Water movement between epidermal cells of barley leaves – a symplastic connection?

W. FRICKE

Dept of Biological Sciences, University of Paisley, Paisley, PA1 2BE, Scotland, UK

ABSTRACT

Using the cell-pressure probe the possibility of symplastic water flow between cells of the upper epidermis of barley leaves was investigated. Cells analysed had either an intact or a more or less damaged cellular environment. Cell damage caused large pressure differentials (0.9 MPa) between damaged and adjacent intact cells. Turgor in cells adjacent to damaged cells decreased significantly. Turgor decreases were the larger the more the adjacent, damaged cell was leaking (decreases by 2.5–4.4%). In cells surrounded by a patch of leaking cells, turgor decreased the most, by 18.1–20.4%. In contrast, half-times of water exchange ($T_{1/2}$) of cells were not affected by a damaged cellular environment. Assuming that in the barley leaf epidermis, plasmodesmata close at pressure-differentials at or exceeding 0.2 MPa as shown for other plant cells (Fricke et al. 1995; Fensom 1987) showed for tobacco leaf hair cells and giant algae cells, respectively, that plasmodesmata close when pressure differentials between neighbouring cells reach or exceed 0.2 MPa. Considering that plant viruses can spread through plant tissues by altering the size exclusion limit of plasmodesmata (Oparka et al. 1997), damage and viral infection of a cell through leaf-boring insects might well lead to changes in symplastic water and solute transport properties. If so, knowing the main pathway of water and solute movement in uninfected tissue, will aid prediction of the potential impact of infection on plant performance during osmotic or transpirational stresses.

The aim of the present study was to measure the effect of cell damage on turgor and half-time of water exchange ($T_{1/2}$) in adjacent, undamaged, epidermal cells. This served two purposes: first, to consider the possibility of symplastic water flows, and second, to evaluate the possibility of cell damage being signalled to adjoining cells hydraulically.

Concerning symplastic water flows, the reasoning was as follows. If plasmodesmata close in a pressure-dependent manner with complete closure at a pressure differential (between adjacent cells) of about 0.2 MPa, then puncturing a cell and reducing its turgor pressure by considerably more than 0.2 MPa should close plasmodesmal connections to adjacent, high turgor cells. Provided that symplastic water flow contributes significantly to water exchange between cells, the $T_{1/2}$ of water exchange of a cell next to a punctured cell should increase (be slower). The magnitude of the effect should increase with the number of punctures per cell or the number of punctured (and leaking) neighbouring cells and should be largest for a cell surrounded by punctured cells. If, on the other hand, water exchange between cells involves mainly transcellular or apoplastic routes, puncture of adjacent cells should not increase $T_{1/2}$ significantly.

Concerning the occurrence of hydraulic signalling between cells, the reasoning was that if turgor in a cell next to a punctured cell is not significantly different from turgor in a cell with an entirely intact cellular environment,
hydraulic signalling can be ruled out as means of communicating cell damage to adjacent cells, at least for the barley leaf epidermis.

Using the cell-pressure probe, turgor and $T_{1/2}$ were measured in cells of the upper epidermis of barley leaves. Within 10–20 s following the removal of the probe from a probed cell, a cell adjacent to the previously probed (and now leaking) neighbouring cell was analysed. The relative location (lateral versus axial), number of leaks per cell and number of leaking cells was varied.

**MATERIALS AND METHODS**

**Plant growth**

Barley, *Hordeum vulgare* L. cv Golf, was grown hydroponically on modified Hoagland solution as described earlier (Fricke, McDonald & Mattson-Djos 1997), except that the Hoagland solution was half-strength. During most experiments, third leaves were analysed. Occasionally, fourth or fifth leaves were analysed. Leaf analyses were carried out during the last few days preceding full expansion, since fully expanded leaves often had turgor pressures too high (> 1–6 MPa) to be recorded with the pressure probe used.

Two types of cells were analysed, both of which were located within the upper (= adaxial) epidermis: cells overlying ridge regions, ridge-cells, and cells located within trough regions, trough-cells (see Fig. 1 in Fricke 1997). Both cell types differ markedly in shape and size. Whereas ridge cells resemble long narrow tubes, with lengths more than a millimetre and volumes approaching 1 nL, trough cells are rather compact, shorter and with volumes in the 400 pL range. Measurements of cell dimensions in the third leaf during previous experiments (Fricke 1997) gave the following values (ridge cell/trough cell): length, 1500/302 μm; width, 25/37·5 μm; depth, 25/37 μm; volume, 736/419 pL; surface, (1·19 × 10⁴)/(4·78 × 10⁴) μm²; and volume : surface ratio, 6·17/8·77 μm².

**Turgor measurements**

Cell turgor was measured with a micropressure probe (Steudle 1993) as described previously (Fricke 1997). Analyses were carried out on intact plants. Apart from dim background (lab-) illumination and scattered daylight, the leaves did not receive any extra illumination to maintain photosynthetic radiation (PAR) at high levels. Therefore, the stomata were opened only little and, by implication, transpiration was low. As a consequence, epidermal cell turgor remained stable over the analysis period.

Probe capillaries were pulled from borosilicate glass tubes (outer diameter, 1·0 mm; inner diameter, 0·58 mm; Clark Electromedical Instruments, Pangbourne, UK) using a capillary puller (type 50–2013, Harvard Apparatus Ltd, Edenbridge, UK) and backfilled with silicon oil (type AS4, Wacker Chemie, München, Germany). The tips were broken by insertion into a leaf cell and sideways (‘brushing’) removal. Most capillaries could be used for between 40 and 60 turgor measurements.

During routine turgor analyses, the cells were punctured with the silicon oil in the probe capillary at near atmospheric pressure. It took less than 20 s to push the cell sap/silicon oil meniscus back to the cell surface and within the following 30–60 s, turgor settled at a stable value. Turgor was recorded for 1–2 min, before $T_{1/2}$ measurements were carried out.

In experiments where the effect of puncturing a cell next to a cell to be pressure-probed was tested, turgor was measured in a cell, hereafter termed ‘cell-1’. The probe was then removed, leaving the cell with a puncture (leak). A cell, hereafter termed ‘cell-2’, adjacent to the leaking cell was analysed for turgor within the next 1–3 min.

**$T_{1/2}$ measurements**

The half-time ($T_{1/2}$) of water exchange across cell membranes was determined by turgor relaxation experiments (Steudle 1993).

To induce an osmotically driven water flow across the cell membrane, cell turgor was changed as quickly as possible by first lowering the pressure in the probe and holding the sap–oil meniscus at its new position (endosmotic water flow) and then moving the sap/oil meniscus back to the cell surface and holding it there (exosmotic water flow). For most cells, this procedure was repeated twice (three individual values) and the average taken as $T_{1/2}$ (endo) and $T_{1/2}$ (exo), respectively. Ideally, during a pressure relaxation experiment, turgor should be changed instantaneously or at least considerably quicker than $T_{1/2}$. This proved difficult for ridge cells. These cells had extremely short half-times of water exchange of about 0·68 s [in fact, in addition to $T_{1/2}$ in growing cortex cells of pea epicotyls (Cosgrove & Steudle 1981), the fastest $T_{1/2}$ so far recorded for any plant cell]. Given the pressure–volume characteristics and the motor-speed adjustment of the pressure probe used and the shape of the capillary tip employed (capillaries with a long, narrow, low-volume-tip could not be used as these were bending away from, rather than penetrating a cell) it was not possible to avoid some water flow across cell membranes during turgor manipulation, at least for ridge cells. However, varying the magnitude of imposed turgor change (< 0·1 MPa to about 0·2 MPa) had no apparent effect on the value of $T_{1/2}$.

**Experimental design**

Two different types of experiment were carried out: turgor and $T_{1/2}$ were measured (i) in cells adjacent to punctured cell(s), and (ii) in cells impaled at elevated probe pressure.

**Turgor and $T_{1/2}$ measurements in cells adjacent to a punctured cell(s)**

Within ridge regions, cells halfway-down or at the base of a ridge were analysed. Turgor and $T_{1/2}$ were measured in a
cell (cell-1). The probe was then removed, leaving the cell with a puncture (leak). Within the next 1–6 min, a cell (cell-2) adjacent to the leaking cell was analysed for turgor and $T_{1/2}$. In one set of experiments, the probed cell was located laterally to the punctured (and leaking) cell and the latter had only one puncture (‘RiLatSi’-experiment); in another set of experiments, the punctured cell had been punctured three times (puncture through probing plus two subsequent punctures; ‘RiLatTri’-experiment); and in a third set of experiments the probed cell was in the same cell file (axial) as the punctured cell (one puncture; ‘RiAxSi’-experiment). Cells were probed at a location as close as possible to the leak in the neighbouring cell. The distance between leak (previously punctured cell) and pressure-probe recording was only about 15–50 μm. Within trough regions, only cells located within the middle of troughs, i.e. cells that had no direct contact with cells next to stomatal pores, were analysed. Turgor and $T_{1/2}$ were measured in a trough cell (cell-1). The capillary was removed and all other cells adjacent to the next cell to be probed were punctured. This left the cell to be probed (cell-2) isolated within a patch of leaking cells. Probing and $T_{1/2}$ measurements were carried out within the following 1–6 min (‘TrIsol’-experiment). None of the underlying mesophyll cells was punctured.

In the above experiments each cell was analysed for both, turgor and $T_{1/2}$. In addition, some experiments were carried out where only cell turgor was measured.

**Turgor and $T_{1/2}$ measurements in cells impaled at elevated probe pressure**

To minimize disturbance of subcellular organization and avoid large, transient pressure changes when impaled, the cells were impaled at a pressure-probe pressure just below the anticipated cell pressure. The pressure difference ranged from 0·027 to 0·211 MPa. Only ridge cells were analysed.

**RESULTS**

**Turgor and $T_{1/2}$ in cells adjacent to a leaking cell(s)**

Turgor and $T_{1/2}$ were measured in cell-1, and after removal of the probe were measured in an adjacent cell, cell-2. Turgor pressure of cells in an undamaged cellular environment (cell-1) was similar for ridge and trough cells and ranged from 0·827 to 1·082 MPa (see 100%-values in Fig. 1). Mean values, pooled from all experiments, were $0·888 \pm 0·157$ and $0·870 \pm 0·080$ MPa for ridge and trough cells, respectively ($n = 87$ and $n = 24$ cells).

Results for cell-2, i.e. cells with a damaged cellular environment, were expressed as percentage of the cell-1-value (Fig. 1). In ridge cells, all treatments resulted in small, but significant turgor decreases in cells adjacent to damaged cells. Turgor in cells located laterally next to a leaking cell was reduced by 3-1%, when the leaking cell had one leak only. When the leaking cell had three leaks turgor was reduced slightly more, by 4-4%. When cells were located axially (same cell file) next to a leaking cell, turgor was reduced by only 2-5%. Turgor in a trough cell surrounded by a patch of leaking trough cells was reduced by 18-1%. Similarly, significance levels by far exceeded those for ridge cells. Experiments in which only trough cell turgor (but not $T_{1/2}$) was measured gave similar results, with a reduction in turgor by 20-4% and $P = 4·21 \times 10^{-13}$ ($n = 38$ pairs of cells; not shown).

In both, cells with an undisturbed (Fig. 2a) and cells with a damaged cellular environment (Fig. 2b), exosmotic water flow was as fast or faster than endosmotic water flow. In cells with an undisturbed cellular environment (cell-1 type cells), differences between $T_{1/2}$ (endo) and $T_{1/2}$ (exo) were in two of four experiments significant (Fig. 2a), whereas in cells with a disturbed cellular environment (cell-2 type cells), differences were always significant (Fig. 2b). The $T_{1/2}$ values were the same or slightly larger (slower) in cells with a disturbed compared to cells with an intact cellular environment (Fig. 2c). However, only in one experiment and then only for $T_{1/2}$ (endo) was the difference significant (at $P = 0·05$).

When data from all experiments and from cell-1 and cell-2 type cells were pooled, ridge cell $T_{1/2}$ was $0·706 \pm 0·119$ s and $0·650 \pm 0·097$ s for endosmotic and exosmotic water flow, respectively ($n = 174$ cells). Values for trough cells
were 1.338 ± 0.601 s and 1.068 ± 0.340 s (n = 48 cells). Differences between endo- and exosmotic $T_{1/2}$ were highly significant with $P = 8.63 \times 10^{-13}$ for ridge, and $P = 7.19 \times 10^{-5}$ for trough cells.

**Turgor and $T_{1/2}$ in cells impaled at elevated probe pressure**

The $T_{1/2}$-values were almost identical in cells impaled at atmospheric probe pressure or a probe pressure just (0.027–0.211 MPa) below turgor pressure (Table 1). Turgor pressure was slightly, but significantly higher in cells impaled at elevated probe pressure. The average increase was 0.034 MPa, corresponding to a rise of 4.1%. Most likely this increase was an artifact and resulted from the experimental approach: when a cell was impaled at elevated probe pressure, silicon oil was flushing out of the capillary tip and some of the silicon oil was entrapped in the cell. Even though most of this oil could be retrieved into the capillary during subsequent turgor measurements, some small oil droplets remained in the cell and increased osmotic pressure, cell volume and turgor.

**DISCUSSION**

The results show that in leaf epidermal cells of barley, the half-time of water exchange is not significantly affected by the presence of a leaking, low-turgor neighbouring cell or cellular environment. In contrast, turgor decreases. The decrease in turgor is always significant and is larger the more the cellular environment leaks.

The response in $T_{1/2}$ can be interpreted in several ways. If plasmodesmata in barley leaf epidermal cells behave similarly to plasmodesmata in tobacco leaf hairs cells and *Chara* and close at pressure differentials of 0.2 MPa (the present pressure differentials were considerably larger), then lack of responses of $T_{1/2}$ to neighbouring-cell puncture suggests that the symplastic pathway plays a minor, insignificant role in water exchange between cells. Alternatively, the symplastic pathway contributes significantly to water exchange between cells, but plasmodesmata between leaf epidermal cells of barley do not close in a pressure-differential-dependent way, even at pressure differentials approaching 1.0 MPa, or, for some reason did not close in the present experiments. A third explanation is that in barley leaf epidermal cells, plasmodesmata neither closed
in a pressure-differential-dependent way nor contributed significantly to intercellular water exchange.

Is it possible that the experimental setup affected the ability to detect plasmodesmatal closure?

Based on the intercellular movement of micro-injected fluorescence dye, Oparka & Prior (1992) concluded that plasmodesmata in tobacco leaf hair cells close completely when pressure differentials between neighbouring cells reach or exceed 0·2 MPa. A similar conclusion was reached by Coté et al. (1987) for internodal cells of Chara, applying electrophysiological approaches. Zawadski & Fensom (1986) observed internodal transport of C14 (photosynthetic) in tandem cells of Nitella, up to pressure differentials of 0·25 MPa. However, transport decreased with increasing pressure differentials. In the present study, pressure differentials between the cell to be analysed and its leaking neighbouring cell(s) were about 0·9 MPa. Thus, pressure differentials should have been large enough to induce plasmodesmatal closure to a detectable level.

Oparka & Prior (1992) made two observations concerning the time scale of plasmodesmatal closure. First, they observed that once plasmodesmatal closure had been initiated through application of pressure differentials, closure remained sustained even after abolition of the pressure differential. Second, the authors observed that once a pressure differential between cells had been applied, it took about 10 min to induce complete plasmodesmatal closure – after 1 min only 0% and after 5 min 66% of plasmodesmatal closure occurred.

In the present study, cells selected for routine turgor and T1/2 measurements were impaled with the pressure in the probe at atmospheric pressure. Thus, upon impalement, large and transient pressure differentials were created between impaled (zero turgor) and intact neighbouring cells. Even though cell turgor was raised to its original value within 10–20 s it is possible that plasmodesmatal closure had already been caused and continued during the subsequent T1/2 measurements. In this case, contribution of symplastic water flows to T1/2 would have remained undetected. Similarly, it is possible that during pressure relaxation experiments itself, imposed pressure changes caused plasmodesmata to close and symplastic water flows to be undetectable. However, this is unlikely, for two reasons. First, there was no apparent relation between the size of imposed pressure change (< 0·1 MPa to > 0·2 MPa) and measured T1/2 (not shown). Second, cells impaled at elevated probe pressure, i.e. at pressures in the probe only 0·027–0·211 MPa lower than turgor pressure (on average 0·110 ± 0·050 MPa lower; n = 22 cells). Both types of cell analyses were carried out on the same leaves, and results are shown as means ± SD of 22 cells

<table>
<thead>
<tr>
<th>Impaled</th>
<th>Cell turgor (MPa)</th>
<th>T1/2 (s)</th>
<th>Endosmotic</th>
<th>Exosmotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>At atmospheric pressure</td>
<td>0·832 ± 0·051</td>
<td>0·46 ± 0·08</td>
<td>0·47 ± 0·08</td>
<td></td>
</tr>
<tr>
<td>At elevated pressure</td>
<td>0·866 ± 0·036</td>
<td>0·44 ± 0·07</td>
<td>0·46 ± 0·08</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Turgor pressure and half-time of water exchange (T1/2) in leaf epidermal cells impaled with the pressure in the probe at atmospheric or at elevated pressure, i.e. a pressure only 0·027–0·211 MPa lower than turgor pressure (on average 0·110 ± 0·050 MPa lower; n = 22 cells). Both types of cell analyses were carried out on the same leaves, and results are shown as means ± SD of 22 cells.
Murphy & Smith (1998) used the pressure-clamp technique to study water transport in mesophyll tissue of Kalanchoë. The authors concluded that symplastic water transport contributes significantly to water exchange of cells during endosmotic water flow, particularly at low pressure differentials (about 0.08–0.16 MPa, see Fig. 8 of Murphy & Smith 1998). Although no quantitative estimate was given, it is possible that differences in the contribution of symplastic water flow between barley leaf epidermis and Kalanchoë mesophyll are linked to differences in the tissues’ physiology or plants’ ecophysiology (C₃ versus CAM). Westgate & Steudle (1985) concluded that during rehydration (osmotic gradients) water flow between maize leaf xylem and midrib-tissue (including epidermis) occurred predominantly along a cell-to-cell-path. This does not necessarily contradict the present conclusion since the cell-to-cell path comprises both a symplastic and transcellular pathway.

Zhang & Tyerman (1991), using the pressure-probe and pressure-clamp technique, observed that the hydraulic conductivity of wheat-root cortical cells decreased by a factor of about three to five (increases in T₁/₂) when roots were treated with azide or analysed under low-oxygen conditions. The authors explained the increases in hydraulic conductivity by an occlusion of plasmodesmata. Further support was gained through the observation that in non-treated roots, the osmotic volume was about twice the geometrical volume of cells (indicative of considerable symplastic solute movement), whereas in azide- or low-oxygen-treated roots, both volumes matched. Malone & Tomos (1990) found for leaf epidermal cells of wheat that osmotic and geometrical volume were about equal. This indicates that in cereals, symplasmic flow of solutes and water plays a more significant role in root cortical compared to leaf epidermal cells and supports the present conclusion, that symplastic water movement contributes insignificantly to water exchange between leaf epidermal cells of barley. Finally, in the present study, only epidermal, but not mesophyll cells were damaged. Therefore, symplastic connections between mesophyll cells and cell-2 may have remained fully functional during experiments and the results do not rule out the possibility of extensive symplastic water (and solute) flow between mesophyll and epidermis.

A large, if not the main proportion of transcellular water flow in plant cells occurs through aquaporins (for review, see Tyerman et al. 1999). The quantitative contribution of water flow through aquaporins was not tested for the epidermal cells analysed. However, the results do suggest that aquaporin function is unaffected by large transcellular pressure gradients. Cosgrove & Steudle (1981) observed for growing cortex cells of pea epicotyl segments that T₁/₂ increased abruptly from short (0.3–1 s) to large (3–20 s) values after cells had been subjected repeatedly (10–30 times) to large pressure relaxations. One of the authors possible explanations was that plasmodesmatal pores had been occluded by the induced water flows. In hindsight, results could have also pointed to a sudden closure of aquaporins. However, if so, the closure of aquaporins might not have been directly pressure-dependent, since other treatments had a similar effect on T₁/₂.

The half-time of water exchange was almost twice as fast in ridge as in trough cells. To a large extent, this difference resulted from a 42% difference in the surface-to-volume ratio of cells. Whether the residual difference in T₁/₂ between ridge and trough cells reflects true differences in the water transport properties of membranes can not be said since other factors such as differences in elastic moduli or sweep-away effects during pressure relaxations were not investigated.

A difference between T₁/₂(endo) and T₁/₂(exo) has also been noted in other plant tissues (Steudle, Smith & Lüttge 1980; Moore & Cosgrove 1991). The present results suggest that in barley, leaf epidermal cells respond quicker to a decrease than to an increase in water potential in the cell environment [T₁/₂(exo) < T₁/₂(endo)]. At the leaf level, this could provide a means to perceive increases in evaporative demand quickly.

A small cell leak, caused by puncture with a microcapillary and of the size of only several microns, was enough to reduce turgor in adjacent cells significantly (even at low-transpiring conditions). However, in absolute terms the reduction in turgor was small (< 0.04 MPa) compared with the short-term fluctuations in leaf turgor in a (natural and) changing water-potential environment (Fricke 1997). Therefore, damage to individual leaf cells by ‘leaf-sucking’ insects such as mites or thrips should be recognizable as turgor changes in the cell environment but it is unlikely that these turgor changes function as signals per se. Rather, the differential in turgor between damaged and intact cells should be important in the wound response. Considering the results from T₁/₂ measurements, it is concluded that the turgor differentials serve primarily to minimize the loss of high-molecular-weight, membrane-impermeable cell components, but not water, to the punctured cell. Small-molecular-weight solutes may well have been lost to the punctured cellular environment: Turgor in trough cells surrounded by a patch of leaking trough cells decreased by 18.1–20.4%, yet cell osmotic pressure as determined by micropile osmometry of extracted cell sap (Malone, Leigh & Tomos 1989) decreased, not increased (!), slightly (437 ± 13 mosmol kg⁻¹; compared to 448 ± 11 mosmol kg⁻¹ in cells with an intact cellular environment; n = 22 cells). Thus, if the 18.1-to 20.4% decrease in turgor was associated with a decrease in cell volume by about 1–3%, solutes must have exited the cell quickly (minutes) and at flux densities (order of 10⁻⁷–10⁻⁶ moles m⁻² s⁻¹) exceeding those in growing leaf epidermal cells and matching those at guard cell membranes (listed in Fricke & Flowers 1998).

Finally, it is intriguing to ask to what degree ‘leaf-sucking’ insects are actually sucking. Provided the stylet penetrates the cell in such a way that it does not cause a leak, the insect performs rather an endosmotic pressure relaxation with the only, and crucial exception, that turgor is kept low.
ACKNOWLEDGMENTS

The author thanks the University of Paisley, particularly Professor Roger O. McLean, for financial support.

REFERENCES


Received 1 March 2000; received in revised form 16 May 2000; accepted for publication 16 May 2000