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Control of leaf cell elongation in barley. Generation rates of osmotic pressure and turgor, and growth-associated water potential gradients

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Abstract. In a previous study on the effects of N-supply on leaf cell elongation, the spatial distribution of relative cell elongation rates (RCER), epidermal cell turgor, osmotic pressure (OP) and water potential (Ψ) along the elongation zone of the third leaf of barley was determined (W. Fricke et al. 1997, Planta 202: 522–530). The results suggested that in plants receiving N at fixed relative addition rates (N-supply limitation of growth), cell elongation was rate-limited by the rate of solute provision, whereas in plants growing on complete nutrient solution containing excessive amounts of N (N-demand limitation), cell elongation was rate-limited by the rate of water supply or wall yielding. In the present paper, these suggestions were tested further. The generation rates of cell OP, turgor and Ψ along the elongation zone were calculated by applying the continuity equation of fluid dynamics to the previous data. To allow a more conclusive interpretation of results, anatomical data were collected and bulk solute concentrations determined. The rate of OP generation generally exceeded the rate of turgor generation. As a result, negative values of cell Ψ were created, particularly in demand-limited plants. These plants showed highest RCER along the elongation zone and a Ψ gradient of at least −0.15 MPa between water source (xylem) and expanding epidermal cells. The latter was similar to a theoretically predicted value (−0.18 MPa). Highest rates of OP generation were observed in demand-limited plants, with a maximum rate of 0.112 MPa · h^{-1} at 16–20 mm from the leaf base. This was almost twice the rate in N-supply-limited plants and implied that the cells in the leaf elongation zone were capable of importing (or synthesising) every minute almost 1 mM of osmolytes. Potassium, Cl^- and NO_3^- were the main inorganic osmolytes (only determined for demand-limited plants). Their concentrations suggest that, unlike the situation in fully expanded epidermal cells, sugars are used to generate OP and turgor. Anatomical data revealed that the zone of lateral cell expansion extended distally beyond the zone of cell elongation. It is concluded that leaf cell expansion in barley relies on high rates of water and solute supply, rates that may not be sustainable during periods of sufficient N-supply (limitation by water supply: Ψ gradients) or limiting N-supply (limitation by solute provision: reduced OP-generation rates). To minimise the possibility of growth limitation by water and osmolyte provision, longitudinal and lateral cell expansion peak at different locations along the growth zone.

Key words: Cell expansion – Continuity equation – *Hordeum* (leaf growth, nitrogen) – Turgor – Water-potential gradient – Xylem

Introduction

Plant growth results from the irreversible enlargement of individual cells. Irreversibility is guaranteed by the plastic properties of the cell wall and enlargement reflects increase in water content. The latter is due to the directional supply of water from the source (typically xylem) to expanding cells. The driving force for water supply, and therefore cell enlargement, is a gradient in water potential ($\Delta\Psi$) between water source and expanding cell (more negative Ψ). The magnitude of $\Delta\Psi$ depends, for a given relative growth rate (RGR), on the hydraulic conductance (L) of the pathway between xylem and expanding cell:

$$RGR = L \cdot \Delta\Psi [s^{-1}]$$

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Abbreviations and symbols: DV = displacement velocity; LER = leaf elongation rate; OP = osmotic pressure; Ψ = water potential; RAR = relative addition rate; RCER = relative cell elongation rate; $t_{1/2}$ = half-time of pressure relaxation (water exchange across membrane) of cell

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Any process affecting either L or $\Delta\Psi$, potentially rate-limits growth. Apart from biochemical processes (Lawlor and Leach 1985), biophysical processes may limit growth, particularly the yielding and extensibility properties of the cell wall and the provision and uptake of water and solutes. Some investigators concluded that the hydraulic conductance between water source and expanding cells was insufficient to match the cell's water demand and would therefore lead to growth limitation and considerable Ψ gradients (−0.2 to −0.3 MPa; Boyer et al. 1985; Nonami and Boyer 1989; but see also Malone and Tomos 1992; Cosgrove et al. 1984). However, most investigators concluded that the hydraulic conductance was sufficiently high and that, instead, control of wall properties was responsible for treatment-associated differences in the expansion of leaf and root cells (e.g. Cosgrove et al. 1984; Tomos and Pritchard 1994; Triboulot et al. 1997). The latter conclusion has its origin in the work of Green et al. (1971) on Nitella and Lockhart's (1965) empirical growth model, which relates RGR to the extensibility properties (m) and the yield threshold (Y) of the cell wall:

$$RGR = m \cdot (P - Y)[s^{-1}]$$

The effective turgor, (P − Y), represents the difference between cell turgor (P) and the yield threshold of turgor below which no cell (wall) expansion occurs. Although attractive in their logic, design and results, it is often overlooked that Green et al. (1971) and Lockhart (1965) studied isolated internodes of giant algae in a well-bathed medium – a situation where water and solute supply are unlikely to be growth-limiting and therefore very different from the situation for a cell within a leaf or root tissue. For example, the above equation demands that two cells, which differ in RGR but not turgor, *must* differ in either m or Y (or both). However, when taking water and solute supply also into account, this need not be the case. Figure 1 illustrates this point. Both cell "A" and cell "B" have to reach the same turgor-yield threshold to initiate a wall-yielding and expansion event.

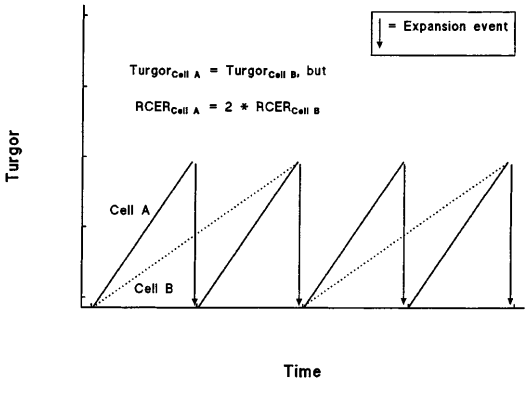


Fig. 1. How differences in (relative) elongation rates between two cells can solely be explained by differences in the rate of turgor generation. Cell "A" and cell "B", elongate at different rates since they generate turgor at different rates. However, their turgors are identical (= integral under curves) as are their turgor-yield thresholds and extensibility properties of the wall

However, cell A generates turgor at twice the rate of cell B (due to twice the rate of water or solute supply), causing twice as many yielding events per time. As a consequence, cell A expands at twice the (relative) rate as cell B – despite both cells having the same turgor (integral under curves) and yield threshold.

Solute (osmolyte) supply has received very little attention as a growth-limiting process (Tomos 1985; Steudle 1985). This may be due partly to the focus on turgor and wall properties and partly to the experimental difficulties of determining solute concentrations and osmotic pressure (OP) at the cell level. Pritchard et al. (1996) observed that cell OP was almost constant and between 0.7 and 0.8 MPa along the growth zone of maize roots. This implied that high rates of solute provision were required to maintain OP in cells elongating at relative cell elongation rates (RCERs) of up to 50% $\cdot$ h^{-1}. Silk and co-workers (Silk et al. 1986; Sharp et al. 1990; Meiri et al. 1992; Bernstein et al. 1995) applied the continuity equation of fluid dynamics (Silk and Erickson 1979) to calculate deposition rates of solutes along the growth zone of maize roots and maize and sorghum leaves. They obtained maximum deposition rates of about 4–40 mM solutes $\cdot$ h^{-1} and generation rates of OP of up to 0.5 MPa $\cdot$ h^{-1} – figures that emphasise the need to consider spatially-and time-constant solute concentrations and OP in growing cells as a dynamic state due to the steady dilution of cell contents by cell volume expansion. Unfortunately, the studies by Silk and co-workers were not carried out at the appropriate cell level, nor was turgor or Ψ measured, rendering it impossible to relate data directly to the processes governing cell expansion.

A recent study on the control of leaf cell expansion in N-limited barley (Fricke et al. 1997) showed that reductions in the leaf elongation rate (LER) resulted from reductions in the rates of cell elongation. Based on the spatial distribution of cell turgor, OP and Ψ along the growth zone it was suggested that, depending on how and how much N plants receive, the rate of solute provision or water supply may limit cell expansion. In the present paper, these suggestions are explored further. The original data were used together with the continuity equation of fluid dynamics to obtain, for the first time, a complete quantification of the rates of turgor, OP and Ψ generation in growing cells. To aid interpretation, anatomical data were collected and bulk-solute concentrations measured.

Materials and methods

Plant growth. Part of the work was carried out at the Agricultural University of Sweden, Uppsala, and part at the University of Sussex, Falmer, Brighton, UK. During both studies, the same cultivar of barley (*Hordeum vulgare* L. cv. Golf) was used and the composition of full-strength (N-sufficient) Hoagland solution was the same. Anatomical data and solute concentrations of bulk leaf extracts were obtained in Sussex. All other measurements, including growth of plants under N-limitation, were carried out in Uppsala. Since the LER of N-sufficient plants in Sussex (2.44 mm $\cdot$ h^{-1}) was comparable to that in Uppsala (2.33–2.60 mm $\cdot$ h^{-1}) the data from both places were combined.

To impose a stationary limitation of N on plants without causing N-deficiency, N was supplied at exponentially increasing rates to plants growing on N-deficient nutrient solution (Ingestad and Lund 1986). The rate constant of addition, the relative addition rate (RAR), was the amount of N added per plant-bound N and day. Theory predicts that when a steady-state rate of growth is achieved, the relative increase in fresh weight of plants will match the RAR of N. This was the case in the present study for the two treatments receiving N at RAR of 16% and 8% N · plant-N^{-1} · d^{-1} (''16%-'' and ''8%-plants'', respectively). Their relative increase in fresh weight during the phase of linear and maximal expansion of the third leaf, the period when all other analyses were carried out, was 15.6% and 8.1% · d^{-1}, respectively. Plants that had all nutrients available in excess throughout development grew in the more conventional, demand-limited way, i.e. they took up as much N as growth demanded (full-strength Hoagland solution; ''Hoagland-plants''). The fresh weight of these plants increased at a relative rate of 18.2% · d^{-1}. The precise conditions for growth and composition of nutrient solution are given in Fricke et al. (1997).

Determination of LER, RCER, cell turgor, OP and Ψ. Details of the experimental approach, setup and analytical techniques are given in Fricke (1997) and Fricke et al. (1997). In brief, the LER of the third leaf was maximal and linear between days 1 and 4 (Hoagland-plants and 16%-plants) and days 1 and 6 (8%-plants) following its emergence from encircling sheaths. All of the other analyses were carried out within this period (mostly between days 1 and 3). Relative cell elongation rates were determined by the pin-pricking method (Schnyder et al. 1987) and corrected for the decrease in LER caused by pricking (ca. 50%; Fricke et al. 1997). Cell turgor and OP (and Ψ) were determined in intact, though manipulated, plants. Manipulation of plants caused about 50% reduction in LER. This was similar to the reduction caused by pricking and assumed to result from similar (wounding) causes. Cell turgor was measured using the micro-pressure-probe technique (Hüsken et al. 1978). Cell OP was determined by picolitre osmometry of extracted cell sap (Malone and Tomos 1992). Cell Ψ was calculated as the difference between cell turgor and OP.

Calculation of displacement velocities (DV), growth trajectories and generation rates. Displacement velocity is the local rate of displacement of a tissue element from the leaf base. Beyond the distal limit of the growth zone, it equals LER. Displacement velocities were calculated from the data on RCER. For each 4-mm segment along the growth zone, the RCER values were multiplied by 4 mm, and the cumulative totals (= DV) were plotted against the respective segment midpoint.

A growth trajectory describes the positional change in time for a tissue element as it is displaced through the growth zone. It indicates how long it will take an element to reach a more distal position or to pass through regions of given RCER. To calculate the displacement time from one segment midpoint to the distally next, e.g. from 10 to 14 mm, the reciprocal of DV of these two segments was multiplied by 2 mm (= half of the segment length) and added together (e.g. 0.5 $DV^{-1}{}_{10\ mm}$ + 0.5 $DV^{-1}{}_{14\ mm}$). The cumulative totals of these local displacement times along the growth zone gave the overall displacement times between the basal starting point and any distal location of interest. Six millimeters from the leaf base was chosen as the starting point (time ''0'') since calculation of growth trajectories for starting points closer to the leaf base may introduce large errors in data derived from RCER curves (Schnyder et al. 1990).

The term ''generation rate'' is used here to describe the rate at which a variable, such as osmotic pressure, is generated during cell volume expansion. It does not refer to the absolute value of that variable. For example, cell osmotic pressure may stay constant in absolute terms during cell elongation. However, due to the accompanying volume expansion, it has to be constantly generated at positive rates. Generation rates of cell turgor, OP and Ψ were calculated using the one-dimensional version of the continuity equation of fluid dynamics (Silk and Erickson 1979; for details of its application to expanding plant tissues see Silk et al. 1986; Sharp et al. 1990). The continuity equation describes the local net deposition or generation rate of a variable (e.g. solutes, turgor, OP) as a function of position (here, mm from leaf base). The equation consists of three additive terms (Sharp et al. 1990): (1) the local rate of change in value of the variable; e.g. here, the change in cell OP with time at a fixed distance from the leaf base; (2) the convective rate of change, which is the product of local DV and change in value of the variable with position, e.g. here, the change in product of the cell OP along the segment 12–16 mm from the leaf base and the local DV; and (3) the growth-dilution term which is the product of value of the variable and RCER at a given position, e.g. here, the product of cell OP and RCER at 14 mm from the base. During steady-state growth, the growth-dilution term is the dominant one quantitatively. Terms (2) and (3) were calculated from the original OP, turgor and Ψ data given in Fricke et al. (1997; Fig. 2B–D) and the RCER and DV data given in Fig. 4A,B. The change in value of the variable (e.g. OP) with position was calculated for each segment by calculating the difference in value of the variable between the adjacent distal and basal segment and dividing it by 4 mm (= segment length). Term (1) was set to zero. It was not determined, since all original data used were means for data obtained within a period of ca. 2 d (typically between days 1–3 after emergence of the third leaf from the encircling sheath). However, the LER during this period was constant. In addition, the ligule was within 4 mm from the leaf base, excluding a major contribution of sheath growth to blade growth. Together with the findings of Schnyder et al. (1990; stage ''A'' and ''B'' leaves) it seems justified to assume that the spatial distribution and values of RCER did not change considerably during the 2-d analysing period. Whether the same applied to the spatial distribution of turgor, OP and Ψ is not known. However, even if, for example, OP had changed by 50% at 16–20 mm from the base during the 2 d of analysis, this would have resulted in an error of only 7–12% in OP-generation rates (0.015 MPa · h^{-1}).

Anatomical and solute analyses. Anatomical data were obtained on fresh, hand-cut leaf sections. Sections were cut and mounted in water and viewed under a light microscope at magnifications of 40–400. Cross-sectional diameters of xylem vessels, epidermal cell widths and surface lengths of epidermal layers were measured using a calibrated graticule. Cross-sectional leaf areas were determined from enlarged photocopies of photographic prints of cross-sections.

Solute concentrations in bulk (= total) leaf extracts were determined by HPLC (Dionex; NO_3^-, Cl^-, SO_4^{2-}; PO_4^{3-}) and atomic absorption spectrometry (K^+). Leaves were at the same developmental stage as those used for other analyses. To obtain bulk leaf extracts, the basal 7 cm of the third leaf was placed in a humid chamber and cut into 1-cm segments. Corresponding segments from four to five plants were combined in a pre-weighed Eppendorf tube. The fresh weight was determined and 400 µl of 25% (v/v) isopropanol was added. The mixture was frozen and thawed twice, and made up to 1 ml with ultrapure water. The sample was centrifuged in a bench centrifuge (45 s at ca. 10 000 *g*) and the supernatant used for analysis after determining its exact volume. The dry weight of the remaining leaf tissue was determined (3–4 d at 70 °C; DW 2–4 mg) and used to calculate the leaf water content from the original leaf fresh weight.

Cell-diffusivity calculations and theoretically predicted Ψ *gradients*. Calculations were only performed for demand-limited, Hoagland-plants, and for the location along the growth zone (30 mm from leaf base) where measured cell Ψ was most negative. Equation A10 of Molz and Boyer (1978) was used. It applies to the one-dimensional Cartesian case of water movement along a file of cells. The cross-sectional geometry of the growth zone of barley leaves is much more difficult to describe and suffers from the uncertainties of water paths. Therefore, the application of Eq. A10 in the present study is limited and applies only to the case of water moving along a file of cells from xylem to epidermis.

Using standard techniques, Eq. A10 of Molz and Boyer (1978) was transformed into:

$$(\Psi_{(x)} - \Psi_{(x=0)}) = -(\epsilon + OP) \cdot (RRWCI \cdot x^2) \cdot (2D)^{-1} \qquad \text{(Eq. E1)}$$

where $\Psi_{(x)}$ is the water potential of an expanding cell at x μm from the water source (unit: MPa), $\Psi_{(x=0)}$ is the water potential at the water source (= Ψ_{xylem}; MPa), ϵ is the elastic modulus of cells (MPa), OP is the osmotic pressure of cells, RRWCI is the relative rate of water-content increase (= RCER; h^{-1}), and D is the tissue free-energy diffusivity for water ($cm^2 \cdot s^{-1}$). Cell OP was 0.775 MPa, RCER was 0.04 h^{-1}, and for ϵ a generally accepted value of 5 MPa was taken. The maximum distance, x, between xylem and epidermal cell was determined as follows. Figure 2 shows cross-sections of the entire leaf (Fig. 2A), of part of the leaf (Fig. 2B) and of large lateral veins (Fig. 2C–F) at various positions along the elongation zone of barley. The leaf consisted of an outer (less rolled-up) and inner (more rolled-up) leaf-"arm" both of which were joined at the midrib. Both leaf-arms contained, besides several small and intermediate veins, three large lateral veins, and only the latter showed fully developed bundles. Thus, together with the bundle of the midrib vein, only seven functional bundles were available to supply the expanding cells with mineral nutrients and water. Water exiting the xylem may have reached the epidermis either via the shortest way, i.e. along the bundle-sheath extensions or equivalent mesophyll tissue, or via diagonal passage through the mesophyll. The former pathway appears to be more likely, since mesophyll cell development may not be strictly in line with epidermal cell development (MacAdam et al. 1989). Considering the epidermal cell layers, this implied that the maximum distance between water source (xylem) and elongating cell would be at least the entire cross-sectional surface length of the epidermis (ca. 3300 μm; Fig. 3) divided by 14, plus the shortest distance between xylem and epidermal layer (about 50–60 μm). At 30 mm from the leaf base, x was therefore at least about 470 μm.

The diffusivity, D, of water was calculated according to Eq. 1 of Nonami et al. (1997) assuming that the cell and tissue diffusivity of water were similar [which requires that water moves mainly along the symplasmic pathway – an assumption that, based on measurements of cell and tisssue $t_{1/2}$ of water exchange, has been confirmed experimentally for fully expanded barley leaves (Fricke 1997)]:

$$D \approx D_{cell} = (a_{cc} \cdot \Delta x^2 \cdot \ln 2) \cdot (2A \cdot t_{1/2})^{-1} \qquad \text{(Eq. E2)}$$

where a_{cc} is the cross-sectional area of the cell normal to the flow (units: m^2), Δx is the width of the cell in the direction of flow (m), A is the surface area of the cell (m^2) and $t_{1/2}$ the half-time of water exchange between cell and surrounding (s). Average cell dimensions were determined as follows. In barley, the epidermis is composed of anatomically different cell types, that may be classified as ridge, trough, near-stomatal and inter-stomatal cells (apart from guard cells) (see Fricke et al. 1995, and Fricke 1997). In the cross-sectional view, the proportion of cells was about 3:2:2:1, respectively. At 30 mm from the leaf base, the (axial) length of cells was about 66% of their final cell size, i.e. 900 μm, 300 μm, 240 μm and 70 μm, respectively. Thus, the average cell length was $[(3 \times 900\ \mu m) + (2 \times 300\ \mu m) + (2 \times 240\ \mu m) + (1 \times 70\ \mu m)]/8 = 481\ \mu m$. The average cross-sectional width of each individual cell was 24 μm (see also Fig. 3B) and the average cross-sectional surface length about 30 μm. For $t_{1/2}$, a value of 2 s was taken (five determinations, ranging from 1 to 3 s). These data were used to calculate a cell (and tissue) diffusivity of $0.37 \times 10^{-10} \cdot m^2 \cdot s^{-1}$. Accordingly, $(\Psi_{(x)} - \Psi_{(xylem)})$ was calculated as −0.182 MPa at 30 mm from the leaf base.

Error propagation. To calculate the propagation of errors from individual measurements (and means) when calculating DV, growth trajectories, cell Ψ and generation rates, Gauss' Law of error propagation was applied. Given the individual measurements (means) "$A \pm SD_A$" and "$B \pm SD_B$", the errors will be: $(A \pm SD_A + B \pm SD_B) = A + B \pm (SD_A^2 + SD_B^2)^{0.5}$; and

Fig. 2A–F. Photo-micrographs of cross-sections of the third leaf of barley (Hoagland-plants) at various distances (mm) from the leaf base. **A** Entire leaf; **B** part of leaf; **C–F** second large lateral vein, counted from the mid-rib, with xylem and phloem elements at various developmental stages. Cross-sections were cut and mounted in water and photographed within 5–15 min. Photographs were taken at microscopic magnifications of × 40 **A**, × 100 **B**, and × 400 **C–F**. Bars represent original dimensions. *Mr*, midrib; LV_{1-3}, 1st, 2nd, 3rd large lateral vein counted from the midrib; *IV*, intermediate vein (one of many); *Ms*, mesophyll; *PSh*, parenchymateous bundle sheath; *MSh*, mesotome sheath; *Ph*, phloem; *MX*, metaxylem; *PX*, protoxylem. The right "leaf-arm", starting at the midrib, in **A** is referred to in the text as "outer leaf arm", and the left arm as "inner leaf arm" since it is more rolled-up. The small, isolated piece of leaf tissue in the middle of **A** and **B** is the developing fourth leaf

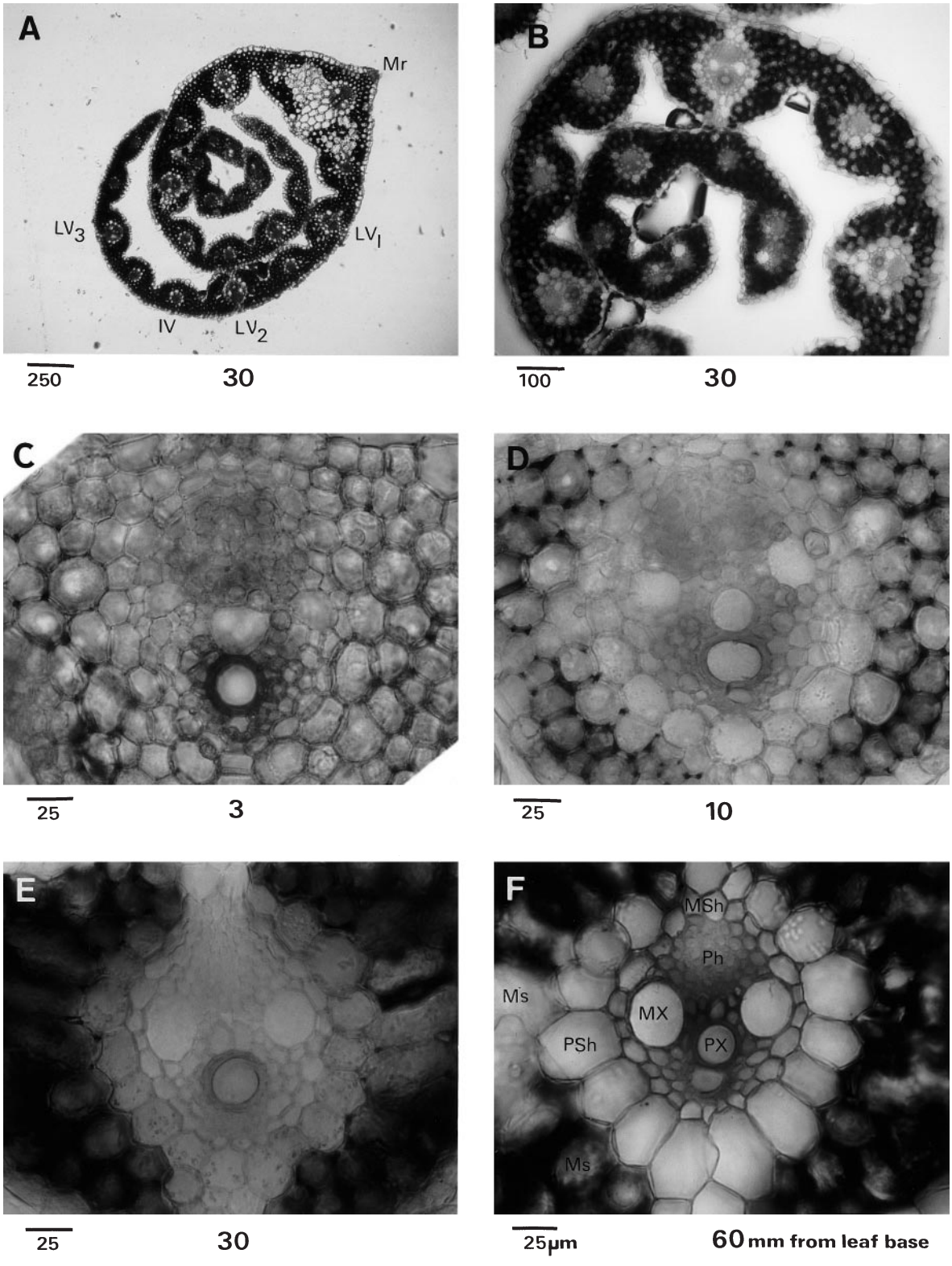
$$(A \pm SD_A \cdot B \pm SD_B) = (A \cdot B) \pm (A \cdot B) \cdot ([SD_A/A]^2 + [SD_B/B]^2)^{0.5}.$$

Results and discussion

Leaf elongation rate, RCER, DV and growth trajectories in barley. A previous study (Fricke et al. 1997) on the control of leaf cell expansion in barley showed that third leaves elongated at reduced rates when plants were supplied at reduced rates with N (8% plants) or when plants were supply- (8% and 16%-plants) rather than demand-limited (Hoagland-plants) in N (for LER data, see legend to Fig. 4). In either case, reductions in LER were due to reductions in RCERs in the distal three-quarters of the elongation zone (Fig. 4A). This was also reflected in the profiles of DV (Fig. 4B). Reductions in LER due to reductions in RCER have also been reported for N-limited tall fescue (Volenec and Nelson 1983; Gastal and Nelson 1994). Despite the differences in RCER, the time it took for a cell to be displaced from 6 mm to the distal end of the growth zone was, within errors of analysis, similar for the three N treatments (29–37 h; Fig. 4C). The displacement times observed for barley leaf cells are comparable to those reported for leaf cells of sorghum (ca. 24–30 h, Bernstein et al. 1995), maize (ca. 40 h, Palmer and Davies 1996), tall fescue (ca. 50 h, MacAdam et al. 1989) and English ryegrass (ca. 30–42 h, Schnyder et al. 1990). This indicates that in grasses the time required or available for a cell to attain its mature size is conserved between species.

Cells passed through the zone of highest RCERs in only 2.7–3.1 h (Fig. 4C). At the same time they increased in length and volume by at least 48% (3 h at 14% in Hoagland-plants). As a consequence, high rates of solute import or synthesis were required to maintain cell OP and the Ψ gradient driving cell expansion.

Rates of OP, turgor and Ψ generation in elongating leaf epidermal cells of barley. The spatial distribution of generation rates of cell OP along the growth zone reflected that of RCER (Fig. 5A). This was so, because, in absolute terms, cell OP underwent only minor changes along the growth zone (Fricke et al. 1997). Generation rates of cell OP were highest in Hoagland-plants,



showing highest RCER, and lowest in 8%-plants, showing lowest RCER. Differences were less pronounced for turgor-generation rates (Fig. 5B). Along the zone of highest RCER, OP was generally generated at higher rates than turgor. As a result, negative Ψ were created. Generation rates of Ψ were largest (most negative) in Hoagland-plants with rates as high as -0.05 MPa $\cdot$ h^{-1} and significantly different from zero (Fig. 5C). Hoagland-plants showed also the most-negative absolute Ψ values. On average, cell Ψ was -0.282 MPa compared to -0.148 MPa and -0.172 MPa in 16%- and 8%-plants, respectively (Fricke et al. 1997). Therefore, cells of Hoagland-plants, elongating fastest, created the driving force for elongation at the highest rate.

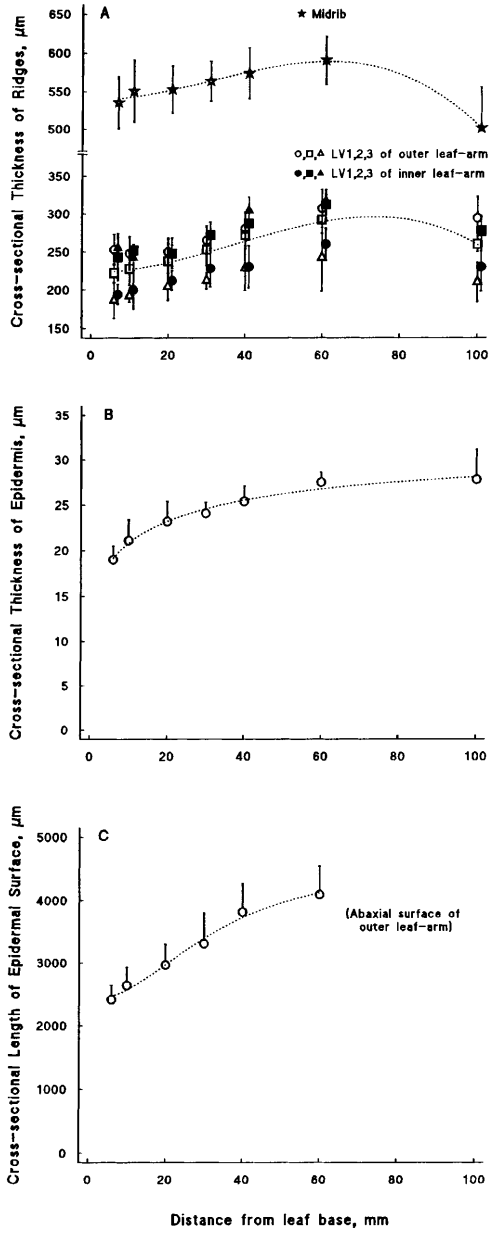


Fig. 3A–D. Cross-sectional thickness of ridges and epidermal layers, and cross-sectional length of epidermal surface at various positions along, and distally beyond the elongation zone of the third leaf of barley (Hoagland-plants). Data were obtained on fresh, hand-cut cross-sections, viewed at magnifications of 40–400. Dimensions were measured using a graticule. Results are means of five to six leaf analyses. Error bars represent SD values

Maximum rates of OP generation ranged from 0.060 MPa · h^{-1} in 8%-plants to 0.112 MPa · h^{-1} in Hoagland-plants. This corresponded to at least 24 to 46 mM solutes deposited · h^{-1} or 0.40–0.77 mM solutes deposited · min^{-1} [the term 'deposition' is used here to refer also to changes in concentrations (= deposition per unit volume)]. An osmotic coefficient of solutes of < 1.00 and lateral cell expansion (see below) would have correspondingly increased the values. The rates of solute deposition deduced here are comparable to maximum deposition rates measured along the growth zone of sorghum and maize leaves. They compare also with the rate of hexose-equivalent import into the elongation zone of tall fescue leaves (Table 1). The latter suggests that *in theory*, solute requirements of expanding leaf epidermal cells of barley could be met by hexoses imported from mature leaf tissues. Table 1 shows that deposition rates of solutes along the elongation zone of grass leaves are about an order of magnitude lower than those along the elongation zone of maize roots and osmotically stressed maize root cortical cells. This difference reflects a 4-times higher maximal RCER in (maize) root cells than in leaf cells (Spollen and Sharp 1991). Thus, either root cells elongate at higher relative rates because they are less restricted in solute supply (through xylem or phloem), or OP generation and solute-deposition rates are higher because higher RCER permit this (while maintaining OP). The former might be more likely since, at least during osmotic stress, root cell elongation seems to be rate-limited by the uptake or supply of solutes (Frensch and Hsiao 1994, 1995).

Table 1 shows that leaf epidermal cells which elongate generate OP and deposit solutes (per unit volume) 10–20 times faster than cells that are fully expanded. Surely, this reflects differences in the cells' solute demand. However, the data of NaCl-treated barley leaves suggest that differences in solute-supply and solute-uptake capacities between expanding and fully expanded cells may be important too. In NaCl-treated plants, with increased solute load of the xylem, there was a priori no reason why cells could not have adjusted osmotically to the external NaCl at rates as high as OP-generation rates in expanding cells to avoid transient turgor loss.

In 8%-plants, the spatial distribution of local DV along the growth zone might have allowed maximum generation rates of OP of 0.06 MPa · h^{-1} and RCER of 8.4% · h^{-1} despite N-limitation. Maintenance of high RCER and DV in the basal 10 mm effectively kept to 3–4 h the time within which cells passed more distally through the zone of highest RCER and OP-generation rates. Thus, keeping the duration of maximum rates of osmolyte-supply short might have enabled maintenance of these maximum rates, and of considerable RCER and the bell-shaped growth profile along the entire growth zone of 8%-plants.

Table 2 shows that within the zone of highest RCER (12–32 mm from base) differences in average OP- and turgor-generation rates were often opposite to those in absolute values between N treatments (absolute values taken from Table 3 in Fricke et al. 1997). The average rate of OP generation was 0.0834 MPa · h^{-1} in Hoagland-plants. This was ca. 70% higher than in 16%-plants, which in turn had a 50% higher rate than 8%-plants, despite having similar OP (0.683 compared to 0.703 MPa). Although having lowest average turgor (0.475 MPa), Hoagland-plants had the highest average rate of turgor generation. The relative rates of OP and turgor generation ranged from 10.2 to 11.0% in Hoagland-, 7.3–7.7% in 16%- and 4.8–5.7% in 8%-plants, and therefore almost perfectly matched relative rates of cell elongation (Table 2).

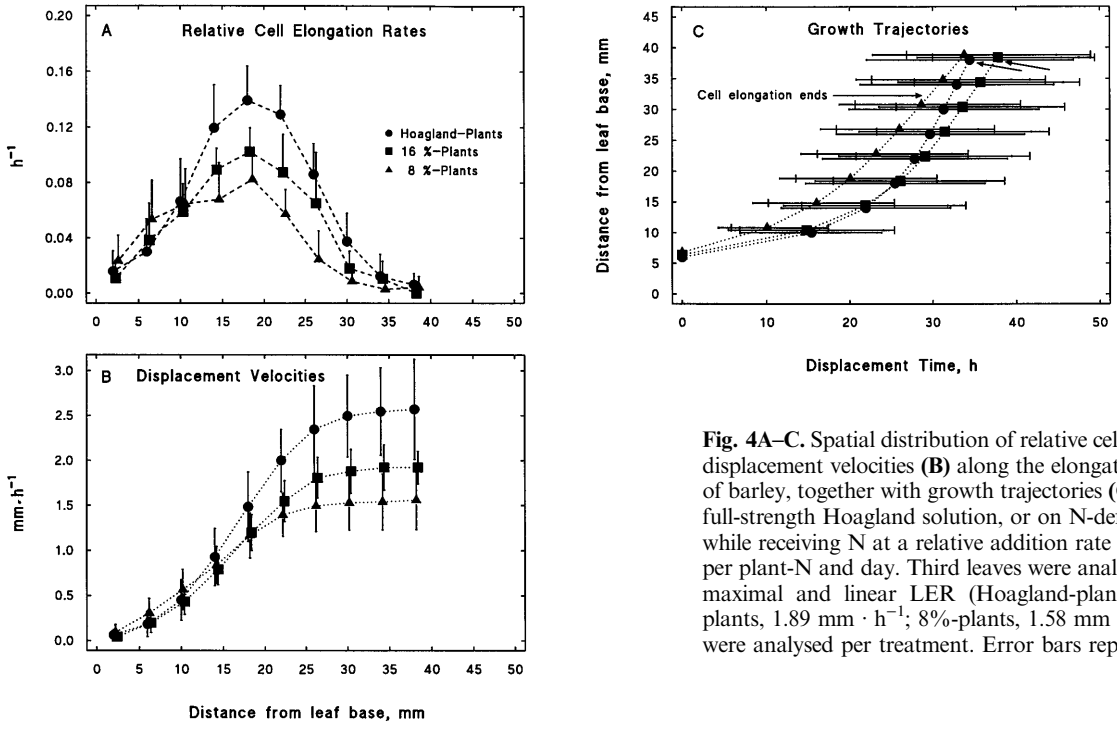


Fig. 4A–C. Spatial distribution of relative cell elongation rates **(A)** and displacement velocities **(B)** along the elongation zone of the third leaf of barley, together with growth trajectories **(C)**. Plants were grown on full-strength Hoagland solution, or on N-deficient Hoagland solution while receiving N at a relative addition rate (RAR) of 16% or 8% N per plant-N and day. Third leaves were analysed during the period of maximal and linear LER (Hoagland-plants, 2.60 mm · h^{-1}; 16%-plants, 1.89 mm · h^{-1}; 8%-plants, 1.58 mm · h^{-1}). Nine to ten plants were analysed per treatment. Error bars represent SD values

Table 2 emphasises the benefit of considering rates of OP and turgor generation, rather than OP and turgor itself, when interpreting changes in the rate of cell elongation at unchanged turgor. For example, considering only turgor might have led to the conclusion that the wall yield threshold in expanding cells of 16%-plants must have been lower than that of 8%-plants. However, considering rates of turgor generation did not lead to such a conclusion (see also Fig. 1). Instead, the data could be interpreted as indicating that cells of 16%-plants elongated at higher relative rates than cells of 8%-plants due to higher rates of solute supply. If so, the difference in externally imposed, growth-dictating RAR of N at *plant level* might have led to corresponding differences in solute supply rates at *cell level*.

The observation that cells of Hoagland-plants expanded at highest RCER, despite having lowest turgor, might suggest that the mechanical yield threshold of their walls was lower than that in 16%- and 8%-plants, allowing higher "effective turgor" (Green et al. 1971). However, this need not be the case. Instead, it could represent a (hormone- or age-dependent) shift in turgor set point of any potentially turgor dependent and rate limiting process involved in elongation growth, e.g. exocytosis of wall material, solute transport across membranes, including acidification (H^+) of the wall space, microtubule orientation (Wymer et al. 1996) or any potential trans-membrane regulation of wall enzymes/proteins such as xyloglucan endotransglycosylase, expansins or peroxidases (see also Zhu and Boyer 1992, for metabolic limitation).

Growth-associated Ψ *gradients and osmolyte provision*. In the present study, Ψ of the nutrient solution was ca. −0.07 MPa in Hoagland and ca. −0.01 MPa in 16%- and 8%-plants. The Ψ of the exposed and mature part of the leaf was between −0.10 and −0.13 MPa (Hoagland-plants; three determinations). The average epidermal-cell Ψ along the growth zone was −0.282 MPa, −0.148 MPa and −0.172 MPa in Hoagland-, 16%- and 8%-plants, respectively (Table 2; data taken from Fricke et al. 1997). Therefore, Hoagland-plants had a Ψ gradient between xylem and elongating leaf epidermal cells of at least −0.15 MPa. This is comparable to the value reported for the elongation zone of soybean hypocotyls (−0.25 MPa; Nonami et al. 1997). In contrast, in 16%- and 8%-plants, the Ψ gradient might have been as small as −0.01 to −0.03 MPa and thus close to zero.

One could argue that the Ψ values obtained for epidermal cells are not representative of the true Ψ values of cells, since (i) turgor and OP were determined in different cells (separate analysis), and (ii) errors of these individual measurements might have propagated beyond xylem Ψ. Figure 6 shows for Hoagland-plants that this was not the case. Errors (standard deviations) calculated according to Gauss' law of error propagation did not reach xylem Ψ; and analysis of turgor and OP in the same cell (combined analysis) gave cell Ψ values that were as negative (−0.3 MPa) as cell Ψ obtained through separate analysis of turgor and OP.

The average RCER (10.2% · h^{-1}) and Ψ-gradient (−0.15 · MPa) in Hoagland-plants, calculated to a tissue hydraulic conductance of $1.89 \times 10^{-4} \cdot s^{-1} \cdot MPa^{-1}$. This is higher, though in the same range as values reported for growing tissue of soybean hypocotyls ($0.78 \times 10^{-1} \cdot s^{-1} \cdot MPa^{-1}$, Boyer et al. 1985) and pea epicotyls (ca. $1.39 \times 10^{-4} \cdot s^{-1} \cdot MPa^{-1}$, Cosgrove and Steudle 1981).

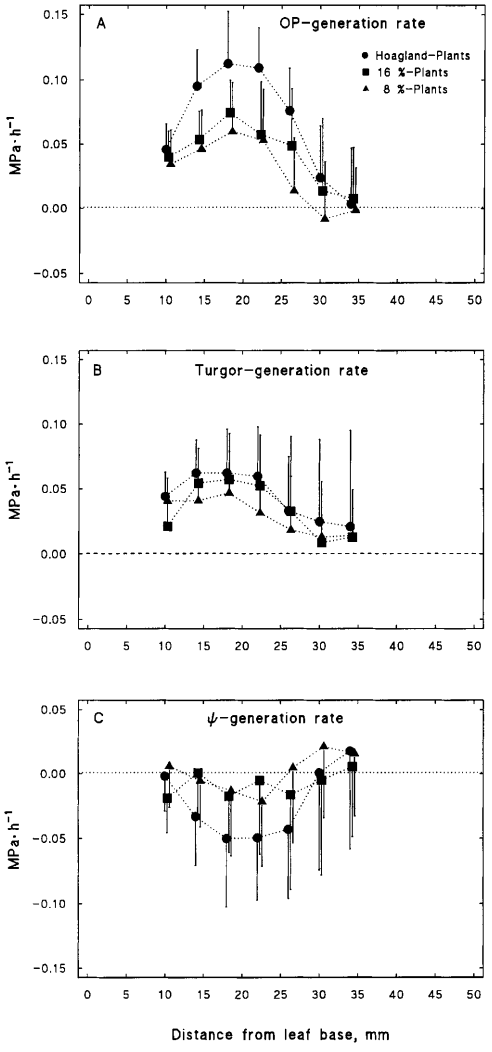


Fig. 5A–C. Rates of OP, turgor and Ψ generation in epidermal cells along the elongation zone of the third leaf of barley. Plants were grown on full-strength Hoagland solution, or on N-deficient Hoagland solution while receiving N at a relative addition rate (RAR) of 16% or 8% N per plant-N and day. Third leaves were analysed during the period of maximal and linear LER. Four to seven plants were analysed per treatment. Error bars represent SD values

The above observations raised several questions. For the water relations the questions are: (1) How can the existence of substantial growth-associated Ψ gradients in Hoagland-plants be brought in agreement with the short half-times ($t_{1/2}$ of 2 s) of water exchange across the membrane of expanding cells? (2) Can the existence of Ψ gradients and their variation along the growth zone be related to anatomical features, for example changes in the ratio of conducting-vessel area to total leaf area, the relative contribution of proto- and metaxylem elements, or the proportion of epidermal to total leaf volume? (3) Do the Ψ gradients result only from longitudinal expansion (elongation) or also from lateral expansion of cells? Concerning solute relations, the questions are: (1) Which are the main osmolytes in the elongation zone of barley? (2) Are these solutes mainly provided through the xylem (inorganic solutes), the phloem (mainly organic solutes) or through the cell itself (synthesis of sugars or degradation of sugar-polymers such as fructans)? (3) If the rate of solute provision limits the rate of cell elongation, does this reflect a limitation through a specific solute (for example nitrate) or a general shortage of solutes? (4) Are the observed generation rates of OP in epidermal cells in agreement with published rates of solute-flux ($mol \cdot m^{-2} \cdot s^{-1}$) across membranes and into cells and tissues?

Water relations. Only nutrient-sufficient, Hoagland-plants were considered. Applying Eq. E2 (*Materials and methods*) to the anatomical data and measured $t_{1/2}$, gave a cell diffusivity of water of $0.39 \times 10^{-10} \cdot m^2 \cdot s^{-1}$ at 30 mm from the leaf base. This is at the lower range of diffusivities obtained for cortical cells of expanding soybean hypocotyls (Nonami et al. 1997). Using this diffusivity value, together with a cell OP of 0.775 MPa and an RCER of 0.04 h^{-1} at 30 mm from the leaf base, Eq. E1 (*Materials and methods*) gave a cell Ψ that was 0.182 MPa more negative than xylem Ψ. Therefore, cell Ψ should have been as negative as [−0.182 + (−0.13 MPa)] = −0.312 MPa, which was almost identical to the experimentally determined Ψ (Fig. 6; 30 mm from leaf base). Although this agreement is remarkable, it should not be forgotten that some of the variables used had not been determined thoroughly, particularly cell elastic modulus and $t_{1/2}$, and could have affected the result easily by a factor of 2–4. Nevertheless, the above calculations show that there is a priori no theoretical reason to reject the measured cell Ψ and experimentally predicted Ψ gradients on the ground of short $t_{1/2}$ (see also Boyer 1985; Boyer et al. 1985; Nonami et al. 1997).

The above calculations, together with Figs. 2 and 3, show that, unlike the elongation zone in soybean hyopcotyls where Ψ gradients are largely due to a layer of small cells between protoxylem and outlying tissues, with very low diffusivities ($0.058 \times 10^{-10} \cdot m^2 \cdot s^{-1}$), Ψ gradients in the elongation zone of barley leaves do not require such a layer of low diffusivity cells. Instead, Ψ gradients may simply result from generally smaller cell dimensions in the direction of water flow compared to soybean tissue and the scarcity of water-supplying elements. Whether specific diffusion barriers, for example wax-like substances in cell walls, contribute to the Ψ gradients was not tested. However, the possibility was tested that the ratio of cross-sectional leaf area (water consumption) to xylem area (water supply) was particularly high in regions of largest Ψ gradients. Figure 7D shows that this was not the case (although see note below). The cross-sectional area of metaxylem vessels increased continuously along the elongation zone (Fig. 7A). At the same time, the area of protoxylem vessels decreased. As a result, total cross-sectional xylem-vessel area increased only slightly. Compared to the elongation zone of primary leaves of barley (Ryan and Matsuda 1988), the total cross-sectional xylem-vessel area was twice as large. The decrease in vessel area beyond 40 mm from the leaf base (Fig. 7A) reflected intrinsic differences in cross-sectional vessel area between parts of the blade that expanded earlier or later

Table 1. Some literature data on the rate at which solutes are deposited (per unit volume; $mM \cdot min^{-1}$) and osmolality is generated ($mosmol \cdot kg^{-1} \cdot min^{-1}$) in individual cells or bulk-tissue samples. Data were calculated from original values given in the cited references and therefore represent approximate values. Cells analysed in barley and wheat were epidermal cells

Specimen	Rate of solute deposition or osmolality generation	Reference
Elongating barley leaf cells	0.770 $mosmol \cdot kg^{-1} \cdot min^{-1}$	Present study
Fully expanded barley leaf cells	0.026 $mosmol \cdot kg^{-1} \cdot min^{-1}$ 0.024 mM $NO_3^- \cdot min^{-1}$	Fricke et al. (1994)
Fully expanded barley leaf cells during NaCl stress	0.050 $mosmol \cdot kg^{-1} \cdot min^{-1}$ 0.046 mM Na and Cl $\cdot min^{-1}$	Fricke et al. (1996)
Elongating sorghum leaf tissue	0.600 mM solutes $\cdot min^{-1}$	Bernstein et al. (1995)
Elongating maize leaf tissue	0.150 mM solutes $\cdot min^{-1}$	Meiri et al. (1992)
Elongating maize root tissue	3.330 mM solutes $\cdot min^{-1}$	Spollen and Sharp (1991)
Elongating maize root cortical cells (osmotic stress)	7.300 mM solutes $\cdot min^{-1}$	Frensch and Hsiao (1995)
Fully expanded wheat leaf cells (excised + illuminated for 24 h)	0.110 $mosmol \cdot kg^{-1} \cdot min^{-1}$ 0.060 mM K $\cdot min^{-1}$ 0.090 mM Cl $\cdot min^{-1}$	Tomos et al. (1992)
Elongation zone of tall fescue (import of hexose-equivalents)	0.520 mM hexose-equiv $\cdot min^{-1}$	Schnyder and Nelson (1989)
Elongation zone of tall fescue	0.027 mM $NO_3^- \cdot min^{-1}$	Gastal and Nelson (1994)
Bundle-sheath (sunflower leaves)	0.660–1.330 mM $\cdot min^{-1}$	Canny (1997)

Table 2. Average RCER, turgor- and OP-generation rates, and turgor and OP in expanding leaf epidermal cells of barley grown under various N-regimes. Plants were grown on full-strength Hoagland solution ("Hoagland-plants"), or on N-deficient Hoagland solution while receiving N at a relative addition rate of 16% or 8% N per plant-N and day ("16%-" and "8%-plants"). Average generation rates were calculated from the values shown in Fig. 5A, B. The zone between 12 and 32 mm from the base (14-, 18-, 22-, 26- and 30-mm values) was considered, since this was the zone of highest RCER and largest differences in RCER between treatments. Absolute values of turgor and OP were taken from Table 3 in Fricke et al. (1997)

	N-treatment		
	Hoagland-plants	16%-plants	8%-plants
RCER (h^{-1})	10.2%	7.3%	4.8%
Turgor pressure			
Absolute value (MPa)	0.475	0.534	0.532
Generation rate ($MPa \cdot h^{-1}$)	0.0484	0.0410	0.0301
Ratio (h^{-1})	10.2%	7.7%	5.7%
Osmotic pressure			
Absolute value (MPa)	0.758	0.683	0.703
Generation rate ($MPa \cdot h^{-1}$)	0.0834	0.0496	0.0330
Ratio (h^{-1})	11.0%	7.3%	4.7%

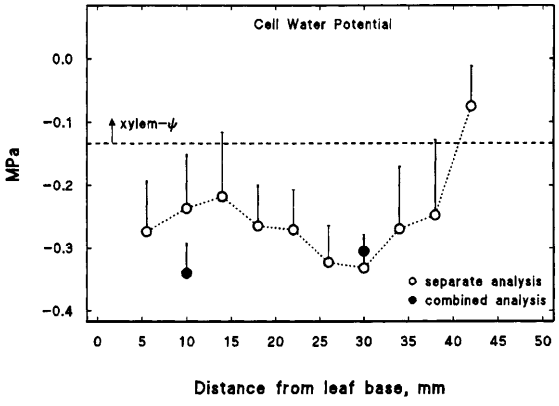


Fig. 6. Water potential of epidermal cells along the elongation zone of the third leaf of barley (Hoagland-plants). Water potentials were calculated from turgor and OP determined in different cells (*separate analysis*) or in the same cell (*combined analysis*). Results are expressed as means ± SD of six plant (*separate analysis*) or four cell analyses (*combined analysis*)

during blade development. Beyond the zone of cell elongation, the ratio of metaxylem-vessel area to protoxylem-vessel area remained constant at about 3.30 (see also Fig. 7C–F). In contrast, leaf cross-sectional area continued to increase beyond the point where cell elongation ceased (Fig. 7C). Therefore, lateral expansion of cells continued until at least 60 mm from the leaf base. As a consequence, the ratio of leaf cross-sectional area to total xylem-vessel area, which ranged between 102 and 164 along the elongation zone, increased to 216 at 60 mm from the base (Fig. 7D). It should be noted that the possibility exists that the metaxylem was not fully functional along the growth zone, in which case the 2.5-fold increase in the ratio of leaf area to protoxylem-vessel area along the elongation zone might have affected its water relations. It is generally assumed that as the protoxylem loses its function during cell elongation due to rupture of walls, the metaxylem gains function. However, it appears from Fig. 2C–E that the

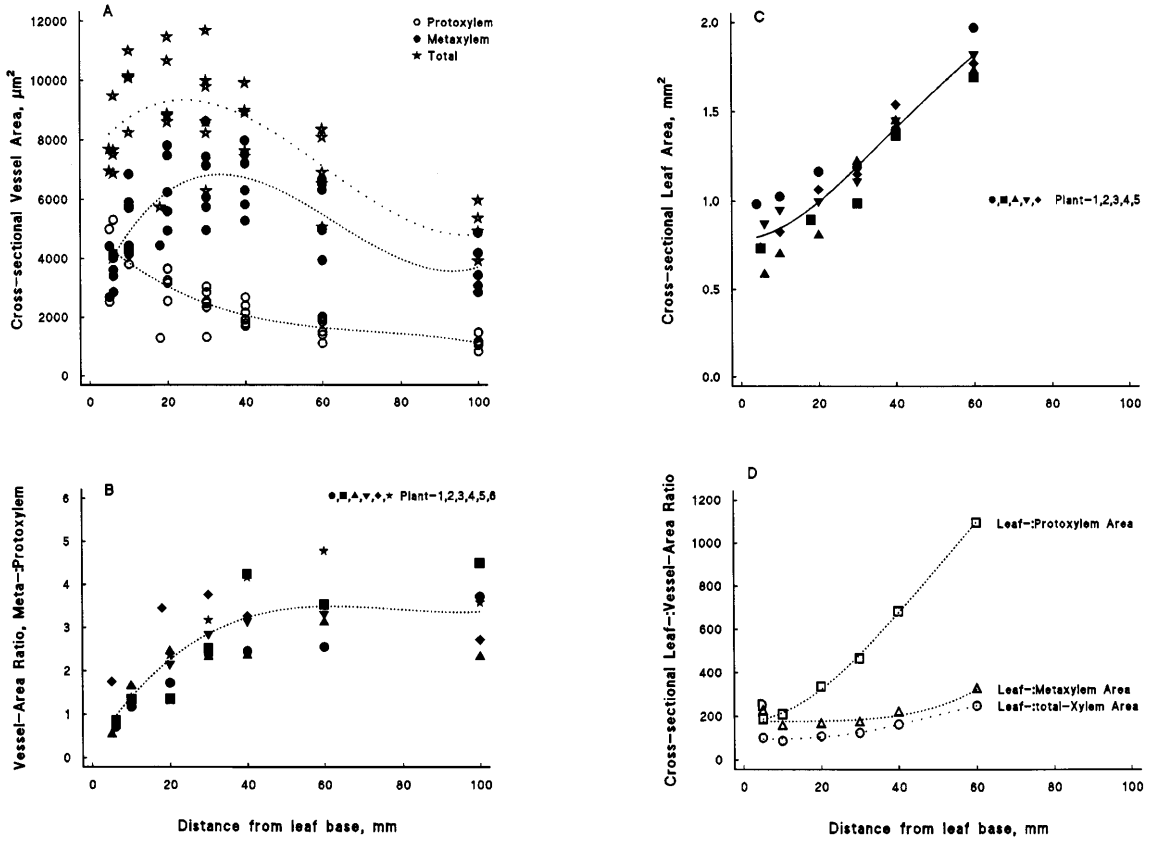


Fig. 7A–D. Cross-sectional areas of proto- and metaxylem-vessel elements **(A)** and their ratio **(B)**, cross-sectional leaf area **(C)** and the ratio of leaf area to vessel area **(D)** at various positions along, and distally beyond the elongation zone of the third leaf of barley (Hoagland-plants). Data were obtained on fresh, hand-cut cross-sections, viewed at magnifications of 40 to 400. Results from five to six leaf analyses are shown

metaxylem might lack lateral wall thickenings during the early stages of development and it is questionable to what degree this could cause walls to collapse at high transpiration rates.

As Fig. 7C shows, lateral expansion accompanied and succeeded longitudinal expansion of cells. Table 3 shows that the relative increase in lateral dimensions peaked at 6.87% $\cdot$ h^{-1} between 30 and 40 mm from the leaf base. This extra increase in volume (extra, compared to elongation), might partly explain why cell Ψ was so negative distally from the zone of highest RCER. It also implies that the generation rates of OP (and turgor), which were calculated solely on the basis of elongation growth, were actually higher: the maximum rate increased by about 22% to 0.137 MPa $\cdot$ h^{-1} (0.931 mM $\cdot$ min^{-1}), the average rate to about 0.102 mPa $\cdot$ h^{-1} (Hoagland-plants). In addition, the decrease in generation rates of OP beyond 16–20 mm from the leaf base was not as pronounced as suggested from Fig. 5 (at least for Hoagland-plants).

Application of the equation of Philip (1958) derived for a plane sheet of tissue to the elongation zone of the third leaf of barley, gave a half-time of Ψ equilibration between xylem and an epidermal cell located at a distance of 60–470 µm (nearest and furthest locations; *Materials and methods*) of 19–1164 s. This would imply that, unlike the situation in the fully expanded blade (Fricke 1997), cell position has a (large) effect on the turgor response-time to externally induced changes in Ψ.

Solute relations. Figure 8 shows the bulk-tissue concentrations of the main inorganic solutes and malate along the basal 70 mm of the third leaf of barley. The main inorganic solute was K^+. Its concentration decreased from 144 mM at 0–10 mm from the base to 90 mM at 40–50 mm from the base. Despite this, K^+ concentrations always at least equalled the sum of concentrations of the (inorganic) anionic solutes. The

Table 3. Relative increase in cross-sectional leaf area (= relative rate of lateral cell expansion) along the basal 60 mm of the third leaf of barley. The zone of cell elongation extended to about 40 mm from the leaf base. For a given leaf segment, the change in cross-sectional leaf area (Fig. 7C) between segment start and end points was related to that at the segment start point (ln[ratio]) and divided by the time taken for an element to be displaced through that segment using data from Fig. 4C (leaves analysed for cross-sectional leaf area elongated at rates (2.44 mm $\cdot$ h^{-1}) similar to those in Fig. 4C)

Leaf segment (mm from leaf base)	Relative increase in cross-sectional leaf area (h^{-1})
6–10	0.72%
10–20	0.95%
20–30	2.78%
30–40	6.87%
40–60	2.69%

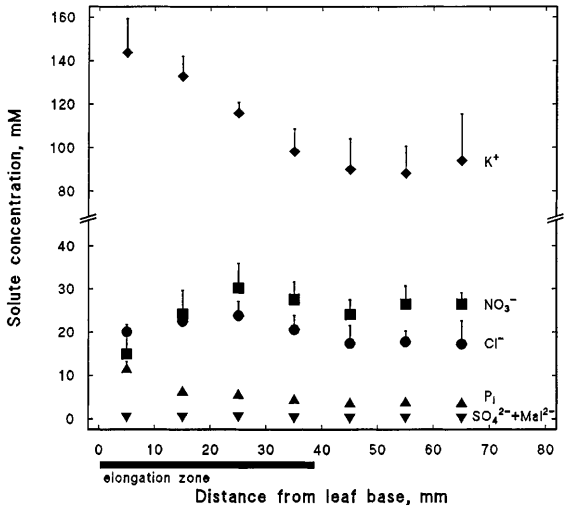


Fig. 8. Bulk solute concentrations at various positions along and distally beyond the elongation zone of the third leaf of barley (Hoagland-plants). Results are means of five experiments, with four to five plants analysed per experiment. Bars represent SD values

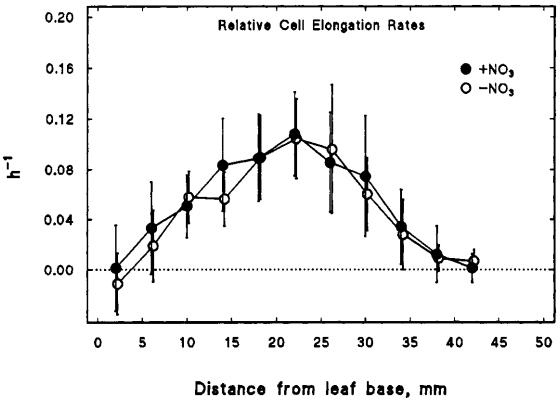


Fig. 9. Relative cell elongation rates along the elongation zone of the third leaf of barley grown with or without added NO_3^-. Plants were grown on Hoagland solution containing 3 mM NH_4^+ and 12 mM NO_3^- ($+NO_3^-$), or solely 4 mM NH_4^+ ($-NO_3^-$) as N-source. Plants were not starved of N since changes in plant density (3–6 plants · l^{-1}) had no effect on results. Third leaves were analysed during the period of maximal and linear LER. Ten plants were analysed per treatment. Error bars represent SD values

latter were mainly represented by Cl^- and NO_3^-. Both ions, particularly NO_3^- increased towards the zone of highest RCER. Solute concentrations generally levelled off beyond 40–50 mm from the leaf base. It is evident from Fig. 8 that at the bulk-tissue level, osmotic pressures of 0.7–0.8 MPa as are found in elongating epidermal cells, cannot be generated by K^+, Cl^- and NO_3^- alone, but that other, organic solutes are required – most likely in the form of hexoses (Barlow 1986; Schnyder et al. 1988; Schnyder and Nelson 1989). As to the situation in epidermal cells, some predictions can be made. Table 4 shows that along the elongation zone, the epidermis comprised between 23.4% and 28.1% of total leaf volume. Together with the bulk-tissue concentration data, these figures allowed estimations of epidermal solute concentrations.

Scenario "A" (30 mm from leaf base). Epidermal cell OP was 0.775 MPa (Fricke et al. 1997). Assuming an osmotic coefficient of 0.82 (Fricke et al. 1994), this would require 385 mM of solutes. If all of the Cl^- and NO_3^- found in bulk leaf extracts were present in the epidermis, then they would contribute 182 mM. To counterbalance this, 182 mM K^+ would be required, leading to a total of 364 mM. This is almost identical to what is needed to build up OP and would not necessarily require use of sugars. However, it would require that NO_3^- is exclusively found in the epidermis and that epidermal K^+ exceeds mesophyll K^+ considerably. This would be unlike the situation in the expanded blade.

Scenario "B" (30 mm from leaf base). If NO_3^- and K^+ were almost evenly distributed between all leaf tissues (as in the expanded blade), then this would amount to 136 mM of epidermal solutes. Together with Cl^-, which is likely to be almost restricted to the epidermis (Fricke et al. 1996), this would give 215 mM of epidermal solutes. This would result in only one excessive negative charge to be counter-balanced, but would leave ca. 170 mM of solutes missing. Most likely, these would be sugars (hexoses; Barlow 1986). Thus, in either scenario, the compartmentation of solutes between epidermis and mesophyll would differ between elongating and fully expanded leaf tissues. Nitrate may constitute up to 27% of epidermal cell OP. However, NO_3^- is not irreplaceable as an osmoticum, since barley grown on nutrient solution without any added NO_3^- showed the same LER and RCER for the third leaf as

Table 4. Cross-sectional area of the leaf and epidermis along the basal 60 mm of the third leaf of barley. The zone of cell elongation extended to about 40 mm from the leaf base. The cross-sectional area of the epidermis was calculated using values of its cross-sectional thickness (all epidermal layers considered; Fig. 3B) and of the surface length of the abaxial layer of the outer leaf arm (Fig. 3C), multiplied by 4 (two leaf arms with adaxial and abaxial epidermal layers; the surface lengths of these were comparable)

mm from leaf base	Cross-sectional area		
	Leaf (mm^2)	Epidermis (mm^2)	% of leaf area
6	0.783	0.183	23.4
10	0.876	0.223	25.4
20	0.986	0.275	27.9
30	1.133	0.319	28.1
40	1.441	0.388	26.9
60	1.796	0.451	25.1

Table 5. Some literature data on the flux density of solutes across the surface of cell membranes, cells and plant organs. The value shown for expanding barley leaf cells is an upper estimate since it is based on the asssumption that OP was exclusively generated by the uptake (and not cell internal synthesis) of solutes

Specimen	Flux density (10^{-8} mol · m^{-2} · s^{-1})	Reference
Expanding barley leaf cells	6.8–6.9 (osmolyte uptake)	Present study
Maize root cortical cells (growing at 100 mM NaCl)	5.0 (Na^+, Cl^- uptake)	Cited in Tomos (1985)
Tonoplast of maize root cells	1.8 (Cl^- uptake)	Cited in Tomos (1985)
Root surface of maize	9–58 (K^+-influx currents)	Kochian et al. (1992)
Suspension-cultured maize cells	0.05–0.10 (K^+ uptake)	Kochian et al. (1992)
Valonia utricularis	10–50 (K^+-influx currents)	Cited in Zimmermann (1977)
Various marine microalgae	4–560 (Cl^-, Na^+ uptake)	Cited in Tomos (1985)
Guard cells (during stomatal closure)	339 (Cl^--efflux currents) 56 (malate-efflux currents)	Schmidt and Schröder (1994)
Isolated barley leaf protoplasts	0.023–0.026 (Cl^- uptake)	Dietz et al. (1992)
Plasma membrane of mesotome sheath next to phloem	230 (sugar flux)	Kuo et al. (1974)

NO_3^--sufficient plants (Fig. 9; LER, 2.43 mm · h^{-1} compared to 2.42 mm · h^{-1}).

If solute provision in general limits the rate of cell expansion, solutes might be "saved" for OP generation in growing cells and not "wasted" for OP generation in fully expanded cells, particularly in 8%-plants. However, this was not the case. Osmotic pressure was measured (five plant analyses with five cell analyses each) in epidermal cells, halfway along the atmosphere-exposed part of the leaf blade. These cells had attained their final size ca. 1 d (Hoagland-plants) or 2 d (8%-plants) before. In Hoagland-plants, the OP of these cells was 0.836 ± 0.027 MPa, i.e. since attaining mature size, OP had increased by about 0.126 MPa and at a rate of 0.0053 MPa · h^{-1} – a rate 15-times lower than the average OP-generation rate during expansion (Table 2). In 8%-plants, mature-cell OP was 1.052 ± 0.050 MPa, i.e. OP had increased since maturation by about 0.372 MPa and at a rate of 0.0078 MPa · h^{-1}; a rate only 4-times lower than during expansion. This apparent paradox, higher solute import after expansion in cells with lower solute import during expansion (final cell length was similar between N-treatments; Fricke et al. 1997), can easiest be solved by suggesting that the proportion and type of solutes that generated OP in expanding leaf epidermal cells differed from that in fully expanded cells – the same conclusion as above. Predicted differences in the intercellular compartmentation of solutes between elongating and mature leaf tissue may partly reflect differences in the way solutes can be carried from the xylem towards the epidermis: in the mature tissue, with the transpiration stream, but not so in the elongating tissue which is surrounded by subtending sheaths (probably 100% relative humidity).

For technical reasons, OP was mainly determined for the largest cells, i.e. ridge and trough cells. At around 20 mm from the leaf base, 1 l of ridge- or trough-cell volume corresponded to about 185 and 188 m^2 cell surface. The maximum rate of OP generation was ca. 46 mM · h^{-1}. This produced, on calculation, a solute flux rate of 6.8×10^8 to 6.9×10^8 mol · m^{-2} · s^{-1}, which is in the range of values reported for several other vascular-plant cells and tissues (Table 5). However, it is (as expected) 1–3 orders of magnitude lower than solute flux rates in guard cells, algae and the mesotome sheath next to the phloem (sugar-loading), and about 2 orders of magnitude larger than values reported for in-vitro systems (protoplasts and suspension cultures). The latter might reflect the potential importance of whole-tissue behaviour and the role of the apoplasm for solute transport at the cell level.

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