Plant Physiol. (1995) 108: 303–312

Rapid Response of the Yield Threshold and Turgor Regulation during Adjustment of Root Growth to Water Stress in *Zea mays*[1]

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Responses of cortical cell turgor (*P*) following rapid changes in osmotic pressure (π_m) were measured throughout the elongation zone of maize (*Zea mays* L.) roots using a cell pressure probe and compared with simultaneously measured root elongation to evaluate: yield threshold (*Y*) (minimum *P* for growth), wall extensibility, growth-zone radial hydraulic conductivity (*K*), and turgor recovery rate. Small increases in π_m (0.1 MPa) temporarily decreased *P* and growth, which recovered fully in 5 to 10 min. Under stronger π_m (up to 0.6 MPa), elongation stopped for up to 30 min and then resumed at lower rates. Recoveries in *P* through solute accumulation and lowering of *Y* enabled growth under water stress. *P* recovery was as much as 0.3 MPa at π_m = 0.6 MPa, but recovery rate declined as water stress increased, suggesting turgor-sensitive solute transport into the growth zone. Under strong π_m, *P* did not recover in the basal part of the growth zone, in conjunction with a 30% shortening of the growth zone. Time courses showed *Y* beginning to decrease within several minutes after stress imposition, from about 0.65 MPa to a minimum of about 0.3 MPa in about 15 min. The data concerning *Y* were not confounded significantly by elastic shrinkage. *K* was high (1.3×10^{-10} m^2 s^{-1} MPa^{-1}), suggesting very small growth-induced water potential gradients.

Roots adjust their growth rate when subjected to water stress. The phenomenon is commonly studied using Lockhart's concept of growth, which relates growth to cell wall mechanics and hydraulic and osmotic properties of the growing tissue (Lockhart, 1965; Hsiao et al., 1976; Boyer, 1985; Cosgrove, 1986). Of the parameters in the Lockhart equations, the yield threshold Y and wall extensibility m are often evaluated from plots (usually linear) of growth rates versus P, with Y representing the intercept on the P axis and m representing the slope for roots (Pritchard et al., 1990b) as well as leaves (Bunce, 1977; Hsiao and Jing, 1987). The underlying assumption is that Y and m do not change when different water regimes are imposed to obtain the different growth rates for the plot. Frequently, the analysis indicated relatively low Y, resulting in a large "growth-effective turgor" ($P - Y$) and low values of m in both roots (Pritchard et al., 1990b) and leaves (Bunce, 1977). Another method of evaluating Y and m is by the stress relaxation technique (Cosgrove, 1986). By withholding water from growing tissue, the cell walls of an excised organ are allowed to yield until P decreases to a pressure at which growth ceases; the rate constant of relaxation yields m. Measured P at that point is taken as Y. Either of the two methods would be questionable if Y changes readily as P varies (Frensch and Hsiao, 1994). A number of studies have shown that root growth and its acclimation to water stress are closely related to processes of cell wall loosening and tightening, with a consequent impact on the values of Y and m (Hsiao and Jing, 1987; Pritchard et al., 1990a, 1991, 1993; Spollen and Sharp, 1991). Indeed, early work on algae (Green et al., 1971) and more recently on roots (Hsiao and Jing, 1987; Frensch and Hsiao, 1994) showed rapid changes of Y with water stress. Measurements of P and root growth during osmotic transitions revealed values of Y much closer to P (Frensch and Hsiao, 1994). This indicates that m is large and factors different from changes in m could be equally or more important in determining rates of elongation.

The rate of cell expansion along the elongation zone is not uniform. For maize (*Zea mays* L.) roots, local relative

[1] This research was supported by grant 91-37100-6671 from U.S. Department of Agriculture to T.C.H. and by the award of a Feodor Lynen Fellowship from the Alexander von Humboldt Foundation (Germany) to J.F.

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Abbreviations: A, surface area (m^2); $\Delta\pi_m$, change of osmotic pressure in the medium (MPa); ϵ, volumetric elastic coefficient (MPa); ER, elongation rate (mm h^{-1}); k, rate constant of water exchange (s^{-1}); K, tissue hydraulic conductivity coefficient (m^2 s^{-1} MPa^{-1}); LRER, local relative elongation rate (h^{-1}); LVDT, linear variable differential transducer; m, cell wall extensibility (MPa^{-1} h^{-1}); P, cell turgor (MPa); P_{min} (P_{max}), minimum (maximum) turgor measured during the response of P to a decrease (increase) in Ψ of the medium; ($P - Y$), growth-effective turgor; π, osmotic pressure (MPa); Ψ, water potential (MPa); r, radius (mm); $t_{1/2}$, half-time of water exchange (s); V, volume (m^3); Y, yield threshold (MPa); $Y_{trans,1}$, Y evaluated from the turgor decline phase during the biphasic pressure-response curve; $Y_{trans,2}$, similar to $Y_{trans,1}$ but evaluated from the turgor recovery instead of the decline phase; z, length of the root elongation zone; subscript "c" associates a parameter with a cell; subscript "m" associates a parameter with the medium; subscript "r" associates a parameter with the root; subscript "t" indicates the dependency of a parameter on time; subscript "z" associates a parameter with respect to its position along the elongation zone.

elongation rates (LRERs) are highest at positions in the middle of the expansion zone (Silk et al., 1986; Sharp et al., 1988; Pritchard et al., 1993). In the absence of significant longitudinal turgor gradients (Pritchard et al., 1991; Spollen and Sharp, 1991; Frensch and Hsiao, 1994), the longitudinal pattern of LRER could be due to gradients of cell wall properties (Tomos et al., 1989; Pritchard et al., 1993). Other factors essential for continuous growth are the supply of water and assimilates to the expanding cell or tissue. A fast LRER must be supported by a sufficient local import of water and solutes (Erickson, 1976; Silk et al., 1986), although the mechanisms and pathways of carbohydrate and water transport into the growth zone are still not clear (Bret-Harte and Silk, 1994).

In a previous paper, we studied adjustment in the growth of maize roots to moderate water stress (π_m = 0.3 MPa) (Frensch and Hsiao, 1994). By measuring P continuously, we observed a biphasic pressure response upon an up-step in π_m. The first phase was a rapid decline of P, attributable to water loss out of the cells. P recovered during the second phase to a level lower than that prior to the up-step in π_m. P recovery and a rapid adjustment of Y compensated for the reduced water availability and maintained a positive difference ($P - Y$) under water stress. In the present study we extended the experiments to a broader range of stress levels. One objective was to determine kinetics of changes in Y and to use the dynamic values of ($P - Y$) to make more accurate estimates of m. Another was to examine the spatial pattern of growth as affected by water stress, particularly the kinetics of turgor recovery, and to determine whether they reflect the observed (Sharp et al., 1988) shortening of the growth zone under water stress. Also included in this paper is a preliminary evaluation of ($P - Y$) and m at different locations along the growth zone. The third objective was to estimate the radial hydraulic conductivity of the root cortex per unit of area and path length and to assess its possible significance in limiting the growth of cortical cells.

MATERIALS AND METHODS

Plant Culture

Seeds of *Zea mays* L. (cv WF9 × Mo17) were germinated on wet filter paper in the dark. After 3 d, seedlings were transferred to containers of aerated culture solution inside a growth chamber (14-h photoperiod, 25°C air temperature, 500 μmol m^{-2} s^{-1} PAR at the lid of growth containers). The culture solution was similar to a one quarter-strength Johnson solution (for composition, see Frensch and Hsiao, 1994) and was replaced by a fresh solution every 3rd d to avoid nutrient depletion and to maintain pH between 6.5 and 6.8. Experiments were performed 2 to 4 d after transfer on seedlings that had developed a primary root 110 to 180 mm long.

Experimental Setup, *P*, and Root Elongation Measurements

Roots were exposed to solutions of different osmolality and the response in growth rate and P was monitored. P and elongation rate (ER) were measured before, during, and after exposure to osmotic stress. A single plant was mounted on a temperature-controlled holder (25°C), which was set up on a vibration isolation table. The primary root was positioned at 70° from the horizontal and bathed continuously by a flowing solution. The procedure used to change rapidly from one solution to another of a different osmotic pressure was identical with that previously described (Frensch and Hsiao, 1994). Because a small volume of the old solution was retained in the experimental apparatus and mixed with the new solution after the change, π_m was measured after mixing and varied slightly from experiment to experiment for the same starting osmotic solution. In contrast to the previous study involving only osmotic solutions of approximately 120 mosmol kg^{-1}, a wide range of osmolalities (between 38 and 240 mosmol kg^{-1}, equivalent to π_m of about 0.1 to 0.6 MPa) was used in this study. To avoid plasmolysis of expanding cells and consequent damage of the tissue, π_m was limited to a maximum of 0.6 MPa. Either mannitol or KCl was used as the osmoticum. The two solutes were considered to be equivalent, since there were no detectable differences between the solutions in the extent of P response or the pattern of P kinetics.

A cell pressure probe (Zimmermann and Steudle, 1978), modified as described by Frensch and Hsiao (1993) and with the capability of monitoring capillary tip position to an accuracy of 10 μm, was used to measure P of elongating cells throughout the cortex (epidermis to inner cortex), 3 to 10 mm behind the root meristem. Root elongation was monitored using a LVDT. The central core of the LVDT was connected to the root cap using a thread (Frensch and Hsiao, 1994). A spring was attached to the other side of the core to keep the thread under a steady tension equivalent to 2 g of weight. The attachment of the LVDT resulted in a transient and slight increase in root growth. ER were calculated directly from a chart recorder using digitizing equipment.

Measurement of LRER

The spatial distribution of root growth as affected by water stress was investigated on a separate batch of plants. Images of root elongation zones were captured at low magnification (×8) using a video camera attached to the phototube of the microscope and connected to a video recorder. Displacement velocities (in mm h^{-1}) were evaluated according to the method of Silk et al. (1986) at increments of 1 mm in length using a digitizing tablet and software (SigmaScan; Jandel Scientific, Corte Madera, CA). Graphite powder was applied to the root surface to aid the tracking of cells on videotape. Prior to an osmotic treatment (π_m = 0.3–0.58 MPa), displacements were measured at intervals of 15 min for 1 h. Following the treatment and starting when root elongation resumed, i.e. P equals $Y_{trans,2}$ (see below), displacements were measured during a period of 1 h at intervals of either 15 min (π_m = 0.3 MPa) or 30 min (π_m = 0.58 MPa). A four-parameter logistic equation was fitted to the plot of displacement velocities versus longitudinal position (Landsberg, 1977). The longitudinal distribution of LRER was obtained upon taking the first derivative of the equation with respect to distance.

Measurement of *Y*, *m*, and (*P* − *Y*)

As in the previous study (Frensch and Hsiao, 1994), *Y* was evaluated directly from time courses of *P* and root elongation (Fig. 1D). Upon the addition of osmoticum to the medium and the subsequent decrease of *P*, the pressure at which root elongation stopped was determined. This $Y_{trans,1}$ was assumed to reflect *Y* prior to the osmotic treatment, since it took *P* in the root only a few seconds to reach this value. $Y_{trans,2}$ was determined from pressure kinetics during the second phase of the biphasic *P* response, when *P* increased above the threshold and root elongation resumed. For concentrations of test solutions <140 mosmol kg^{-1} ($\pi_m < 0.35$ MPa), $Y_{trans,2}$ was evaluated from *P* kinetics at positions 3 to 10 mm behind the root tip. To avoid complications due to shortening of the growth zone, $Y_{trans,2}$ was determined from *P* kinetics of cells at positions 3 to 5 mm for $\pi_m > 0.35$ MPa. Possible interferences of elastic responses are examined in "Discussion."

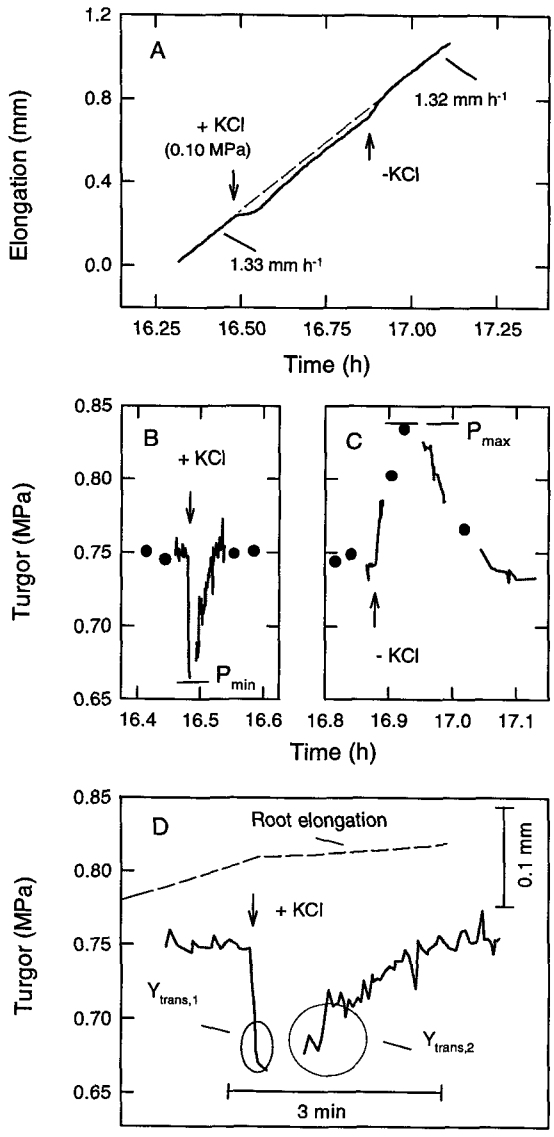

Figure 1. Effects of small changes in π_m (addition and withdrawal of 0.1 MPa KCl) on root elongation and *P*. A, Recorder tracing of the elongation (solid line) and extrapolated hypothetical growth of the root if left untreated (dashed line). Details of the biphasic turgor responses after the addition (B) and the withdrawal (C) of KCl, reflecting the water and solute responses of the tissue, are shown. Solid lines represent continuous measurements of *P* in a single cell during a period, and closed symbols represent short-term measurements of *P* of different cells. Horizontal dashed lines indicate P_{min} and P_{max}, respectively. $t_{1/2}$ values were evaluated from the *P* decline following the application of KCl and from the *P* increase following the withdrawal of KCl to calculate *K*. In D, transient *P* and elongation after the addition of KCl are shown to illustrate the determination of $Y_{trans,1}$ and $Y_{trans,2}$. *P* was measured 4 to 5 mm behind the root tip 30 to 120 μm inside the cortex. Length of root = 180 mm.

For each longitudinal position, (*P* − *Y*) was calculated before the imposition of osmoticum as the difference between the steady-state *P* prior to the osmotic treatment and $Y_{trans,1}$. After the imposition of osmoticum, (*P* − *Y*) was calculated as the difference between the near steady-state *P* at the end of the biphasic pressure response and $Y_{trans,2}$. Hence, changes of *P* and *Y* during the treatment were integrated in one single variable, (*P* − *Y*). Among individual seedlings, it is also more logical to compare their (*P* − *Y*) instead of their *Y*, because *P* varies somewhat from plant to plant (Frensch and Hsiao, 1994) and *Y* tends to increase with increasing *P*. Using the near steady-state *P* and $Y_{trans,2}$ to calculate (*P* − *Y*) introduces some inaccuracy because $Y_{trans,2}$ was determined 3 ($\Delta\pi_m$ = 0.1 MPa) to 20 min ($\Delta\pi_m$ = 0.6 MPa) before the near steady-state *P* was reached. Our opinion is that *Y* is likely to increases slightly during this period of *P* recovery. Hence, (*P* − *Y*) would be slightly overestimated.

Since (*P* − *Y*) varied along the growth zone, *m* was also calculated locally according to

$$m = \frac{\text{LRER}}{(P - Y)_z} \quad (1)$$

where LRER replaces the product of $ER \times z^{-1}$ (*z* = length of the elongation zone) of the original equation given by Lockhart (1965). Although $(P - Y)_z$ was measured locally, the criterion of zero growth at which point *Y* was taken is based on growth of the whole root. In that sense, the values of neither *Y* nor *m* are strictly local.

Calculation of *K*

The hydraulic conductivity of the root cylinder per unit of cross-sectional area and path length in the radial direction of water flow (*K*, $m^2\ s^{-1}\ MPa^{-1}$) was determined from measurements of transient *P* responses of individual cortical cells. Water transport across the root can be described using a general equation for a steady-state radial flow of cylindrical geometry (Nobel, 1991), modified to account for a rapid increase of osmotic pressure in the medium ($\Delta\pi_m$) using a nonpermeating solute (Steudle and Tyerman, 1983) for which cells exhibit a reflection coefficient of 1:

$$J_V = \frac{dV}{dt}\frac{1}{A_r} = -\frac{K}{r \ln (r/r_c)}(P_{(t)} - \pi_{c(t)} + \pi_m + \Delta\pi_m) \quad (2)$$

where J_V (m s^{-1}) = volume flux (volume flow [dV/dt] per unit of root surface area [A_r]) and r = radius of the root. The term in brackets on the far right side is the Ψ difference between a cell at the radial position r_c ($\Psi_{c(t)} = P_{(t)} - \pi_{c(t)}$) and the medium ($\Psi_m = -\pi_m - \Delta\pi_m$) just after the addition of the osmoticum. Subscripts "t" denote the dependency of P and π_c on time during the transition. Under conditions of $\Psi_m < \Psi_c$, water is lost to the medium and J_V is negative. Using Equation 2, the evaluation of K is limited to a model that treats the root as a simple two-compartment system, with an interior compartment separated from an exterior compartment by a structureless barrier. Thus, Equation 2 does not distinguish between different local pathways of water transport. For a theoretical treatment that considers the apoplast and symplast as separate pathways, see Molz and Ikenberry (1974) and Steudle (1992). These models, however, use water relations coefficients for each pathway, which are technically difficult to assess.

In this study, mannitol and KCl were used to induce a water flow either into or out of the root. Although K^+ and Cl^- must be transported across the cell membrane, our results (Frensch and Hsiao, 1994) show that mannitol and KCl are both essentially nonpermeating (a reflection coefficient of near unity) and have osmotic effects indistinguishable from each other on the short time scale used in this study. Following a perturbation of π_m, a cell as well as the entire root respond with transient volume changes until a new steady state is achieved. For small changes in V, the root dimensions may be assumed to be constant and Equation 2 can be solved for the initial conditions of $t = 0$ and $P = P_0$ (Dainty, 1963; Steudle and Tyerman, 1983):

$$P_{(t)} - P_0 = \frac{\epsilon}{\epsilon + \pi_c} \Delta\pi_m (e^{-kt} - 1) \quad (3)$$

with

$$k = \frac{\ln(2)}{t_{1/2}} = \frac{A_r}{V_r}(\epsilon + \pi_c)\frac{K}{r \ln(r/r_c)} \quad (4)$$

where ϵ = elastic coefficient of a single cell and A_r/V_r = ratio of surface area to volume of the elongation zone. The ratio $\epsilon/(\epsilon + \pi_c)$ in Equation 3 is derived from the relationship between changes of cell volume and cell osmotic pressure to replace $\pi_{c(t)}$ in Equation 2 (Dainty, 1963). Thus, effects of $\pi_{c(t)}$ on $P_{(t)}$ during the transition are incorporated. Measured P kinetics were digitized and the rate constant (k) or the half-time of P kinetics ($t_{1/2}$) was determined according to Equations 3 and 4. For a single cortical cell in the growth zone, ϵ is approximately 4 MPa (Pritchard et al., 1990a) and π_c is approximately 0.9 MPa at high Ψ (Sharp et al., 1990). Therefore, a total of 5 MPa was assumed for the sum of ($\epsilon + \pi_c$) to calculate K; a value of 0.8 was assumed for the ratio $\epsilon/(\epsilon + \pi_c)$ in Equation 3. K was evaluated from plotting $t_{1/2}$ versus $\ln(r/r_c)$. A mean value of r = 0.4 mm was measured using a microscope 5 mm behind the root tip.

RESULTS

Response of *P* and Root Growth to Low Ψ

As reported previously (Frensch and Hsiao, 1994), replacing the nutrient solution with test solutions of higher osmotic pressure (π_m = 0.3 MPa) caused biphasic pressure kinetics in root cells: a fast decline in P constituted the first phase, followed by a slow increase in P ("turgor recovery") after the P minimum. Here we studied the effects of test solutions (KCl, mannitol) with π_m higher or lower than 0.3 MPa. With more dilute test solutions (π_m = 0.1 MPa), the two phases were shorter than they were when using 0.3-MPa test solutions, and the initial P and ER were restored completely approximately 10 min after the onset of the stress treatment (Fig. 1). Replacing the test solution with the original nutrient solution resulted in a second biphasic pressure response with a pressure maximum (Fig. 1C). The temporary increase of growth associated with the decrease of π_m fully accounted for the previous reduction of growth during the exposure of roots to the osmoticum, as indicated by the dashed line in Figure 1A. Figure 1D illustrates the determination of $Y_{trans,1}$ and $Y_{trans,2}$ from the time courses of P and growth and the degree of uncertainty in the procedure.

The response of P and root elongation was different in experiments (n = 8) in which π_m was altered by approximately 0.6 MPa. Increasing π_m to 0.58 MPa (Fig. 2) caused a larger decrease of P, a longer period of zero growth, and a substantially slower rate of elongation when growth resumed. The period of zero growth ranged between 19 and 31 min. After the initial decrease in P when π_m was stepped up, P increased gradually with time, approaching a new but lower steady-state value at a position 4 to 5 mm from the root tip after about 1 h (Fig. 2C). The rate and extent of P recovery varied with location in the growth zone. Recovery was most marked near the root tip and gradually decreased toward the basal portion of the growth zone (Fig. 2B). Following the withdrawal of the osmoticum, growth increased transiently to very high values, but the final root length never reached the extrapolated position of an undisturbed root (dashed line, Fig. 2A). ERs were substantially smaller than the initial rates following the release of the osmotic treatment (0.75 compared to 1.15 mm h^{-1} in Fig. 2A) and never recovered completely up to 2 h after the release.

Spatial Distribution of Root Growth

Spatial analysis of growth carried out on a separate batch of plants revealed the common sigmoidal shape of displacement velocities along the root growth zone. Fastest acceleration rates were found 4 to 6 mm behind the apex in the two osmotic treatments and in the control (Fig. 3A). However, the total amount of displacement decreased with increasing π_m, resulting in a smaller ER under water stress. The pattern of LRER (Fig. 3B), calculated from the fitted data of displacement velocities, showed that LRERs at positions apical to 6 mm were affected by π_m = 0.3 MPa and higher and that LRERs in more basal parts were reduced at π_m = 0.6 MPa. The latter caused shortening of the elonga-

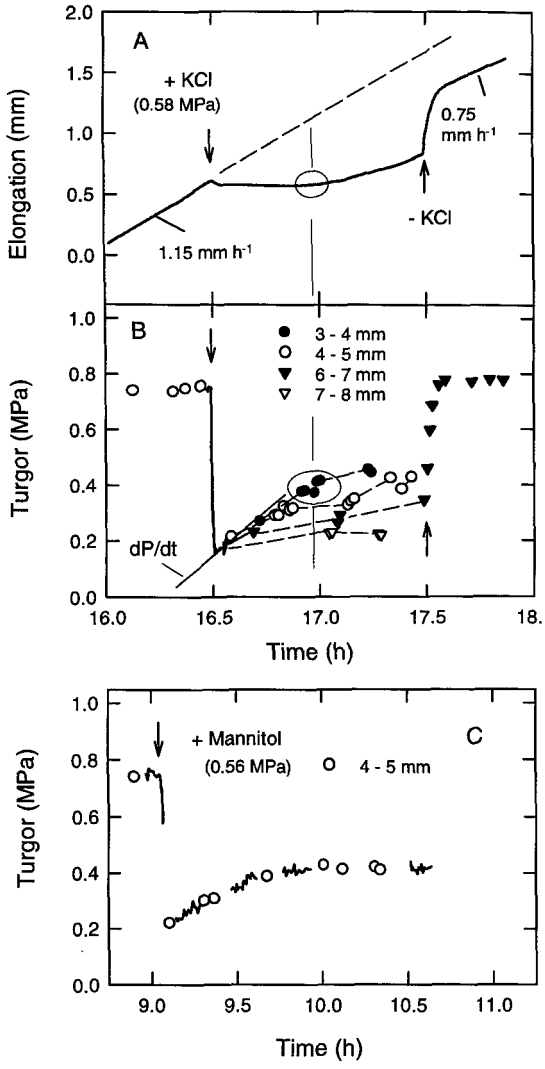

Figure 2. Effects of large changes in π_m (addition and withdrawal of 0.58 MPa KCl) on root elongation and *P*. A, Recorder tracing of the elongation (solid line) and extrapolated hypothetical growth of the root if left untreated (dashed line). B, Turgor response to changes in π_m with respect to various positions (●, ○, ▼, ▽) along the elongation zone. The solid line between the open circles represents a continuous measurement of *P* in a single cell during a period, and symbols represent short-term measurements of *P* of different cells. For better identification, measurements within indicated intervals are connected by a dashed line. The measurements show that turgor recovery gradually decreased from younger to older parts of the elongation zone. The maximum rate of turgor recovery (*dP/dt*), which is a measure of solute accumulation in cells, was determined from the increase of *P* (straight line) subsequent to P_{min}. The two circles in A and B at approximately 17 h indicate how $Y_{trans,2}$ was derived. *P* was measured 4 to 5 mm behind the tip 20 to 180 μm inside the cortex. Length of root = 180 mm.

tion zone from the original 10 mm to 7 mm. Earlier work on maize roots (Sharp et al., 1988; Pritchard et al., 1993) showed that LRERs in the basal region were inhibited by water stress, whereas those in the apical region were not significantly affected. The present results are apparently different in that LRERs in the apical region also appeared to be reduced. The difference may not be significant, however, in light of the relatively large SE values in our data (Fig. 3A). Our seedlings may also be different because they were older and autotrophic and, thus, not relying on the seed reserve for growth as in the previous studies.

Root Hydraulic Conductivity

$t_{1/2}$ was determined from *P* kinetics upon the addition (open symbols) and the withdrawal (closed symbols) of an osmoticum (Fig. 4A). $t_{1/2}$ increased with increasing radial distance from the root periphery and was generally smaller for the process of water loss than water gain. Longer $t_{1/2}$s were probably caused by fast elongation during the increase of *P*, which supposedly attenuated the *P* increase. Supporting this conclusion is the observation that in mature root tissue $t_{1/2}$ for the process of water loss was essentially the same as $t_{1/2}$ for water gain (Frensch and Hsiao, 1994). Therefore, *K* was evaluated from *P* kinetics upon the addition of osmoticum, which occurred to a large extent after the root stopped growing. Using the slope in Figure 4B (slope = 83 s), we calculated *K* to be 1.3×10^{-10} m^2 s^{-1} MPa^{-1}. The linear fit in Figure 4B was based on the assumption that *K* is constant across the cortex.

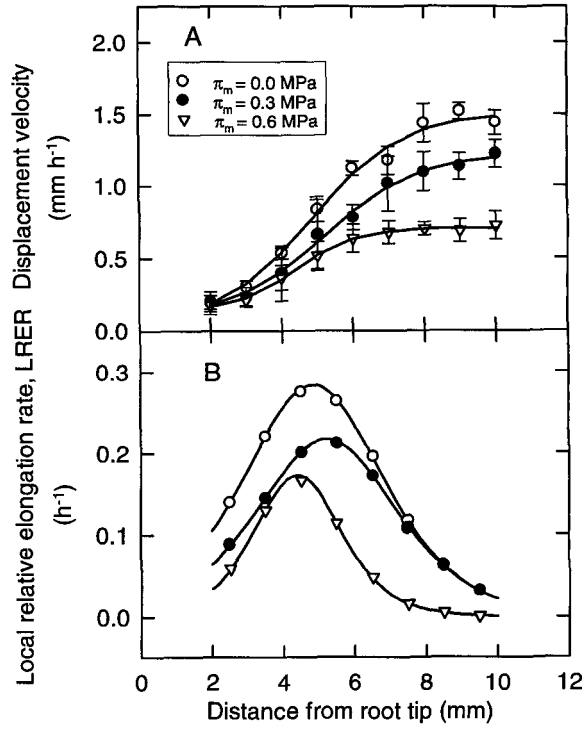

Figure 3. Spatial distribution of the rate of cell displacement (A) and of the LRER along the root axis (B) following changes in π_m using KCl. Displacement velocities of cells at 1-mm intervals (means ± SE, *n* = 3 to 7 roots) were evaluated from video images of root growth and were fitted by a logistic equation. The derivative of this equation was used to calculate LRER. Symbols indicate the LRER values used for calculation of *m* (see Fig. 7).

Response of *P* Recovery to Water Stress

With increasing water stress, the maximum slope of P recovery (dP/dt) gradually decreased by a factor of about 5 (Fig. 5). The scatter among data points, which represent individual experiments on separate roots (n = 32), largely resulted from measuring cells at different radial positions: cells deeper inside the tissue showed a higher rate of recovery than those at the root periphery, indicative of a solute supply from the phloem (Frensch and Hsiao, 1994). Figure 5B shows a profile of dP/dt along the root axis. P recovery ceased upon exposure of roots to strong water stress (open triangles: π_m approximately 0.6 MPa) at positions basal of about 7 mm, which was associated with the shortening of the growth zone (Fig. 3B).

Cell Wall Rheology

After the onset of water stress, Y decreased from initial values ($Y_{trans,1}$) between 0.6 and 0.7 MPa to adjusted values ($Y_{trans,2}$) as low as 0.3 MPa (Fig. 6A). Adjustment of Y was not detectable in experiments in which π_m was increased to only 0.1 MPa (Fig. 1). The data in Figure 6A indicate a minimal pressure for irreversible cell expansion of 0.3 MPa. Replotting the data of Figure 6A with respect to the time after the application of the osmoticum (Fig. 6B) showed that Y decreased rapidly within the first 10 min. Apparently, decline of $Y_{trans,2}$ between 0.4 and 0.3 MPa took longer than between 0.65 and 0.4 MPa, although the time course of $Y_{trans,2}$ could have been confounded by the slow rate of P recovery at high π_m (Fig. 5A). Thus, the adjustment of Y may have been faster than indicated by the solid line in Figure 6B.

The $(P - Y)$ was calculated for each measured position along the root axis. The means ± SE for the three treatments are given in Figure 7B. To better show the plant-to-plant variations and the number of measurements making up the mean, the individual data points taken from a total of six plants are also given (Fig. 7A). Considering that P is fairly constant along the growth zone and across the cortex at steady-state growth rates before perturbation of π_m (Frensch and Hsiao, 1994), larger values of $(P - Y)$ at positions 4 to 6 mm behind the apex indicate a low Y rather than a high P. That is, Y appeared to be lowest in the middle of the elongation zone before roots were exposed to osmotic solutions. Afterward, $(P - Y)$ declined significantly at positions closer to the root tip. In contrast to $(P - Y)$, m, although exhibiting rather large SE values, was fairly con-

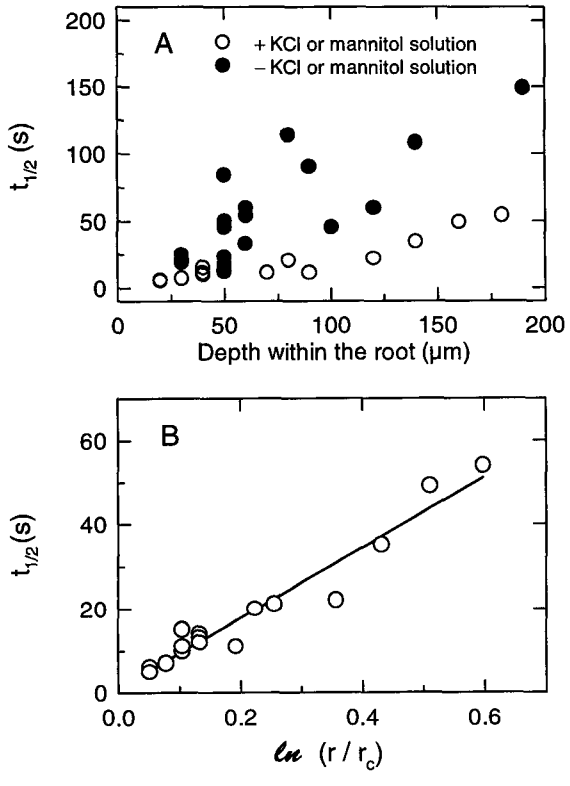

Figure 4. $t_{1/2}$ determined from the pressure kinetics following the application (○) and the withdrawal of osmotica (●). Each data point represents a kinetic that was measured either completely in a single cell or in two nearby cells. In A, $t_{1/2}$ is plotted as a function of the radial distance from the root periphery. In B, data obtained from the turgor decline (○) are shown transformed according to Equation 3, to account for the cylindrical geometry of the root. The hydraulic conductivity coefficient of the radial pathway (K) was calculated from the slope. r, Radius of the root; r_c, radius of the position of the cell at which $t_{1/2}$ was measured.

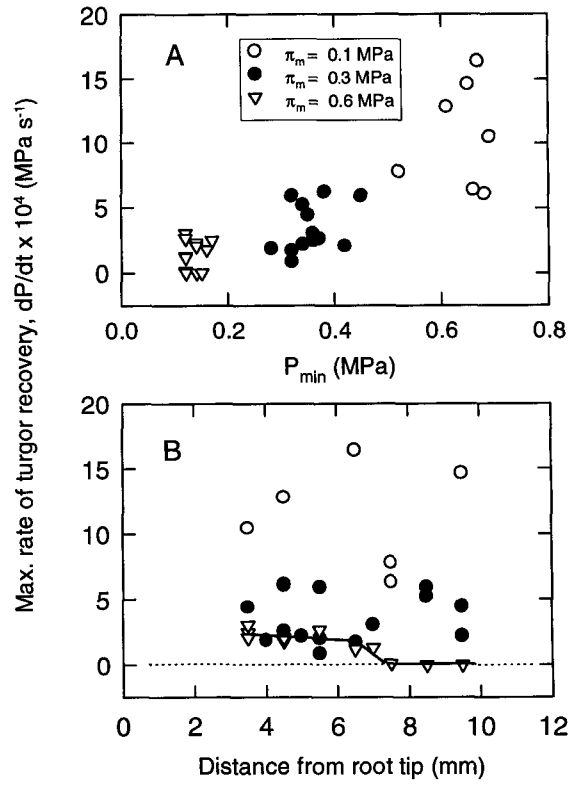

Figure 5. A, Correlation between the maximum rate of turgor recovery and P_{min} (see Fig. 1). π_m was perturbed using KCl or mannitol solutions of various strengths as indicated by the symbols. B, Turgor recovery along the elongation zone as affected by changes in π_m. The solid line indicates that turgor recovery did not occur at distances more basal than 7.5 mm behind the root tip when π_m was increased to about 0.6 MPa (▽).

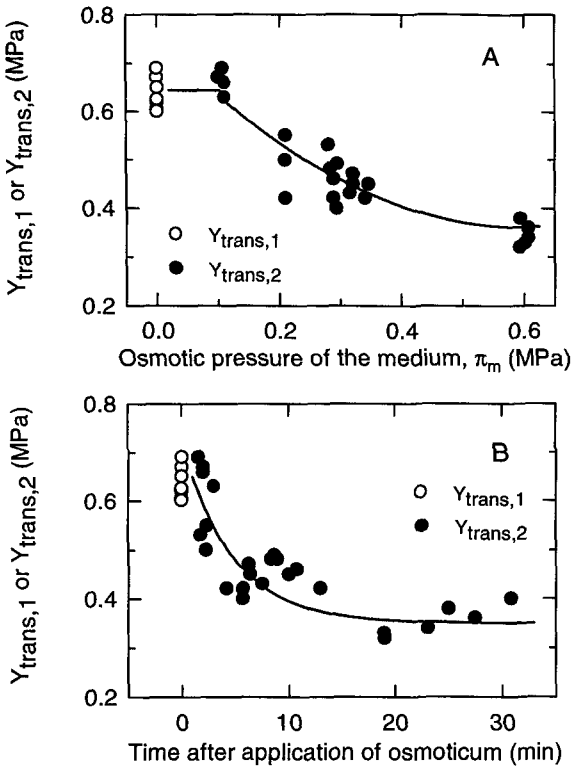

Figure 6. Response of Y to changes in Ψ. In A, Y is shown as affected by a change in π_m. In B, the same data are plotted as a function of the time between the application of test solutions (KCl, mannitol) and the actual measurement of Y. $Y_{trans,1}$ was determined as the turgor at which elongation stopped after the addition of osmoticum (π_m = 0.1, 0.3, and 0.6 MPa) to the medium and presumably reflected Y at π_m = 0 MPa. $Y_{trans,2}$ was determined as the turgor at which elongation resumed during the recovery of P. Y was determined 3 to 10 mm behind the root tip, except for treatments at π_m = 0.6 MPa, for which Y was measured 3 to 5 mm behind the tip. Some of the data for π_m = 0.3 MPa were adapted from Frensch and Hsiao (1994).

stant along the root axis (Fig. 7, C and D) and means of m varied between 2 and 3 MPa^{-1} h^{-1}. No significant change of m was observed during exposure of roots to water stress for 1 to 2 h.

DISCUSSION

The processes controlling root elongation respond to changes in π_m to compensate for a decrease in P and, consequently, to prevent a complete stoppage of elongation. Primary processes that enable the recovery of root growth include turgor maintenance through osmotic response (Hsiao and Jing, 1987; Sharp et al., 1990; Pritchard et al., 1991, 1993) and enhanced extending ability of the cell wall (Hsiao and Jing, 1987; Spollen and Sharp, 1991). A previous paper (Frensch and Hsiao, 1994) demonstrated conclusively that a rapid reduction in Y is one of the key compensatory responses. The results also suggested the phloem as the likely source of the solutes responsible for the turgor recovery.

The present study delineated the speed of the adjustment in Y and the apparent maximum adjustment of Y in roots of maize seedlings (Fig. 6). A minimum P of 0.3 MPa was found to be necessary for cell expansion under water stress. This limit corresponds well with Y determined from relaxation experiments in other organs such as excised stem segments of pea (Cosgrove, 1985; Matyssek et al., 1988) and soybean (Matyssek et al., 1988). Normal P for those tissues before excision, however, were 0.1 to 0.3 MPa lower than the P we measured in maize roots. The immediate decrease of Y following an increase in π_m (Fig. 6B) illustrates that cell expansion responds rapidly to changes of environmental conditions. Therefore, it is questionable that Y for well-watered conditions could be correctly determined from pressure relaxation over periods of up to several hours when water supply to the tissue was cut off (Cosgrove, 1985, 1987). The technique assumes constant cell wall properties during the pressure relaxation and takes the lower limit of the relaxation to be the original Y. Our data on maize roots indicate that pressure relaxation determines an already adjusted value of Y that would be equivalent to our minimum Y. Based on less direct evidence, Matyssek et al. (1988) reached a similar conclusion for soybean stems, in which the tissue excised for the relaxation experiment included sufficient mature cells to serve as a water reservoir to slow the development of water deficits during a number of hours. They also concluded that $(P - Y)$ is small, in agreement with our results, and that the "true Y" can be determined by pressure relaxation if no mature tissue is included to allow a fast progression of water deficits. Our finding that Y declined detectably within minutes after stress was imposed to stop growth (Fig. 6B), however, would assert that the pressure relaxation technique determines a Y always lower than that in the intact plant.

Monitoring Y with sufficient time resolution is important to calculate the $(P - Y)$ accurately, which is a prerequisite for the analysis of limitations of growth. For example, if Y is small and $(P - Y)$ is large, the resulting small volumetric extensibility m would be a major factor determining growth (see Eq. 1). In the present study, $(P - Y)$ was shown conclusively to be small, giving rise to high values of m (Fig. 7, C and D) in comparison to that reported in much of the literature (Cosgrove, 1985, 1986; Pritchard et al., 1990b).

Although the parameters of Lockhart's equation serve as a framework in this study, the drawbacks of the equation are recognized. In addition to the fact that the rapid adjustment in Y necessitates the repeated evaluation of Y under changing conditions, there is also a lack of correspondence between growth and P in some situations (Shackel et al., 1987; Serpe and Matthews, 1992; Zhu and Boyer, 1992). Indications are, however, that this lack of correspondence is confined more to conditions in which water is ample and P is very high and, thus, growth is probably limited by other factors. It is hoped that data such as those reported here will lead to better models of expansive growth that are as readily amenable to experimental study as the equation of Lockhart.

The determination of Y from transient changes of growth and P may be questioned on the basis of possible interference by elastic (reversible) length changes of the root (Cosgrove, 1986). When P is stepped down, an elastic shrinkage

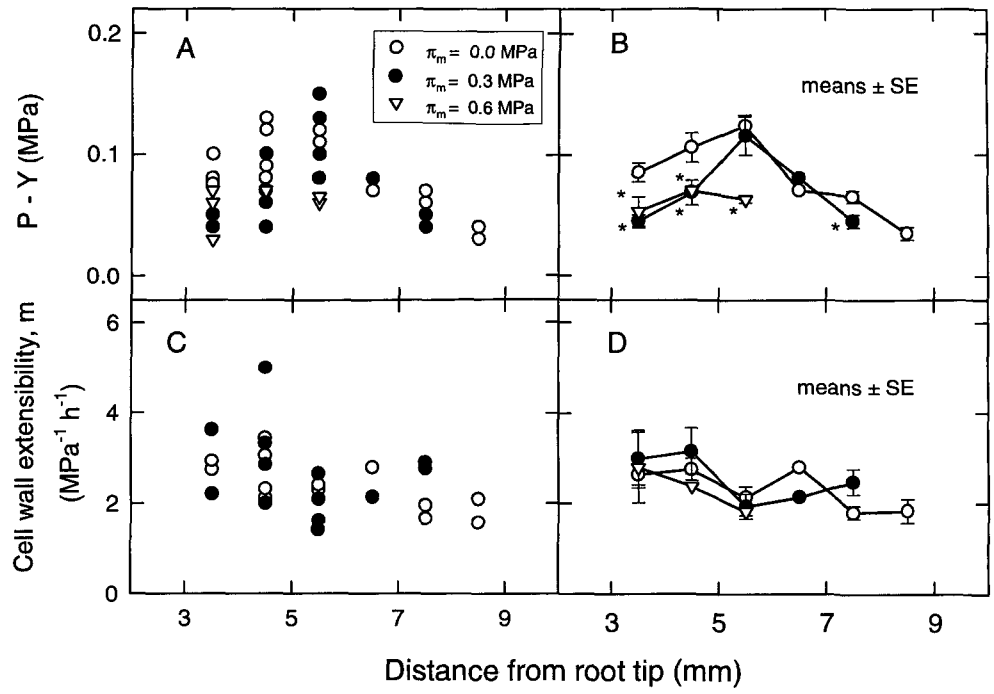

Figure 7. Spatial distribution of $(P - Y)$ (A and B) and m (C and D) along the root axis. $(P - Y)$ was calculated as the difference between the steady-state P before the addition of test solutions and $Y_{trans,1}$ for π_m = 0 MPa and as the difference of the near steady-state P at the end of the biphasic pressure response and $Y_{trans,2}$ for π_m = 0.3 MPa and π_m = 0.6 MPa. m was calculated according to Equation 1 and LRER of Figure 3. Data points in A and C represent individual experiments; data points in B and D represent means ± SE. Asterisks indicate significant differences (P = 0.05, t test) between osmotic treatments and the untreated controls at π_m = 0 MPa.

in length could mask the irreversible plastic extension defined as growth, leading to apparently but erroneously high values of $Y_{trans,1}$. This possibility cannot be ruled out unequivocally, although we believe the resulting error should be almost negligible for the following reasons. First, $Y_{trans,2}$ should be largely free from errors due to elastic changes because it is determined after substantial time has elapsed since the increase in π_m. Elasticity can be delayed as has been observed in frozen and thawed maize coleoptiles using unidirectionally applied force of large magnitudes (Hohl and Schopfer, 1992). There is, however, no indication of significant retarded elastic behavior in our living roots. Second, in our roots there were no detectable elastic length changes in the mature part (basal to apical 12–15 mm) when π_m was suddenly altered by approximately 0.3 MPa (Frensch and Hsiao, 1994). Last, in principle and as shown by experimental evidence, $Y_{trans,1}$ values obtained in this study could be affected only minimally by elastic changes. Bulk modulus of elasticity of tissues is known to be highest at the highest P and to decline with decreasing P (Steudle et al., 1977). Therefore, elastic shrinkage per unit of reduction in P would be very slight at the beginning of the P downstep when P is the highest but would increase as P decreases lower and lower. Since $Y_{trans,1}$ was determined in this study only when P was near its maximal value, complication due to elastic changes should have been slight. That conclusion is borne out by the similarity in value between the experimentally determined $Y_{trans,1}$ and $Y_{trans,2}$ when π_m was reduced by approximately 0.1 MPa (Figs. 1 and 6A). Because $Y_{trans,2}$ is more free from complication due to elastic changes, $Y_{trans,2}$ should have been significantly lower than $Y_{trans,1}$ if there were substantial shrinkage during the time $Y_{trans,1}$ was determined. The lack of difference between the two Ys determined during the same osmotic step (Figs. 1 and 6) can be taken as strong evidence for the absence or minimal presence of errors in $Y_{trans,1}$ due to elastic changes.

The lack of elastic changes in length of the mature part of the root when subjected to π_m of 0.3 MPa is inconsistent with calculations based on published values of volumetric modulus of elasticity for maize root cells assuming isotropy (Frensch and Hsiao, 1994). We had concluded previously that elastic shrinkage must occur, but mostly in the radial direction of the root.

Pritchard et al. (1990b) found that wheat roots undergo considerable elastic shrinkage when exposed to π_m of approximately 0.35 MPa. Possibly, this contrasting behavior to our system is due to a difference between the two species or is the result of different growth conditions. Methanol-boiled maize roots have also been shown to be substantially elastic when subjected to unidirectionally applied force (Pritchard et al., 1993). The characteristics of killed tissue, however, may not be relevant when considering the behavior of the living system (Cosgrove, 1993), as already pointed out.

The LRERs determined on a separate set of plants were combined with the $(P - Y)$ data along the growth gradient to calculate m (Fig. 7). The results suggest that m was

constant along the root axis and did not change during the course of short-term exposure to stress. Therefore, turgor maintenance and the adjustment of Y are probably more important than m in controlling LRER and the overall ER of the root. A gradient of $(P - Y)$ was measured along the elongation zone, indicative of lower Y at positions 4 to 6 mm behind the apex (Fig. 7, A and B). Independent of π_m, the longitudinal distribution of $(P - Y)$ was closely associated with that of LRER (Fig. 3), which reflects their functional relationship. Furthermore, the data concerning wall rheology (Y, m) and LRER are consistent with the data concerning turgor recovery, which is a measure of solute accumulation in cells. The rate of turgor recovery, dP/dt, decreased with increasing water stress, which could have caused the observed smaller $(P - Y)$ with sustained high values of m. The effect of water stress on dP/dt indicates that the mechanisms regulating P could be a key process of root adaptation to water stress.

The present data and more importantly the approach used to obtain local growth parameters represent an improvement over Y and m calculated from plots of growth rate versus P for the whole growth zone. Repeating the experiment with more plant replications and measuring Y at more locations should yield results more clear-cut than those in Figure 7. On the other hand, truly local values of Y and m can only be obtained when the determination of Y is based on LRER. Measuring LRER with sufficient resolution for the evaluation of Y represents a challenge for the current technology.

For roots exposed to π_m = 0.6 MPa, P decreased well below the minimal Y of 0.3 MPa and solute accumulation was essential to establish a growth-effective turgor. At π_m = 0.6 MPa, and in contrast to treatments with smaller perturbations of π_m, turgor recovery continued in younger parts of the growth zone but ceased in the more basal parts. The lack of turgor recovery was associated with a reduction of the length of the zone of approximately 30%. Water stress caused a shortening of the elongation zone in roots of maize (Sharp et al., 1988; Pritchard et al., 1993) and wheat (Pritchard et al., 1991), both of which were associated with changes of cell wall mechanics. Combining those published results with our measurements, it appears that the early maturation of the elongation zone is triggered by a reduction of solute accumulation followed by changes of cell wall rheology. In this zone, the tightening of the cell wall corresponded with a decreased activity of the enzyme xyloglucan endotransglycosylase in one study (Pritchard et al., 1993) but not in another (Wu et al., 1994). Xyloglucan endotransglycosylase is possibly linked to the metabolism of cell wall loosening and tightening (Smith and Fry, 1991).

In addition to cell wall rheology, Boyer and co-workers (Nonami and Boyer, 1990, 1993) attributed an important limitation of cell expansion in soybean hypocotyls to low hydraulic conductance of the growing tissue. In the hypocotyl, however, water is not as readily available as it is in roots, and gradients in Ψ across the tissue could be larger compared to roots because of its larger diameter. Silk and Wagner (1980) modeled Ψ gradients for the growth zone of maize roots using a K value of 4×10^{-11} m^2 s^{-1} MPa^{-1}. According to their calculations, Ψ gradients across the cortex ranged between 0.01 and 0.1 MPa, with the highest values present in the innermost part of the root cylinder 4 mm behind the root tip. In the present study, K has been determined by a novel method, monitoring turgor kinetics of cells at various depths in the growth zone of the root. Our value of K (1.3×10^{-10} m^2 s^{-1} MPa^{-1}) is about 3 times higher than that used by Silk and Wagner (1980). Thus, Ψ gradients across the cortex may be expected to be in the order of 0.03 MPa at maximal ER. These small gradients of Ψ would be difficult to assess with current techniques.

Our results indicate that only a small $(P - Y)$ is required for cell expansion and that the root can readily reduce Y in the face of moderate water stress. Yet, values of $(P - Y)$ declined under water stress (Fig. 7, A and B) and the growth rate was only near maximum when high P was maintained. It is tempting to argue that pressure-sensitive transport processes, particularly for solutes, may be involved. Turgor-sensitive processes have been proposed for Suc transport (Oparka and Wright, 1988) in potato tubers as well as for assimilate transport across the seed coat of beans (Wolswinkel, 1990; Patrick, 1994). Apparently, pressure-sensitive regulation of symplastic solute transport through plasmodesmata (Robards and Lucas, 1990) may not explain completely the observed high ERs of roots (Bret-Harte and Silk, 1994). Alternatively, pressure-sensitive transport proteins (Kirst, 1990; Alexandre and Lassalles, 1991) could have mediated some of the radial solute transport. This alternative, however, would involve the apoplast as a pathway for solute transport, which has yet received only little attention.

Received November 4, 1994; accepted December 20, 1994.

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