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Opinion

Electrical Wiring and Long-Distance Plant Communication

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Electrical signalling over long distances is an efficient way of achieving cell-to-cell communication in living organisms. In plants, the phloem can be considered as a 'green cable' that allows the transmission of action potentials (APs) induced by stimuli such as wounding and cold. Measuring phloem potential changes and separating them from secondary responses of surrounding tissues can be achieved using living aphids as bioelectrodes. Two glutamate receptor-like genes (*GLR3.3* and *3.6*) were identified as being involved in the propagation of electrical activity from the damaged to undamaged leaves. However, phloem APs are initiated and propagated independently of these glutamate receptors. Here, we propose new screening approaches to obtain further information on the components required for electrical signalling in phloem cables.

Trends

The phloem of higher plants serves as a cable for long-distance electrical signalling.

Aphids can be used as bioelectrodes to study plant electrical communication.

Leaf wounding and cold stimuli induce phloem-travelling APs.

APs do not require the GLR 3.3/3.6 glutamate receptor pair.

Remote Signalling in Animals and Plants

Long-range signalling in higher organisms is essential for flexible responses to environmental threats and challenges. Animals have a nervous system that allows fast transmission of electrical signals between different parts of the body. The sensing of a stimulus, such as heat when accidentally touching an oven, is converted into the contraction of muscles, pulling the wounded hand away. These electrical circuits are based on a dense network of nerve cells connected via their axons. The axons operate as cables that conduct information encoded by the number and frequency of transitory APs.

By contrast, higher plants do not have specialised nerve cells with axons, but similar to animals, they do operate long-distance electrical signalling. Indeed, mechanically induced transient electrical AP-like waves in Venus flytrap (*Dionaea muscipula*) were reported by Darwin [1], long before Cole and Curtis recorded similar signals in squid [2]. Later, Hodgkin and Huxley established the ion channel basis of the AP in the squid giant axon [3]. The patch clamp technique, in combination with advanced molecular cell biology and genomics, has provided a detailed picture of the channels and receptors of the human nervous system. Although similar studies have been conducted in plants, the cellular and molecular nature of the green circuits remain largely unknown.

In this opinion, we show that the phloem network conducts long-distance electrical signals and argue that they are carried by voltage-dependent plant-specific ion channels. We propose that screens for mutants in phloem electrical signalling using phloem-expressed genetically encoded voltage-sensing dyes will provide new insights into plant whole-body communication and the underlying membrane receptors and channels operating the green circuit.

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Electrical Circuits: Cable Features of Phloem Sieve Tubes

In animals, electrical excitability initiates APs. These signals travel from the site of initiation to the site of integration and coordinate appropriate responses via neuronal networks. Nerve conduction velocity (the speed at which an electrochemical impulse propagates down a neural pathway) is an important aspect in timing information flow within the circuit. Each axon, which connects two nerve cells in the neuronal network, can be treated as an electrical cable. The propagation of an AP, which spreads along the axonal surface, depends on the excitability of the membrane and the conductive properties of the intracellular fluid [4]. In addition, myelin-rich glial cells (Schwann cells) shroud the axons of motor and sensory neurons to form the myelin sheath. The myelination of the axonal membrane further shields the axon with a dielectric layer, which both reduces leakage of current out of the axon and decreases the negatively inferring membrane capacitance. These two effects increase the speed and distance that an AP can travel along an axon. The conductive cytoplasm of an axon is dominated by a 100-mM K^+ electrolyte, shielded by the plasma membrane from an extracellular Na^+-based electrolyte of approximately the same ionic strength. In nervous systems, axonal wiring is periodically interspersed by Nodes of Ranvier, which are unmyelinated segments. At these sites, APs can be induced and re-amplified, and information flow altered.

In plants, the phloem can be thought of as representing a comparable anatomy of a wired network. The network is visible when GFP is expressed under the control of the phloem-specific pSUC2 promoter (see Figure IA in Box 1). The phloem sieve tube pipeline is formed by a chain of cylindrical, cytoplasmically connected cells. During phloem development, sieve tube cells undergo partial programmed cell death. In this process, the central vacuole, the nucleus, and common plastids degenerate, thus allowing the flow of solutes [5]. Just like the axon interior, the sieve tube cytoplasm is dominated by an electrolyte of approximately 100 mM K^+, separated from the exterior (approximately 1 mM K^+) by the plasma membrane. Analogous to the glial cells that provide support and protection for neurons, the sieve elements are

Box 1. How to Monitor Phloem Excitability

Accessing the sieve elements for electrophysiological recordings is no easy task, given that this cell cable is embedded deep in the plant vasculature tissue. The conventional technique using glass electrodes [60,61] is invasive, so wounding side effects cannot be ruled out. Furthermore, the exact location of the electrode is not known; it records the 'local field potential' (i.e., the summed electrical signals from all the cells with which it is in contact). An ingenious alternative to the conventional glass microelectrode approach is the 'aphid bioelectrode method' [17] (Figure I). Briefly, aphids are caged overnight on the desired part of the plant. The next day, an aphid is selected, and its stylet cut with a focussed laser beam or with a brief radiofrequency pulse, delivered via a fine tungsten needle [17,62,63]. If the aphid is in phloem-feeding mode just before stylectomy, sap continues to ooze along the cut stylet, and the tip of a KCl electrode can then be immersed into the sap to gain electrical access to the sieve element. The aphid method is minimally invasive and guarantees that recordings are unequivocally made from sieve elements and absolute phloem potentials are recorded. Nonetheless, it is a low-throughput method and three conditions need to be met for a successful experiment: (i) the aphid must be in a stable phloem-feeding phase at the time of stylectomy, and this cannot be predicted; (ii) stylectomy must be successful; and (iii) an effective electrical connection with the sieve element must be established by immersing a KCl glass electrode into the sap that oozes from the cut stylet.

Alternatively, phloem monitoring can be achieved via the body of living aphids using a procedure termed 'electrical penetration graph' (EPG) [12] (Figure IB). EPG was originally devised for studying the feeding cycle of hemipteran insects [64,65]. These insects have long mandibulae and maxillae, which assemble to form a long and flexible tube with salivation and ingestion canals running along its length. The assembled stylet is thin, enabling navigation between the cells of the plant without causing significant damage. In the direct current (DC) version of EPG, there are two voltage levels that correspond to the extracellular and intracellular position of the aphid stylet (Figure IC). The lower voltage level corresponds to the intracellular position and reflects the relatively negative interior of the sieve element. The insect selectively searches for amino acids and finds them enriched in sieve tubes. When inserted, the fine stylet is kept in place for long periods of time. The entering of the stylet of the EPG-wired aphid into a sieve element appears in the EPG recording as an abrupt voltage drop (Figure IC). First, the aphid salivates for 1–3 min into the sieve element, and then enters into the E2 phase, which is the phloem-feeding phase, lasting normally for several hours. After reaching this configuration, the desired stimulus can be applied to the plant as close as a few millimetres from the EPG-recorded aphid.

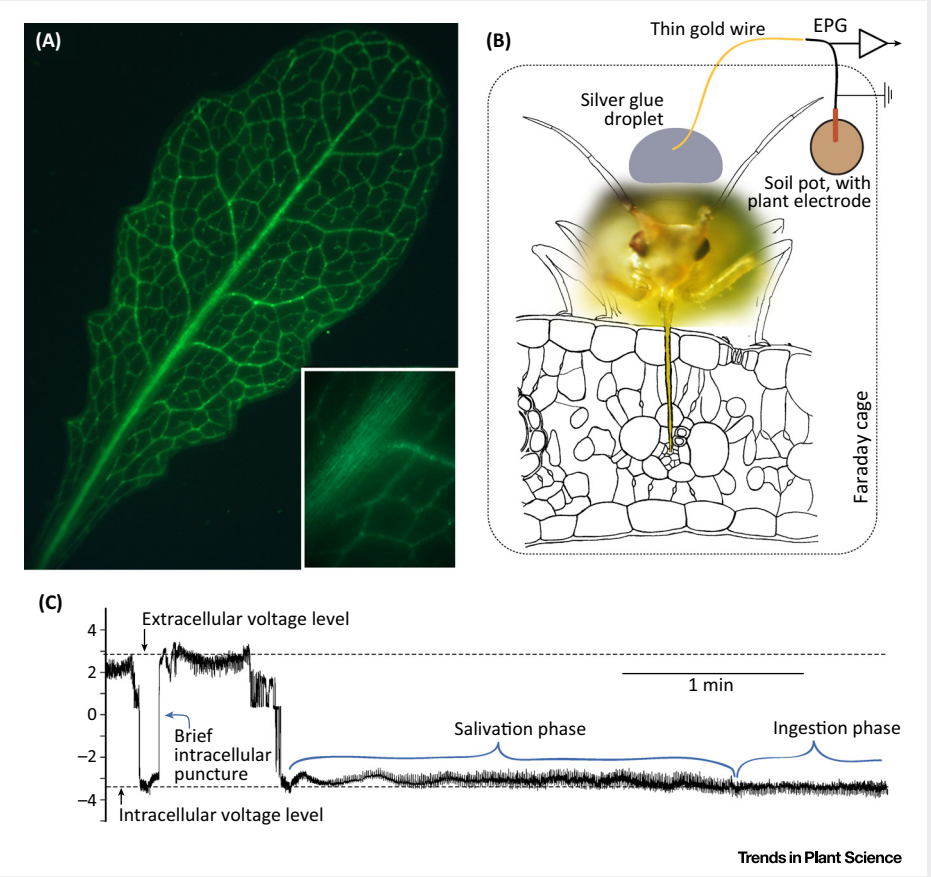


Figure I. The Electrical Penetration Graph (EPG) as a Method for Intracellular Electrophysiology of Sieve Elements. (A) The phloem network of an *Arabidopsis thaliana* leaf. Image of a leaf of a transgenic plant expressing GFP under the control of the phloem-specific promoter of the *SUC2* gene. (B) Diagram of the experimental configuration of the EPG circuit. A hemipteran insect, here a *Brevicoryne brassicae* aphid, is connected to the EPG equipment via a gold filament that is glued to a droplet of silver glue placed on the abdomen of the insect (more details can be found in [12,18]). (C) The feeding cycle of the aphid comprises two long-lasting phases: (i) the pathway phase, which is largely an extracellular phase in which the aphid stylet navigates between cells and makes numerous brief punctures into cells; and (ii) the phloem or intracellular phase, which corresponds to the stable phloem-feeding phase in which the stylet is inserted into a sieve element. The output from the direct current (DC) version of EPG used here shows a relatively high voltage level that corresponds to the pathway phase and a relatively low voltage level that corresponds to the phloem-feeding phase, which reflects the negative membrane potential of the sieve element. After the stylet reaches a suitable sieve element, there is a short period of salivation into the phloem (E1 phase in EPG terminology), followed by a long period of sap ingestion (E2 phase in EPG terminology). While in E2 phase, the EPG-recorded aphid can be effectively used as an intracellular electrode. Modified from [16] (B).

accompanied by companion cells. This anatomy of the 'green cable' should, theoretically, allow generation and modification of APs travelling along the phloem network. However, when compared with an axon, the phloem cable appears not to be optimised for electrical signalling (Figure 1). This is because: (i) sieve plates with pores constitute a non-negligible resistance for the passive diffusion of an AP by constricting regularly the cable surface; and (ii) companion cells with their electrolyte-rich cytoplasm cannot function as a myelin-like insulator. Consequently, companion cells neither reduce the membrane capacitance nor electrically shield the sieve tube. Instead, they can dissipate part of the electric signal and mediate gross exchange of electrolytes and metabolites with the adjacent cytoplasmically connected sieve element. Given that some features of the axon are shared with sieve elements, we ask whether the phloem can provide an electric wire for long-distance information flow.

Phloem Highway for Solute and Information Flow

In the classical view, the phloem network is the primary pathway for sugars, amino acids, and other metabolites from the site of photosynthesis (source) to emerging roots, shoots, or fruits (sink), but there is more to it than that. The multicellular phloem highway channels all kinds of information, such as flowering time and notification of stresses, ranging from herbivore attack wounds, pathogens, and salt or osmotic stress, to water and nutrient limitation [6,7]. Stimuli involved in phloem-based systemic signalling appear to include key factors, such as peptides, RNAs, hormones, metabolites, and ions [8–10].

Foraging herbivorous insects cut leaves with their razor-like chewing mouthparts, causing collateral damage. Molecules released from the wounded cells appear to act as damage-associated molecular patterns (DAMPs). DAMP elicitors are recognised by receptors, which in turn, activate plasma membrane-bound kinases, ion channels, and NADP oxidases [11]. The induced electrical signals, reactive oxygen species (ROS), and calcium waves move from the wound to unwounded areas that can be several centimetres away [12–14]. These systemic signals are associated with the induction of defence responses, including the expression of defence genes for the biosynthesis of toxins or deterrents for herbivores (reviewed in [15]).

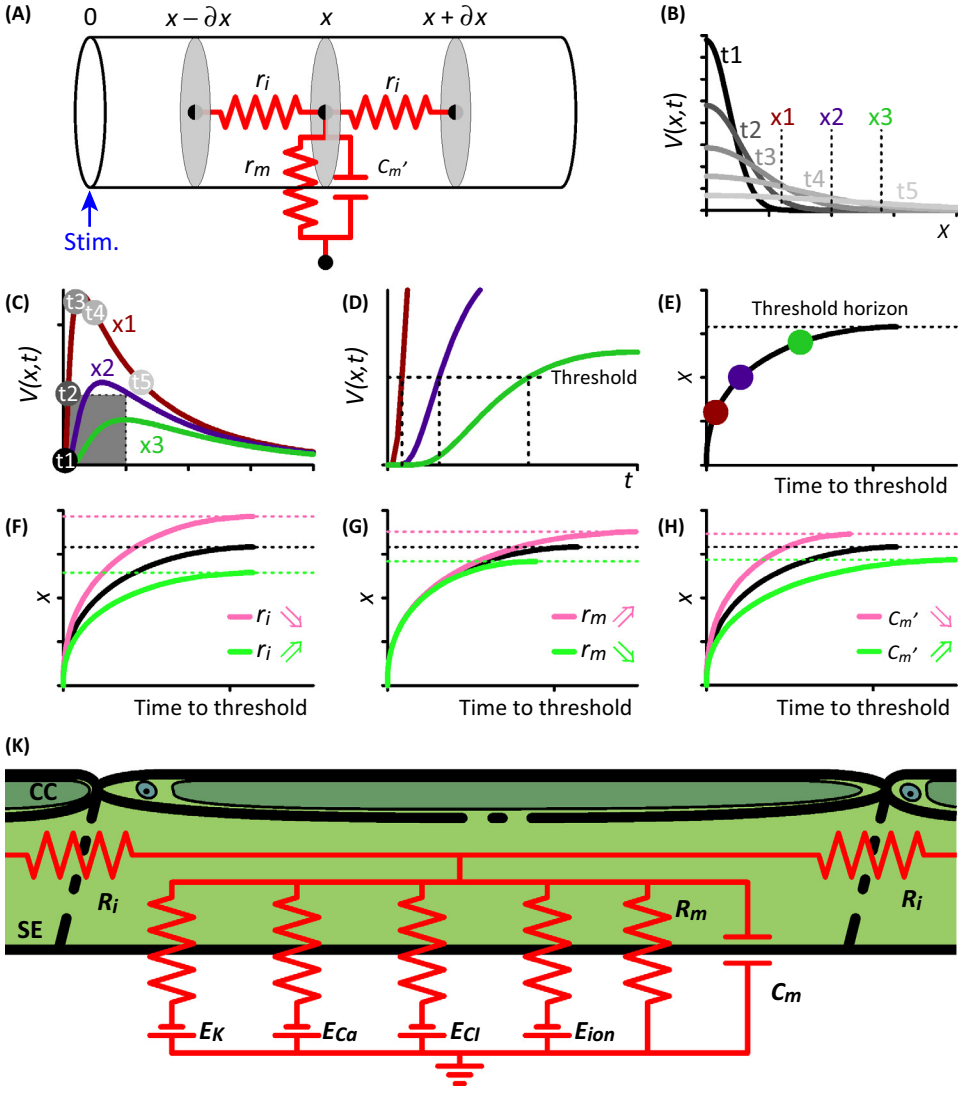
Electrical Signals in the Phloem

Electrical signals moving along the vascular tissue in plants can be registered using electrophysiological techniques [16]. Surface electrodes non-invasively measure relative changes in the electrical potential propagating at the leaf surface, which are associated with the excitement of cells within and outside the vascular bundle. This technique is unspecific in local resolution, because it measures the transient phloem signal together with the systemic response of the neighbouring tissue. In comparison, microelectrodes that pick up absolute membrane potential changes can be inserted into cells of or near the phloem network, albeit at the cost of tissue damage. A more cell-specific method uses sucking insects, which feed on amino acid-rich sieve tubes. This aphid-based electrophysiology (Box 1) [12,17,18] provides phloem-precise positioning of the stylet-based 'bioelectrode'. This enables direct measurement of the stimulus-dependent changes in phloem potential [19].

Here, we concentrate on the phloem signal measured with aphid-based electrodes using the direct current (DC) version of the electrical penetration graph (EPG). The voltage transients associated with wounding or a cold stimulus come in two parts: (i) a fast signal with a duration of approximately 15 s and relative amplitude of approximately 60–100 mV; and (ii) a slow signal of several minutes duration, with an approximate 40 mV amplitude [12]. While the slow component is only observed upon wounding, the fast component is induced by cold and wounding in arabidopsis (*Arabidopsis thaliana*) (Figure 2). In maize (*Zea mays*), a fast component has only been observed in response to a cold stimulus, rather than in response to wounding [20]. The signals propagate with a speed in the range of approximately 1 mm s^{-1} [12,21] along the phloem. This electrical wave can also spread systemically to more distant leaves. In plants, the velocity of signal propagation is approximately three to five orders of magnitude slower than electrical transmission in nerves (approximately1–100 m s^{-1}). Thus, even though the phloem represents a rather imperfect axon, it does transmit electrical signals. This leads to the question: are these signals passively propagating waves or do they have 'AP-like' features based on voltage-dependent ion channels, as seen in animals?

Phloem Circuit Excitation without Axon-Type Na^+ and Ca^{2+} Channels

As mentioned above, the phloem has similar tube-like features to those of an axon. However, the electrical resistance caused by narrowing the membrane surface at the sieve plates and the relatively large capacitance of the unmyelinated membrane are severe obstacles for the passive propagation of an electrical wave (Figure 1). Without re-amplification of the signal as in axonal



Trends in Plant Science

Figure 1. The Phloem Cable. The anatomy of the phloem allows for conducting electrical signals, as in a cable. Consequently, the general cable equation also applies to the phloem (Equation 1):

$$cm' \cdot \frac{\partial V(x,t)}{\partial t} = \frac{1}{r_i} \cdot \frac{\partial^2 V(x,t)}{\partial x^2} - i_{ion} \qquad [1]$$

where r_i [$\Omega \cdot m^{-1}$] is the internal resistance in axial direction per unit length and $c_{m'}$ [$F \cdot m^{-1}$], the capacitance of the surface of the cable slice per unit length; i_{ion} denotes the current per unit length that flows across the membrane; and $V(x,t)$ is the voltage at position x and time t. If we first consider the phloem as a passive cable (A), i_{ion} can be expressed as $V(x,t)/r_m$, with the transmembrane resistance of a cable segment r_m [$\Omega \cdot m$]. This allows the evaluation of important physical properties of the green cable. In computational simulations, a standard cable is stimulated by a very short pulse at $t = 0$ at position 0 [(A) 'stim.']. The dissipation of the depolarisation $V(x,t)$ is calculated over time. (B) The spatial distribution of $V(x,t)$ for five different time points ($t1<t2<t3<t4<t5$, cross-referenced by different shades of grey). (C) Time course of $V(x,t)$ for the three positions x1, x2, and x3 indicated in (B) and cross-referenced by colour. For position x1, the time points $t1$–$t5$ are indicated. (D) Magnification of the grey box in (C). To induce downstream reactions, $V(x,t)$ usually needs to overcome a certain threshold. This threshold is reached at the three positions at three different time points. (E) Time to reach the threshold at distance x. The values for the three positions x1, x2, and x3 are indicated by colour-coded dots. The slope of the curve reflects the velocity of signal propagation; with increasing distance x, not only the amplitude (C), but also the velocity of the signal diminishes. Note: due to signal damping, $V(x,t)$ will, after some distance, no longer reach the threshold, thus defining the 'threshold horizon', the range of the signal. (F–H) The computational simulations repeated with modified parameters

APs, an electrical wave along the phloem would not reach far. Consequently, for long-distance propagation, ion channels with special features are required.

APs along an axon are transmitted from one segment to the neighbouring one. The major drivers of this process are voltage-dependent ion channels, which cluster at high densities at specific sites along the axon. Along the wire, the incoming depolarising stimulus activates voltage-gated sodium (NaVs) or calcium (CaVs) channels. Na^+ or Ca^{2+} influx, along their transmembrane gradients, further depolarises the membrane, amplifying the incoming signal. The resulting signal is large enough to propagate passively to the next segment, where it again activates NaVs or CaVs for re-amplification. Subsequent opening of depolarisation-activated K^+ channels is key to the repolarisation of the membrane.

NaVs and CaVs, together with voltage-gated K^+ channels, constitute a superfamily of voltage-gated ion channels, characterised by their ability to rapidly respond to changes in the membrane potential. Sodium and calcium currents are also found in algae [22,23], indicating that voltage-activated Na^+ channels have the capacity to similarly generate fast Na^+-based APs in unicellular photosynthetic organisms. However, vascular plants, and even the model plant for electrical excitability, the Venus flytrap *D. muscipula*, lack NaVs and CaVs.

How Do Plant Cells Become Excited?

Early electrophysiological studies on giant algae, such as *Chara*, suggested that an AP is initiated by the opening of anion channels [24]. Nonetheless, the molecular entities of this are still obscure. The model plant *A. thaliana* expresses QUAC1, a voltage-dependent R-type anion channel [25–28]. Just like NaV channels, R-type anion channels activate rapidly in response to depolarisation and undergo slow inactivation during ongoing voltage stimulation. Thus, R-type anion channels may be suited for participating in the initial phase of the plant AP. Alternatively or in addition, Ca^{2+} channel opening may contribute to the depolarisation phase in two ways: (i) the influx of Ca^{2+} ions directly contributes to depolarisation [29]; or (ii) cytosolic Ca^{2+} increases stimulate Ca^{2+}-activated anion channels through channel phosphorylation by Ca^{2+}-activated protein kinases, as is the case for the CPK and CBL/CIPK-dependent gating of SLAC/SLAH anion channels [30,31]. The Nernst potentials for anions and Ca^{2+} are significantly more positive than for K^+ (E_K), which is the primary determinant of the resting potential of the cell. Thus, as in *Chara*, the transmembrane anion and Ca^{2+} gradients are well suited for locally amplifying the incoming depolarisation signal.

Hodgkin and Huxley discovered that the falling phase of APs in the squid giant axon is initiated by the opening of depolarisation-activated K^+ release (Kv) channels, which causes a repolarisation and finally terminates the Na^+ channel-initiated animal AP [3]. Archaebacteria also have a Kv channel that is both functionally and structurally similar to eukaryotic Kv channel family members, indicating that the molecular structures underlying both ion selectivity and voltage-dependent gating in Kv channels are highly conserved [32,33]. Indeed, similar voltage-gated K^+ channels are found in higher plants. For instance, *A. thaliana* has nine Kv-like channels [34]; two (SKOR and GORK) are outward rectifiers, just like animal Kv channels [35–37]. GORK is ubiquitously

r_i (F), r_m (G), or $c_{m'}$ (H), but otherwise under identical global conditions. Parameter changes reflect the conditions in the 'red cable', axon, and 'green cable', the phloem sieve elements. For comparison, the results from (E) with the initial parameters are shown in black. In axons, r_i is minimised, and the myelin sheath increases r_m and decreases $c_{m'}$ in the red cable. Sieve elements are connected via sieve pore-containing sieve plates, which represent a significant contribution to r_i, while connections via plasmodesmata to peripheral cells reduce r_m and increase $c_{m'}$ in the green cable. All red- or green-specific adjustments produce the same effects: the red cable is characterised by faster signal propagation and a larger signal range (higher threshold horizon), whereas in the green cable, signal propagation is slowed and its range is reduced. (K) The active phloem cable is recharged by different batteries, that is, ion gradients across the membrane. An important role has already been uncovered for the 'potassium battery' E_K.

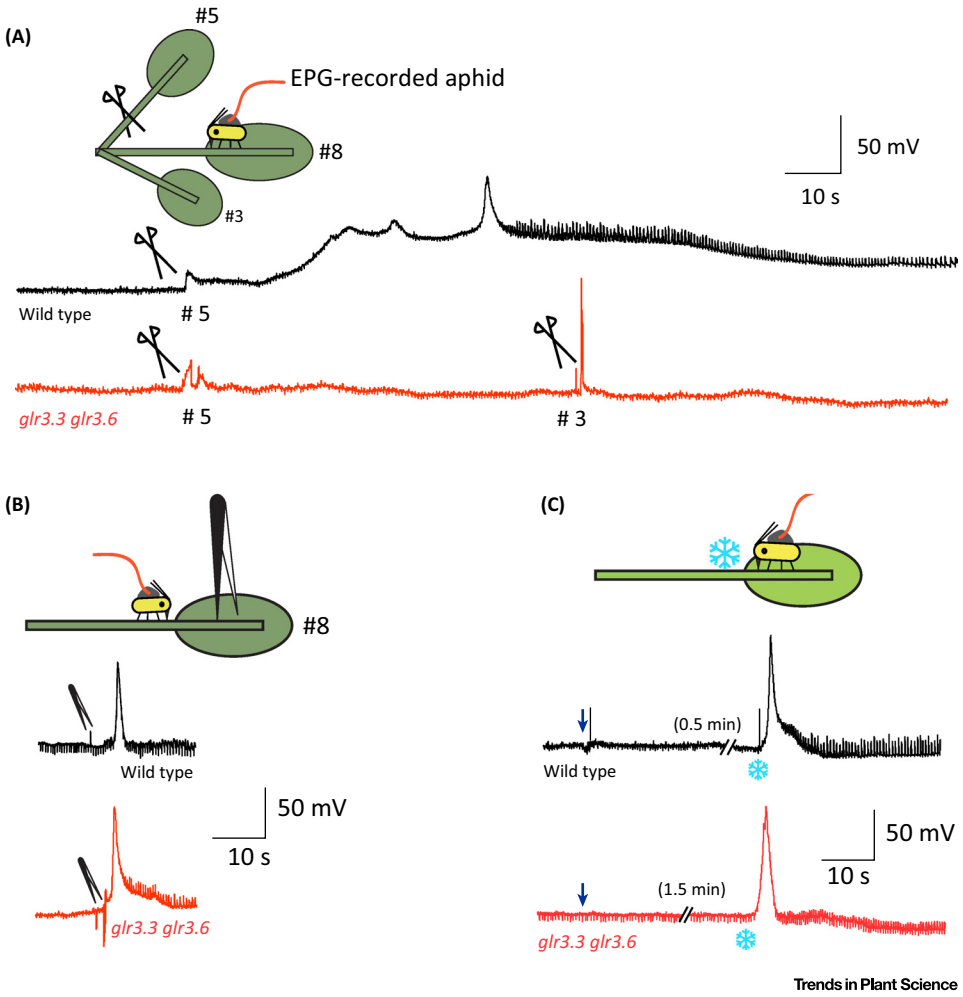


Figure 2. Wound and Cold-Induced Electrical Signals in the Phloem of *Arabidopsis thaliana*. (A) Leaf-to-leaf phloem transmission of wound-induced electrical signals. Black trace: segment of an electrical penetration graph (EPG) trace in E2 phase (ingestion phase) from a wild-type arabidopsis leaf sieve element, recorded during the cutting of a neighbour leaf. Cutting-induced typical electrical signal with slow and fast depolarisations. Red trace: segment of an EPG trace in E2 phase from a glutamate receptor-like (*glr*)*3.3 glr3.6* arabidopsis leaf sieve element, recorded during the cutting of two neighbour leaves. In this case, no electrical signals were induced in the EPG-recorded sieve elements in unwounded leaves. (B) Small wounds inflicted on the midvein in the vicinity of the EPG-recorded aphid (<1 cm away) induced fast action potentials in the sieve elements of both wild-type and *glr3.3 glr3.6* plants. (C) Representative action potentials in wild-type and *glr3.3 glr3.6* plants induced by application of a droplet of cold water <1 cm away from the EPG-recorded aphid in the sieve element-feeding mode. Cartoons illustrate the implementation of the experimental design. The times of application of the stimuli are indicated by corresponding symbols.

present in the vascular tissue of the shoot and root, and another Kv-like channel, the phloem-expressed AKT2/3, is able to mediate both potassium uptake and efflux. AKT2/3 has been shown to contribute to phloem reloading of photo-assimilates (Box 2) and is proposed to be involved in membrane repolarisation [38]. Phloem electrophysiology with channel loss-of-function mutants should tell us whether AKT2/3 and/or GORK contribute to electrical waves travelling along the green cable.

The plant AP could be generated by depolarisation via anion release through QUAC1-type channels, with the subsequent repolarisation resulting from K^+ efflux mediated by depolarisation-activated SKOR/GORK-type potassium channels. Nevertheless, we still lack data that: (i) allow reliable predictions regarding the molecular bases of the processes underlying the initiation

Box 2. The 'Potassium Battery'

Voltage-gated K^+ channels are essential entities of the electrical circuits in axons; they are involved in the repolarisation of APs. Voltage-gated K^+ channels are also found in the phloem cable in plants [66]. Among the nine Kv-like channels from arabidopsis, the inward-rectifying K_{in} channels KAT1 and KAT2 and the outward-rectifying K_{out} channel GORK are associated with this tissue by signals from promoter–reporter fusion experiments and phloem cell-specific expressed sequence tags (ESTs) [66–68]. However, the most abundant expression in the phloem is observed for AKT2/3 [19,69–71], a K^+ channel type that appears to have an important role in phloem loading and unloading [19,72,73]. AKT2/3 has unique gating properties and can operate in two different modes. In mode 1, it is an inward-rectifying channel that allows H^+-ATPase-energised K^+ uptake, while in mode 2, it is an open, K^+-selective channel [74]. It can switch between the two modes via reversible phosphorylation affecting the voltage sensor of the channel [75–77]. Toggling AKT2-like channels from mode 1 to the voltage-independent mode 2 taps a 'potassium battery' (E_K, Figure 1K, main text), providing additional energy for transmembrane transport processes. The battery is charged under energy (ATP) consumption by a hyperpolarising proton pump (AHA2) and inward-rectifying K^+ channels (AKT2/3 in mode 1 and/or KAT1/2 [67]). The K^+ ions are then circulated in the phloem and serve as a decentralised energy store. This energy can be exploited to overcome local energy limitations by regulation of AKT2/3-like channels [78].

and propagation of the plant AP; and (ii) provide detailed information about voltage-gated channels clustering in plant cells in general, and in phloem cells or zones in particular. Are there regions equivalent to the axonal nodes of Ranvier? To figure out which ion channels are essential for signal re-amplification, a first step would be to test loss-of-function mutants in voltage-dependent K^+ and anion channels for impaired phloem excitability.

Signals and Receptors in Plant Electrical Circuitry

When leaves are crushed, nucleotides and other metabolites are released from the wound. In mammals, extracellular ATP is an essential signalling molecule that is perceived by P2-type purinoceptors in the plasma membrane. Extracellular ATP also has a critical role in plants in stress responses. Interestingly, the arabidopsis mutant does not respond to nucleotides 1 (*dorn1*) is insensitive to ATP [39]. *DORN1* encodes a legume-type lectin receptor kinase that is structurally distinct from the mammalian pain P2X-type ATP receptors. Nevertheless, DORN1 binds ATP with high affinity and is required for the ATP-induced calcium response to wounding.

In addition to wounding, cold, salt, and osmotic stresses also trigger electrical and calcium waves that can travel long distances [40]. In contrast to animals, plants do not have heat- or cold-activated channels of the 'transient receptor potential' (TRP) protein type [41–43]. Nonetheless, in arabidopsis mesophyll cells, cold transiently activates calcium-permeable channels [44,45] and in rice, the protein COLD1 confers chilling tolerance. COLD1 localises to the plasma membrane and, at low temperatures, it may be the as yet unknown calcium permeable channel or at the least, a cold-sensing auxiliary subunit of it [46]. It will be interesting to see whether and how the loss of COLD and DORN1 feed back on phloem electrical cold and wound signalling.

Salt stress-induced Ca^{2+} waves are associated with long-distance root-to-shoot signalling in plants [47,48]. These Ca^{2+} waves seem not to propagate along the phloem, but instead along the cortex and endodermal cell layers, and this movement appears dependent on the Two-Pore-Channel 1 (TPC1) [48]. TPC1 homologues are found in animals and plants, and, in both branches of the tree of life, TPC1 is located in endomembrane systems (reviewed in [49]). In plants, TPC1 operates as a vacuolar voltage-dependent nonselective cation channel that is blocked by luminal calcium ions [50]. The arabidopsis mutant *fou2* expresses a hyperactive TPC1 version (TPC1-D454N), which is insensitive towards calcium in the vacuole lumen. It has elevated jasmonate levels and behaves like a constitutively wounded plant [51].

GLRs Mediate Wound Electrical Phloem Excitement But Do Not Drive Phloem APs

Wounded leaves communicate their damage via distal production of jasmonates into defence responses. Ted Farmer's group discovered that the GLUTAMATE RECEPTOR-LIKE genes

GLR3.2, *GLR3.3*, and *GLR3.6* mediate leaf-to-leaf wound signalling in arabidopsis [13,52]. Plant glutamate receptor-like genes (GLRs) are homologous to the genes for mammalian ionotropic glutamate receptors (iGluRs) [53]. Using surface electrodes, the authors mapped surface potential changes after wounding and the movement of electrical signals from the wounded leaf to an adjacent undamaged leaf. Mutations in the genes *GLR3.2*, *3.3*, or *3.6* reduced the duration of wound-induced surface potential changes in both the damaged and the undamaged neighbouring leaf. Furthermore, in a *glr3.3 glr3.6* loss-of-function double mutant, wounding still transiently depolarised the wounded leaf with the same amplitude as in the wild type, but did not affect the nonwounded neighbour leaves [13]. In addition, jasmonate-response gene expression in leaves distal to wounds was reduced. Using EPG aphid electrodes for phloem-selective recording, Salvador-Recatalà *et al*. [12] demonstrated that leaf wounding of the *glr3.3 glr3.6* double mutant was not electrically transmitted to no-damaged leaves, while in wild-type plants, wounding of one leaf caused a long-lasting depolarisation superimposed by an AP in the neighbouring leaf. (Figure 2A) [12]). When measuring the phloem potential directly in the wounded leaf, both the wild type and the *glr3.3 glr3.6* double mutant produced fast transient electrical depolarisations (Figure 2), consistent with the results of Mousavi *et al*. [13] using surface electrodes. These findings suggest that GLR3.3 and 3.6 are not essential for the elicitation and propagation of fast APs, but are important in channelling the signal from damaged to undamaged leaves [52]. Thus, the questions in this context are whether the ligand of the two GLRs is released at the junction between the wounded leaf and nonwounded stem, and whether it travels through the phloem.

Is the Vacuolar Cation Channel TPC1 Involved in Phloem APs?

Sieve elements do not have vacuoles, so it is unlikely that the ubiquitously expressed vacuolar cation channel TPC1 [49] has a major role in phloem electrical signalling. Nevertheless, the vacuolar cation channel TPC1 has been reported to be essential for the systemic propagation of cytosolic Ca^{2+} waves elicited by wounding or salt stress [14,48]. In leaves, wounding induces a local increase in the cytosolic Ca^{2+} level, while a systemic response is only observed when the midrib is wounded. This systemic response is exclusively found in wild-type plants; it is undetectable in the *tpc1*-knockout plant. Interestingly, the local response is not depressed, but the local signal appears to be of longer duration in the mutant. It has been proposed that the TPC1-dependent Ca^{2+} wave propagation is channelled through the cortex and endodermal cell layers with a velocity of up to approximately 400 $\mu m\ s^{-1}$ [54].

The observation that wounding induces both electrical signalling and Ca^{2+} waves in symplastically connected tissues is intriguing because it points to a complex electrochemical interplay. Ca^{2+} channels activated by an electrical stimulus can contribute to the Ca^{2+} signal, which in turn can induce or reinforce an electric signal by ion channels being directly or indirectly activated by Ca^{2+}. Ca^{2+} may not be the only chemical signalling ion or molecule. By using diverse membrane transporter proteins, manifold mixed chemical signals might be conjectured. The chemical stimulus may even enable membrane proteins in endomembranes, such as TPC1, to contribute to the systemic plant response. This would allow the specific release of compounds stored in organelles.

Concluding Remarks and Future Directions

Here, we have shown and discussed that stress-induced electrical signals are transmitted along the phloem cable network. It is likely that the network operates input sensors and ion channels to depolarise the phloem in stimulated, distinct zones of affected leaves. Once the strength-related depolarisation reaches a threshold, another set of ion channels is most likely activated, which initiates the AP and excites the phloem. APs that propagate from the affected leaf to the unaffected leaves have to be translated into a systemic signal. This process has been shown to involve calcium signalling. Deeper insights into these phenomena will be gained by obtaining further detailed information on the phloem signalling network (see Outstanding Questions).

Outstanding Questions

What is the molecular nature of the phloem network? Our knowledge of the players involved in phloem signalling is still rudimentary.

What does the 4D pattern of phloem excitement look like? Despite significant progress, measurements of electrical phloem signals still lack satisfying spatial and temporal resolution.

Answering these questions will require a combination of methods and the development of new tools: (i) how can we identify the molecular nature of the phloem network? In leaves from *Plantago* species, vascular bundles can be mechanically isolated and examined for the expression of receptors and channels [55] at rest and after long-term stimulation via wounding (ATP), cold, and osmotic stress. Isolated plantago bundles could be used to study the characteristics of AP transmission in the phloem cable. With the model plant arabidopsis and other plants where vascular strands resist quick isolation, laser microdissection may be ideal [56]. In addition, localisation studies can be carried out with tagged candidate receptors and channels (e.g., with a GFP tag) that are expressed under their own promoter. Properties of phloem-expressed channels need to be scrutinised in plant and nonplant expression systems; (ii) how can we monitor the 4D pattern of phloem excitement? The phloem network of a leaf is branched and comprises a midvein as well as first- and second-order strands (see Figure IA in Box 1). To monitor AP transmission with high spatial and temporal precision, video imaging would be the technique of choice. This approach takes advantage of genetically encoded membrane potential sensors (voltage-sensing fluorescent proteins, VSFPs) [57,58], where the membrane-bound voltage-sensing part of ion channels or phosphatases is fused to a tandem of fluorescent proteins, such as CFP and YFP, to create a FRET sensor. This sensor construct can be expressed under the phloem-specific SUC2 promoter; promoters for phloem-adjacent cell types would add additional information about the electrical activity within the vasculature and common leaf cells. When coexpressed with genetically encoded calcium sensors [59], the electrical and calcium waves could be monitored in parallel; (iii) screens for mutants defective in electrical signalling. There are two approaches to associate a certain receptor or ion channel to stimulus-dependent phloem-travelling APs: the first is a biased approach testing loss-of-function mutants and overexpressor plants for AP abnormalities, whereas the second is an unbiased approach that tests mutant populations of VSFP-expressing plants for different electrical phenotypes. It is likely that these screens for mutants in phloem electrical signalling would provide new insights into the membrane receptors and channels of the green circuit.

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