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RESEARCH PAPER

Rapid hydropassive opening and subsequent active stomatal closure follow heat-induced electrical signals in *Mimosa pudica**

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Abstract

In *Mimosa pudica* L., heat stimulation triggers leaflet folding in local, neighbouring and distant leaves. Stomatal movements were observed microscopically during this folding reaction and electrical potentials, chlorophyll fluorescence, and leaf CO_2/H_2O-gas exchange were measured simultaneously. Upon heat stimulation of a neighbouring pinna, epidermal cells depolarized and the stomata began a rapid and pronounced transient opening response, leading to an approximately 2-fold increase of stomatal aperture within 60 s. At the same time, net CO_2 exchange showed a pronounced transient decrease, which was followed by a similar drop in photochemical quantum yield at photosystem (PS) II. Subsequently, CO_2-gas exchange and photochemical quantum yield recovered and stomata closed partly or completely. The transient and fast stomatal opening response is interpreted as a hydropassive stomatal movement caused by a sudden loss of epidermal turgor. Thus, epidermal cells appear to respond in a similar manner to heat-induced signals as the pulvinar extensor cells. The subsequent closing of the stomata confirms earlier reports that stomatal movements can be induced by electrical signals. The substantial delay (several minutes) of guard cell turgor loss compared with the immediate response of the extensor and epidermal cells suggests a different, less direct mechanism for transmission of the propagating signal to the guard cells.

Key words: Chlorophyll fluorescence, electrical signals, guard cells, *Mimosa pudica*, stomata.

Introduction

In *Mimosa pudica*, wounding by cutting or burning triggers the propagation of a signal, which causes upfolding of leaflets of neighbouring pinnae and distant leaves. Responsible for this folding is a rapid loss of turgor in the extensor cells in the pulvini of leaves, pinnae, and leaflets (Weintraub, 1952). The transmission of the stimulus is associated with systemic electric, hydraulic, and chemical responses (Braam, 2005). The nature of the primary signal, however, is still uncertain. An electrical signal with variable form and duration is propagated, which is involved in local (leaf level) and distant responses (Houwink, 1935; Sibaoka, 1953, 1966, 1969). However, propagation of the response may also occur through dead tissue (Houwink, 1935; Ricca, 1916), which points against an exclusive role of action potentials in long-distance signalling. In addition, distant responses could be elicited by hydraulic signals, which arise from a rapid release of xylem tension caused by release of water into the apoplast at the wounded site and by deflating extensor cells (Malone, 1994; Ricca, 1916). Such changes in xylem pressure are reported to cause local depolarizations similar to a propagating electrical signal (Stahlberg and Cosgrove, 1997). In this view, the electrical events are not the travelling stimulus but rather the effects of the hydraulic signals (Malone, 1994; Mancuso, 1999). The hydraulic dispersal of solutes released to the apoplast could also distribute chemical signals (Ricca, 1916) at a speed sufficient to explain the propagation of the leaf folding response (Malone, 1994; Rhodes *et al.*, 1999).

The mechanism by which the extensor cells lose and recover their turgor is only partly understood (Braam, 2005). The cellular mechanisms of both processes are very

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similar to those responsible for guard cell swelling and shrinking. In pulvinar motor cells, Cl^- efflux causes a rapid depolarization, outward rectifying K^+-channels open and release K^+, which then causes a loss of turgor (Allen, 1969; Stoeckel and Takeda, 1993). This basic process also causes guard cell turgor loss (MacRobbie, 1998). In *Mimosa* extensor cells, however, the velocity of water release is much higher than expected from known membrane permeabilities and, therefore, mechanisms such as solute-water co-transporters or aquaporins are suggested (Fleurat-Lessard *et al.*, 1997*a*, *b*; MacRobbie, 1999; Morillon *et al.*, 2001). The mechanisms of turgor build-up during the regeneration of extensor cells is analogous to the processes involved in turgor increase during stomatal opening. In brief, in both cases, proton pumping of an H^+-ATPase (Fleurat-Lessard *et al.*, 1997*b*; Serrano, 1989) is the driving force, which then promotes the uptake of K^+ and an increase of the osmotic potential. Despite those similarities, the question if guard cells respond to the propagating heat-induced signal in a similar manner to extensor cells has not been examined.

This study was motivated by a previous study on chlorophyll fluorescence, gas exchange, and electrophysiological responses of *M. pudica* (Koziolek *et al.*, 2004), where a strong decrease in assimilation rate during leaf folding and a concomitant decrease in photochemical quantum yield at PSII was observed. While net CO_2 exchange dropped, leaf transpiration first rapidly increased, reaching a peak after approximately 150 s, and subsequently declined to a low level (Koziolek *et al.*, 2004). The reason for this extraordinarily fast transient increase in water loss has not been commented on in previous work. With current knowledge, both a true stomatal opening response as well as a release of water into the extracellular spaces appeared possible. In this study this question was addressed by microscopic observation of stomatal aperture and the sequence of events in the lamina physiology upon heat-induced electrical signals is reported.

Materials and methods

Plant culture and experimental set-up

Plants were cultivated from seeds under standard greenhouse conditions and with optimal water and nutrient supply.

Stomatal aperture, chlorophyll fluorescence, electrical potential, and leaf gas exchange were recorded simultaneously on the same, attached pinna of *Mimosa pudica* plants (Fig. 1). In order to allow these simultaneous measurements, a gas exchange cuvette was designed into which one leaf of the plant was inserted. At the tip of an adjacent leaf outside the cuvette, heat stimulation using a flame was carried out. In order to allow for microscopic observation, assessment of chlorophyll fluorescence, and measurement of membrane potential it was necessary to fix most of the leaflets of one pinna with the adaxial side to a Perspex plate with double-sided transparent adhesive tape (Tesa 56661–2, Tesa, Hamburg, Germany). Some terminal leaflets of the same pinna were left unfixed to determine the timing of leaflet folding. Subsequently, the plate was mounted inside the cuvette, which allows observation of the lower leaf surface with

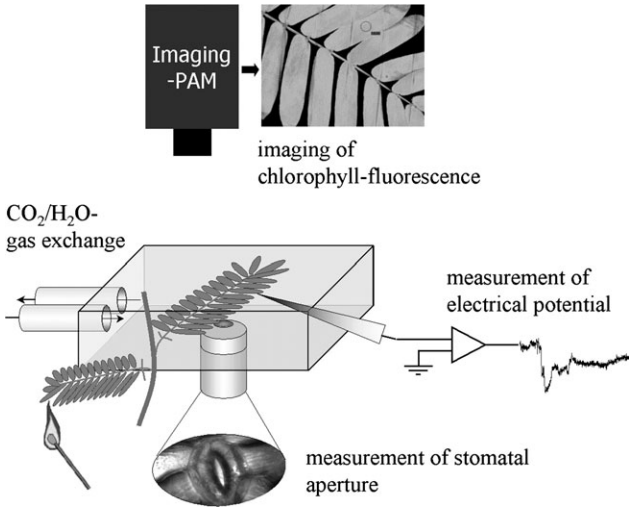

Fig. 1. Schematic plan of the experimental set-up allowing assessments of leaf gas exchange (CO_2, H_2O), chlorophyll fluorescence, epidermal electrical potential, and stomatal aperture at a pinna of a *M. pudica* plant.

a microscope lens mounted in the bottom of the gas-exchange cuvette (Kaiser and Kappen, 2001). Temperature and air humidity were set to 22 °C and a leaf–air mole fraction of water vapour (Δ_W) of 5 mmol mol^{-1}, respectively. Irradiance (*PPFD* of approximately 400 μmol photons m^{-2} s^{-1}) was provided by a halogen light source (LS2, Hansatech, UK). After inserting one pinna into the gas-exchange cuvette, the whole plant was allowed to relax and adjust to measuring conditions for at least 24 h.

Membrane potential

In the same experimental set-up (Fig. 1) and simultaneously with the other measurements, changes in the electric membrane potential of abaxial epidermal cells were monitored using glass microelectrodes with tip diameters of less than 1 μm, back-filled with 3 M KCl. These electrodes were inserted into the leaf epidermis using a micromanipulator. Electrical potential was recorded between the microelectrode and an Ag/AgCl reference electrode, which was connected to a cut end of another twig of the plant by immersion into artificial pond water (APW). Potentials were recorded by a FD23 electrometer (WPI Inc, USA) connected to a datalogger (CR10, Campbell Scientific, UK).

Stomatal aperture

Digital images of stomatal apertures on the lower leaf surfaces were recorded at a maximum rate of 1 image per 10 s during leaflet folding with a modified inverted microscope (Axiovert 25CFL, Zeiss; Kaiser and Kappen, 2001) equipped with a long-distance lens (×50) and a video camera. Subsequently, apertures (stomatal pore areas) were measured with custom image-analysis software.

Chlorophyll fluorescence

Simultaneously with the other measurements, fluorescence imaging of several leaflets covering the area of aperture and electrical measurements was used to assess the spatiotemporal variations of the photochemical quantum yield of energy conversion in PSII (Siebke and Weis, 1995) by means of an IMAGING-PAM Chlorophyll fluorimeter (Heinz Walz GmbH, Effeltrich, Germany). This system allows non-invasive determination of the photochemical quantum yield at PSII by the saturation pulse method (Genty *et al.*, 1989; Schreiber *et al.*, 1986). Saturation pulses were given every 12 s

and photochemical quantum yield at PSII was calculated as $(F'_m - F)/F'_m$ (for nomenclature, see Kooten and Snel, 1990).

Leaf gas exchange

Gas-exchange measurements allowing calculation of stomatal conductance (g_{H_2O}) and net CO_2-exchange rate (J_{CO_2}) were either performed simultaneously with the other measurements by means of a modified gas-exchange system (Heinz Walz GmbH, Effeltrich, Germany, Fig. 1, for details see Kaiser and Kappen, 2001) or carried out independently using a Li-Cor 6400 system (Li-Cor, Nebraska, USA).

Results

Approximately 40 s after heat stimulation of a neighbouring leaf at a distance of approximately 8–11 cm, the net CO_2 gas exchange of the observed leaf started to decline transiently to nearly zero within 1 min. This transient reduction started at the instant of leaflet upfolding. Subsequently, net CO_2 exchange recovered within the following 20 min to the initial levels (Fig. 2). Stomatal conductance rapidly increased during the first 2 min after heat stimulation and subsequently declined to approximately half of the values before the heat stimulation. Similar to the net CO_2 gas exchange, stomatal conductance recovered in the following 20 min to values similar to the initial rates.

Aperture measurements revealed a fast opening movement leading to a doubling of aperture. Although there was considerable variability in stomatal aperture between plants and experiments (cf. Figs 3, 4), the transient stomatal opening was consistently observed when leaves folded. This opening movement was completed within 1–2 min and followed by a pronounced closing movement approximately 1–2 min later (Fig. 3; a movie of these stomatal responses is available as supplemental material from http://jxb.oxfordjournals.org/). Four to five minutes after heat stimulation, observed stomata appeared completely closed. The sequence of reactions in electrical potential, leaflet upfolding, stomatal movement, and photochemical quantum yield at PSII is presented in Fig. 4. The measurements shown were conducted at the same spot of one leaflet as indicated in Fig. 5A. Within 1 min after stimulation of an adjacent leaf, the electrical potential dropped sharply at the observed leaflet, simultaneously with leaflet upfolding (which was prevented at the observed pinna by fixing of leaflets, see Materials and methods). At the same time, the opening response of the stomata started, leading to an increase of stomatal aperture of 80–100% within the next 60 s (cf. Fig. 3). However, a decline of photochemical quantum yield at PSII occurred only 2 min after heat stimulation, when the stomatal opening response was nearly completed (Fig. 5C) and 3.5 min after stimulation it reached a minimum of approximately 0.2. Chlorophyll fluorescence imaging revealed a high heterogeneity in photochemical quantum yield of 0.1 or lower (Fig. 5; a movie is available as supplemental material from http://jxb.oxfordjournals.org/). Recovery of photochemical quantum yield started in parallel with the recovery of electrical potential and was completed within 2 to 3 min (Figs 4, 5D).

Discussion

In the leaf of *Mimosa pudica*, not only the pulvinar motor cells respond to heat-induced signals, but the physiology of the entire leaf lamina is strongly affected (Fig. 5). When the electrical signal arrived at the observed leaf area, net

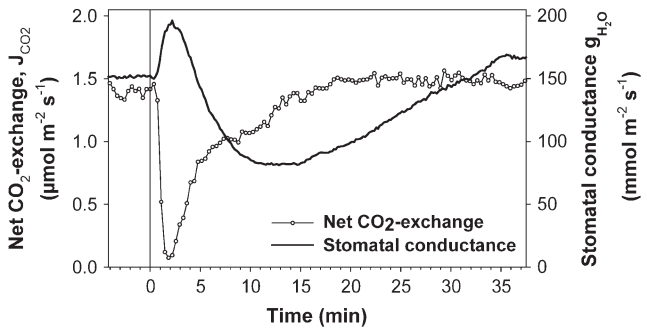

Fig. 2. Stomatal conductance (g_{H_2O}) and net CO_2 exchange (J_{CO_2}) during leaflet folding. The measured leaflets were fixed and not allowed to fold. The vertical line (time zero) indicates the time of heat stimulation of an adjacent pinna.

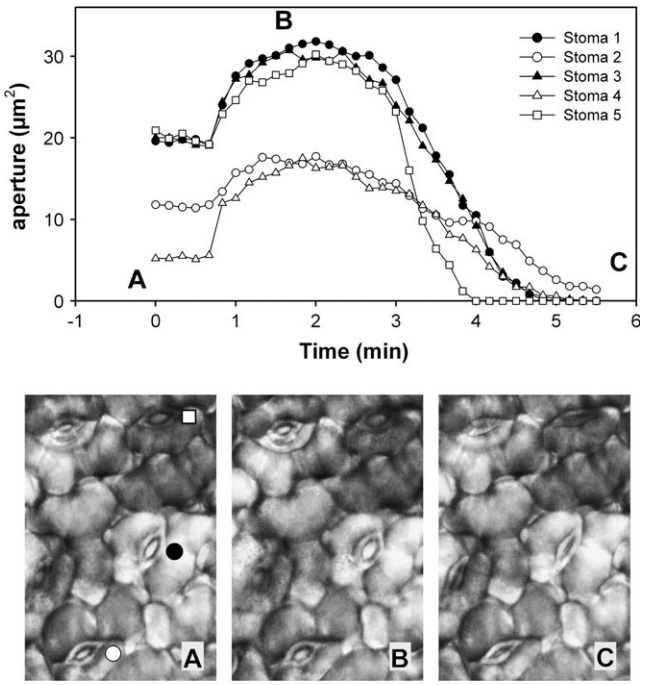

Fig. 3. Time-course of stomatal apertures during leaf folding and images of stomata from the same experiment taken at three different times (A, B, C). Legend symbols in (A) mark three stomata whose responses are plotted in the graph. Time zero is the time of heat stimulation of an adjacent pinna.

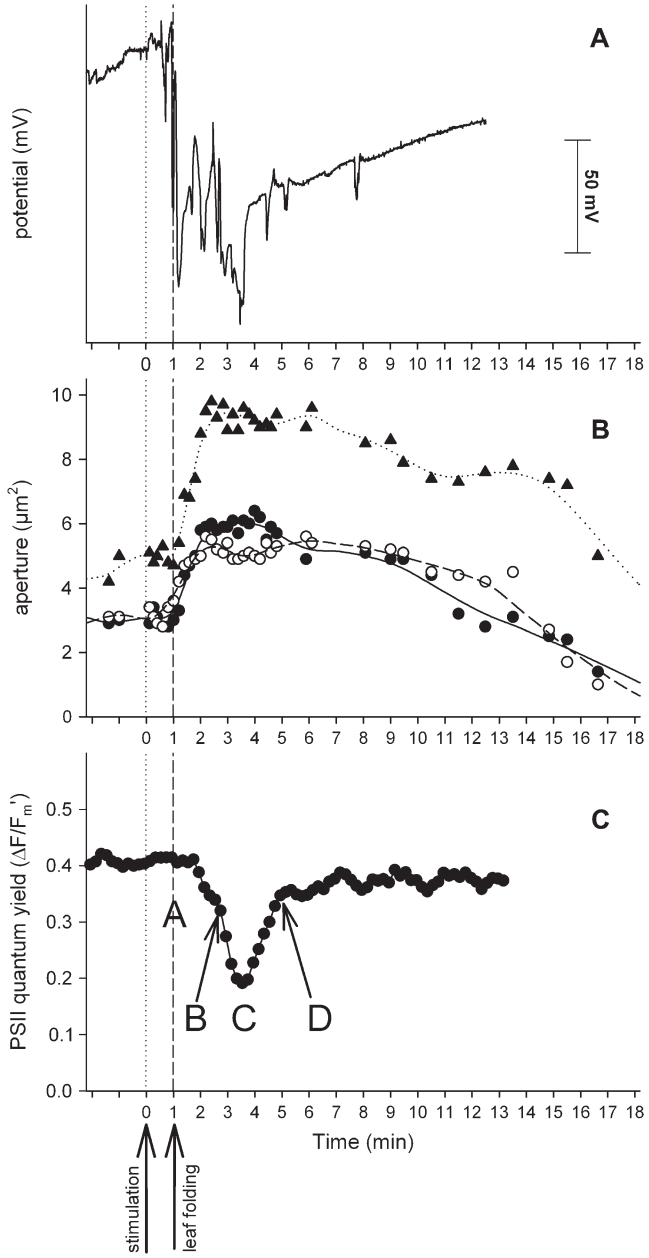

Fig. 4. Heat stimulation of an adjacent leaf caused the following responses: (a) changes of membrane potential measured with a microelectrode inserted into an epidermal cell on the abaxial leaf surface. Measurements were performed a few millimetres apart from the site of stomata observation, (b) aperture response of three stomata, and (c) photochemical quantum yield at PSII. Assessment of chlorophyll fluorescence was performed on the same spot as the aperture measurements. The vertical lines mark the times of heat stimulation (dotted) and leaf folding (dashed). Letters indicate the times of the yield measurements shown in Fig. 5.

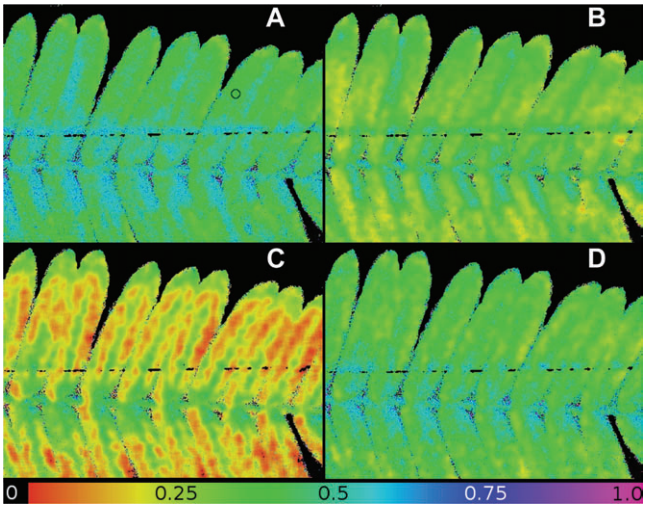

Fig. 5. Images of photochemical quantum yield at PSII in the moment of stimulation (A) and after 72 s (B), 156 s (C), and 228 s (D) in the experiment shown in Fig. 4. The circle in (A) indicates the spot where the fluorescence and aperture measurements shown in Fig. 4 were performed.

CO_2 exchange started to decline transiently (Fig. 2). Approximately 1 min later, photochemical quantum yield at PSII was also decreased to values of 0.1–0.2 (Figs 4, 5). This observed transient knockout of the dark and light reactions of photosynthesis are in accordance with a previous report (Koziolek *et al.*, 2004) and are similar to recently published observations in poplar (Lautner *et al.*, 2005).

Koziolek *et al.* (2004) also reported a rapid transient increase of transpiration when leaves were prevented from reducing their transpiring area by overlapping their leaflets during the upfolding reaction. From the gas-exchange measurements alone, Koziolek *et al.* (2004) could not identify the reason for the transient increase in water loss. In this work, direct evidence for a fast and substantial stomatal response triggered by heat stimulation of distant leaves is presented. This stomatal response is complex and consists of two phases, (i) a fast opening response which is followed by (ii) a pronounced reduction of stomatal aperture. In principle, stomata can open for two reasons: an increase in guard cell turgor or a decrease in epidermal turgor. An increase in guard cell turgor requires energy and is inherently slow. By contrast, 'hydropassive' stomatal movements are caused by an epidermal turgor loss without any energy-dependent osmotic increases of guard cell turgor and, thus, can be quite fast. The best known example for such hydropassive movements is the Iwanoff-effect (Iwanoff, 1928), which occurs after cutting a transpiring leaf, and the transient stomatal opening caused by a sudden increase in leaf-to-air mole fraction water difference (Kappen *et al.*, 1987; Kappen and Haeger, 1991). Due to the quickness of the opening movement (phase 1), it is concluded that it is not osmotically driven but caused by a sudden loss of epidermal turgor. The sequence of events supports this interpretation. Stomatal opening started exactly when epidermal cells depolarized and leaflets folded. The most likely explanation for the initial opening response (phase 1) is that epidermal cells, similar to the pulvinar motor cells, respond with depolarization and a decrease of turgor. Another possible

explanation for passive stomatal movements might be the hydraulic pressure wave which travels through the mimosa plant (Hooke, 1667; Malone, 1994). However, an increase in pressure should result in stomatal closure rather than opening because it would increase epidermal backpressure on guard cells. In fact, Malone's measurements (1994) give evidence that the pressure wave leads to a transient increase of leaf thickness. However, during leaf folding this initial increase of leaf thickness was reversed and followed by a sharp decline of leaf thickness (by approximately 16 %). Although Malone did not interpret these measurements in this functional manner, they are in accordance with the proposition of epidermal turgor loss.

During the second phase of stomatal response following the initial opening, aperture declined at various rates (cf. Fig 3, 4). In some cases, an extremely fast response led to full closure of previously widely opened stomata within 1.5 min (Fig. 3). As far as is known, such fast closing responses have not previously been reported. Stomatal closure can be caused either by a decrease in guard cell turgor or a recovery of epidermal turgor and it is difficult to differentiate between these two mechanisms. However, since stomata closed further than their initial aperture before the heat stimulation, it is likely that guard cell osmotic potential decreased.

Stomata of *M. pudica* apparently do not respond directly to the depolarization of the epidermal membrane potential as their closing response was delayed for several minutes. This is not surprising, when considering the fact that they are electrically isolated by the lack of plasmodesmata to the adjacent epidermal cells (Palevitz and Hepler, 1985). Therefore, guard cell deflation is most likely not triggered directly by the electrical signal traveling through the leaf tissue, but by indirect factors. These could include apoplastic factors as well as an increased intercellular CO_2-concentration due to the inhibition of photosynthesis. Although substantially delayed, the closing response is, nevertheless, a stomatal response, which is ultimately triggered by propagating heat-induced signals. These results underline the possible involvement of electrical signals in long-distance signalling for co-ordinating leaf gas exchange on the whole plant level (Fromm, 1998; Fromm and Eschrich, 1993; Van Sambeek and Pickard, 1976).

When the heat-induced signal reaches a *Mimosa* leaf, both the pulvinar and epidermal cells lose turgor at the same time. This demonstrates that, although pulvinar motor cells are specialized to perform a very fast and substantial turgor loss upon depolarization, this ability is also present in epidermal cells. In addition to the evidence from chlorophyll fluorescence and gas-exchange measurements, these results underline that the entire leaf and not only the pulvini undergo intense physiological responses. These results present evidence that the heat-induced signal causes (i) a rapid, hydropassive stomatal opening response as a result of turgor loss of the surrounding epidermal cells, and (ii) an active stomatal closure concomitant with a loss of net CO_2 uptake.

Supplementary data

Supplemental material to this paper is available at http://jxb.oxfordjournals.org/

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