Evidence for the Involvement of Electrical, Calcium and ROS Signaling in the Systemic Regulation of Non-Photochemical Quenching and Photosynthesis

Maciej Bialasek1, Magdalena Górecka1, Ron Mittler2 and Stanislaw Karpinski1,*

1Department of Plant Genetics, Breeding and Biotechnology, Faculty of Horticulture, Biotechnology and Landscape Architecture, Warsaw University of Life Sciences SGGW, Nowoursynowska 159, 02-776 Warsaw
2Department of Biological Sciences, College of Arts and Sciences, University of North Texas, Denton, TX 76203-5017, USA
*Corresponding author: E-mail, stanislaw_karpinski@sggw.pl; Fax, +48225932152.
(Received May 9, 2016; Accepted December 24, 2016)

In contrast to the function of reactive oxygen species, calcium, hormones and small RNAs in systemic signaling, systemic electrical signaling in plants is poorly studied and understood. Pulse amplitude-modulated Chl fluorescence imaging and surface electrical potential measurements accompanied by pharmacological treatments were employed to study stimuli-induced electrical signals in leaves from a broad range of plant species and in Arabidopsis thaliana mutants. Here we report that rapid electrical signals in response to a local heat stimulus regulate systemic changes in non-photochemical quenching (NPQ) and PSII quantum efficiency. Both stimuli-induced systemic changes in NPQ and photosynthetic capacity as well as electrical signaling depended on calcium channel activity. Use of an Arabidopsis respiratory burst oxidase homolog D (RBOHD) mutant (rbohD) as well as an RBOH inhibitor further suggested a cross-talk between ROS and electrical signaling. Our results suggest that higher plants evolved a complex rapid long-distance calcium-dependent electrical systemic signaling in response to local stimuli that regulates and optimizes the balance between PSII quantum efficiency and excess energy dissipation in the form of heat by means of NPQ.

Keywords: Calcium signaling • Chlorophyll fluorescence • Electrical signals • Photosynthesis • Reactive oxygen species • Systemic acquired acclimation.

Abbreviations: AP, action potential; DMSO, dimethyl sulfoxide; DPI, diphenyleneiodonium chloride; NPQ, non-photochemical quenching; PAM, pulse amplitude-modulated; PET, photosynthetic electron transport; PQ, plastoquinone; QA, quinone A; Qb, quinone B; qP, photochemical quenching; RBOHD, respiratory burst oxidase homolog D; ROS, reactive oxygen species; SAA, systemic acquired acclimation; SAR, systemic acquired resistance; SP, system potential; VP, variation potential; WT, wild type; Y(II), PSII quantum efficiency.

Introduction

Being constantly tied to one habitat, plants developed complex physiological and molecular mechanisms that enable them to communicate efficiently between their different leaves and organs. Systemic signaling pathways such as systemic acquired resistance (SAR) and systemic acquired acclimation (SAA) have been intensively studied, with many reports supporting a substantial role for reactive oxygen species (ROS), hormones and, recently, small RNA molecules in long-distance signaling. Nevertheless, the role of electric signals has in recent years been neglected. Electric signals in plants were discovered in the 19th century in Dionaea muscipula (Burdon-Sanderson 1872). Subsequent research revealed that in contrast to animals, plants evolved only a few types of electrical communication, which have distinct characteristics. At least three types of electrical signals have been described to date: action potential (AP), variation potential (VP) and system potential (SP) (Zimmermann et al. 2009).

An AP consists of depolarization, repolarization and hyperpolarization phases, and requires a stimulus strong enough to reach a particular threshold. The mechanism of AP generation relies on ion transport across the membranes, particularly Ca2+, K+ and Cl−, and it is the fastest known form of electrical communication in plants. It is propagated, within a few seconds, over a long distance and is responsible, for example, for the immediate reaction of traps in carnivorous plants. The preferred potential medium for AP signals are sieve elements, as they ensure continuous, good communication from cell to cell via plasmodesmata (van Bel and Ehlers 2005). AP generation is usually associated with non-damaging stimuli, which can ultimately affect phloem transport, gas exchange or gene expression (Fromm and Bayer 1994, Fromm and Lautner 2007).

A VP is a slow propagating type of signal, which is generated upon an injurious stress treatment. The membrane depolarization and repolarization cycle takes several minutes. In contrast to AP, VP amplitude positively correlates with the stimulus strength. VP propagation is correlated with changes in the hydraulic pressure mainly in xylem vessels, and fades away with distance from the point of origin (Malone 1992, Stankovic et al. 1997, Stahlberg et al. 2005). The underlying mechanism of VP employs mainly perturbations of H+-ATPase activity (Stahlberg et al. 2006). VP affects hormone emission and gene expression (Wildon et al. 1992, Dziubinska et al. 2003).
Unlike the initial depolarization that accompanies the generation of AP and VP, SP primary polarity is reversed (Zimmermann et al. 2009). SP can be evoked by wounding as well as heat stimulation (scorching), and its induction and spread depend mainly on cations (Zimmermann and Mithöfer 2013). It is noteworthy that a close relationship between SP propagation and NADPH oxidase was recently reported, as plants devoid of functional NADPH oxidase (rbohD) had a suppressed capability to mediate SP (Miller et al. 2009, Suzuki et al. 2013). This finding suggested a close link and cross-talk between ROS and this type of electrical signaling.

There are two main approaches for measuring plant electrical signaling, which deliver different information. The intracellular method uses a glass microelectrode, filled with electrolyte. The microelectrode is carefully inserted into a living cell and can be used for the measurement of absolute values of cell voltage potential (Krol et al. 2004, Szczynska-Hebda et al. 2010, Sukhov et al. 2014). In contrast to intracellular measurements, the extracellular approach delivers only relative values of electrical potentials. Extracellular measurements include invasively inserting the electrode directly into the plant material, usually employed with woody plants (Rios-Rojas et al. 2014), or non-invasively placing the electrode on the surface of the plant with an intermediate ensuring good electrical contact (Mousavi et al. 2013, Suzuki et al. 2013). The second method is widely used, as it eliminates wounding and is relatively easy.

Systemic changes in Chl fluorescence parameters were reported for the first time during the discovery of SAA (Karpinski et al. 1999). However, the cause and the effects of these changes are poorly understood. For example, it is unknown whether the transient systemic suppression of photosynthesis upon local stimulation is just a symptom of stress in plants, or whether it carries any information to the unstressed parts of the plant, alerting them to an upcoming danger. An association between electrical signaling, calcium channel activity, ROS and photosynthetic parameters, and propose their reciprocal link and cross-talk between ROS and this type of electrical signaling.

Results and Discussion

We assessed values of PSII quantum efficiency [Y(II)], non-photochemical quenching (NPQ) and photochemical quenching (qP) upon a local and punctual, damaging heat stimulus. Y(II) values decreased in a concentric manner, while NPQ and qP values increased starting from the site of treatment. Changes in these parameters were initially observed in veinal tissues of leaves and then spread through the mid-veinal regions (Fig. 1; Supplementary Video S1).

Proper functioning of PSII, reflected in normal Chl a fluorescence parameters, depends on accurate electron transport via the photosynthetic electron transport (PET) chain. The electron transport chain is in turn susceptible to temporary perturbations, and at the same time has a broad range of plasticity to ensure efficient operation of further photosynthetic processes. Due to the many carriers involved, there are several different factors which can truncate PET (for reviews, see DeEll and Toivonen 2003, Foyer et al. 2012).

To test whether electrical signaling accompanied the changes in photosynthetic parameters, surface potential (Mousavi et al. 2013, Suzuki et al. 2013) and photosynthesis changes were monitored in dandelion (Figs. 1, 2). The results showed that following the initial hyperpolarization recorded, repolarization occurred at different time points with respect to the proximal and distal electrodes (in reference to the site of stress; while the electrodes were on the same leaf; Fig. 2a). Potential changes recorded by electrodes placed at different distances from the site of stress application indicated that the electrical signal spread within a few minutes, and that the hyperpolarization, and then repolarization was delayed on the distal electrode. This, together with a reduced amplitude of hyperpolarization detected by the distal electrode in comparison with that recorded on the proximal electrode (Fig. 2a), suggested that the tracked electrical events are SPs. Similar measurements were performed with Arabidopsis. Despite no indication, or weak insignificant changes of fluorescence disturbance upon local treatment, surface potential changes were clearly detectable with the same trends as in dandelion (Fig. 2c).

A link between surface potential changes and photosynthesis was studied using LaCl3, diphenyleneiodonium chloride (DPI) and DCMU. LaCl3 is a widely known Ca2+ channel blocker, and is commonly used in electrophysiology (Sharma et al. 1992, Friedman et al. 1998). Moreover the ROS-generating activity of respiratory burst oxidase homolog D (RBOHD) is regulated by Ca2+ ions directly (Takeda et al. 2008, Kimura et al. 2012) as well as via post-translation modification by kinase activity (Drerup et al. 2013, Li et al. 2014, Monaghan et al. 2014). Our results clearly show that regulation of PSII operation in response to the focused heat stimuli depended on calcium channel activity, as LaCl3 application to the leaf led to a significant delay in systemic changes of Chl a fluorescence (Fig. 3; Supplementary Fig. S1a–c), that was accompanied by surface potential delay and amplitude reduction (Fig. 2a, b; Supplementary Fig. S1d).

To confirm that the surface potentials measured in our experiments were SPs, rbohD mutants were exposed to a local heat stimulus. Similar to the findings of Miller et al. (2009) and Suzuki et al. (2013), electrical signals in rbohD mutants were significantly less pronounced than in the wild type (WT), but definitely detectable (Fig. 2d; Supplementary Fig. S1e). These observations indicated that the stimulus applied in our work generated a similar type of electrical signal associated with RbohD function. In order to examine the RbohD function in dandelion, we used the flavin oxidase inhibitor DPI. The application of DPI blocked the spreading of Chl fluorescence changes following heat stimulation (Fig. 4); however, it did not stop...
These results suggest that extracellular propagation of the systemic electrical signaling depends on plasma membrane calcium channels, while intracellular retransmission of these signals into PSII reaction centers may depend on ROS signaling. Such an interpretation is the simplest one to explain cross-talk between electrical and ROS systemic signaling and is in agreement with a recently published integrated model of the ROS, calcium and electrical wave-like systemic signaling presented by Gilroy et al. (2016).

In a previous work, we showed that partial illumination of leaves with excess light led to the generation of intercellular electrical potential changes from the exposed leaf to systemic shaded leaves (Szechynska-Hebda et al. 2010). To exclude any contribution of PET activity to the measured surface potential, we employed a pharmacological treatment with DCMU, a known and specific PET inhibitor. DCMU binds to the quinone B- (Q_B) binding pocket and blocks the oxidation of quinone A (Q_A) and subsequent reduction of plastoquinone (PQ). It results in a disturbance in F_m and F_o, which leads to strong...
reduction of NPQ (Escoubas et al. 1995, Kulasek et al. 2016). In our experiments, DCMU application also resulted in a strong decrease of NPQ in the leaf area exposed to this inhibitor; however, it did not disturb spreading of Chl fluorescence changes across the treated region (Fig. 5). This result suggests that the specific inhibition of PET at the Q_b side does not inhibit propagation of calcium- and RbohD-dependent systemic electrical and ROS signaling from local to systemic PSII reactions centers.

In an attempt to examine how widespread the link is between electrical signaling and changes in photosynthetic parameters, we measured it in a variety of different plants. In these studies, an effort was made to check many species from different families for the response of photosynthesis to the stimulus mediated by electrical signals. Using the same experimental design, photosynthetic parameters were evaluated in plants from natural and laboratory conditions exposed to local heat stress. The velocity and area of the plants’ reaction varied in different species. Clear similar effects were observed in Aesculus hippocastanum, Hieracium pilosella, Plantago major, Populus maximowiczii, Rumex acetosa, Syringa vulgaris,

![Fig. 2](image_url)

**Fig. 2** Leaf surface electrical potential changes following heat stimulation of the tip of the leaf. (a and b) Effect of lanthanum chloride (LaCl_3) on electrical signal propagation. Representative potentials recorded with two electrodes placed on the same leaf irritated by heat: the proximal electrode on the area not treated directly (Non-treated) and the distal electrode on the area directly treated by solution: (a) without inhibitor (Mock) or (b) with LaCl_3. (c) Comparison of electrical signals in Arabidopsis and dandelion. Representative potentials recorded on Arabidopsis and dandelion leaves. (d) Effect of Arabidopsis RBOHD gene mutation on electrical signal propagation. Representative potentials recorded on rbohD plants vs. the WT. The arrows indicate the moment of heat stimulation of the tip of the leaf for 1 s by the flame of a lighter. Statistical significance of observed changes is shown in Supplementary Fig. S1.
Taraxacum officinale, Tilia cordata and Tilia platyphyllos (Supplementary Fig. S2). In contrast, there was weak or no detectable reaction in A. thaliana, Capsicum annuum, Cucurbita pepo, Ginkgo biloba, Nicotiana benthamiana and Nicotiana tabacum (Supplementary Fig. S2). Using the same experimental design for different plant species from evolutionarily distant families, we revealed that there are substantial differences between various species of higher plants in PSII sensitivity or in response to the local stimulation that triggered systemic electric, calcium and ROS signaling that regulates NPQ changes in a non-directly stressed PSII reaction centers. We further examined whether systemic fluorescence changes are characteristic for plants grown in natural, variable conditions.

We found that Arabidopsis and tobacco did not exhibit spreading of transient photosynthesis signals when grown in the field (data not shown). In contrast, dandelion retained the ability to accommodate systemic changes of fluorescence parameters upon local stimulus even when grown in a controlled, laboratory environment (data not shown). These results may suggest that plants such as Arabidopsis and tobacco lost their ability to accommodate systemic changes in photosynthesis as a result of many years of cultivation of subsequent generations in non-natural, controlled conditions. It is also possible that differences in leaf architecture (i.e. thickness, cuticle layer, presence of trichomes, etc.), photosynthetic apparatus composition and chloroplast morphology could have led to the observed differences in systemic signaling while responding to a local stimulus or affected the surface potential recordings. In addition, it is possible that the methods used in our study were not sufficient to detect significant responses of insensitive plants.

Fig. 3 Changes in Chl a fluorescence of a dandelion leaf treated with a calcium channel blocker (LaCl3) following heat stimulation. (a) Spatiotemporal changes of NPQ, Y(II) and qP assessed by Chl fluorescence imaging. Arrow indicates the area stimulated for 1 s with a flame-heated metal wire. The black rectangle shows the area treated with LaCl3. The time is shown in mins format (heat stimulation was performed at 0:00). Scale bar = 1 cm. The false color scale represents values of assessed parameters. (b) NPQ, (c) Y(II) and (d) qP changes measured by Chl fluorescence imaging at representative areas [shown in (a) as black circles] of treated (Area 2 represented by circle 2) and untreated (Area 1 represented by circle 1) parts of the leaf at a similar distance from the stimulated region. Representative data of five independent experiments are shown. The statistical significance of the observed changes is shown in Supplementary Fig S1.

Speculations on the mechanism of calcium influence on photosynthesis are rather difficult. For example, it is hard to explain how the disturbance of NPQ occurs, because the precise mechanism of quenching is still under discussion (Duffy and Ruban 2015). The agricultural significance of these processes is extremely important, as recent studies clearly showed that adequate manipulations of the quenching machinery may lead to a substantial increase in biomass production (Kromdijk et al. 2016).

The nature of electrical signaling in plants is very complex, and its role remains poorly understood. It seems to interact with some of the other main players in rapid signaling such as ROS and calcium waves (Gilroy et al. 2016). Its involvement
in SAA and SAR and impact on photosynthesis and systemic communication between photosystems of remote chloroplasts and cells strongly suggests that proper electrical communication as well as cross-talk with ROS signaling is essential for plant survival under conditions of natural multivariable stresses and stimuli. Our research shows that at the molecular level calcium ion channels directly link stimuli-induced transient changes in photosynthesis with electrical signaling and these findings are in agreement with the recently presented model (Gilroy et al. 2016).

**Materials and Methods**

**Plant material and growth conditions**

*Arabidopsis thaliana* Col-0 and the rbohD mutant (Torres et al. 2002) were grown on Jiffy pots (Jiffy products) in a growth chamber (23 °C, 8 h light/16 h dark with a light intensity of 100 ± 15 µmol photons m⁻² s⁻¹). *Nicotiana benthamiana*, *N. tabacum* and *T. officinale* were grown on soil mixed with perlite (2:1) in a growth chamber (23 °C, 16 h light/8 h dark with a light intensity of 100 ± 15 µmol photons m⁻² s⁻¹). Plants at 6–8 weeks old were used for experiments. Whole, fully developed and undamaged detached leaves of *A. hippocastanum*, *C. annum*, *C. pepo*, *G. biloba*, *H. pilosella*, *P. major*, *P. maximowiczii*, *R. acetosa*, *S. vulgaris*, *T. officinale*, *T. cordata* and *T. platyphyllos* growing in their native environment or field conditions (collected from June to August) were used for Chl a fluorescence imaging. After detachment, the petiole of the leaf was put in tap water for 30 min to 1 h before the beginning of the experiment.

**Chl a fluorescence imaging**

Spatiotemporal Chl a fluorescence was measured using an imaging Chl fluorometer (IMAGING-PAM MINI, Walz). Imaged leaf area was 32 mm x 24 mm. The plants were kept in darkness for 30 min, then blue (470 nm) actinic light (60 µmol photons m⁻² s⁻¹) was switched on for 12 min. To measure *Fₘ* and *F₅ₐₐₜ*, saturating pulses were applied (6,000 µmol photons m⁻² s⁻¹, duration 800 ms). Non-photochemical quenching, NPQ = (*Fₘ* - *F₅ₐₐₜ*)/*Fₘ*, effective quantum yield of PSII, Y(II) = (*F₅ₐₐₜ* - *Fₐₐₜ*)/*Fₘ*, and photochemical quenching, qP = (*Fₘ* - *Fₐₐₜ*)/*Fₘ* were determined according to the manufacturer’s instructions and as described previously (Baker 2008, Gawroński et al. 2014). Changes in these parameters during the experiment were monitored by applying saturating pulses at 10 s intervals. Four minutes following the beginning of the measurement the leaf was stimulated by touching for about 1 s with a 1 mm thick metal wire heated with the flame of a lighter for approximately 3 s. For control measurement, an unheated metal wire was used. To determine the transience of the fluorescence changes after heat stimulation, the same measurement procedure was carried out twice after stimulation, each preceded by 15 min of dark adaptation.

![Fig. 4](image-url) Changes in Chl fluorescence of a dandelion leaf treated with a RBOHD inhibitor (DPI) following heat stimulation. (a) Spatiotemporal changes of NPQ, Y(II) and qP assessed by Chl fluorescence imaging. The arrow indicates the area stimulated for 1 s with a flame-heated metal wire. The black rectangle shows the area treated with DPI. Time is shown in mins format (heat stimulation was performed at 0:00). Scale bar = 1 cm. The false color scale represents values of assessed parameters. (b) NPQ, (c) Y(II) and (d) qP changes measured by Chl fluorescence imaging at representative areas [shown in (a) as black circles] of treated (Area 2 represented by circle 2) and non-treated (Area 1 represented by circle 1) parts of the leaf at a similar distance from the stimulated region. Representative data of five independent experiments are shown.
Pharmacological treatments

LaCl₃ (1 mM; Acros), 500 μM DPI (DPI, Sigma Aldrich) or 500 μM DCMU (Sigma Aldrich) solutions were made in deionized water and contained in addition 0.2% (v/v) dimethyl sulfoxide (DMSO; Sigma Aldrich) and 0.1% (v/v) Tween-20 (Sigma Aldrich). Half of the surface of the leaf was submerged in LaCl₃ solution for 18 h prior to the experiment. DPI and DCMU were sprayed on the leaf surface 30 min prior to the experiment. DPI was used on half of the surface of the leaf and DCMU was used on a smaller area in the center of the leaf. For control experiments, a mock solution that contained only DMSO and Tween-20 was used. The concentrations of agents were chosen based on the literature and adjusted to our particular experimental design.

Surface potential recordings.

Surface potential was measured using a glass microelectrode filled with 1 M KCl connected to the leaf through a drop (10 μl) of 1 M KCl in 1% (w/v) agar placed 25 mm from the tip of the leaf, so that the electrode did not damage the cuticle. The ground electrode (Ag/AgCl) was placed in the soil. An Axoclamp 900A amplifier (Molecular Devices) was used to record the surface potential. Experiments were conducted in a Faraday cage in room temperature (22–25°C). A few minutes after connection of the electrodes to the leaf, the tip of the leaf was heat stimulated by the flame of the lighter for 1 s. The experimental set-up for electrophysiological measurements made it very difficult to touch the leaf during electrical potential recordings using the metal wire, avoiding any disturbance during assessment of the parameters.

Additionally, an ImagingPAM device was used for Chl fluorescence measurements which hampered the access of microelectrodes to the leaf.

Statistics and data analysis

All data analysis and statistics were performed using R (R Core Team, 2015). For data visualization, the R package ‘ggplot2’ was used (Wickham 2009).

Supplementary data

Supplementary data are available at PCP online.

Funding

This study was supported by funding from the National Science Centre project [UMO-2012/07/B/NZ3/00228 and UMO-2014/14/A/NZ1/00218 to S.K., M.B. and M.G.]; the POKL.04.03.00-00-042/12-00 programme co-financed by the European Social Fund [to M.B.]; the USA National Science Foundation [IOS-1353886, IOS-0639964 and IOS-0743954 to R.M.] and the University of North Texas, College of Arts [to R.M.]. The funders had no role in the design, data collection, analysis, decision to publish, or preparation of the manuscript.
Disclosures

The authors have no conflicts of interest to declare.

References

Baker, N.R. (2008) Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu. Rev. Plant Biol. 59: 89–113.

Burdon-Sanderson, J. (1872) Note on the electrical phenomena which accompany irritation of the leaf of Dionaea muscipula. Proc. R. Soc. Lond. 21: 495–496.

DeEll, J.R. and Toivonen, P.M.A. (2003) Practical Applications of Chlorophyll Fluorescence in Plant Biology. Springer US, Boston, MA.

Drezup, M.M., Schülling, K., Hashimoto, K., Manishankar, P., Steinhorst, L., Kuchitsu, K., et al. (2013) The Calcineurin B-like calcium sensors CBL1 and CBL9 together with their interacting protein kinase CIPK26 regulate the Arabidopsis NADPH oxidase RBOHF. Mol. Plant. 6: 559–69.

Duffy, C.D.P. and Ruban, A.V. (2015) Dissipative pathways in the photosystem-II antenna in plants. J. Photochem. Photobiol. B 152: 215–226.

Dziubinska, H., Filek, M., Koscielniak, J., and Trebacz, K. (2003) Variation and action potentials evoked by thermal stimuli accompany enhancement of ethylene emission in distant non-stimulated leaves of Vicia faba minor seedlings. J. Plant Physiol. 160: 1203–1210.

Escoubas, J.-M., Lomas, M., LaRoche, J. and Falkowski, P.G. (1995) Light intensity regulation of cab gene transcription is signaled by the redox state of the plastoquinone pool. Proc. Natl. Acad. Sci. USA 92: 10237–10241.

Fey, C.H., Neukermans, J., Queval, G., Noctor, G. and Harbinson, J. (2012) Photosynthetic control of electron transport and the regulation of gene expression. J. Exp. Bot. 63: 1637–1661.

Friedman, H., Meir, S., Rosenberger, I., Halevy, A.H., Kaufman, P.B. and Philosoph-Hadas, S. (1998) Inhibition of the graviresponse of snapdragon spikes by the calcium-channel blocker lanthanum chloride. Plant Physiol. 118: 483–492.

Fromm, J. and Bauer, T. (1994) Action potentials in maize sieve tubes change phloem translocation. J. Exp. Bot. 45: 463–469.

Fromm, J. and Lautner, S. (2007) Electrical signals and their physiological significance in plants. Plant Cell Environ. 30: 249–57.

Gawroński, P., Witó, D., Vashchuka, K., Ederska, M., Betliński, B., Rusacczonek, A., et al. (2014) Mitogen-activated protein kinase 4 is a salicylic acid-independent regulator of growth but not of photosynthesis in Arabidopsis. Mol. Plant. 7: 1151–1166.

Gilroy, S., Bialasek, M., Suzuki, N., Górecka, M., Devireddy, A., Karpinski, S., et al. (2016) ROS, calcium and electric signals: key mediators of rapid systemic signaling in plants. Plant Physiol. 171: 1606–1615.

Hlaváčková, V., Krchnák, P., Naus, J., Novák, O., Spundová, M. and Strnad, M. (2008) Electrical and chemical signals involved in short-term systemic photosynthetic responses of tobacco plants to local burning. Planta 225: 235–244.

Jabs, T., Dietrich, R.A. and Dangl, J.L. (1996) Initiation of runaway cell death in an Arabidopsis mutant by extracellular superoxide. Science 273: 1853–1856.

Karpinski, S., Reynolds, H., Karpinska, B., Wingsle, G., Creissen, G. and Mullineaux, P. (1999) Systemic signaling and acclimation in response to excess excitation energy in Arabidopsis. Science 284: 654–657.

Kimura, S., Kaya, H., Kawarazaki, T., Hiraoka, G., Senzaki, E., Michikawa, M., et al. (2012) Protein phosphorylation is a prerequisite for the Ca2+-dependent activation of Arabidopsis NADPH oxidases and may function as a trigger for the positive feedback regulation of Ca2+ and reactive oxygen species. Biochim. Biophys. Acta 1823: 398–405.

Kolář, V., Krchnák, P., Naus, J., Novačkova, O., Strnad, M. and Karpinski, S. (2013) Temporal–spatial interaction between reactive oxygen species and abscisic acid regulates rapid systemic acclimation in plants. Plant Cell 25: 3553–3569.

Kulašek, M., Bernacki, M.J., Ciszak, K., Witoń, D. and Karpinski, S. (2016) Contribution of PsbS function and stomatal conductance to foliar temperature in higher plants. Plant Cell Physiol. 57: 1495–1509.

Kronía, J., Głowacka, K., Bernacki, M., Malinowska, M. and Ciszak, K. (2012) RbohD to control plant immunity. Cell Host Microbe 15: 329–38.

Kostka, J.M. (2014) Kinetics of wound-induced hydraulic signals and variation potentials in wheat seedlings. Planta 187: 505–510.

Kulasek, M. (1992) Characteristics of electrical signals in poplar and responses in photosynthesis. Plant Physiol. 108: 2200–2209.

Li, L., Li, M., Yu, L., Zhou, Z., Liang, X., Liu, Z., et al. (2014) The FLS2-associated kinase BIK1 directly phosphorylates the NADPH oxidase RbohD to control plant immunity. Cell Host Microbe 15: 605–615.

Mousavi, S.A.R., Chauvin, A., Pascaud, F., Kellenberger, S. and Farmer, E.E. (2013) GLUTAMATE RECEPTOR-LIKE genes mediate leaf-to-leaf wound signalling. Nature 500: 422–426.

Mühlenbock, P., Chyžynska-Hebda, M., Plaszczyna, M., Baudo, M., Mateo, A., Mullineaux, P.M., et al. (2008) Chloroplast signalling and LESION SIMULATING DISEASE1 regulate crosstalk between light acclimation and immunity in Arabidopsis. Plant Cell 20: 2339–56.

R Core Team (2015) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rios-Rojas, L., Tapia, F. and Gurovich, L.A. (2014) Electrophysiological assessment of water stress in fruit-bearing woody plants. J. Plant Physiol. 171: 799–806.

Sharma, S.S., Sharma, S. and Rai, V.K. (1992) The effect of EGTA, calcium channel blockers (lanthanum chloride and nifedipine) and their interaction with abscisic acid on seed germination of Brassica juncea cv. RLM-198. Ann. Bot. 70: 295–299.

Stahlberg, R., Cieland, R.E. and Van Volkenburgh, E. (2005) Decrement and amplification of slow wave potentials during their propagation in Helianthus annuus L. shoots. Planta 220: 550–558.

Stahlberg, R., Cieland, R. and Van Volkenburgh, E. (2006) Slow wave potentials—a propagating electrical signal unique to higher plants. In Communication in Plants. Edited by Baluška, F., Mancuso, S. and Volkmann, D. pp. 291–308. Springer, Berlin.

Stankovic, B., Zawadzki, T. and Davies, E. (1997) Characterization of the variation potential in sunflower. Plant Physiol. 115: 1083–1088.

Sukhov, V., Shershteina, O., Surova, L., Katicahe, L. and Vodeneev, V. (2014) Proton cellular influx as a probable mechanism of variation potential influence on photosynthesis in pea. Plant Cell Environ. 37: 2532–2541.

Takeda, S., Gapper, C., Kaya, H., Bell, E., Kuchitsu, K. and Dolan, L. (2008) Local positive feedback regulation determines cell shape in root hair cells. Science 319: 1241–1244.

Arabidopsis thaliana, Helianthus annuus and Vicia faba. Physiol. Plant. 120: 265–270.

Kromdijk, J., Głowacka, K., Leonelli, L., Gabbily, S.T., Iwai, M., Niyogi, K.K., et al. (2016) Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. Science 354: 857–861.

Monaghan, J., Matschi, S., Shorinola, O., Rojemich, H., Matei, A., Segonzac, C., et al. (2014) The calcium-dependent protein kinase CIPK28 buffers plant immunity and regulates BIK1 turnover. Cell Host Microbe 16: 605–615.

Moussavi, S.A.R., Chauvin, A., Pascaud, F., Kellenberger, S. and Farmer, E.E. (2013) GLUTAMATE RECEPTOR-LIKE genes mediate leaf-to-leaf wound signalling. Nature 500: 422–426.
Torres, M.A., Dangl, J.L. and Jones, J.D.G. (2002) Arabidopsis gp91phox homologues AtRbohD and AtRbohF are required for accumulation of reactive oxygen intermediates in the plant defense response. Proc. Natl. Acad. Sci. USA 99: 517–522.

van Bel, A.J.E. and Ehlers, K. (2005) Electrical signalling via plasmodesmata. In Plasmodesmata. Edited by Oparka, K.J. pp. 263–278. Blackwell Publishing Ltd., Oxford.

Wickham, H. (2009) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.

Wildon, D.C., Thain, J.F., Minchin, P.E.H., Gubb, I.R., Reilly, A.J., Skipper, Y.D., et al. (1992) Electrical signalling and systemic proteinase inhibitor induction in the wounded plant. Nature 360: 62–65.

Zimmermann, M.R., Maischak, H., Mithöfer, A., Boland, W. and Felle, H.H. (2009) System potentials, a novel electrical long-distance apoplastic signal in plants, induced by wounding. Plant Physiol. 149: 1593–1600.

Zimmermann, M.R. and Mithöfer, A. (2013) Electrical long-distance signaling in plants. In Long-Distance Systemic Signaling and Communication in Plants. Edited by Baluska, F. pp. 291–308. Springer, Berlin.