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 $-0.15 \text{ MPa}$ between water source (xylem) and expanding epidermal cells. The latter was similar to a theoretically predicted value ($-0.18 \text{ MPa}$). Highest rates of OP generation were observed in demand-limited plants, with a maximum rate of $0.112 \text{ MPa} \cdot \text{h}^{-1}$ 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:

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

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

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-expanding and expansion event. 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 (RCEs) 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-dependent 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 \( \Psi \) 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 \( \Psi \) 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 \( \Psi \) 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.