*Plant, Cell and Environment* (2009) **32**, 319–326 doi: 10.1111/j.1365-3040.2008.01922.x

# Heat-induced electrical signals affect cytoplasmic and apoplastic pH as well as photosynthesis during propagation through the maize leaf

THORSTEN E. E. GRAMS[1], SILKE LAUTNER[2], HUBERT H. FELLE[3], RAINER MATYSSEK[1] & JÖRG FROMM[2]

[1]*Ecophysiology of Plants, Department of Ecology and Ecosystem Sciences, Technische Universität München, Am Hochanger 13, Freising, Germany,* [2]*Department of Wood Biology, University of Hamburg, Leuschnerstrasse 91, Hamburg, Germany and* [3]*Botanisches Institut I, Justus-Liebig-Universität Gießen, Senckenbergstrasse 17, Gießen, Germany*

## ABSTRACT

**Combining measurements of electric potential and pH with such of chlorophyll fluorescence and leaf gas exchange showed heat stimulation to evoke an electrical signal (propagation speed: 3–5 mm $s^{-1}$) that travelled through the leaf while reducing the net $CO_2$ uptake rate and the photochemical quantum yield of both photosystems (PS). Two-dimensional imaging analysis of the chlorophyll fluorescence signal of PS II revealed that the yield reduction spread basipetally via the veins through the leaf at a speed of 1.6 ± 0.3 mm $s^{-1}$ while the propagation speed in the intervein region was c. 50 times slower. Propagation of the signal through the veins was confirmed because PS I, which is present in the bundle sheath cells around the leaf vessels, was affected first. Hence, spreading of the signal along the veins represents a path with higher travelling speed than within the intervein region of the leaf lamina. Upon the electrical signal, cytoplasmic pH decreased transiently from 7.0 to 6.4, while apoplastic pH increased transiently from 4.5 to 5.2. Moreover, photochemical quantum yield of isolated chloroplasts was strongly affected by pH changes in the surrounding medium, indicating a putative direct influence of electrical signalling via changes of cytosolic pH on leaf photosynthesis.**

*Key-words*: *Zea mays*; chlorophyll fluorescence imaging; electron quantum yield; gas exchange.

## INTRODUCTION

Electrical excitability and signalling, frequently associated with rapid responses to environmental stimuli, are ubiquitous features of higher plants (Davies 2004). A plethora of physiological responses have been discovered recently to trail electrical signals in plants (Trebacz, Dziubinska & Krol 2006; Fromm & Lautner 2007), i.e. following mechanically induced action potentials or wound-induced variation potentials (Sibaoka 1969; Pickard 1973; Stahlberg & Cosgrove 1992, 1994). However, generation of electrical signals upon exciting stimuli and their physiological consequences are still a poorly understood feature of higher plants.

With regard to photosynthesis, a series of recent studies have focused on local and systemic effects of electrical signals on light and dark reactions in higher plants (Herde *et al.* 1999; Koziolek *et al.* 2004; Lautner *et al.* 2005; Hlavackova *et al.* 2006; Kaiser & Grams 2006; Grams *et al.* 2007). In particular, heat-induced electrical signals caused a strong local as well as systemic reduction in net $CO_2$ uptake and quantum yield of electron transport at photosystem II (PS II) and hydropassive responses in stomatal aperture (Koziolek *et al.* 2004; Kaiser & Grams 2006). In more detail, Lautner *et al.* (2005) analysed the spread of a heat-induced signal in the phloem of *Populus trichocarpa* and showed that the signal depends on the availability of calcium.

Similar to action potentials in animals, the propagation of electrical signals in plants is mediated by ion channels. While the ionic mechanism of excitation in animals is mainly based on $Na^+$ and $K^+$ fluxes, in plants, $Ca^{2+}$, $Cl^-$ and $K^+$ play a significant role during the action potential (Samejima & Sibaoka 1980; Kikuyama & Tazawa 1982; Felle & Zimmermann 2007). In addition, active transport of $H^+$ across the plasma membrane could also play an essential role in generation of action potentials (Opritov, Pyatygin & Vodeneev 2002; Vodeneev, Opritov & Pyatygin 2006). Upon wounding, such as heating of leaves, plants elicit an electrical signal with distinct consequences on $H^+$-fluxes at the plasma membrane involving transient shutdown of the P-type $H^+$-ATPase (Stahlberg & Cosgrove 1996; Stahlberg, Cleland & Van Volkenburgh 2006). It is also reported that enzymes in the cell wall, the plasma membrane and the cytoplasm modify their activities during local changes in ion concentrations (Davies 1987). However, to date, changes in pH after heat-induced signalling, in particular in the cytoplasm, remain obscure. Action potentials that arise at the plasma membrane could affect cross-membrane $H^+$-transport, which results in a transient alkalization of surrounding experimental media (Bulychev & Kamzolkina 2006a,b; Vodeneev *et al.* 2006) and, supposedly, result in acidification of the cytoplasm. Similar to findings in *Mimosa*

*Correspondence: J. Fromm. Fax: +49 (0)40 428912835; e-mail: j.fromm@holz.uni-hamburg.de*

The first two authors contributed equally to this work.

and poplar (Koziolek *et al.* 2004; Lautner *et al.* 2005), these results provided evidence that electrical signals are transmitted to the level of thylakoid membranes and are repressing $O_2$ evolution and quantum yield of electron transport at PS II.

The aim of the present study was to clarify an involvement of both apoplastic and cytoplasmic pH changes in heat-induced electrical signals and their photosynthetic response. In addition, if the speed of the photosynthetic response travelling along the veins is faster than the propagation in intervein region as shown in *Mimosa* (Koziolek *et al.* 2004) and poplar (Lautner *et al.* 2005), the $C_4$ grass *Zea mays*, due to its leaf morphology with strictly parallel veins, appeared to be a rewarding object to determine the different velocities in veins and intervein regions. The Kranz anatomy of $C_4$ plants facilitates tracing the signal propagation along the veins because PS II activity is deficient in bundle sheath cells (Woo *et al.* 1970; Hatch 1992) and, thus, PS I should immediately be affected by the travelling signal.

## MATERIALS AND METHODS

### Plant material

Plants of *Z. mays* L. var. *Mozart* were grown in a greenhouse during fall/winter from seeds in pots (3 L; Fruhstorfer Erde, Typ P; Archut, Germany). Additional lighting was provided by mercury vapour lamps ensuring a photosynthetic photon flux density (PPFD) of >200 $\mu$mol $m^{-2}$ $s^{-1}$ and 14/10 h day/night period at air temperature 20–22 °C, while relative air humidity fluctuated with outside conditions. Plants of 80–140 cm in height were transferred into a climate-controlled phytotron (York, Germany; air temperature of 22 °C, relative air humidity of 60%, PPFD c. 150 $\mu$mol $m^{-2}$ $s^{-1}$, 14/10 h day/night period) and measurements were performed on mature, 3- to 4-week-old leaves. Plants were fertilized with macro- and micronutrients in a Hoagland solution (Hoagland & Arnon 1950).

### Isolation of chloroplasts from leaf material

Seventy-five grams of young leaves were rinsed off with distilled water and dissected from their midribs. The whole isolation was conducted at 0 °C. In isolation medium [composed of 330 mM sorbitol, 10 mM Na-pyrophosphate (pH 6.5), 5 mM $MgCl_2$, 4 mM Na-ascorbate], leaf material was mixed for 5 s and filtered through mull and nylon cloths. The filtrate was centrifuged (1 min 4000 *g*) and washed twice [in 330 mM sorbitol, 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.6) 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM ethylenediaminetetraacetic acid (EDTA)] to gain a purified chloroplast suspension. For further details, see Heldt (1997).

### Electrical measurements

For intracellular membrane potential measurements, a microelectrode, filled with 100 mM KCl, was inserted into a mesophyll cell of a leaf. The reference electrode was immersed into artificial pond water (APW) where the cut cross-section of the excised leaf was also submerged. The APW was composed of 1.0 mM NaCl, 0.1 mM KCl, 0.1 mM $CaCl_2$ and 1.0 mM MES, adjusted with Tris to a pH value of 6.0. Prior to each experiment, both electrodes had been calibrated (0 mV) in APW and were connected to a differential amplifier (WPI, Model 750, World Precision Instruments, Sarasota, FL, USA). After microelectrode insertion, the tip of the leaf was heat-stimulated at 10–12 cm distance to the site of electric potential measurements and data were recorded by a chart recorder. Heat stimulation was performed by the flame of a lighter (c. 1000 °C) for 1 to 2 s.

For apoplastic measurements, four-leaved whole maize plants were placed inside a Faraday cage and part of the second leaf was fixed horizontally while a long-distance microscope objective (20×) permitted the positioning of the electrodes at an angle of approximately 45° (according to Felle & Zimmermann 2007). The tip of another leaf was cut off and the cut end of the leaf was connected to earth (zero voltage) submersed in a basal solution which comprised 1–5 mM KCl, 0.1 mM $CaCl_2$, 0.1 mM NaCl, and a 1 mM Mes/Tris buffer solution, adjusted to pH 5.5. Voltage- and pH-selective microelectrodes were positioned in the substomatal cavity of open stomata. Their preparation and insertion were carried out as described by Felle *et al.* (2000, 2004). Electrodes were connected with a high-impedance ($10^{15}$ $\Omega$) amplifier (FD223, World Precision Instruments, Sarasota, FL, USA) and kinetics were recorded on a chart recorder (L2200, Linseis, Selb, Germany). Signals picked up by pH-selective electrodes consist of both apoplastic voltage and pH-specific voltage. To obtain the pH-specific net signal, traces were subtracted from each other by a differential amplifier. As soon as the electrode tip had contact with the aqueous phase of the stomatal cavity, the electrical circuit was closed and the tip of the leaf was heat-stimulated as described above at a distance of 10 cm.

For cytoplasmic pH measurements, the same experimental set-up and stimulation procedure was used as described for apoplastic recordings (see above). However, voltage- and pH-selective microelectrodes were positioned in mesophyll cells. Fabrication of the pH-sensitive microelectrodes was performed as described in detail by Felle & Bertl (1986).

### Leaf gas exchange measurements

Assessment of leaf gas exchange was performed using an open-flow porometer (Li 6400, Li-Cor Inc, NE, USA) at constant $CO_2$ concentration of 400 $\mu$L $L^{-1}$, relative air humidity of c. 60%, air and leaf temperature of 25.1 and 25.5 °C, respectively, and PPFD of c. 100 $\mu$mol $m^{-2}$ $s^{-1}$.

### Chlorophyll fluorescence measurements of PS II

The spatio-temporal variations in the PS II chlorophyll fluorescence of heat-stimulated leaves was assessed using

an Imaging pulse-amplitude modulation (PAM) chlorophyll fluorometer (Heinz Walz GmbH, Effeltrich, Germany). This method allows non-invasive determination of photochemical quantum yield of PS II by the saturation pulse method (Schreiber, Schliwa & Bilger 1986). For simultaneous measurements of leaf gas exchange, the Imaging-PAM system was mounted directly on the top of the broadleaf cuvette of the Li-Cor 6400. Prior to heat stimulation, the imaged leaf area was adapted to a PPFD of c. 100 $\mu$mol m$^{-2}$ s$^{-1}$ until gas exchange rates became stable (typically after 20–30 min). Saturating light pulses were given every 10 or 20 s to determine the images of effective quantum yield of PS II electron flow, calculated as $\Delta F/F'm = (F'm - F)/F'm$. Here, F and F′m designate actual and maximum yield of PS II chlorophyll fluorescence in a light-adapted leaf, respectively (for nomenclature, see van Kooten & Snel 1990). Intensive testing proved the observed effects of electrical signals on photochemical quantum yield of electron transport at PS II not to be affected by applying the saturation pulses at high frequency of 20–30 s intervals.

Assessment of effective quantum yield of PS II in isolated chloroplasts was performed immediately after isolation with the Imaging-PAM system although, here, the spatial mean value of the slowly stirred chloroplast suspension in a Petri dish was evaluated. Measurements were done in darkness at room temperature and saturation pulses were applied every 180 s. Under these conditions, chloroplasts displayed a stable PS II quantum yield of c. 0.4 for at least 1 h. pH changes in the chloroplast suspension were achieved by addition of 30–60 $\mu$L 0.1 M NaOH or HCl.

### Simultaneous measurements of PS I and PS II chlorophyll fluorescence

Assessment was performed on intact leaves by means of a new generation of PAM-system (Dual-PAM-100, Walz, Effeltrich, Germany) using blue light for PS II excitation ($\lambda = 440$ nm) and far-red light ($\lambda = 715$ nm) for preferential excitation of PS I (Klughammer & Schreiber 2008). This system allows for simultaneous determination of the quantum yields of photochemical energy conversion at PS I and PS II at the same leaf area. Measurements were done on pre-illuminated (c. 100 $\mu$mol m$^{-2}$ s$^{-1}$) attached leaves.

## RESULTS

### Response of photosynthesis to heat stimulus

Heat stimulation of the leaf tip of *Z. mays* plants resulted in a transient drop of net $CO_2$ uptake rate in the investigated central part of the leaf lamina (Fig. 1). One hundred fifty seconds after heat stimulation, net $CO_2$ uptake rate decreased from 3.8 ± 0.2 $\mu$mol m$^{-2}$ s$^{-1}$ (mean ± SE, $n = 11$) to about 2.3 ± 0.2 $\mu$mol m$^{-2}$ s$^{-1}$, before recovery set in towards 3.1 ± 0.2 $\mu$mol m$^{-2}$ s$^{-1}$. While net $CO_2$ uptake rate declined, stomatal conductance ($g_{H2O}$) first rapidly increased (from 35 ± 3 before to 79 ± 7 mmol m$^{-2}$ s$^{-1}$ upon

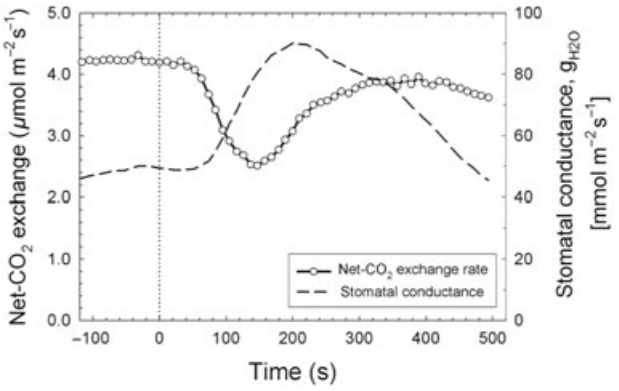


**Figure 1.** Response of net $CO_2$ uptake rate (open circles) and stomatal conductance (dashed line) of a leaf upon heat stimulation of its tip at c. 10 cm distance. Data shown are representative for a total of 11 measurements.

stimulation), reaching a peak after about 200 s, prior to declining towards 20 ± 7 mmol m$^{-2}$ s$^{-1}$ (Fig. 1, $n = 5$).

Similar to the response in leaf gas exchange, heat stimulation of the leaf tip caused a substantial decrease in the photochemical quantum yield of PS II (Fig. 2) indicating a retardation of the non-cyclic electron transport. This effect began 50 s after heat stimulation in the lamina regions along veins, at a distance of c. 10 cm from the heat-induced leaf tip. The transient drop of photosynthesis spread basipetally throughout the leaf and passed through a minimum after c. 150 s, prior to incipient recovery (Fig. 2). The reduction of the photochemical quantum yield occurred by two distinct steps: A fast response propagated along the veins with a speed of 1.6 ± 0.3 mm s$^{-1}$ (mean ± SE, $n = 7$; cf. signal spreading from right to left in Fig. 3a), which was followed by proliferation into the intervein regions, at a velocity retarded by a factor of c. 50 (propagation speed of 0.03 ± 0.01 mm s$^{-1}$, cf. Fig. 3b).

Due to the chloroplast dimorphism in $C_4$ plants such as *Z. mays* with bundle sheath chloroplasts (close to veins) lacking PS II activity (Woo *et al.* 1970; Hatch 1992), simultaneous assessment of PS I and PS II chlorophyll fluorescence of the leaf gave further evidence for the propagation of the signal via the veins (Fig. 4). Mean travelling time of the signal from the site of heat stimulation to the onset of PS I drop at the monitored leaf area corresponded to 1.1 ± 0.2 mm s$^{-1}$ (mean ± SE, $n = 5$). In all investigations, heat stimulation affected quantum yield of PS I before PS II. However, due to the small distance between chloroplasts in bundle sheath cells (lacking PS II activity) and mesophyll cells, the time interval between the decline in quantum yield of PS I and PS II was short (i.e. 10 s) but significant.

### Electrical signalling

After inserting a microelectrode into a mesophyll cell of a mature leaf, its tip was heat-stimulated at a distance of 10–12 cm and an electrical signal with an amplitude of 30 mV (±4 mV, $n = 5$) and a speed of 3–5 mm s$^{-1}$ was

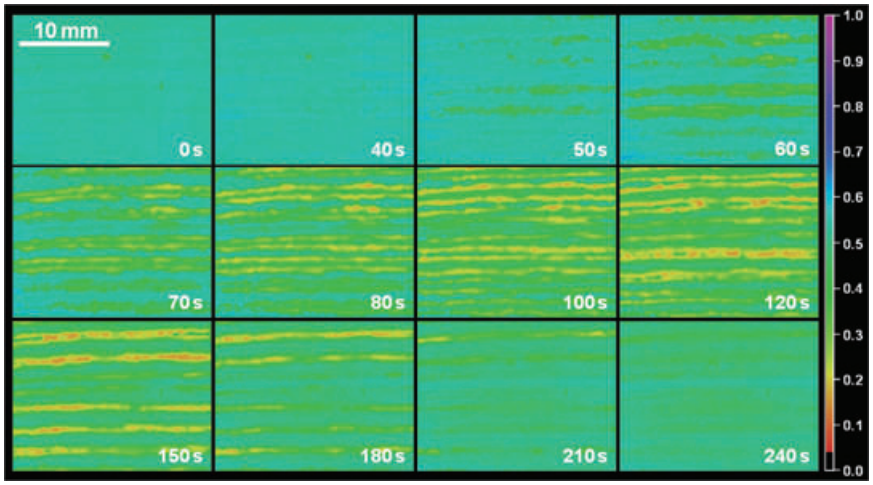


**Figure 2.** Spatio-temporal changes of photochemical quantum yield of photosystem II (PS II) (ΔF/F′m) assessed by PS II chlorophyll fluorescence imaging. The imaged area (l: 22 mm; w: 17 mm) covers the central part of the leaf lamina. The leaf tip was heat-stimulated at a distance of c. 10 cm. Time intervals given after the instant of heat stimulation (at time = 0 s). Changes in ΔF/F′m took 50 s to become apparent. The decrease in PS II quantum yield is indicated by a false-colour shift from light blue to yellow/orange (equivalent to a lowering of ΔF/F′m from 0.6 to about 0.2–0.1). Data shown are representative for a total of seven measurements.

released (Fig. 5a). For comparison, electrical signals were also measured by apoplastic voltage microprobes placed non-invasively into sub-stomatal cavities. After heating of the leaf tip, an electrical signal with an amplitude of 60 mV (±9 mV, $n = 5$) and a speed of 3–5 mm $s^{-1}$ was observed (Fig. 5b). Due to the apoplastic location of the microelectrode, the electric potential hyperpolarized transiently in contrast to the depolarizing signal measured in the cytoplasm (Fig. 5a). Surprisingly, cutting the leaf tip did not trigger electrical signals. Furthermore, we never observed spontaneous signals.

## The pH response

Both depolarization and repolarization of an electrical signal are caused by ion fluxes. In case of a typical action potential transmembrane ion fluxes ($Ca^{2+}$, $Cl^-$, $K^+$) move down their electrochemical gradient after their channels have been activated (Fromm & Lautner 2007). Although the ionic background of action potentials has already been identified in some plant species, e.g. in *Characeae* (Kikuyama & Tazawa 1982; Williamson & Ashley 1982), in the liverwort *Conocephalum conicum* (Favre *et al.* 1999), in maize (Fromm & Bauer 1994) and barley (Felle & Zimmermann 2007), for heat-induced signals in maize, the problem seems largely unresolved. In order to examine if a heat-induced electrical signal is caused by a transient shutdown of the $H^+$-ATPase (Stahlberg & Cosgrove 1996; Stahlberg *et al.* 2006), pH-sensitive microprobes have been applied to study cytoplasmic pH changes. After inserting the electrode into a mesophyll cell of an attached leaf, cytoplasmic pH rests between 7.0 and 7.4 (7.2 ± 0.2, $n = 5$). It rapidly

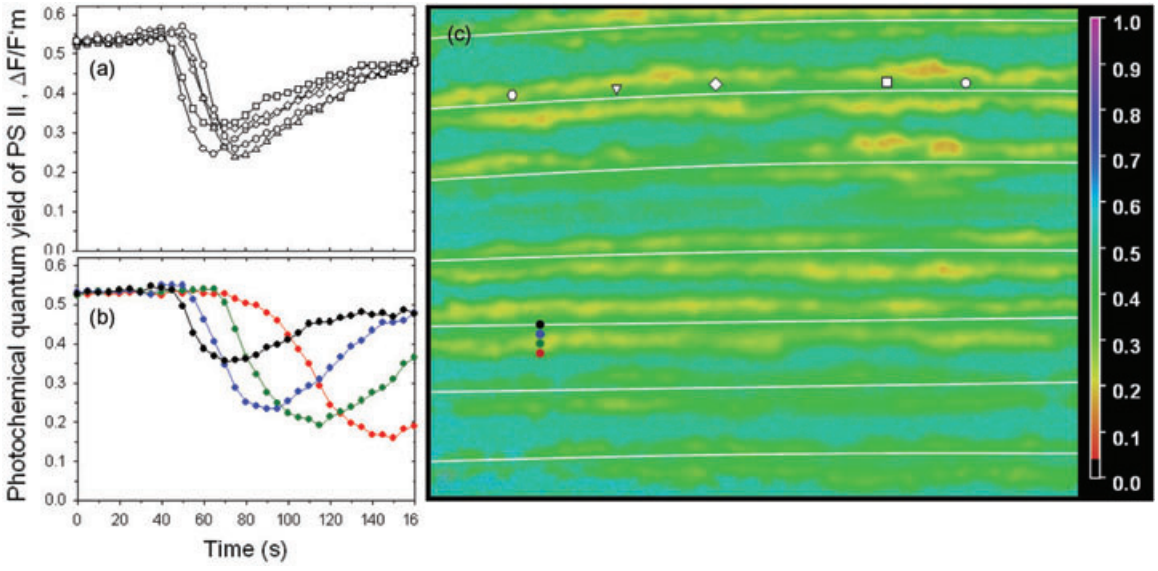


**Figure 3.** Kinetic changes of photochemical quantum yield of photosystem II (PS II) (ΔF/F′m) measured by chlorophyll fluorescence imaging after heat stimulation of the leaf tip. (a) Spreading of ΔF/F′m along a leaf vein. (b) Spreading of ΔF/F′m from veins to intervein regions. (c) Detail of Fig. 2, giving the position of measurements shown in (a) and (b). White lines indicate positions of veins. PS II quantum yield is expressed by a false-colour scale (right, see Fig. 2).

 

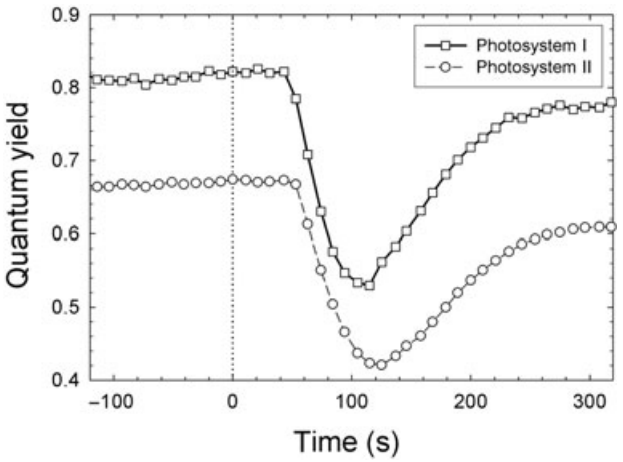


**Figure 4.** Quantum yield of photosystem I (PS I) and PS II upon heat stimulation of a leaf tip at time 0 and a distance of c. 7 cm. Data shown are representative for a total of five measurements.

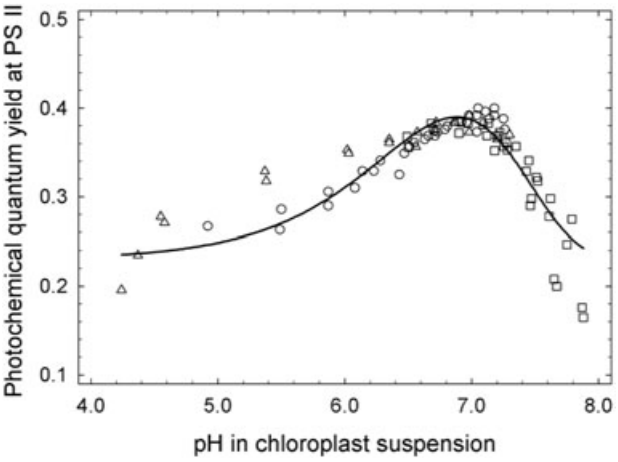


**Figure 6.** pH dependence of photosystem II (PS II) quantum yield of isolated *Z. mays* chloroplasts. Different symbols represent independent isolations and subsequent measurements.

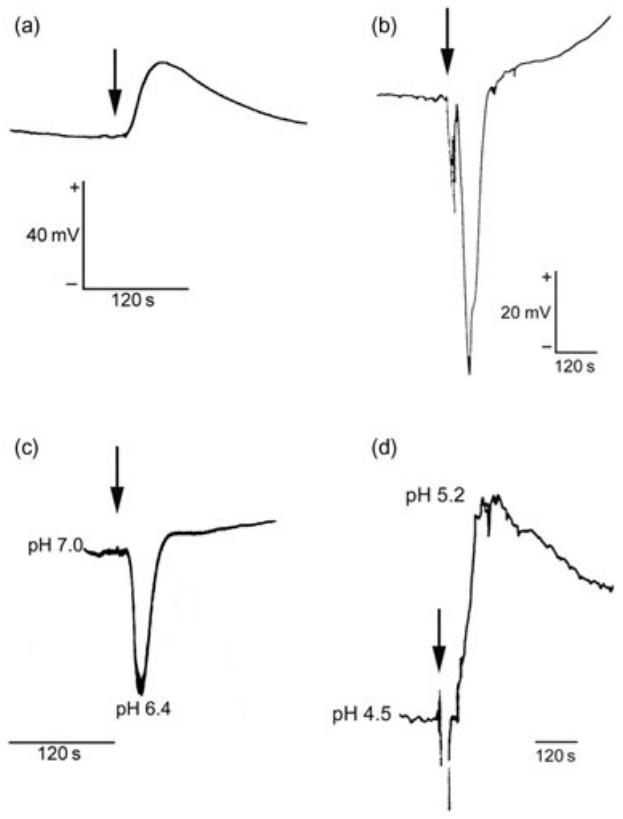


**Figure 5.** (a) Electrical signals measured with a microelectrode in the leaf mesophyll induced by heat stimulation (arrow) of the leaf tip at a distance of 10 cm. Typical signal time courses out of a total of five measurements. (b) Action potential measured in the apoplast of maize leaves after heating (arrow) of the leaf tip (at 10 cm distance). (c) Transient change of cytoplasmic pH in the mesophyll after heating the leaf tip. (d) Kinetic change of pH accompanying an electrical signal in the apoplast of the leaf after heat stimulation of the leaf tip at a distance of 10 cm.

decreases after heating the leaf tip at 10–12 cm distance by about 0.6 pH units (Fig. 5c). In addition, the non-invasive approach with pH-selective microprobes inserted in the sub-stomatal cavity provides a valuable hint because they measure continuous $H^+$ movements as soon as protons are extruded from cells. Similar to the change in cytoplasmic pH, apoplastic pH responded distinctly during the electrical signal. pH increased rapidly from 4.5 to 5.2 and returned slowly close to the value measured prior to stimulation (Fig. 5d). Interestingly, the velocity of the transient change in pH was similar to that of the electrical signal.

Clearly, heat-induced electrical signals yielded distinct pH responses in the cytoplasm as well as in the apoplast. In correlation to the acidification of the cytoplasm during the signal, the apoplast alkalizes which points to a possible deactivation of the $H^+$ pump.

## pH dependence of chlorophyll fluorescence

To check for potential effects of a pH change on chlorophyll fluorescence, chloroplasts were isolated from the leaves. They displayed a strong pH dependence of photochemical quantum yield of PS II as presented in Fig. 6. Maximum quantum yield of c. 0.4 occurred around pH 7, while the yield level decreased sharply at pH > 7 (e.g. 0.18 at pH of 7.8). Upon acidification, PS II quantum yield more gradually declined towards c. 0.3 at pH of 6.0.

## DISCUSSION

Two hypotheses were posed at the beginning: (1) heat-induced electrical signals are propagated through the leaf along the veins, which represent a signalling highway with higher travelling velocities than in the intervein regions of the lamina; and (2) heat-induced variation in $H^+$ flux across the plasma membrane causes pH changes which in turn affects net $CO_2$ uptake and photochemical performance of both PS.

 

Recent studies on local and systemic signal transmission in *Mimosa pudica* (Koziolek *et al.* 2004; Kaiser & Grams 2006) and poplar (Lautner *et al.* 2005) demonstrated a link between heat-induced electrical signals and photosynthetic responses. Likewise, upon heat stimulation, we observed a transient rise in stomatal conductance which was accompanied by a drop of net $CO_2$ uptake (Fig. 1). Thus, neither the rapid suppression of net $CO_2$ uptake nor its recovery appears to be a direct consequence of a changing stomatal aperture. Our findings support the view of Kaiser & Grams (2006) that the increase in stomatal conductance is due to hydropassive stomatal movement caused by sudden loss of epidermal turgor, whereas the reduction of net $CO_2$ uptake is, at least partially, due to a disturbance of the light reactions at PS I and II (Figs 2 & 3, Koziolek *et al.* 2004; Lautner *et al.* 2005). The extent to which a parallel increase in mitochondrial respiration is affecting the net $CO_2$ uptake remains unclear (cf. Dziubinska, Trebacz & Zawadzki 1989; Filek & Koscielniak 1997). In the monocot *Z. mays*, we observed the spatio-temporal dynamics of the heat-induced, transient drop in photochemical quantum yield of PS II through chlorophyll fluorescence imaging (Figs 2 & 3). The photosynthesis reduction emerged first along the veins and subsequently intruded into the intervein regions, suggesting that the electrical signal spreads through the leaf lamina via the veins and from there to the mesophyll cells as previously indicated in *Mimosa* and poplar (Koziolek *et al.* 2004; Lautner *et al.* 2005). In *Z. mays*, the bundle sheath cells that enclose the leaf vessels contain chloroplasts which, contrasting the mesophyll chloroplasts, neither possess grana thylakoids nor PS II (Woo *et al.* 1970; Hatch 1992). Hence, electrical signals travelling within the leaf bundles would first affect PS I in the bundle sheath chloroplasts before encountering PS II when the signal arrives in the leaf mesophyll cells. Accordingly, simultaneous assessment of chlorophyll fluorescence in both PS revealed PS I to be consistently affected first (Fig. 4), giving additional evidence that the signal is propagated through the veins. Moreover, the speed of the signal spread through the veins of $1.6 \pm 0.3$ mm $s^{-1}$ is about 50 times faster than the subsequent velocity in the intervein regions. These findings support our hypothesis 1 as posed above.

Upon heat stimulation, we measured an electrical signal in both the cytoplasm of the mesophyll and the substomatal cavity (Fig. 5a,b). In comparison with the so-called variation potentials (Roblin 1985) or slow waves (Julien *et al.* 1991), which were observed after wounding and show a prolonged transient of repolarization (Stankovic *et al.* 1998), the signals measured in the present study show a short repolarization phase. Hence, their affiliation is still a matter of discussion.

Concerning ion fluxes during electrical signals, a strong initial depolarization is the main releasing factor that first causes $Ca^{2+}$ influx and then anion efflux, which causes the typical voltage 'breakthrough' in action potentials. Subsequently, $K^+$ efflux starts after the depolarizing voltage has passed the $K^+$ equilibrium potential (Fromm & Bauer 1994; Felle & Zimmermann 2007). Anion channels do not only release depolarizing anions during an action potential but also organic acid anions which bind protons under acidic conditions and therefore cause a pH increase in the apoplast. Recently, Felle *et al.* (2005) demonstrated apoplastic alkalization in general to indicate stress. In the present study, heat stimulation of a maize leaf increased apoplastic pH while cytoplasmic pH decreased. The question if the heat-induced electrical signal shown in Fig. 5 is generated by $H^+$ pump deactivation or by other mechanisms (e.g. proton leakage through plasma membrane) is currently under investigation in our laboratory. In characean algae and pumpkin seedlings, action potentials arising at the plasma membrane also affected cross-membrane $H^+$-transport and resulted in changes of the surrounding pH milieu (Opritov *et al.* 2002; Bulychev & Kamzolkina 2006a,b). The work by Bulychev & Kamzolkina (2006a,b) also indicates that a change in cytoplasmic $Ca^{2+}$ concentration may affect the chloroplast in a way that both $O_2$ evolution and quantum yield of electron transport at PS II are reduced. These transient reductions in photosynthesis upon induction of action potentials appear to be linked to an increase in $\Delta$pH across the thylakoid membrane (Bulychev & Kamzolkina 2006a,b). In isolated *Z. mays* chloroplasts, the photochemical quantum yield of PS II was strongly affected by pH changes in the surrounding medium (Fig. 6). This indicates a putative direct influence of the electrical signal on photochemical quantum yield and net $CO_2$ uptake (cf. Figs 1–3) via lowered cytoplasmic pH, which is in line with our hypothesis 2. However, in contrast to the hypothesis of Bulychev & Kamzolkina (2006b), in *Z. mays*, cytoplasmic $Ca^{2+}$ concentrations might not be involved in the transient drop in photosynthesis, as increase in $Ca^{2+}$ concentration had no effect on the photochemical quantum yield of isolated chloroplasts (data not shown).

In conclusion, we confirm earlier findings that heat-induced electrical signals spread via the veins through the leaf lamina with a speed of 3–5 mm $s^{-1}$. In comparison, the speed of the photosynthetic response is $1.6 \pm 0.3$ mm $s^{-1}$, which appears to be 50 times faster along the veins than in intervein regions. Therefore, evidence is given that the photosynthetic response is caused by the electrical signal. Upon signal induction through the heat stimulus, cytoplasmic pH is decreased. Moreover, photochemical quantum yield of isolated chloroplasts was strongly affected by pH changes in the surrounding medium, indicating a putative direct influence of electrical signalling via changes of cytosolic pH on leaf photosynthesis. However, the mechanism underlying photosynthetic limitation upon decreasing cytosolic pH requires further investigations. pH-dependent enzymes, e.g. carbonic anhydrase, might be strongly involved in this process; it is known that an important part of the regulation of mesophyll conductance to $CO_2$ seems to have a metabolic origin, possibly related to carbonic anhydrase (Flexas *et al.* 2008). Therefore, changes in enzyme activity upon cytosolic pH decrease might play a main role in photosynthetic limitation upon electrical signalling.

## ACKNOWLEDGMENTS

The authors would like to thank Dr Markus Löw for help with the simultaneous measurements of leaf gas exchange and chlorophyll fluorescence. We are also thankful to the company H. Walz for lending the Dual-PAM equipment for simultaneous measurements of PS I and PS II chlorophyll fluorescence. This work was supported by Deutsche Forschungsgemeinschaft (FR 955/10-3 and B5 of SFB 607).

## REFERENCES

Bulychev A.A. & Kamzolkina N.A. (2006a) Differential effects of plasma membrane electric excitation on $H^+$ fluxes and photosynthesis in characean cells. *Bioelectrochemistry* **69,** 209–215.

Bulychev A.A. & Kamzolkina N.A. (2006b) Effect of action potential on photosynthesis and spatially distributed $H^+$ fluxes in cells and chloroplasts of *Chara corallina*. *Russian Journal of Plant Physiology* **53,** 1–9.

Davies E. (1987) Action potentials as multifunctional signals in plants – a unifying hypothesis to explain apparently disparate wound responses. *Plant, Cell & Environment* **10,** 623–631.

Davies E. (2004) New functions for electrical signals in plants. *New Phytologist* **161,** 607–610.

Dziubinska H., Trebacz K. & Zawadzki T. (1989) The effect of excitation on the rate of respiration in the liverwort Conocephalum conicum. *Physiologia Plantarum* **75,** 417–423.

Favre P., Zawadzki T., Dziubinska A., Trebacz K., Greppin H. & Degli Agosti R. (1999) Repetitive action potentials induced in the liverwort Conocephalum conicum. *Archives des Sciences* **52,** 187–198.

Felle H.H. & Bertl A. (1986) The fabrication of $H^+$-selective liquid-membrane micro-electrodes for use in plant cells. *Journal of Experimental Botany* **182,** 1416–1428.

Felle H.H. & Zimmermann M.R. (2007) Systemic signaling in barley through action potentials. *Planta* **226,** 203–214.

Felle H.H., Hanstein S., Steinmeyer R. & Hedrich R. (2000) Dynamics of ion-activities in the apoplast of the sub-stomatal cavity of intact *Vicia faba* leaves during stomatal closure evoked by ABA and darkness. *The Plant Journal* **24,** 297–304.

Felle H.H., Herrmann A., Hanstein S., Hückelhoven R. & Kogel K.-H. (2004) Apoplastic pH-signalling in barley leaves attacked by the powdery mildew fungus (*Blumeria graminis* f.sp. *hordei*). *Molecular Plant–Microbe Interactions* **17,** 118–123.

Felle H.H., Herrmann A., Hückelhoven R. & Kogel K.-H. (2005) Root-to-shoot signalling: apoplastic alkalinization, a general stress signal and defence factor in barley (*Hordeum vulgare*). *Protoplasma* **227,** 17–24.

Filek M. & Koscielniak J. (1997) The effect of wounding the roots by high temperature on the respiration rate of the shoot and propagation of electric signal in horse bean seedlings (*Vicia faba* L. minor). *Plant Science* **123,** 39–46.

Flexas J., Ribas-Carbo M., Diaz-Espejo A., Galmes J. & Medrano H. (2008) Mesophyll conductance to $CO_2$: current knowledge and future prospects. *Plant, Cell & Environment* **31,** 602–621.

Fromm J. & Bauer T. (1994) Action potentials in maize sieve tubes change phloem translocation. *Journal of Experimental Botany* **45,** 463–469.

Fromm J. & Lautner S. (2007) Electrical signals and their physiological significance in plants. *Plant, Cell & Environment* **30,** 249–257.

Grams T.E.E., Koziolek C., Lautner S., Matyssek R. & Fromm J. (2007) Distinct roles of electric and hydraulic signals on the reaction of leaf gas exchange upon re-irrigation in *Zea mays* L. *Plant, Cell & Environment* **30,** 79–85.

Hatch M.D. (1992) $C_4$ photosynthesis – an likely process full of surprises. *Plant and Cell Physiology* **33,** 333–342.

Heldt H.W. (1997) *Plant Biochemistry & Molecular Biology*. Oxford University Press, Oxford, UK; New York, USA; Tokyo, Japan.

Herde O., Pena-Cortes H., Fuss H., Willmitzer L. & Fisahn J. (1999) Effects of mechanical wounding, current application and heat treatment on chlorophyll fluorescence and pigment composition in tomato plants. *Physiologia Plantarum* **105,** 179–184.

Hlavackova V., Krchnak P., Naus J., Novak O., Spundova M. & Strnad M. (2006) Electrical and chemical signals involved in short-term systemic photosynthetic responses of tobacco plants to local burning. *Planta* **225,** 235–244.

Hoagland D.R. & Arnon D.I. (1950) *The Water Culture Method for Growing Plants without Soil, Vol Circular 374*. California Agricultural Experimental Station, Berkeley, CA, USA.

Julien J.L., Desbiez M.-O., De Jaegher G. & Frachisse J.M. (1991) Characteristics of the wave of depolarization induced by wounding in *Bidens pilosa* L. *Journal of Experimental Botany* **42,** 131–137.

Kaiser H. & Grams T.E.E. (2006) Rapid hydropassive opening and subsequent active stomatal closure follow heat-induced electrical signals in *Mimosa pudica*. *Journal of Experimental Botany* **57,** 2087–2092.

Kikuyama M. & Tazawa M. (1982) Transient increase of intracellular $Ca^{2+}$ during excitation of tonoplast-free *Chara* cells. *Protoplasma* **117,** 62–67.

Klughammer C. & Schreiber U. (2008) Saturation pulse method for assessment of energy conversion in PS I. *PAM Application Notes* **1,** 11–14.

van Kooten O. & Snel J.F.H. (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynthesis Research* **25,** 147–150.

Koziolek C., Grams T.E.E., Schreiber U., Matyssek R. & Fromm J. (2004) Transient knockout of photosynthesis mediated by electrical signals. *New Phytologist* **161,** 715–722.

Lautner S., Grams T.E.E., Matyssek R. & Fromm J. (2005) Characteristics of electrical signals in poplar and responses in photosynthesis. *Plant Physiology* **138,** 2200–2209.

Opritov V.A., Pyatygin S.S. & Vodeneev V.A. (2002) Direct coupling of action potential generation in cells of a higher plant (*Cucurbita pepo*) with the operation of an electrogenic pump. *Russian Journal of Plant Physiology* **49,** 142–147.

Pickard B.G. (1973) Action potentials in higher plants. *Botanical Review* **39,** 172–201.

Roblin G. (1985) Analysis of the variation potential induced by wounding in plants. *Plant and Cell Physiology* **26,** 255–261.

Samejima M. & Sibaoka T. (1980) Changes in the extracellular ion concentration in the main pulvinus of *Mimosa pudica* during rapid movement and recovery. *Plant and Cell Physiology* **21,** 467–479.

Schreiber U., Schliwa U. & Bilger W. (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynthesis Research* **10,** 51–62.

Sibaoka T. (1969) Physiology of rapid movements in higher plants. *Annual Review of Plant Physiology* **20,** 165–184.

Stahlberg R. & Cosgrove D.J. (1992) Rapid alterations in growth-rate and electrical potentials upon stem excision in pea-seedlings. *Planta* **187,** 523–531.

Stahlberg R. & Cosgrove D.J. (1994) Comparision of electric and growth-responses to excision in cucumber and pea-seedlings: 1. Short-distance effects are a result of wounding. *Plant, Cell & Environment* **17,** 1143–1151.

Stahlberg R. & Cosgrove D.J. (1996) Induction and ionic basis of slow wave potentials in seedlings of *Pisum sativum* L. *Planta* **200,** 416–425.

Stahlberg R., Cleland R.E. & Van Volkenburgh E. (2006) Slow wave potentials – a propagating electrical signal unique to higher plants. In *Communication in Plants: Neuronal Aspects of Plant Life* (eds F. Baluska, S. Mancuso & D. Volkmann) pp. 291–308. Springer Verlag, Berlin, Heidelberg, Germany; New York, USA.

Stankovic B., Witters D.L., Zawadzki T. & Davies E. (1998) Action potentials and variation potentials in sunflower: an analysis of their relationships and distinguishing characteristics. *Physiologia Plantarum* **103,** 51–58.

Trebacz K., Dziubinska H. & Krol E. (2006) Electrical signals in long-distance communication in plants. In *Communication in Plants: Neuronal Aspects of Plant Life* (eds F. Baluska, S. Mancuso & D. Volkmann) pp. 277–290. Springer Verlag, Berlin, Heidelberg, Germany; New York, USA.

Vodeneev V.A., Opritov V.A. & Pyatygin S.S. (2006) Reversible changes of extracellular pH during action potential generation in a higher plant *Cucurbita pepo*. *Russian Journal of Plant Physiology* **53,** 481–487.

Williamson R.E. & Ashley C.C. (1982) Free $Ca^{2+}$ and cytoplasmic streaming in the alga *Chara*. *Nature* **296,** 647–651.

Woo K.C., Downton W.J.S., Osmond C.B., Anderson J.M., Boardman N.K. & Thorne S.W. (1970) Deficient photosystem II in agranal bundle sheath chloroplasts of $C_4$ plants. *Proceedings of the National Academy of Sciences of the United States of America* **67,** 18–25.

*Received 14 October 2008; received in revised form 10 November 2008; accepted for publication 11 November 2008*

 