPs only (Stahlberg and Cosgrove 1997b). The observed stronger  $\text{Ca}^{2+}$  release was arrestingly demonstrated by the  $\text{Ca}^{2+}$ -dependent forisome dispersion within sieve elements of *Vicia faba* plants after a heat stimulus (Furch et al. 2007, 2009).

The transmission of VPs was demonstrated for both directions—acropetal and basipetal (van Sambeek and Pickard 1976a)—and is even able to pass dead tissue being in good accordance with the concept of a running hydraulic wave along xylem vessels (Roblin 1985; Roblin and Bonnemain 1985). However, a recent study observed discrimination between the acro- and basipetal pathway. Following a cut at a petiole of *Cucurbita maxima* plants, the recorded VPs were stronger at the basal side (basipetal direction) (Zimmermann et al. 2013). In addition, it could be shown that the electrical reaction after application of heat was strongest in phloem cells (sieve elements and companion cells) in comparison to other cell types, i.e., epidermal, cortical, and vascular parenchyma cells. This may be founded by the intensive interaction of xylem and phloem (Eschrich et al. 1988; Rhodes et al. 1996).

Heat is a favored stimulus to provoke electrical signals (see Tables 1 and 2). After a heat stimulus, a combined spatiotemporal appearance of APs and VPs has been reported (Roblin 1985; Furch et al. 2007, 2009). Typically, electrical recordings close to the stimulus site are characterized by an overlap of AP and VP. This phenomenon complicates an exact determination of the particular signaling type and is referred to as electropotential wave (EPW; Fig. 3) (Furch et al. 2007). Any increase of the distance between stimulus and recording site thus



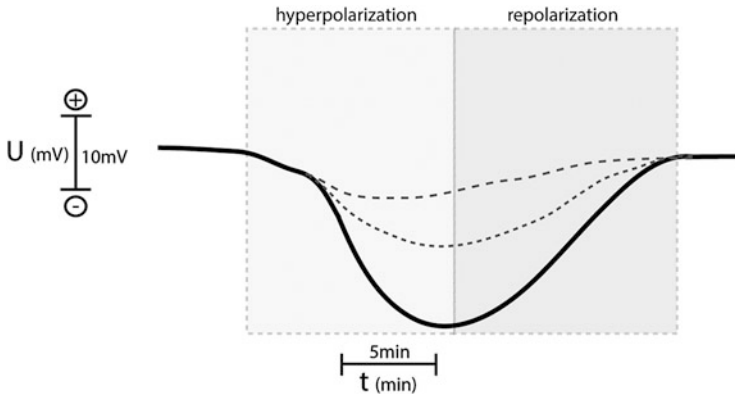
**Fig. 3** Illustration of an intracellular recorded electropotential wave (EPW) in higher plants. An EPW reflects a mixed potential of AP and VP, generally observed after a heat stimulus close to the application site. Voltage pattern of EPWs is also variable and depends on the distance to the stimulus site, indicated here with different lines (*continuous, dashed, and dotted lines*). With increasing distance to the stimulus site, VPs are diminished and the voltage pattern of APs is solidified.  $U$  = voltage

strongly decreases the part of VP and in many cases the AP remains. Consequently, systemic measurements in a distant leaf only showed an AP (Zimmermann and Felle 2009).

### 2.3 System Potential

System potentials (SPs), in contrast to APs and VPs, reflect a systemic self-propagating hyperpolarization of the plasma membrane or depolarization of apoplastic voltage, respectively (Fig. 4) (Zimmermann et al. 2009). The term SP considers the striking fact this electrical signals can be reliably recorded on systemically, in contrast to APs and VPs. An occurrence of SPs was demonstrated for monocots as well as for dicots indicating a general ability of higher plants to transmit hyperpolarizations systemically (Zimmermann et al. 2009; Zimmermann 2010).

The combined application of wounding (cutting) and different chemical substances (e.g., glutamic acid,  $\text{LiCl}_2$ ,  $\text{CuCl}_2$ , aspartic acid, glutamate) as well as stimulation with heat (scorching) evoked SPs (Zimmermann 2010). However, closer analysis was executed with diverse salt solutions ( $\text{KCl}$ ,  $\text{NaCl}$ ,  $\text{MgCl}_2$ , and  $\text{CaCl}_2$ ). SPs were systemically recorded with a noninvasive approach of substomatal-placed microelectrodes (Felle et al. 2000; Zimmermann et al. 2009)



**Fig. 4** Illustration of an intracellular recorded system potential (SP) in higher plants. SPs are self-propagating hyperpolarizations with variable shapes displayed here by different line types (*continuous, dashed, and dotted*). The slope of the hyperpolarization is mainly steeper than the repolarization.  $U$  = voltage

by giving a stimulus at one leaf and recording of the electrical reaction at a distant leaf. A constant propagation rate of SPs was detected with  $5\text{--}6\text{ cm min}^{-1}$  for both directions acropetal and basipetal. The determined range of velocity indicates rather an electrotonic propagation of SPs than a possible induction of wound-allocated electrogenic substances, which has been mentioned for VPs and was determined to be approximately  $0.8\text{--}1.7\text{ cm min}^{-1}$  (Canny 1975).

Studies with various chemical stimuli (KCl, NaCl,  $\text{MgCl}_2$ , and  $\text{CaCl}_2$ ) at different concentrations (10–100 mM) showed that (1) cations rather than anion trigger SPs, (2) shapes of SPs are adapted to the mode of the stimulus, and (3) strength (in terms of concentration) of the stimulus; all of which indicate the possibility of encoding more sophisticated information (Zimmermann et al. 2009).

An analysis of diverse ion activities ( $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{H}^+$ ,  $\text{Cl}^-$ ) within the apoplast of substomatal cavity during a SP again indicated the participation of proton pumps. This hypothesis was proven with the application of fusicoccin, a toxin of the phytopathogenic fungus *Fusicoccum amygdali*, causing an activation of proton pumps (Marrè 1979; Hager 2003). Besides the induction of SPs by fusicoccin, the transmission of the electrical signal could be prevented with the application of the proton pump inhibitor vanadate (Zimmermann et al. 2009). Hence, these results suggest that the activated status of proton pumps may be relayed from cell to cell reasoning the self-propagation of SPs. Although the same applied stimuli are well known to provoke APs (i.e., glutamic acid, KCl,  $\text{CaCl}_2$ ) or VPs (heat), SPs were systemically recorded in the most cases, indicating a higher probability of long-distance transmission for SPs in comparison to APs and VPs (Zimmermann 2010). However, it seems contradictory that the same stimuli evoke different electrical signaling types; but the depolarizations of APs and VPs can be seen as “regulated” disturbances of the membrane potential that has to be recovered with the activation of proton pumps; the latter, on the other hand, possess a sufficient electrical

coupling for long-distance transmission. Without a doubt, the diverse stimuli trigger APs and/ or VPs at the site of application but the depolarization gets “lost” on the systemic spreading due to the pronounced decrement of VPs and/or the obvious suboptimal electrical coupling of plant cells for APs (Zimmermann et al. 2009).

Hence, SPs represent the first affirmation of previously proposed concept of propagating signals basing upon fast changes of active pumps (refs. see Stahlberg and Cosgrove 1992) and support previous results of systemically recorded hyperpolarizations (Lautner et al. 2005).

### 3 Downstream Signaling and Physiological Responses

The evidence of electrical signals in plant tissues per se, generated upon artificial treatments such as heat or high salt concentrations, does not necessarily mean that those signals are of physiological relevance. However, up to now various studies demonstrated an involvement of electrical signals in various physiological reactions in higher plants (Table 3). Numerous indications were given for a proper role of electrical signals within intra- and distant intercellular communication and for the regulation of physiological processes at the molecular, cellular, and the organism level (Davies 1987; Fromm and Bauer 1994; Fromm and Lautner 2007). The most obvious results were obtained for plants with motor activity, i.e., tentacle bending in *Drosera spp.*, or leaf movements in *Mimosa pudica* or *Dionaea muscipula*. The necessity of mechanically triggered action potentials for the induction of rapid, thigmonastic leaf movement has been well established (Braam 2004; Volkov et al. 2007, 2009, 2010b). All these motor activities depend on the generation of an AP (Sibaoka 1969; Williams and Pickard 1972; Fromm and Eschrich 1988).

Heat induces both AP and VP that accompany the enhancement of systemic ethylene emission, for example, in leaves of *Vicia faba* seedlings (Dziubinska et al. 2003). Other studies focused on general metabolic processes (photosynthesis respiration, gas exchange, stomata movements) following diverse abiotic stimuli (Table 3).

Encouraging results have been published in the field of plant defense supporting the idea of electrical signaling as an integral signaling event in the initiation of defensive reactions (Stahlberg and Cosgrove 1994; Favre et al. 2001). Electrical signals have been shown to cause effects in systemic leaves, for example, the regulation of various genes (Graham et al. 1986; Wildon et al. 1992; Stanković and Davies 1997; Herde et al. 1998). In tomato (*Lycopersicon esculentum*), the first results described that proteinase inhibitor (*pin*) as well as calmodulin genes were upregulated due to wounding and heat stimuli. Consequentially, plants that generated no electrical signal could not accumulate *pin* mRNA (Stanković and Davies 1997). Moreover, a heat-induced and VP-mediated accumulation of proteinase inhibitor genes (*pin II*) and jasmonic acid was described in potato (*Solanum*

**Table 3** Electrical signal types and proposed mediated physiological impacts

Species	Stimulus	Signal type	Physiological reaction	Reference
<i>Dionaea muscipula</i>	Mechanical	AP	Trap closure, delivery of enzymes for digestion	Sibaoka (1969)
<i>Drosera spec.</i>	Mechanical	AP	Movement of tentacles to catch insects	Williams and Pickard (1972)
<i>Mimosa pudica</i>	Cold shock, mechanical	AP	Regulation of leaf movement	Fromm and Eschrich (1988), Sibaoka (1969)
<i>Salix viminalis</i>	Wounding	AP	Transpiration and photosynthesis	Fromm and Eschrich (1993)
<i>Zea mays</i>	Irrigation	AP	Increase of gas exchange	Fromm and Fei (1998)
<i>Z. mays</i>	Cold shock	AP	Reduction of phloem transport	Fromm and Bauer (1994)
<i>L. esculentum</i>	Electrical	AP	Induction of <i>pin2</i> gene expression	Stanković and Davies (1996)
<i>Cucumis sativus</i>	Wounding	VP	Growth inhibition	Stahlberg and Cosgrove (1994)
<i>Pisum sativum</i>	Wounding	VP	Drop of growth rate	Stahlberg and Cosgrove (1992)
<i>Solanum tuberosum</i>	Heat	VP	Induction of jasmonic acid biosynthesis and <i>pin2</i> gene expression	Fisahn et al. (2004)
<i>Populus nigra</i>	Heat	VP	Transient reduction of photosynthesis	Lautner et al. (2005)
<i>Nicotiana tabacum</i>	Heat	VP	Systemic induced stomatal closure and increase of ABA and JA	Hlaváčková et al. (2006)
<i>Bidens pilosa</i>	Heat	AP/VP	Calmodulin mRNA accumulation	Vian et al. (1996)
<i>Vicia faba</i>	Heat	AP/VP	Induce systemic enhancement of ethylene emission	Dziubinska et al. (2003)
<i>Vicia faba</i>	Heat	EPW	Occlusion of sieve elements via forisome dispersion	Furch et al. (2007, 2009)
<i>Cucurbita maxima</i>	Heat	EPW	Occlusion of sieve elements via proteins	Furch et al. (2010)
<i>L. esculentum</i>	Mechanical wounding	?	Accumulation of mRNA encoding proteinase inhibitor	Wildon et al. (1992)
<i>L. esculentum</i>	Heat	?	Accumulation of PIN2, JA; involvement of ABA	Herde et al. (1998)

Wounding includes damage, excision, and cutting. Data are arranged to the category signal type. AP action potential, VP variation potential, ? electrical signal is not specified

*tuberosum*) plants (Fisahn et al. 2004). Proteinase inhibitors negatively affect the digestion of herbivores and jasmonic acid is a well-established regulator within herbivory-induced plant defense responses (Mithöfer and Boland 2012). A previous report of a general decline of phloem content translocation (Fromm and Bauer

1994) has been specified in recent studies where a distinct relationship of transmitted electrical signals and the release of  $\text{Ca}^{2+}$  into sieve elements were shown. The consequence was the occlusion of sieve elements via  $\text{Ca}^{2+}$ -dependent forisome dispersion in *Vicia faba* plants or protein plugs in *Cucurbita maxima* (Furch et al. 2007, 2009, 2010). Sealing of sieve elements can be seen as an early response to pathogen and/or herbivore attack to prevent transmission of attacker-released toxins and loss of valuable phloem sap.

The biological significance of SPs as a common signal asks for a natural trigger and an involvement within distinct physiological processes. First indications were found following feeding of herbivorous lepidopteran caterpillars (*Spodoptera littoralis*). Various hyperpolarizations could be recorded in distant leaves indicating SPs. Moreover, preliminary results suggest a systemic decrease of jasmonic acid accumulation after induction of SPs (Zimmermann 2010).

## 4 Conclusions and Outlook

For higher plants, the existence of electrical long-distance signals is well established and accepted. The three types of electrical signaling, AP, VP, and SP, have been demonstrated for various plants, monocots as well as dicots. Regardless of how the different types of electrical signals are generated, realized, and propagated, this indicates that this kind of signaling is widespread and a general feature in plants, though the best-noticed studies have been done with specialized plants such as the carnivorous *Dionaea muscipula* or touch-sensitive *Mimosa pudica*.

Although there is a growing body of evidence strongly suggesting that electrical signals in plants are indeed constituents of abiotic and biotic stress-induced signaling cascades, a final proof is still missing. Unfortunately, still very often the applied stimuli used to induce electrical signals are artificial and far from being physiological. There is no question that such experiments clearly showed the ability of plants to generate electrical signals, but it is difficult to generally conclude that these signals are naturally occurring and involved in plant's physiology, particularly in the communication between distant cells or organs. Thus, in order to understand electrical long-distance signaling in plants, the elucidation of a signaling cascade that includes or depends on an electrical signal and leads to the initiation of a defined physiological response is still a task that urgently needs to be addressed. For sure, this will remain a major scientific challenge for plant physiologists in the future.

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