The short-term growth response to salt of the developing barley leaf

Wieland Fricke1,*, Gulya Akhiyarova2, Wenxue Wei1,†, Erik Alexandersson3, Anthony Miller4, Per Ola Kjellbom3, Andrew Richardson1, Tobias Wojciechowski1, Lukas Schreiber5, Dima Veselov2, Guzel Kudoyarova2 and Vadim Volkov1,6

1 Division of Biological Sciences, University of Paisley, Paisley PA1 2BE, UK
2 Russian Academy of Sciences, Ufa Research Centre, 450054 Ufa, Russia
3 Department of Plant Biochemistry, Lund University, PO Box 124, SE-22100 Lund, Sweden
4 Crop Performance and Improvement Division, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK
5 Institute for Cellular and Molecular Botany, Bonn University, Kirschallee 1, D-53115 Bonn, Germany
6 Institute of Biomedical and Life Sciences, Division of Plant Sciences, University of Glasgow, Glasgow G12 8QQ, UK

Received 21 July 2005; Accepted 12 December 2005

Abstract
Recent results concerning the short-term growth response to salinity of the developing barley leaf are reviewed. Plants were grown hydroponically and the growth response of leaf 3 was studied between 10 min and 5 d following addition of 100 mM NaCl to the root medium. The aim of the experiments was to relate changes in variables that are likely to affect cell elongation to changes in leaf growth. Changes in hormone content (ABA, cytokinins), water and solute relationships (osmolality, turgor, water potential, solute concentrations), gene expression (water channel), cuticle deposition, membrane potential, and transpiration were followed, while leaf elongation velocity was monitored. Leaf elongation decreased close to zero within seconds following addition of NaCl. Between 20 and 30 min after exposure to salt, elongation velocity recovered rather abruptly, to about 46% of the pre-stress level, and remained at the reduced rate for the following 5 d, when it reached about 70% of the level in non-stressed plants. Biophysical and physiological analyses led to three major conclusions. (i) The immediate reduction and sudden recovery in elongation velocity is due to changes in the water potential gradient between leaf xylem and peripheral elongating cells. Changes in transpiration, ABA and cytokinin content, water channel expression, and plasma membrane potential are involved in this response. (ii) Significant solute accumulation, which aids growth recovery, is detectable from 1 h onwards; growing and non-growing leaf regions and mesophyll and epidermis differ in their solute response. (iii) Cuticular wax density is not affected by short-term exposure to salt; transpirational changes are due to stomatal control.

Key words: Abscisic acid, aquaporin, cell elongation, cuticle, cytokinin, epidermis, Hordeum vulgare, leaf development, membrane potential, solute and water transport.

Introduction
Salinity affects leaf growth in the short and long term. The causes of growth reduction differ (Munns, 1993; Munns et al., 1995), but it is not clear which mechanisms plants employ to maintain residual growth and to what extent these mechanisms differ between short- and long-term responses. The immediate effect of addition of salt to the root medium is a lowering in external water potential and a reduction in water uptake of plants, similar to the situation under drought (Munns, 2002). As salinity continues, Na and Cl are taken up and, increasingly, displace mineral nutrients such as K, Ca, and nitrate. Although accumulation of Na and Cl aids osmotic and water potential adjustment...
of cells, it increases the risk of long-term ion toxicity, if not compartmentalized appropriately, exported, or secreted.

Leaf elongation velocity (LEV) in grasses is a measure of growth and can be recorded continuously and at high resolution using linear variable displacement transducers (LVDT). Many studies have shown that LEV responds within seconds to changes in external water potential or evaporative demand (Hsiao et al., 1970; Acevedo et al., 1971; Cutler et al., 1980; Cramer and Bowman, 1991; Passioura and Munns, 2000). Under saline conditions, LEV may be reduced to zero or close to zero, depending on the stress level (Thiel et al., 1988; Yeo et al., 1991). The most obvious explanation for the rapid cessation in leaf growth is that salinity reduces xylem water potential in the elongation zone below the water potential of growing, peripheral cells. Since water cannot be pumped actively against a gradient in water potential, growth stops.

To resume water uptake into growing cells and leaf elongation, the gradient in water potential between xylem (higher water potential) and peripheral cells (lower water potential) has to be restored. In principle, there exist two strategies to achieve this. Either the water potential of peripheral cells, in the epidermis and mesophyll, decreases through an increase in osmolality or a decrease in turgor; or xylem water potential increases through a decrease in transpiration (reduced water loss) or an increase in root hydraulic conductance (increased water supply for a given driving force) (Fig. 1). Similarly, the rate of water movement between leaf xylem and peripheral growing cells depends on both the magnitude of the gradient in water potential (driving force) and the hydraulic conductivity (conductance) between the two locations. Water from the xylem has at some point to cross membrane(s) to reach peripheral cells, and it is possible that hydraulic conductivity is controlled by water channel activity. The latter is affected by a range of internal factors (Johansson et al., 2000), including abscisic acid (ABA) (Hose et al., 2000), a plant hormone also known to affect transpiration. The dual function of ABA as a regulator of whole-plant water balance through transpiration and a regulator of tissue water transport through water channel activity makes this hormone a prime candidate to be involved in growth responses to disturbances in external water potential.

It is obvious from the considerations above that a plant has multiple options to respond to salt. The ideal alternatives would be not to take up salt and achieve osmotic adjustment through accumulation of solutes other than Na and Cl in cells—solutes that are less cytotoxic—or to take up Na and Cl and use these solutes as osmolytes in the vacuole of cells, while maintaining cytosolic levels of Na and Cl in a non-toxic range and accumulating solutes which are compatible with cell functions (e.g. proline, sugars, glycine betaine) in the cytoplasm. Both of these options require regulation of transport processes and provision of solutes other than Na and Cl at osmotically significant levels.

The developing barley leaf, as leaves of other grasses, offers an ideal experimental system to study growth. An intercalary meristem produces cells that undergo elongation in a spatially defined elongation zone. In leaf 3 of barley, the elongation zone stretches to about 40 mm from the point of leaf insertion. Above the elongation zone is a zone in which cells have ceased to elongate yet continue some lateral expansion. This ‘non-elongation’ zone is still enclosed by sheaths of older leaves, unlike the uppermost portion of the growing leaf, which has emerged from surrounding sheaths. The micro-environment between enclosed and emerged leaf portions differs with respect to humidity, light and, probably, tissue-levels of carbon dioxide. Therefore, the growing grass leaf represents an inherent combination of growth, developmental, and micro-environmental zones, and this combination can be used to identify signalling events which are involved in the regulation of growth and growth responses to stress.

This review deals, in particular, with the roles which water transport, ABA, cytokinins, and solute accumulation play in the growth response to salt of the growing barley leaf within the first minutes to days of exposure to salt. Key solutes used for the generation of osmolality have been shown to be distributed differently between leaf tissues such as the epidermis and mesophyll (Leigh and Tomos, 1993; Fricke et al., 1994, 1996).
Materials and methods

The materials and methods employed have been described in detail previously (Veselov et al., 1992; Fricke et al., 1994, 1997; Tomos et al., 1994; Fricke and Peters, 2002; Fricke, 2004; Richardson et al., 2005). All experiments were carried out with barley (Hordeum vulgare L. cv. Golf (Svalöf Weblab AB, Svalöv, Sweden), on the elongating leaf 3, between 1 and 4 d following its emergence from the sheath of leaf 2. Plants were grown hydroponically on modified Hoagland solution (Fricke et al., 1997; Fricke and Peters, 2002) in a growth chamber (Microclima MC1000HE, CEC Technology, Glasgow, UK). Photosynthetically active radiation at third-leaf level was 350–400 μmol photons m⁻² s⁻¹, the relative humidity 70%, the temperature 21/15 °C (day/night), and the light/dark period 16/8 h. At the time of the analyses, leaf 3 elongated at a (near-) maximum velocity, which was typically between 2.5 and 3 mm h⁻¹. Leaves elongated mainly through the elongation of blade tissue, and sheath elongation contributed little.

Leaf elongation was measured either with a ruler, as an increase in total leaf length with time (hour to day range), or with a linear variable differential transformer (LVDT; model DFG 2.5, RS-components Ltd, Corby) over the second to minute range. The latter method allows leaf elongation to be monitored continuously at a resolution of a few micrometres and is ideally suited to follow sudden changes in growth rates in response to environmental disturbances. The length of the elongation zone of leaf 3 and the distribution of relative elemental growth rates (REGRs) was determined by pin-pricking (Schnyder et al., 1987; Fricke and Peters, 2002). Transpiration was measured as gravimetric loss of water from pots containing plants, where pots were sealed such that water loss could only occur through the shoot surface area. Hormone concentrations were determined by immunoassay (Veselov et al., 1992), and expression and tissue-localization of a water channel was monitored by northern analysis and in-situ PCR, respectively. Cuticular waxes were analysed and quantified by GC-FID analyses of chloroform extracts of dried leaf segments (Richardson et al., 2005). Epidermal surface areas of leaf segments that were used for wax analyses were calculated from cross-sectional surface lengths of abaxial and adaxial epidermal layers. Cellular water relationships were evaluated in the abaxial epidermal layer, using the cell-pressure probe (turgor), picolitre osmometry (osmolality), and energy-dispersive X-ray analysis of picolitre-sized cell extracts (solute concentrations) (Hüsken et al., 1978; Tomos et al., 1994; Fricke, 2004). The two latter methods were also employed to measure osmolality and solute concentrations, respectively, in bulk leaf extracts. These extracts were obtained by rapid centrifugation of frozen leaf tissue. Mesophyll solute concentrations were calculated from values for epidermis and bulk leaf by assuming that the epidermis occupied about 26% of leaf symplast volume, while the mesophyll occupied the majority of the remaining volume (Fricke and Flowers, 1998; Fricke, 2004).

Membrane potentials of epidermal cells were measured with microelectrodes. In short, single-barrelled microelectrodes were prepared using filamentated borosilicate glass as described previously (Zhen et al., 1992; Miller, 1996). The microelectrodes were back-filled with a 100 mM KCl solution using a 70-mm-long Microfil needle (World Precision Instruments Inc., Stevenage, UK). Leaf 3 of intact barley plants was mounted in a chamber with a reference electrode (100 mM KCl on 3% agar) contacting 10 mM KCl+1 mM CaCl₂ solution at the edge of the leaf. A stereomicroscope was used to view the impalement. The leaf was illuminated during the course of experiments. For salinity experiments, NaCl (100 mM final concentration) was added to the root medium, while membrane potential was recorded in the emerged portion of the growing blade.

The elongation zone of leaf 3 was accessed for cell analyses by either peeling off or cutting a window into the sheaths of leaves 1 and 2. The exposed portion of the elongation zone was covered with moist tissue paper to minimize water loss; leaves continued to elongate, even though at a reduced rate (~50–80%; Fricke and Peters, 2002) compared with undisturbed plants. It cannot be ruled out that this reduction in leaf elongation velocity, which occurred in addition to that caused by salinity, affected turgor or membrane potential, since these two variables could change significantly within seconds to minutes—by contrast to osmolality or concentrations of individual solutes.

Within each experiment, different leaf zones were compared from the same plants. All experiments were repeated on independent batches of plants, and results were pooled to calculate averages (±standard deviation). Whenever possible, a paired t test was used to determine the statistical significance of differences in values between leaf regions.

Results

Leaf growth

Addition of NaCl to a final concentration of 100 mM to the root medium of barley plants caused an immediate reduction in elongation velocity of the growing leaf 3. A typical experiment is shown in Fig. 2. In most plants, leaf elongation velocity was zero or so close to zero that it was difficult to distinguish between ‘no growth’ and residual extension caused by the counter weight of the LVDT. In the experiment shown in Fig. 2A, LEV averaged (five plants) 0.11 mm h⁻¹, or 6% of the pre-stress level, at 10 min after salt addition. Between 20 and 30 min after adding salt, elongation velocity suddenly recovered, to 1.13 mm h⁻¹ (see also Fig. 2B). This corresponded to 46% of the pre-stress level. Elongation velocity increased only slightly during the following day and, by day 5 of stress, had increased to about 70% of the level in non-stressed plants (data not shown; see Fricke and Peters, 2002). Figure 2B shows traces of two LVDT recordings. Leaf elongation velocity recovered suddenly, in an ‘on-off’ manner. Some threshold, either in terms of resistance (mechanical, hydraulic) or driving force (direction of water potential gradient) must have been overcome.

When plants were kept inside a humid polythene bag to reduce transpiration for 20 h prior to the addition of salt, leaf elongation took longer to resume and at greatly reduced velocities. It took 52±15 min for growth to resume in plants stressed under conditions of reduced transpiration (humid polythene bag), compared with 27±4 min in plants stressed under ‘ambient’ (standard growth) conditions. Once growth resumed, it recovered to 48±8% of the pre-stress level in ‘ambient’ plants, but to only 17±4 % in plants kept inside a humid polythene bag (Fricke et al., 2004).

The length of the cell elongation zone of leaf 3 and the distribution of relative elemental growth rates (REGRs) was determined by the ‘pin-pricking’ approach, through making small holes at known distances along the leaf and analysing their displacement with time. This approach requires a minimum incubation period of several hours.
to obtain sufficiently large displacement of holes for reliable measurements under the stereomicroscope. Therefore, it was not possible to measure elongation zone length and distribution of REGR within minutes to hours following the addition of salt to the root medium. Instead, measurements were carried out at 1 d and 3 d following salt addition. At both time points, salt neither affected the length of the elongation zone nor the distribution pattern of REGR. Instead, salt reduced leaf elongation velocity through a general reduction in REGR along the elongation zone (Fig. 3).

**Transpiration**

Transpiration was measured for the whole plant, and results represent the sum of transpirational water loss from mature leaves 1 and 2 and growing leaf 3 (Fig. 4). No attempts were made to distinguish between transpirational water loss of growing and mature leaves when measuring transpiration within the first hour(s) of salinity.

Addition of salt caused a rapid (1 min) and almost 3-fold increase in transpiration, which lasted 1–1.5 min (not shown in Fig. 4A). Thereafter, transpiration returned to the control level, until about 15 min when it decreased by almost 20% below the rate in control plants (Fig. 4A, B). This preceded growth resumption by 5–15 min (compare parts A and B in Fig. 2). Transpiration in salinized plants remained below the level in control plants for the remainder of the experimental period (Fig. 4A, B).

Addition of salt to the root medium caused a decrease in stomatal conductivity of leaf 3 (Fricke et al., 2004). This was already apparent after 10 min of salt treatment. By contrast, leaf 2 responded to salinity first with a slight increase in stomatal conductivity, and it took 40 min until stomatal conductivity decreased below the level in nonsalinized plants (Fricke et al., 2004).

**ABA**

Addition of NaCl to the root medium caused rapid and tissue-specific changes in ABA (Fig. 5; see also Fricke et al., 2004). Within 10 min, ABA increased 6-fold in the distal, but hardly changed in the proximal half of the leaf elongation zone. In the adjacent, enclosed non-elongation zone, ABA almost doubled, while in the emerged portion of the blade, ABA increased almost 3-fold. Twenty minutes later, 30 min after the addition of salt, ABA was still highest in the distal half of the elongation zone, but had doubled in the proximal half. In the
emerged blade, ABA had decreased by about one-third, yet was still twice as high as before stress. Between 30 min and 1 h, ABA in the emerged portion of the blade continued to decrease towards the pre-stress level. In the elongation and adjacent non-elongation zone, ABA concentrations levelled out at about 300% of the concentration before stress. Between 1 h and 2 h following the addition of salt, ABA in enclosed leaf tissue decreased. Twenty hours after the addition of salt, ABA levels in salted plants were similar to those in non-salted plants.

Cytokinins

Two cytokinins were tested: zeatin, which is a major active cytokinin; and zeatin-riboside, which may act as the precursor or transport form of zeatin. Salinity caused a rapid (in 10 min) decline in levels of zeatin and zeatin-riboside in the leaf elongation zone, particularly in the proximal (bottom) half (Fig. 6). Levels of zeatin and zeatin-riboside remained low until 30 min, and thereafter, increased towards the level in non-stressed plants. The emerged portion showed opposite changes in cytokinins. Zeatin levels increased significantly throughout the experiment (Fig. 6A), while levels of zeatin-riboside increased significantly after 30 min of exposure to salt (Fig. 6B). In the non-elongation zone, zeatin increased towards the end of experiment, while zeatin-riboside remained near the control level throughout.

Water and solute relationships

There was no significant increase in total solute load (osmolality) at bulk leaf level within 30 min following addition of salt to the root medium (Table 1). After 1 h, the solute load had increased significantly in the proximal half of the leaf elongation zone. The significance of solute accumulation increased within the next 4 h, and solute accumulation also occurred progressively in the distal
portion of the elongation, the non-elongation, and the emerged zones (Table 1). There was a clear base-to-tip gradient in the ability of tissues to increase total solute load in response to salt. Between 8 h and 20 h after addition of salt, solute accumulation in the elongation zone was complete, matching almost perfectly the external increase in osmolality. By contrast, in the emerged zone, solute load had not even increased to the lowest significance level; and after 1 d, the increase in bulk osmolality in the emerged zone accounted for <50% of the increase in external osmolality.

The increase in osmolality in response to NaCl was not due to a decrease in water content, but to a net increase in solute content. Water content of leaf sections did not change during the first hour of stress (Fricke, 2004). If salt addition had caused any rapid and significant loss of water from leaf tissues, this would have been expected to show during the first hour.

Osmolality in epidermal cells was determined after 20 h of stress. Epidermal osmolality responded to salinity in the same way as bulk osmolality (Table 2). This suggests that the major leaf tissues—epidermis and mesophyll—respond to salt with a similar increase in total solute load.

The term ‘osmotic adjustment’ refers to a situation where total cell solute load (osmolality) increases by the same amount as external osmolality increases, while cell turgor is maintained at the control or pre-stress level. Turgor was not measured within the first day, but between 3 d and 5 d following addition of salt (75 mM or 120 mM) to the root medium (Fricke and Peters, 2002). Salt did not alter

<table>
<thead>
<tr>
<th>Time after addition of NaCl (h)</th>
<th>Leaf region</th>
<th>Elongation zone</th>
<th>Non-EZ</th>
<th>Emerged</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proximal</td>
<td>Distal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>388</td>
<td>402</td>
<td>398</td>
<td>491</td>
</tr>
<tr>
<td>0.5</td>
<td>405</td>
<td>423</td>
<td>409</td>
<td>493</td>
</tr>
<tr>
<td>1</td>
<td>432*</td>
<td>423</td>
<td>426</td>
<td>492</td>
</tr>
<tr>
<td>2</td>
<td>455***</td>
<td>447*</td>
<td>425</td>
<td>487</td>
</tr>
<tr>
<td>Experiment II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>452</td>
<td>457</td>
<td>434</td>
<td>496</td>
</tr>
<tr>
<td>0.5</td>
<td>462</td>
<td>440</td>
<td>427</td>
<td>464</td>
</tr>
<tr>
<td>1</td>
<td>510**</td>
<td>488</td>
<td>471</td>
<td>512</td>
</tr>
<tr>
<td>2</td>
<td>518**</td>
<td>490</td>
<td>455</td>
<td>509</td>
</tr>
<tr>
<td>8</td>
<td>556***</td>
<td>557***</td>
<td>499**</td>
<td>513</td>
</tr>
<tr>
<td>20</td>
<td>644***</td>
<td>643***</td>
<td>591**</td>
<td>562**</td>
</tr>
<tr>
<td>72</td>
<td>609***</td>
<td>605***</td>
<td>548***</td>
<td>591***</td>
</tr>
</tbody>
</table>

Table 1. Osmolality (mosmol kg\(^{-1}\)) in bulk leaf extracts of barley, which were harvested at different times following addition of 100 mM NaCl to the root medium

Third leaves were analysed within the elongation zone, in the proximal and distal half, within the adjacent, enclosed non-elongation zone (Non-EZ), and within the emerged portion of the blade. Results from two independent experiments are shown, and values are means of four to seven plant analyses. The significance of osmolality increases in response to salt is indicated by asterisks (*, \(P<0.05\); **, \(P<0.01\); ***, \(P<0.001\)).
turgor significantly in the elongation zone and, as a result, growing cells adjusted osmotically and behaved like perfect osmometers. By contrast, cells in the emerged leaf zone adjusted to the lowered external osmolality through both an increase in cell osmolality and a decrease in turgor (Fricke and Peters, 2002). Whether cells in the leaf elongation zone also maintained turgor and adjusted osmotically within the first hours of stress cannot be said. This needs to be tested.

Previous studies have shown that leaf epidermal cells of barley, like epidermal cells of other grass leaves, use, almost exclusively, inorganic solutes to generate osmolality (Fricke et al., 1994). Under standard growth conditions, plants that grow on hydroponics (e.g. Hoagland solution) use K and nitrate as the main cationic and anionic osmolytes, respectively. Under conditions of high external ratios of Na:K or Cl:nitrate (salinity) or development-related increasing ratios of Ca:K (leaf ageing), K is replaced by either Ca or Na, and nitrate is replaced by Cl. There exist different types of epidermal cells in the barley leaf epidermis and some cell types—small ones located next to stomata—can accumulate large concentrations of malate (Fricke et al., 1996). However, the larger epidermal cells analysed in the present study accumulate little, if any, malate.

In the present study, concentrations of Na, K, and Cl were followed in response to salt in epidermal cells and in bulk leaf extracts; mesophyll concentrations were calculated by subtraction. Solute concentrations changed rapidly, within 7–8 h of stress. Chloride concentrations almost doubled in the epidermis of the elongation and emerged zone, but changed little in the mesophyll (Fig. 7A; precise values with standard deviations are given in Table 3; statistical significances are given in Table 4). Chloride continued to increase in the epidermis and by day 3 was four to six times as high as in the mesophyll. Sodium was close to zero in all tissues and leaf zones before the application of stress and increased to detectable levels during the first 7 h only in epidermal cells of the elongation zone (Fig. 7B). Thereafter, Na increased in all tissues. Increases

<table>
<thead>
<tr>
<th>Extract</th>
<th>Control treatment</th>
<th>NaCl treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf region</td>
<td>Leaf region</td>
</tr>
<tr>
<td></td>
<td>EZ</td>
<td>Emerged blade</td>
</tr>
<tr>
<td></td>
<td>EZ</td>
<td>Emerged blade</td>
</tr>
<tr>
<td>Epidermal cell</td>
<td>386 (22)</td>
<td>423 (22)</td>
</tr>
<tr>
<td>Bulk leaf</td>
<td>428 (16)</td>
<td>451 (15)</td>
</tr>
</tbody>
</table>

Table 2. Osmolality (mosmol kg⁻¹) in bulk leaf and epidermal cell extracts of barley at 20 h following addition of 100 mM NaCl to the root medium

Values for non-stressed plants (control) are also shown. Third leaves were analysed within the elongation zone (EZ; between 10 and 30 mm from the point of leaf insertion) and within the emerged portion of the blade. Averages (and standard deviations) of eight leaf analyses are shown. Data originally published in Fricke (2004, Rapid and tissue-specific accumulation of solutes in the growth zone of barley leaves in response to salinity. Planta 219, 515–525), and reproduced by kind permission of Springer Science and Business Media.

Fig. 7. Changes in concentrations of (A) Cl, (B) Na, and (C) K in epidermal (Epi) and mesophyll (Mes) cells of the elongation zone (EZ) and emerged blade (EmBl) of the elongating leaf 3 of barley in response to 100 mM NaCl. Results are presented in a scheme; concentrations of solutes are represented by circles, and are closest approximations of averages of four leaf analyses. The precise concentrations are given in Table 3, and the statistical significance of differences between leaf zones, tissues, and treatments is given in Table 4.
Table 3. Concentrations of Na, K, and Cl in epidermal and mesophyll cells of leaf 3 of barley subjected to short-term treatment with 100 mM NaCl

Plants were grown under control-conditions until the third leaf stage. At time zero ($t_0$, Control), NaCl was added to nutrient media to reach a final concentration of 100 mM NaCl. Salinized plants were harvested at 7 h, 20 h, and 72 h following addition of salt. Leaves were analysed within the elongation zone (EZ, mid-portion showing highest relative elemental growth rates) and half-way along the portion of the growing leaf 3 which had emerged from the sheath of leaf 2 (EmBl, emerged blade portion). Sap from individual epidermal cells was extracted and analysed by energy-dispersive X-ray analysis as detailed previously (Tomos et al., 1994; Fricke, 2004). Together with values of bulk-leaf extracts, which were also analysed by the energy-dispersive X-ray technique, and anatomical data (tissue volumes), mesophyll concentrations of Na, K, and Cl were calculated (for details, see Fricke, 2004). Averages (± standard deviation) of four leaf analyses are shown. The statistical significance of difference in solute concentrations is shown in Table 4. Data originally published in Fricke (2004, Rapid and tissue-specific accumulation of solutes in the growth zone of barley leaves in response to salinity. Planta 219, 515–525), and reproduced by kind permission of Springer Science and Business Media.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Leaf zone</th>
<th>Tissue</th>
<th>Control $t_0$</th>
<th>NaCl-treatment $t_7$ h</th>
<th>$t_{20}$ h</th>
<th>$t_{72}$ h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>EZ</td>
<td>Epidermis</td>
<td>3 (6)</td>
<td>19 (9)</td>
<td>50 (10)</td>
<td>98 (20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mesophyll</td>
<td>−7 (16)*</td>
<td>−2 (8)</td>
<td>23 (8)</td>
<td>61 (22)</td>
</tr>
<tr>
<td>EmBl</td>
<td>Epidermis</td>
<td>0 (10)</td>
<td>1 (2)</td>
<td>28 (22)</td>
<td>133 (31)</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>EZ</td>
<td>Epidermis</td>
<td>186 (8)</td>
<td>236 (38)</td>
<td>211 (32)</td>
<td>126 (11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mesophyll</td>
<td>147 (13)</td>
<td>177 (13)</td>
<td>185 (18)</td>
<td>209 (12)</td>
</tr>
<tr>
<td></td>
<td>EmBl</td>
<td>Epidermis</td>
<td>264 (29)</td>
<td>306 (17)</td>
<td>264 (68)</td>
<td>221 (17)</td>
</tr>
<tr>
<td>Cl</td>
<td>EZ</td>
<td>Epidermis</td>
<td>113 (28)</td>
<td>124 (27)</td>
<td>97 (28)</td>
<td>160 (10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mesophyll</td>
<td>71 (14)</td>
<td>158 (36)</td>
<td>210 (41)</td>
<td>183 (44)</td>
</tr>
<tr>
<td></td>
<td>EmBl</td>
<td>Epidermis</td>
<td>16 (7)</td>
<td>25 (24)</td>
<td>10 (14)</td>
<td>43 (23)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mesophyll</td>
<td>111 (29)</td>
<td>144 (10)</td>
<td>230 (67)</td>
<td>292 (13)</td>
</tr>
</tbody>
</table>

* Slightly negative mesophyll values can occur at concentrations close to zero: this suggests that the assumed proportions of tissue volumes (epidermis, 26%; mesophyll, bulk of remaining volume), which were used to calculate mesophyll from epidermal and bulk-leaf solute concentrations, differed by a few per cent from the true proportions in the leaves analysed.

Membrane potential

Membrane potentials of epidermal cells from the emerged region of the leaf were measured in response to the addition of salt to the root medium in 10 plants. Within the population of membrane potential measurements some, most probably, are recordings from the vacuole and some from the cytoplasm. Those from the vacuole will be more positive (about +20 mV) than the actual trans-plasma membrane voltage (Cuin et al., 2003). There is no evidence for two populations of microelectrode measurements, but the data set is small. Therefore, in the following, reference is made to ‘membrane potential’, and trans-plasma membrane and tonoplast potential are not differentiated; pH microelectrode measurements could identify the compartments and resting potentials across each membrane. Two representative recordings are shown in Fig. 8 and relevant parameters of all recordings are summarized in Table 5. The pre-stress membrane potential in the emerged blade ranged from −67 to −92 mV, averaging −76 mV. Plasma membrane potential started to depolarize 2–5 min following the addition of NaCl to the root medium. The maximum depolarization ranged from 3 to 38 mV (average 13 mV) and occurred 5–10 min after the addition of salt (average 7.1 min). Depolarization ended at 15–40 min (average 24.6 min). The final membrane potential was slightly more negative than before stress (−81 mV compared with −76 mV; Table 5).

Measurements of potential differences have proved difficult in the leaf elongation zone since cells have to be impaled for 20–50 min following the addition of salt, and growth displacement causes the microcapillary to move, which in turn causes leaks of cell contents. Recordings have proved difficult even for shorter times (5–10 min), unlike the situation in non-stressed plants, yet will be essential to link changes in membrane potential to changes in growth rate of cells.

Expression of water channel

Recently a clone was identified that encodes a plasma membrane intrinsic protein, belonging to the subgroup of PIP1s (W Wei, E Alexandersson, D Golldack, A Miller, O Kjellbom, W Fricke, unpublished results; EMBL accession number CO720031). This clone has been termed HvPIP1;6. HvPIP1;6 shows water channel activity when expressed in oocytes and its expression is higher in elongating compared with non-elongating leaf tissue; expression is confined to the epidermis. When 100 mM NaCl was added to the root medium of plants, expression of HvPIP1;6 increased within 10 min, remained elevated at 30 min, but was lower than before application of stress at 4 h (Fig. 9; densitometric results of northern analysis of
two experiments are shown). Expression in non-salinized plants, which were analysed in parallel, was slightly higher at 4 h compared with the start of experiment (data not shown).

Table 4. Statistical significance of differences in solute concentrations between (A) leaf tissues (epidermis, Epi; mesophyll, Meso), (B) leaf zones (elongation zone, EZ; emerged blade, EmBl), and (C) control (t0) and salinized plants (t7 h, t20 h, t72 h)

Concentrations are shown in Table 3.
(A) Comparison between epidermis and mesophyll
+, Significantly higher in epidermis compared with mesophyll (+, P < 0.05; ++, P < 0.01; ++++, P < 0.001); −, significantly lower in epidermis compared with mesophyll (−, P < 0.05; −−, P < 0.01; −−−, P < 0.001); ns, not significant.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Treatment and leaf zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t0</td>
</tr>
<tr>
<td>Na</td>
<td>ns</td>
</tr>
<tr>
<td>K</td>
<td>+</td>
</tr>
<tr>
<td>Cl</td>
<td>++</td>
</tr>
</tbody>
</table>

(B) Comparison between elongation zone and emerged blade
+, Significantly higher in elongation zone compared with emerged blade (+, P < 0.05; ++, P < 0.01; ++++, P < 0.001); −, significantly lower in elongation zone compared with emerged blade (−, P < 0.05; −−, P < 0.01; −−−, P < 0.001); ns, not significant.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Treatment and leaf tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t0</td>
</tr>
<tr>
<td>Na</td>
<td>ns</td>
</tr>
<tr>
<td>K</td>
<td>+</td>
</tr>
<tr>
<td>Cl</td>
<td>+</td>
</tr>
</tbody>
</table>

(C) Comparison between salinized plants (t7 h, t20 h, t72 h) and control (t0)
+, Significantly higher in salinized plants compared with control plants (+, P < 0.05; ++, P < 0.01; ++++, P < 0.001); −, significantly lower in salinized plants compared with control plants (−, P < 0.05; −−, P < 0.01; −−−, P < 0.001); ns, not significant.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Leaf tissue</th>
<th>NaCl treatment and leaf zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t7 h</td>
<td>t20 h</td>
</tr>
<tr>
<td>Na</td>
<td>Epidermis</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Mesophyll</td>
<td>ns</td>
</tr>
<tr>
<td>K</td>
<td>Epidermis</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Mesophyll</td>
<td>ns</td>
</tr>
<tr>
<td>Cl</td>
<td>Epidermis</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Mesophyll</td>
<td>ns</td>
</tr>
</tbody>
</table>

Fig. 8. Typical recordings of membrane potential in cells of the emerged blade of the growing leaf 3 of barley, and the response of membrane potential to changes in (A) illumination and (B) addition of NaCl (100 mM) to the root medium. Twelve NaCl stress experiments were carried out, with similar results.

Cuticle
The aim of cuticle analyses was to determine whether plants exposed to salinity respond to decreases in external water availability by increasing their epidermal wax load and, by implication, decrease cuticular water loss (Schreiber and Schönner, 1993). The deposition and density of cuticular waxes was studied in plants grown under control conditions and in plants exposed to salinity for 2–3 d. Previous analyses on leaf 3 of barley have shown that wax deposition commences within the portion of the leaf that is enclosed by sheaths of older leaves and continues into the portion that has emerged from sheaths (Richardson et al., 2005). Wax was determined for two 1-cm-long sections along the elongating leaf 3; section 1 was 10–20 mm below the point of emergence from the sheath of leaf 2, and section 2 was located 40 mm closer to the leaf tip, between 20 and 30 mm above the point of emergence. The time it took for a tissue element at position 1 to be displaced (through cell elongation at the leaf base) to position 2...
was calculated using light and dark elongation velocities of leaf 3 of control and salinized plants. It took a leaf segment only slightly longer to move from position 1 to position 2 in salinized plants (25 h) than in non-salinized plants (20.8 h), due to an almost identical elongation during the dark period until around 11 h after light onset when a slight increase occurred (Table 6). This calculates to a deposition rate of wax of 0.25 and 0.22 μg cm⁻² h⁻¹ in control and salinized plants, respectively (Table 6). Therefore, salinity reduced the wax deposition rate and leaf elongation velocity by a similar proportion, resulting in comparable wax densities per epidermal surface area.

Discussion

In the present paper, the short-term growth response to salt of the elongating leaf 3 of barley has been reviewed by combining data from various studies. Variables most likely to be involved in the regulation of cell expansion were reported and an attempt made to link responses at the cellular level (solute, osmolality, turgor) to changes in hormones (ABA, cytokinins) and water balance (transpiration). Analyses were carried out between 10 min and 5 d of exposure to salt. Responses within this short term reveal immediate stresses and mechanisms to cope with these stresses. Investigations into the ability of barley to cope with longer-term exposure to salt may require longer experimental periods (15–20 d; see Flowers and Hajibagheri, 2001). In the present study, the short-term response was divided further, into a response occurring within minutes to hours and a response occurring within hours to days. The aim of this distinction was to test the significance of solute accumulation in the early response: to cope with salinity, growing plant cells must increase their solute load, in order to avoid loss of turgor (i.e. turgor close to zero). Turgor in growing leaf cells, including leaf 3 of barley, is typically between 0.4 and 0.5 MPa (Fricke, 2002).

Table 6. Cuticular wax density and deposition rate in response to salinity along the developing leaf 3 of barley

Plants were exposed to 100 mM NaCl for 2–3 d prior to harvest. Non-stressed, control plants were also harvested. Leaves were analysed at two locations, one being 10–20 mm below (Segment 1) and one being 20–30 mm above (Segment 2) the point of emergence of leaf 3 from the sheath of leaf 2. Based on the time it took for leaf segments to be displaced from segment 1 to segment 2 (20.8 h for control; 25 h for NaCl treatment), deposition rates of wax were calculated. Averages (and standard deviations) of five to six leaf analyses are shown.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wax density (μg cm⁻²)</th>
<th>Wax deposition rate (μg cm⁻² h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Segment 1</td>
<td>Segment 2</td>
</tr>
<tr>
<td>Control</td>
<td>1.99 (0.53)</td>
<td>7.22 (0.93)</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.96 (0.42)</td>
<td>7.34 (1.14)</td>
</tr>
</tbody>
</table>

Fig. 9. Expression of the barley water channel HvPIP1:6 in response to 100 mM NaCl along the growing leaf 3 of barley. Expression was determined just before (t₀) and at 10 min, 30 min and 4 h following application of stress. Results of densitometric analyses of northern results of two experiments are shown. Densitometric data for HvPIP1:6 were first related to that of the expression reference 28S-rRNA and the resulting values of salinized plants related to that of non-stressed plants at the start of the experiment. Between 20 and 30 plants were harvested at each time point in each experiment. EZ, Elongation zone; NEZ, non-elongation zone; EmBL, emerged blade.
and a stress of 100 mM NaCl decreases external water potential by 0.46 MPa. Without solute accumulation in cells, such a stress would lead to a complete loss of turgor and hence growth.

Once it is accepted that solute accumulation is pivotal during the growth response to salt, the first question to ask is: Do solutes accumulate rapidly enough to aid initial growth recovery or do they accumulate after this initial period to aid further recovery or maintenance of growth? Intimately linked to this question is: What other mechanisms could plant (cells) employ to regain or maintain growth should solute accumulation not occur quickly enough? Furthermore, if solute accumulation is pivotal for growth recovery, it would be expected that growing tissues, in particular, are sinks for solutes, and are supplied in preference to non-growing, mature tissues. Finally, it matters which solutes accumulate in which cell type or cell compartment, and the question arises from when solute accumulation becomes discriminative.

**ABA and cytokinins**

ABA has been shown to influence both tissue hydraulic and stomatal conductivity (Collins and Kerrigan, 1974; Davies and Zhang, 1991; Freundl et al., 2000; Hose et al., 2000), and has been proposed to influence growth in response to drought or salinity through changes in cell wall extensibility (Dodd and Davies, 1996; Thompson et al., 1997; Cramer et al., 1998; Bacon, 1999) or apoplastic pH (Bacon et al., 1998). Much attention has been paid to the growth-inhibitory role of ABA in leaves (Cramer and Bowman, 1991; Dodd and Davies, 1996; He and Cramer, 1996; Thompson et al., 1997; Cramer et al., 1998). Surprisingly little attention has been paid to a potential role of ABA in facilitating growth resumption following the application of stress (Thompson et al., 1997).

ABA accumulated in leaf tissue >6-fold within 10 min in response to salinity. The time-course of ABA accumulation in transpiring leaf tissue, together with its known effect on stomatal physiology, suggests that ABA causes xylem water potential to rise and leaf growth to recover. However, ABA accumulated even more and faster in non-transpiring, growing tissue, and there must be other mechanisms through which ABA affects cell and leaf expansion. This is not surprising, considering that ABA has been implicated in the control of every biophysical variable relevant to growth at cell and tissue level: solute transport (Roberts and Snowman, 2000) and, by implication, turgor (Jones et al., 1987), hydraulic conductivity (Collins and Kerrigan, 1974; Freundl et al., 2000), wall properties (Cramer et al., 1998; Bacon, 1999), and photosynthetic import (Jones et al., 1987; for a discussion, see Munns and Cramer, 1996). For example, the pronounced increase in ABA in the distal portion of the elongation zone might increase peroxidase activity and stiffen walls in this leaf region and, by so doing, aid preferential growth recovery in the proximal portion of the leaf growth zone. Here, ABA might promote growth through an increase in transcellular water flow by gating water channels (Wan et al., 2004).

The two cytokinins studied, zeatin and zeatin-riboside, showed similar changes in response to salt. Both cytokinins decreased rapidly in growing tissues and increased with a delay in non-growing and emerged tissues. Percentage changes in content of zeatin and zeatin-riboside were far smaller than for ABA. However, in absolute terms, changes were either similar or larger. This resulted from very different absolute levels of ABA (30–50 ng g⁻¹ DW) and cytokinins (~800–1000 ng g⁻¹ DW) before stress. The two types of hormones showed opposite changes in response to salt in the elongation zone, but similar changes in emerged tissue. The decrease in zeatin after 10 min and 30 min of salinity in the proximal portion of the elongation zone possibly reflects a reduction by salt of cell division (less zeatin present to stimulate cell division). Although the bottom 2 mm of the leaf, which contains the leaf ‘apical’ meristem, were not included in the sample of the proximal elongation zone, epidermal and, particularly, mesophyll cell division proceeds well into the sampling zone (MacAdam and Nelson, 1987).

**Osmolality and membrane potential**

Solute accumulation played no significant part in growth recovery between 20 and 30 min following the addition of salt to the root medium. The sensitivity of the method used to determine osmolality is below 5 mosmol kg⁻¹, which corresponds to about 0.01 MPa of osmotic pressure. If the leaf-to-leaf variation in osmolality is taken into account, a change in osmolality of around 10 mosmol kg⁻¹ should have been detected as a significant increase. This corresponds to a change in cell osmolality of around 0.02 MPa. Such recovery of water potential may have occurred unnoticed, but should still have been insufficient to restart cell-elongation growth, considering that external water potential had changed by 0.46 MPa.

However, solute accumulation contributed, from 1 h onwards, to the recovery of growth, and by 20 h had proceeded to such an extent that it could account for the entire growth recovery. This would enable the leaf to abandon mechanisms employed previously, for example, to recover turgor, wall properties, or transpiration to the pre-stress level. Furthermore, solute accumulation in growing leaf tissue almost perfectly matched changes in external osmolality caused by salt addition.

Membrane potential is both a driving force and possible regulator, and is a consequence of trans-membrane solute transport and permeation. Membrane potential depolarized in response to salt within 2–5 min and returned to its pre-stress level between 15 min and 40 min. The majority of
the plants recovered membrane potential between 18 min and 30 min following addition of salt. These data suggest that recovery in membrane potential coincides with growth recovery and precedes solute accumulation at osmotically significant levels. Turgor in mature leaf epidermal cells of barley starts to decrease within seconds in response to salt (Fricke, 1997) and, therefore, precedes membrane depolarization—as does the bulk of transpirational burst in response to salt. It is possible, therefore, that epidermal membranes depolarize as a direct result of altered cell water relationships or membrane tension (e.g. stretch-activated channels), or that the transpirational burst causes a transient increase in apoplastic K, which in turn depolarizes the plasma membrane. Apoplastic K can rise through two mechanisms: through a decrease in apoplastic water content (if not all of the transpirational burst is transmitted as an increase in apoplastic tension) or through a release of guard-cell K to counter the transpirational burst. At least the latter mechanisms cannot occur in the (non-transpiring) leaf elongation zone.

A more detailed study, paying special attention to membrane permeability and individual ion channels in the elongation zone, is needed to understand the importance of membrane potential changes for growth inhibition and recovery under salt treatment. Membrane potentials reported here for mature barley leaf epidermal cells are in the same range as those obtained by Cuin et al. (2003), also on barley leaf cells, and for root cells of barley (Van der Leij et al., 1998; Walker et al., 1998). Although potentials of −70 to −80 mV are significantly less than those generally assumed for plasma membrane potentials of higher plant cells, they do not result from leaky seals around the microcapillary as evidenced from responses to light (Fig. 9A) and changes in external K (results not shown).

It is not clear why growth failed to return to 100% of the pre-stress level but stayed reduced despite sufficient osmolality increases in cells. One possibility is that rates of solute accumulation to sustain osmotic adjustment in expanding cells were limiting growth. By reducing the rate of cell expansion (and dilution of cell contents), growing cells were able to take up solutes rapidly enough to guarantee osmotic adjustment and maintain turgor at the level observed in non-salinized plants (Delane et al., 1982; Fricke and Peters, 2002). In this context, growth reduction is not so much a detrimental consequence of, but a means to cope with, stress.

Why did osmolality increase first in the proximal portion of the growth zone? If solute accumulation plays a vital part in the maintenance of residual elongation growth during salinity, the present data suggest that cell elongation resumed first (1 h) in the proximal portion of the elongation zone. This is difficult to prove directly. Methods employed to characterize the spatial distribution of relative elemental growth rates along the base of grass leaves are destructive (e.g. pin-pricking; Schnnyder et al. 1987), require incubation periods of several hours, and reduce elongation velocity substantially. However, theory predicts that the relative elemental growth rate must recover first in the basal portion of the growth zone: cells are displaced through the elongation zone through the cumulative elongation of cells at more proximal positions. The closer a cell is located to the point of leaf insertion, the fewer cells are located at more proximal (basal) positions which can displace this cell distally (tip-wards). Therefore, it takes a cell close to the point of leaf insertion much longer to be displaced a certain distance than a cell which is located, for example, halfway along the elongation zone. At the extreme, displacement velocity close to the point of leaf insertion approaches zero, while at the distal end of the elongation zone it matches leaf elongation velocity. If REGR and growth were maintained high in the distal, rather than the proximal portion of the growth zone, elongation of a leaf of a stressed plant took weeks, not days. Therefore, theory predicts that any accumulation of solutes, facilitating growth recovery in response to salinity, must set in first in the proximal portion of the growth zone and, from here, proceed towards the tip of the leaf. This is exactly what was observed in the present study. The question is not so much ‘why’ did growth and solute accumulation occur preferentially in the proximal half of the growth zone, but ‘how’ this was achieved.

After 20 h, osmolality in the proximal and distal half of the elongation zone had increased by the same amount. According to the above reasoning, this suggests that at 20 h, growth had recovered in both halves of the elongation zone to a similar extent. The data on spatial distribution of REGR support this conclusion.

**Solute**

Salinity affected the elongation zone and mature zone, and epidermis and mesophyll, differently in their K and Na relationships. At the same time, the basic distribution of Na, K, and Cl between epidermis and mesophyll was not affected by stress, with concentrations being either non-significantly different or significantly higher in the epidermis (Table 4A; the only exception being K in the elongation zone at 72 h) than the mesophyll. During the first hours of stress, K accumulated substantially in all tissues, probably as a result of increased import via the phloem (Wolf and Jeschke, 1987). By contrast, Na accumulated significantly only in epidermal cells of the elongation zone (Table 4C). As stress continued, between 20 h and 72 h, Na:K ratios were affected differentially in epidermis and mesophyll. This was due primarily to changed K relationships of tissues. Whereas K increased significantly in mesophyll cells, it started to decrease significantly in epidermal cells, particularly in the elongation zone (Table 4C). It appears that transport properties for Na and K differ between leaf tissues and change with the cell.
Leaf cell elongation and salinity

Leaf cell elongation and salinity

The continued and substantial accumulation of Na, but not Cl, in all tissues of the leaf growth zone suggests that, of the two ions, Na poses the bigger challenge to ion homeostasis in cells of barley. This is by contrast to observations on salinized wheat, where Na accumulated in the elongation zone to only one-fifth of the concentration reported here (Hu and Schmidhalter, 1998). Chloride accumulated significantly at all times in the epidermis, while mesophyll concentrations were kept low and increased significantly only past 20 h of stress (Table 4C). The present data confirm earlier reports for mature barley leaf tissue (Fricke et al., 1994, 1996) and show that the strict compartmentalization of Cl between epidermis and mesophyll is expressed early during barley leaf cell development and independently of growth and micro-environment. Whether Na or Cl affects leaf growth through direct cytotoxicity has been questioned recently (Hu et al., 2005). The present observation that concentrations increased in all tissues examined after 72 h of stress, yet leaf elongation velocity was higher compared with 20 h of stress, argues against a cytotoxic effect of Na and Cl.

Aquaporins

Authors’ unpublished data show that a member of the PIP1 subfamily, HvPIP1;6, was expressed highly in growing leaf tissue of barley. Hollenbach and Dietz (1995) observed that expression of Emip1, a PIP1, is high in the basiplast of leaf 1 of barley. The authors did not test whether Emip encoded a water channel. However, Emip has high sequence homology to HvPIP1;6 and it is possible that the two genes are alleles of the same gene and encode the same gene product. Chaumont et al. (2000) studied two PIP1s in maize, ZmPIP1a and ZmPIP1b, and concluded that these aquaporins were not involved in the regulation of cell expansion in leaves. Schünemann and Ougham (1996) studied the expression of a tonoplast intrinsic protein (TIP) in roots and in leaf 1 of wild-type barley and the barley slender mutant. In leaves, expression was highest in the elongation zone and even higher in root tissue, yet the biochemical function of this TIP was not tested. It remains to be shown whether and how HvPIP1;6 aids growth-associated water transport between leaf xylem and peripheral tissues. The transient increase in expression in response to salinity (Fig. 9) suggests some involvement of HvPIP1;6 in maintaining residual growth.

Zhu et al. (2005) recently studied the response to salinity (100 and 200 mM) of the entire set of maize TIPs and PIPs from root tissues. The response to stress varied between genes and could be observed within 2 h of stress application. The timing of regaining osmotic gradients for uptake of water through root tissues coincided with the transient expression of three PIPs. This suggests that PIP expression (and presumably water channel activity) can contribute to water uptake in roots and growth recovery in salinized plants.

Cuticle

After several days of growth at 100 mM NaCl, the rate of cuticular wax deposition along the growing leaf 3 was reduced by a similar proportion to the reduction in elongation velocity. As a result, wax density, the amount of wax per unit epidermal surface area, did not change (Table 6). The leaf cuticle consists of two major components, waxes and cutin. It is generally assumed that waxes rather than cutin provide the main diffusion barrier for water through the leaf cuticle (Schreiber and Schönherr, 1993). If this also holds for the developing barley leaf, acclimation to salinity of plants through reduction in transpirational water loss must be due to stomatal regulation.

Model

The short-term growth response to 100 mM NaCl of the developing leaf 3 of barley can be divided into four stages (Fig. 10).

(i) Before stress: Before application of salinity, cells were elongating and were at maximum velocity. Water moved into cells along a gradient in water potential between leaf xylem (less negative) and peripheral growing cells (more negative water potential). About 98–99% of water entering the growing leaf via the xylem was lost through transpiration; the rest was consumed (or stored) through cell expansion in the basal growth zone (Fricke, 2002; recycling between shoot and root not considered).

(ii) First 1–1.5 min following addition of salt: Addition of 100 mM NaCl to the root medium caused two immediate effects: it lowered the water potential of the root medium by 0.46 MPa and it caused a transient burst in transpirational water loss, which lasted 1–1.5 min. It is unlikely that the water potential gradient between xylem and growing leaf cells exceeded 0.3 MPa prior to salt application (Fricke, 2002). Therefore, addition of 100 mM NaCl levelled or reversed this gradient, stopping water supply to peripheral cells. As previously growing cells underwent their last expansion event, walls relaxed and turgor decreased to the yield threshold (Matyssek et al., 1988) without being regenerated by water entry. As a result, water potential of peripheral growing cells decreased too—the possibility that the leaf epidermis is hydraulically isolated as recently concluded for the epidermis of growing hypocotyl tissue of soybean (Passiourea and Boyer, 2003) does not contradict this idea.

A sudden burst in transpiration in response to osmotic shock has been reported previously for grasses (Falk,
Epidermal turgor in mature barley leaves decreases within seconds to minutes following addition of salt (Fricke, 1997), and release of back-pressure from guard cells and transient stomatal opening could easily explain the observed increase in transpiration (Raschke, 1970; Meidner, 1990). Such a response of guard cells could be viewed as a design fault of stomata—increased water loss in response to reduced water availability—but it also has two advantages: it amplifies the original signal (change in water potential) and it aids the recovery of water and hormone supply from root to shoot following application of osmotic stress (Fricke et al., 2004).

(iii) Growth recovery between 20 and 30 min following addition of salt: As salinity continued, increased ABA in the emerged portion of the growing leaf blade caused stomatal aperture to decline and transpiration to decrease. Xylem water potential became less negative. At some point (20–30 min), the gradient in water potential between xylem and peripheral cells in the growth zone again favoured water uptake into the latter. Leaf growth resumed in an ‘on-off’ manner. This idea is further supported by the observation that resumption of growth took longer in plants kept under conditions of no or reduced transpiration: the less plants were transpiring, and the less negative xylem water potential was before the addition of salt, the more difficult it was to recover xylem water potential sufficiently through a reduction in transpiration. The observation that plants kept under low or non-transpiring conditions recovered leaf elongation at greatly reduced velocities suggests that other growth variables such as hydraulic conductivity were also affected by the transpiration environment of plants.

An ‘on-off’ resumption of growth can also be explained by lowering the yield-threshold of walls below cell turgor, as proposed for roots of osmotically stressed maize plants (Frensch and Hsiao, 1994). It is possible that leaves respond in the same way (Serpe and Matthews, 1992), but at 100 mM NaCl, turgor in a growing cell could easily approach zero if adjustment of wall properties was the only means to regain growth. In addition, ABA is thought to cause an increase, not a decrease in yield threshold of leaf tissues in response to salt (Cramer and Bowman, 1991; Munns and Cramer, 1996) yet, in the present study, ABA accumulated throughout the growth zone.

In the distal portion, where ABA initially accumulated the most, ABA might have suppressed growth resumption through an increase in peroxidase activity (Bacon et al., 1997; Bacon, 1999). This would have aided channelling of resources (water, solutes) to the proximal portion of the elongation zone and ensured that cell elongation resumed here first. If this were the case, proximal and distal tissues responded to the same stimulus (ABA) differentially.

Not all plants (leaves) stopped elongating during the period immediately following salt addition. Some plants...
(40% of population) continued to elongate at reduced rates, and two plants elongated at 17–18% the velocity before salt addition. This could be explained by reduced water potential gradients—driving force for water transport—between xylem and growing leaf tissues. If so, why did plants that had shown a complete cessation of growth resume leaf elongation at substantially higher velocities? Assuming that growth resumed the moment water potential gradients favoured elongation growth, these gradients must have been at least as small as in plants that never ceased elongation. There are two possible explanations: the idea that restoration of water potential gradients causes growth to resume is wrong; or, during the initial 20–30 min when water potential gradients were gradually restored to drive water uptake into growing tissues, additional changes in growth variables such as an increase in tissue hydraulic conductivity in response to ABA occurred (Hose et al., 2000).

(iv) Between 1 h and 1 d, and onwards, following addition of salt: From 1 h onwards, solutes accumulate to osmotically significant levels in the growing leaf 3. Initially, solute accumulation is confined to the proximal portion of the leaf elongation zone but, with time, proceeds towards the tip of the leaf. Between 8 h and 20 h of stress, solute accumulation in the leaf elongation zone is large enough to account for the entire recovery of water potential gradient and growth. This could explain why ABA, which affected growth through other variables, returned to near control levels between 2 h and 20 h of stress, yet elongation continued at the same velocity.

One day after application of stress, elongation velocity was still reduced and only half that in non-salinized plants. It is possible that the rate of solute accumulation to achieve osmotic adjustment in expanding leaf cells became the growth-limiting factor (Delane et al., 1982; Fricke and Peters, 2002; however, see Michelena and Boyer, 1982).

Wax deposition rate was reduced by a similar proportion as leaf elongation velocity and, as a result, wax density was not altered by stress. It needs to be tested whether the co-reduction in wax deposition rate and elongation velocity is due to a common signal caused by salt or whether the wax deposition rate is linked through some other mechanism to epidermal cell elongation rate. In either case, any adjustment of transpiration rate to salt does not involve cuticular waxes.

Acknowledgements

This study was supported by grants from the Biotechnology and Biological Sciences Research Council (BBSRC), UK (grants P/15151 and P/18283; and International Scientific Interchange Scheme, ISIS, grant no 1070); a study visit grant from the Royal Society of Edinburgh to WF; the Russian Foundation for Basic Research (RFFR; grant No 03-04-49780 to GK); a Royal Society of London study visit grant to DV and WF, and a grant from The Leverhulme Trust to WF. The authors would like to thank the editor and two anonymous referees for their very helpful and constructive comments.

References


