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Transpiration Induces Radial Turgor Pressure Gradients in Wheat and Maize Roots[1]

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Previous studies have shown both the presence and the absence of radial turgor and osmotic pressure gradients across the cortex of roots. In this work, gradients were sought in the roots of wheat (*Triticum aestivum*) and maize (*Zea mays*) under conditions in which transpiration flux across the root was varied. This was done by altering the relative humidity above the plant, by excising the root, or by using plants in which the leaves were too young to transpire. Roots of different ages (4–65 d) were studied and radial profiles at different distances from the tip (5–30 mm) were measured. In both species, gradients of turgor and osmotic pressure (increasing inward) were found under transpiring conditions but not when transpiration was inhibited. The presence of radial turgor and osmotic pressure gradients, and the behavior of the gradient when transpiration is interrupted, indicate that active membrane transport or radial solvent drag may play an important role in the distribution of solutes across the root cortex in transpiring plants. Contrary to the conventional view, the flow of water and solutes across the symplastic pathway through the plasmodesmata cannot be inwardly directed under transpiring conditions.

Several studies during the last 10 years have failed to show turgor pressure gradients across the cortex of cereal and bean roots (Steudle and Jeschke, 1983; Jones et al., 1987; Pritchard et al., 1989, 1991; Spollen and Sharp, 1991). Recently, however, such gradients have been demonstrated for the two halophytes *Mesembryanthemum crystallinum* (Rygol and Zimmermann, 1990) and *Aster tripolium* (Zimmermann et al., 1992). All previous studies were performed on plants differing in their age of development and conditions of growth, which makes it difficult to compare the results and requires experiments to be performed on glycophytic cereals under conditions identical to those used in the study of the halophytes. This would show whether the difference in behavior was a feature of the glycophyte/halophyte distinction or whether it was due to different experimental conditions.

One such condition would be the transpiration state of the plant. Transpiration influences the water relations of the roots by altering the bulk flow rate through the xylem column linking roots and leaves. The precise relationship between transpiration and the parameters that drive volume flow in the radial direction across the root cortex remains unclear. For example, solvent-solute interactions may be significant, as suggested for the maintenance of pressure gradients in the halophytes (Zimmermann et al., 1992). Information regarding these interactions can be obtained by direct flow-velocity measurements in the xylem vessels of the root using the xylem pressure probe (Benkert et al., 1991) in combination with simultaneous turgor and π gradient measurements in the root tissue on the single-cell level. However, such measurements are difficult to perform in glycophytic cereals. If the gradients do depend on water flow through the plant, cessation of transpiration should ultimately stop or greatly diminish bulk water flow in the xylem, and thus turgor and pressure gradients would ultimately disappear. These experiments are much easier to perform than combined xylem/cell turgor pressure probe measurements.

In this paper, we report results of measurements of turgor and π across the cortex of roots of nontranspiring seedlings of wheat (*Triticum aestivum*) and maize (*Zea mays*) as well as of older plants under differing transpiration conditions (induced by 20–100% RH or by excision of the roots). The experiments show that turgor and π gradients are apparently a general phenomenon in plant roots that can be induced by transpiration.

MATERIALS AND METHODS

Plant Material

Seeds of *Triticum aestivum* and *Zea mays* were germinated in the dark on damp tissue paper in a greenhouse at 22 to 25°C for 4 d. Young seedlings were then transferred to aerated hydro-culture (Rygol and Zimmermann, 1990) and placed in a growth cabinet (12 h:12 h light:dark; light intensity 400 μmol m^{-2} s^{-1}; RH 50%:80% during the day:night regime). The culture medium was changed every 5 to 7 d.

For the experiments, a single plant was taken and the primary seminal root was carefully mounted in the perspex measuring chamber filled with growth medium (Rygol and Zimmermann, 1990). For measurements under varying humidity, the leaves were enclosed in a commercial transpiration cuvette (Miniküvettensystem, Zentraleinheit CMS 400, Firma Walz, Effeltrich, Germany) that allowed control of temperature and humidity as well as the measurement of

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Abbreviations: π, osmotic pressure; σ, reflection coefficient.

transpiration rate. To achieve 100% RH it was necessary to wrap the leaves in wet paper towels enclosed in a polythene bag. In all cases, plants were left for 1 h before measurement.

Turgor and π

Turgor pressures of individual cells were measured radially across each root cortex using the pressure probe (Hüsken et al., 1978; Pritchard et al., 1989; Rygol and Zimmermann, 1990). In contrast to the work on *Aster*, the very tip of the microcapillary of the pressure probe was filled with cell sap taken from the overlying rhizodermal cell before being inserted into the underlying cortical cells. Radial penetration of successive cells was achieved as described by Pritchard et al. (1989) or by Zimmermann et al. (1992). Cell types were identified from the directly measured depth of the tip and the known dimensions of the cells involved were determined from hand-made sections (see Fig. 1).

Extraction of sap from single cells and the measurement of its π were made as described by Zimmermann et al. (1992). The method for maize was as described by Malone et al. (1989) and Tomos et al. (1992). Both procedures produced the same results.

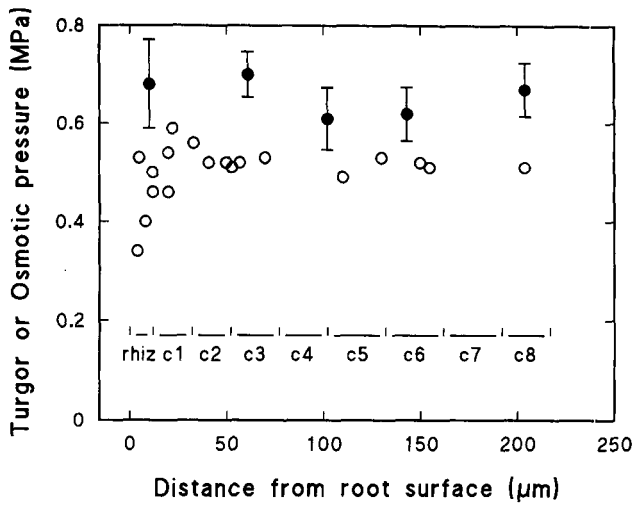

Figure 1. Turgor (O) and osmotic (●) pressure of rhizodermal and cortical cells of 4- to 7-d-old maize root measured at 7 mm from the root tip. The turgor pressure measurements are single measurements, and error bars on the π data indicate the SD values from eight independent experiments. c, Cortical cells; rhiz, rhizodermal cell.

RESULTS

Radial Turgor and Osmotic Profiles in Young, Nontranspiring Seedlings

Roots of 4- to 7-d-Old Maize

These seedlings possessed only a partially expanded first leaf in which stomatal transpiration would be expected to be negligible and cuticular transpiration was small. No turgor or π gradients were observed across the cortex at 7 mm from the tip of such roots (Fig. 1). The π was consistently higher (by some 0.2 MPa) than the corresponding turgor pressures. Turgor pressure in the rhizodermis was lower than in the cortical cells.

Maize plants have nine layers of cells between the rhizodermis and the endodermis (Jones et al., 1988). Almost the entire profile is represented in Figure 1. However, the difficulty of successfully measuring very deep cells is reflected in the diminishing number of turgor pressure data points with depth into the tissue. In most of the following experiments in maize, only the outer four to six layers were measured often enough to allow statistical analysis.

In a parallel experiment, the turgor pressure profiles were measured at 20 mm from the tip (Fig. 2A). To suppress transpiration completely, the plant was submerged in the bathing solution for a short period before and throughout the measuring period. Again, no turgor gradient was detected across the first three layers of the cortex, with a mean cell turgor pressure of 0.68 ± 0.07 MPa (n = 38). In the rhizodermis, the pressure was lower (0.34 ± 0.12 MPa [n = 15]).

Roots of 4- to 6-d-Old Wheat

Seedlings in which the leaf had not yet emerged from the coleoptile were totally immersed in the bathing solution prior to measurement. As with the maize, no gradient in turgor pressure was observed between the first and the fourth cortical cell (Fig. 2B) at 20 mm from the tip (average 0.47 ± 0.10 MPa [n = 100]). In contrast to maize, wheat roots have only four to five layers of cells between the rhizodermis and the endodermis (Jones et al., 1988). The rhizodermal cell turgor pressure was lower at 0.33 ± 0.07 MPa (n = 13).

Radial Turgor Profiles in Older Plants

In older plants, transpiration has a significant role in water relations. These plants were studied under both transpiring and nontranspiring conditions; the pressure profiles were measured 5 or 20 to 30 mm from the root tip. (At 5 mm, the cells are still actively expanding.)

Roots of 11- to 13-d-Old Transpiring Maize

As shown in Figure 3, gradients in turgor pressure were measured at 5 mm from the tip of roots at both 20 and 60% RH (corresponding to transpiration rates of 0.58 and 0.15 mmol m^{-2} leaf s^{-1} on a leaf area basis, respectively). At 20% RH, turgor increased from 0.16 MPa in the cells of the first cortical layer to about 0.45 MPa in cells of the fourth layer. At 60% RH, the same gradient of turgor pressure was observed despite a reduction in the transpiration rate by a factor of 4 (Fig. 3).

Qualitatively similar behavior was observed at 20 to 30 mm from the tip for plants at 20, 50, and 60% RH (Fig. 4). For plants at 50% RH, π was also measured and a gradient was again found (Fig. 4). π rose from 0.65 MPa in the first cortical layer to 0.95 MPa in the fourth. As noted above for nontranspiring tissue (Fig. 1), π was significantly higher than the corresponding turgor pressures. Here the difference is

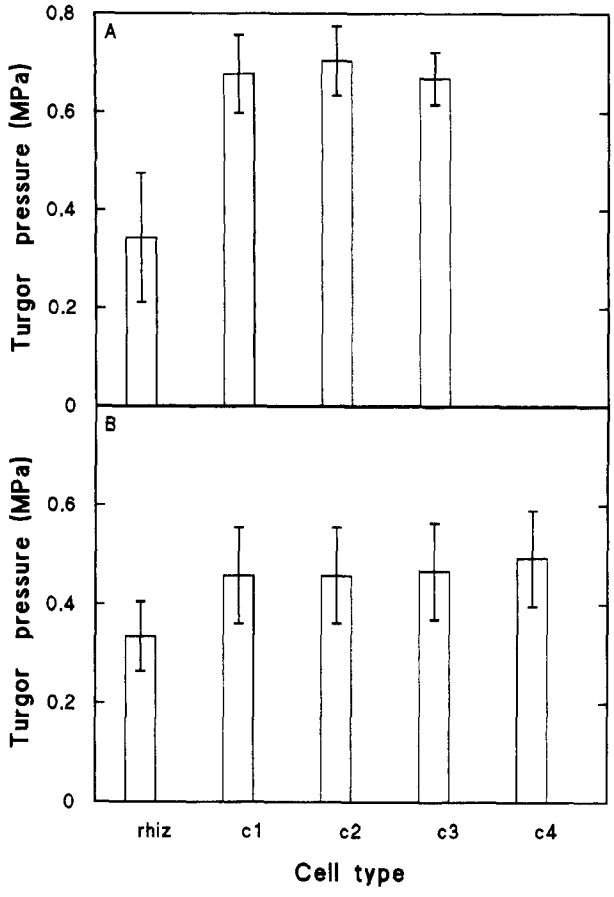

Figure 2. Turgor pressure profiles across the rhizodermis and cortex of young maize and wheat roots. A, Four- to 7-d-old maize seedlings. B, Four- to 6-d-old wheat seedlings. Error bars indicate the SD values from 5 to 29 independent measurements performed 20 mm from the root tip.

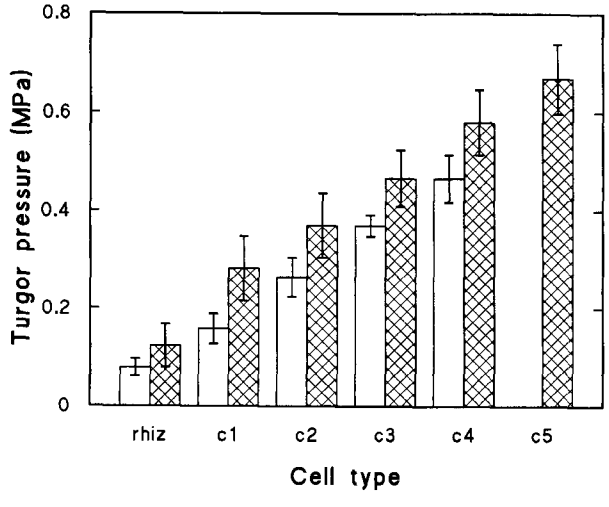

Figure 3. Turgor pressure gradients across the root cortex of 11- to 13-d-old transpiring maize 5 mm from the root tip. Twenty percent RH (open columns); 60% RH (cross-hatched columns). Error bars indicate the SD values of 15 to 21 independent experiments.

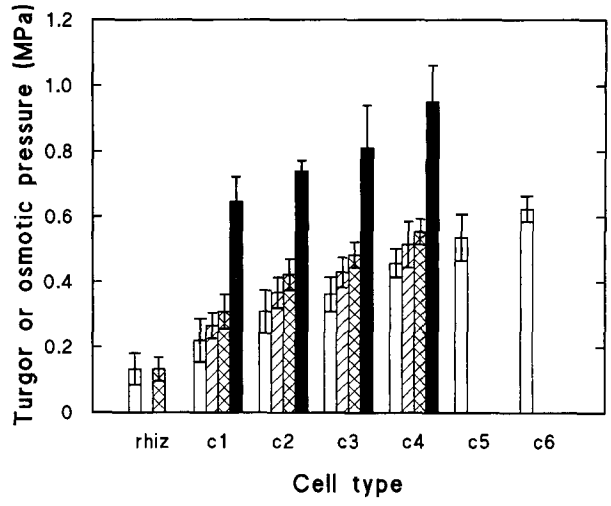

Figure 4. Turgor and π gradients across the root cortex of 11- to 13-d-old transpiring maize at 20 to 30 mm from the root tip. Turgor pressure at 20% RH (open columns), at 50% RH (hatched columns), and at 60% RH (cross-hatched columns). π at 50% RH (black columns). Error bars indicate the SD values of 3 to 16 independent experiments.

approximately 0.4 MPa (i.e. twice that observed in the non-transpiring seedlings).

Roots of 65-d-Old Transpiring Maize

At 65 d, the gradients persisted at 20 and 60% RH at both 5 mm and 20 to 30 mm from the tip (data not shown).

Roots of 26- to 33-d-Old Wheat Plants

At 20% RH, the turgor pressure was measured across the cortex at 30 mm from the root tip (Fig. 5). There was a marked gradient in pressure from the cells of the first cortical layer (0.16 ± 0.06 MPa [n = 18]) to the fourth (0.50 ± 0.08 MPa [n = 22]). Quantitatively similar results were obtained when the RH within the cuvette was raised to 60% (Fig. 5). Continuous monitoring of the transpiration rate throughout the experiment indicated that there was a water flow through the plant roughly similar to that observed in 11- to 13-d-old maize.

Radial Turgor Profiles in Roots of Older, Nontranspiring Plants

To distinguish the effects of age from those of transpiration, turgor profiles were measured in older plants (in which gradients had been found at lower RH values) under conditions of 100% RH.

Roots of 11- to 13-d-Old Nontranspiring Maize

The 11- to 13-d-old maize plants were covered in wet paper and enclosed in a plastic bag. The turgor pressures in all four measured cortical cell layers were very similar to the mean value of 0.60 ± 0.05 MPa (n = 35) (Fig. 6A). Again, the rhizodermal cells showed significantly lower turgor pres-

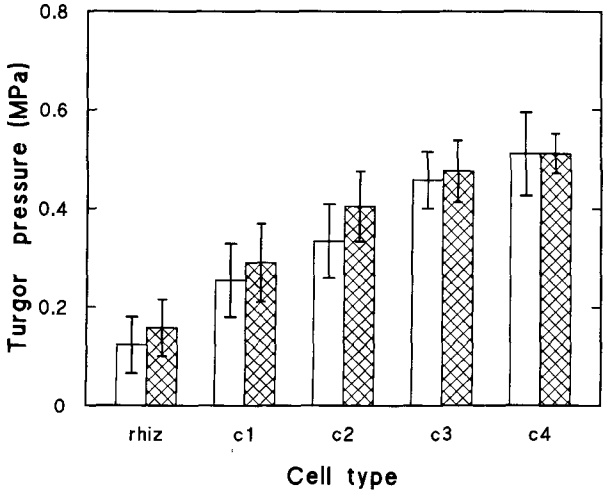

Figure 5. Turgor pressure gradients across the root cortex of 26- to 33-d-old transpiring wheat 30 mm from the root tip. (Symbols have the same meaning as in Figs. 3 and 4.) Twenty percent RH (open columns); 60% RH (cross-hatched columns). Error bars indicate the SD values of 2 to 32 independent experiments.

sure by a factor of 2 (0.28 ± 0.07 MPa [n = 16]). The π measurements indicated a difference of about 0.2 MPa between the first and fourth cortical layers (Fig. 6A); however, the scatter in the data makes it difficult to identify a continuous gradient across the cortex. Despite this scatter, the π in the cells of all layers remained significantly higher than the corresponding turgor pressures.

Roots of 25- to 28-d-Old Nontranspiring Wheat Plants

Similarly, the transpiration rate of 25- to 28-d-old plants was suppressed by enclosing the leaves in wet paper in a polythene bag (Fig. 6B). No significant increase in turgor was observed from the first cortical cell to the fourth, with an average pressure of 0.56 ± 0.09 MPa (n = 91).

Radial Turgor Gradients in Excised Roots

A third approach used to prevent transpiration-driven water flow through the roots was to excise them from the leaf system (Zimmermann et al., 1992). Data were obtained either with the pressure probe inserted before excision and left in the same cell throughout the experiment or by single measurements after a new steady state was reached (after 30–120 min). Both procedures gave similar results.

Figure 7 shows the kinetics of the change in turgor pressure of individual wheat cells in cortical layers 1 and 4 (30 mm from the tip) following excision. The turgor pressures of cells in layer 1 rose while those in layer 4 dropped. The final steady-state turgor pressure of cells in both layers appeared to reach a similar intermediate value.

For maize, the π profiles (at 30 mm from the tip) were measured in addition to turgor pressure after reaching steady state following excision (Fig. 8). Excision of maize roots resulted in the loss of the turgor and π gradient. As in wheat (Fig. 7), the turgor pressure in maize cells of cortical layer 1

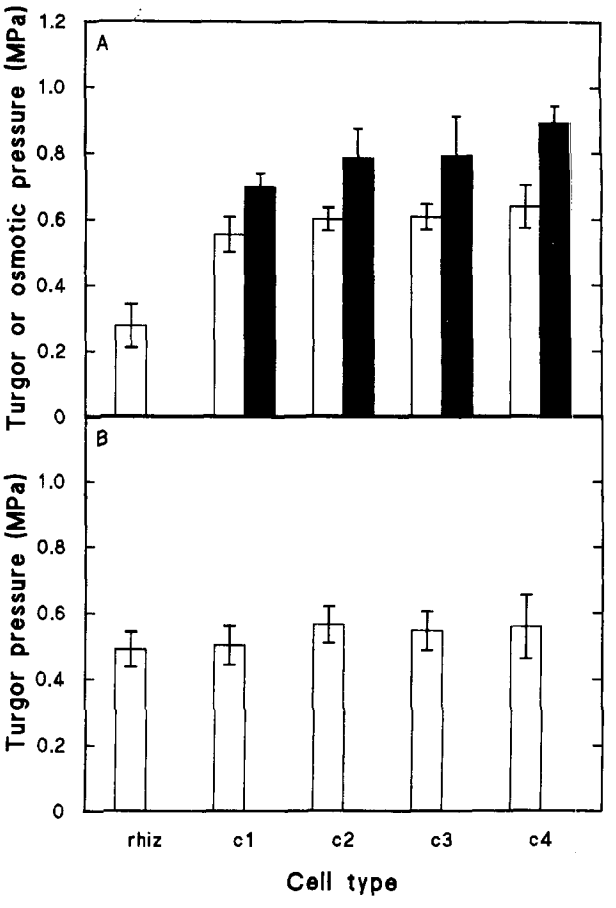

Figure 6. Turgor and π across the root cortex of nontranspiring maize and wheat plants at 30 mm from the root tip at 100% RH. A, Eleven- to 13-d-old maize. B, Twenty-five- to 28-d-old wheat. Turgor pressure, open column; π, black column. Error bars indicate the SD values of 6 to 25 independent experiments.

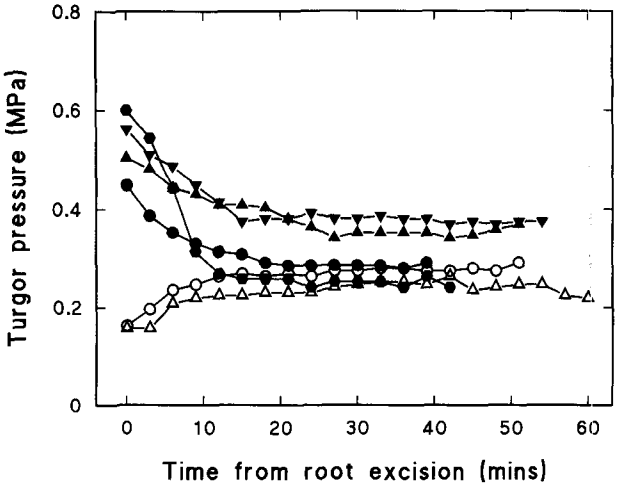

Figure 7. Time course of turgor pressure from 28- to 32-d-old wheat plants following excision of the root. Each trace represents data from a single cell of either cortical layer 1 (○, △) or 4 (●, ▲, ▼, and ●). Prior to excision, the leaves were in 20 to 40% RH.

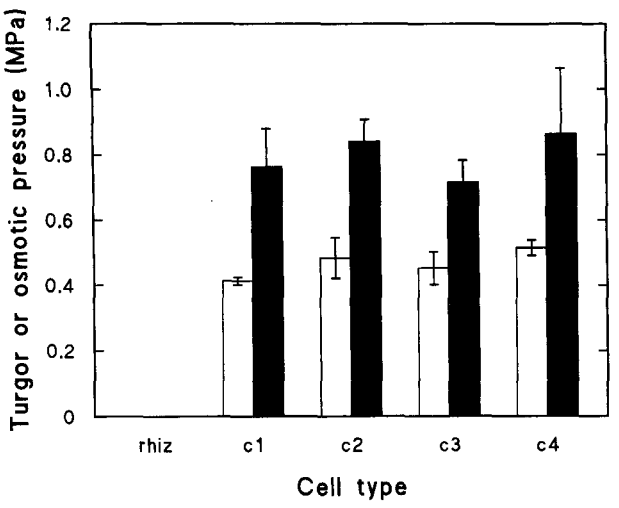

Figure 8. Turgor and π profiles across the cortex at 30 mm from the root tip of 11- to 13-d-old maize plants, 30 to 120 min after excision. Error bars indicate the SD values of 3 to 13 independent experiments.

increased (compare Figs. 4 and 8) by about 0.2 MPa and the π also increased. In apparent contrast to wheat, the turgor pressures of cortical layer 4, however, did not change significantly; the π also appeared to be unchanged.

DISCUSSION

Turgor Pressure Gradients

The distinct gradients in turgor pressure described in this work are in contrast to previous descriptions of root cortices of the same species (Steudle and Jeschke, 1983; Jones et al., 1987; Pritchard et al., 1989; Spollen and Sharp, 1991) and to other detailed data presented by Steudle's group (e.g. Zhu and Steudle, 1991). In none of these previous studies was a gradient detected. Similarly, in the present work turgor gradients were not found under conditions of 100% RH, nor following excision, nor in plants without mature leaves. The common feature of each of these would appear to be a lack of transpiration flow across the root cortex. In the reports of Steudle and Jeschke (1983) and Zhu and Steudle (1991), excised tissue was used, whereas in the work of Jones et al. (1987), Pritchard et al. (1989), and Spollen and Sharp (1991), it could be argued retrospectively that the seedlings used were too young to display transpiration (7–10, 6, and 4 d, respectively) or that transpiration was low under the measuring conditions (Spollen and Sharp, 1991).

Recent results on the halophytes *M. crystallinum* (Rygol and Zimmermann, 1990) and *A. tripolium* (Zimmermann et al., 1992) did describe turgor pressures increasing at depth into the root. The information presented here demonstrates that such gradients are not a feature of halophytes alone but are shared by at least two glycophytes (maize and wheat). The arrangement of cortical cells in radial strings as observed in *Aster* and *Mesembryanthemum* seems, therefore, not to be essential for the establishment of the gradient.

In light of the data presented here, we find that the gradients are induced by transpiration and, therefore, appear to depend upon bulk flow across the root cortex. Since there was no significant difference between the magnitude of the gradient between 20 and 60% RH (and a 4-fold decrease in transpiration rate), the relationship between the two is clearly nonlinear, which has also been shown for tobacco stems by Benkert et al. (1991).

Similar gradients were observed at both 5 and 20 to 30 mm from the tip. This observation might appear to be inconsistent with reduced transpiration-driven flow across the tissue close to the tip due to hydraulic isolation of this tissue; however, as we have shown, the magnitude of the gradient is not directly proportional to the transpirational flow rate, and some xylem continuity would appear to extend to within 8 mm of the tip (Steudle and Frensch, 1989).

Turgor and π under Differing Conditions

As in *Aster*, gradients of π were measured wherever a turgor pressure gradient was found. However, in contrast to the halophyte, the π of each cell measured was always found to be considerably higher than the turgor pressure, which indicates one or more of the following conditions. (a) The π of the apoplastic space has a value of up to the difference between turgor and intracellular π. (b) The σ of the cell membranes is considerably less than unity. (c) The hydrostatic pressure of the apoplast/xylem is less than atmospheric pressure by a value of up to the difference.

Based on the observations on maize cortex, we can make some speculations regarding this characteristic. Excision has been assumed to reduce the bulk of water flow across the cortex, but following this treatment it is unlikely that a hydrostatic pressure difference could be maintained between the root medium and the apoplast of the cortex (and the xylem), and the apoplast will approach atmospheric pressure. Upon excision of the root, the difference between turgor and intracellular π changed from an average of 0.4 to an average of 0.33 MPa (data calculated from Figs. 4 and 8). This implies that the effective apoplast π was of the order of 0.33 MPa and that the original hydrostatic tension in the apoplast was no greater than about 0.07 MPa. This corresponds to an absolute pressure in the apoplast of about +0.03 MPa, a value that agrees well with average xylem pressures measured directly with the xylem probe in tobacco (Balling and Zimmermann, 1990; Benkert et al., 1991), maize, and other plants (U. Zimmermann, unpublished data).

The analogous analysis of the data for maize plants in which transpiration was prevented by 100% RH appears to lead to a similar conclusion. However, in this case the average difference between turgor and π is 0.2 MPa (data calculated from Fig. 6A) compared with 0.4 MPa at 50% RH, i.e. a change of 0.2 MPa. This may imply an absolute xylem pressure of −0.1 MPa, again not inconsistent with the range of xylem probe data (Balling and Zimmermann, 1990; Benkert et al., 1991). Alternatively, the effective apoplast π is decreased from 0.33 to 0.2 MPa due to dilution or removal of solutes by transport processes, a process that does not appear to occur in the excised root. A possible mechanism for this is an outwardly directed apoplasmic flow of water and solutes under conditions in which xylem pressure exceeds that of the

atmosphere (root pressure), as recently discussed by Steudle (1993). If this were the case, it would indicate that the π of the apoplast represents a steady state rather than an equilibrium, which suggests that the origin of the high π (0.33 MPa) in the apoplast of transpiring roots is due to ultrafiltration of the root medium (π approximately 0.03 MPa) at the endodermis or stele.

Cause of the Pressure Gradients across the Cortex

If, for the moment, we neglect electrical coupling, there are three components that influence the net flow of solutes across the cortex: a passive diffusion component, an active component, and a component arising from the coupling between water and solute flow (see Zimmermann et al., 1992, for refs.). For two compartments separated by a barrier, these are linked according to the following equation:

$$J_s = \omega\Delta\pi + J_a + (1 - \sigma)c_sJ_v$$

where J_s is net solute flow; ω is thermodynamic diffusion permeability; $\Delta\pi$ is π difference across the barrier; J_a is active component; c_s is average concentration of the compartments; and J_v is net water (volume) flow. According to this equation, a stationary gradient in π (and therefore in turgor pressure) can be maintained only in the presence of active transport and/or solvent drag.

There are various ways in which these processes can be arranged. For example, we can propose two (not mutually exclusive) spatial arrangements. In the first, the flows J_v and J_s are inwardly directed across the cell-to-cell pathway of the cortex. This leads to a build up of solutes in the inner cells of the cortex and an outwardly directed diffusive component ($\omega\Delta\pi$). A stable steady-state gradient will be set up due to solvent drag. (J_a may also be radially directed at the tangential membranes.) This cell-to-cell pathway generally has been discounted in the past on the basis that such a pathway is energetically expensive. Such calculations ignore the solvent drag component ($[1 - \sigma]c_sJ_v$). However, we must admit that at present we cannot propose a mechanism for such a thermodynamically derived solution in which the membrane σ plays the key role. However, the solution may lie in the properties of the membrane channel proteins.

Wayne and Tazawa (1990) have described the characteristics of a channel in the giant-celled alga *Nitellopsis* that would appear to conduct both K^+ ions and water. Such a property could well provide a mechanism for a value of σ of less than unity. Wayne and Tazawa (1990) suggest that this channel plays a dynamic role in regulating both ion and water transport.

In the second arrangement, the solute gradient observed within the cortical cells is set up by active transport of solutes from the apoplast into the innermost cortical cells. The π of these cells increases, resulting in an increased turgor pressure and back flow of ions in an outward direction across the cortex (the pathway will be discussed below). This gradient must also be stabilized at the rhizodermis by export of solutes into the external medium. In this arrangement, inward solute and water flow across the cortex will be in the apoplast and driven by the pressure step between the exterior and the xylem. This arrangement would explain the insensitivity of the gradients observed (between 20 and 60% RH) to the absolute transpiration rate, since the gradient is only indirectly dependent upon this. However, it would conflict with reports that show that active uptake of solutes occurs at the epidermal plasma membrane. Such reports range from the demonstration of electrogenic pumps (Dunlop and Bowling, 1971) to the immunolocalization of plasmamembrane H^+-ATPase in the epidermis (Parets-Soler et al., 1990). As pointed out by Drew (1987) and Cortes (1992), however, such observations do not rule out an additional role for solute uptake across the plasmamembrane deep within the apoplast.

Some information regarding these proposals can be obtained from the behavior of the root following inhibition of transpiration. A feature of the gradient in *Aster* and wheat is that upon collapse of the gradient, the turgor pressure (and π) of all cells approached the mean value of the original gradient across the entire cortex. The data for maize roots would appear to indicate a different behavior. The cells in the outermost layers assumed the value of that of the innermost layers measured (cortical layer 4). However, the observations are, in fact, not inconsistent with those for *Aster* and wheat, since we can expect the gradient to extend over the entire nine layers of the cortex, only four of which are represented. The average of this would then be equivalent to layer 4 as observed.

For the solvent-drag arrangement, this behavior would be due to the cessation of J_v and the dissipation of the gradient due to the diffusive term within the cortex. In this case, the total collapse of the gradient would show that any active component (J_a) between the cells is negligible. (This statement does not include the outer rhizodermal or innermost cortical cell membranes.)

For the arrangement that is dependent on active transport from the apoplast, the basis would be different. It is envisioned that the collapse of the gradient is due to the cessation of bulk flow in the apoplast either diminishing (or stopping) the supply of solutes from the medium to the site of uptake or having a direct effect on the active solute transport system at the inner cortical cells. (Such a process would be related to pressure-dependent transport processes as described elsewhere [e.g. Zimmermann and Steudle, 1978; Wyse et al., 1986].) Indeed, the observed behavior of the system would require both the active transport steps at the inner cortex and at the rhizodermis to respond to the flow rate. Inhibition of the flow rate must decrease both rates in parallel.

We emphasize that these processes are not mutually exclusive and may well occur simultaneously. Additionally, as mentioned above, electrical effects need to be considered. In principle, it is certainly possible to set up a solute (and hence turgor pressure) gradient using electrical forces generated by electrogenic membrane pumps, and gradients of membrane potential across the cortex of roots have recently been invoked in a published model (Cortes, 1992). The only published results of cortical electrical profiles clearly show the absence of any such gradients (Bowling, 1972; Dunlop, 1973), but were measured on excised tissue and, therefore, are not necessarily applicable here. However, it is difficult to envision a reasonable arrangement in which gradients of both anions and cations (as well as of nonelectrolytes) could be stabilized against concentration and pressure gradients.

A third possible basis for the π gradient, efflux of a solute from the stele that is either exported from the epidermis or metabolized in the outer cortex, appears to be discounted by the following observations. (a) Following excision, it would be expected that the export or metabolic "sink" behavior would continue and the gradient would collapse to the lowest pressure values. (b) It is not clear why such a system should be associated with transpiration.

Implications for Radial Symplasmic Flow

Regardless of the mechanism of their formation, the presence of the turgor and π gradients across the cortex has implications for our understanding of the role of plasmodesmata and symplastic transport in roots. According to the conventional view (e.g. Salisbury and Ross, 1985; Drew, 1987; Cortes, 1992), the symplastic pathway through the plasmodesmata plays a role in the movement of solutes across the cortex of roots. Most workers assume that solutes from the rhizosphere enter the symplast across a single membrane. (The site of absorbance may be at any point within the apoplast up to the endodermis, although the outermost cells are favored [Drew, 1987].) Movement through the plasmodesmata must therefore be from outside to inside (centripetal). For this to be true, solutes must pass each plasmodesma driven either down a concentration gradient or by (centripetal) pressure-driven bulk flow. It was implicitly assumed that either a pressure and/or a concentration step exists at each plasmodesma to drive this flow. The data presented in this paper and in the previous reports on *Aster* and *Mesembryanthemum* show explicitly that the required pressure gradients not only do not occur, but are actually directed against such bulk flow. The conventional view of centripetal solute flow through the symplast of the root cortex driven by hydrostatic pressure (e.g. Cortes, 1992) cannot be correct under transpiring conditions.

We also need to ask whether the required concentration steps occur. Only small steps in concentration would be required across each plasmodesma for diffusion rate to overcome pressure-driven bulk flow. Within each individual cytoplasm, active streaming could transport the solutes faster over the longer distances involved. Since no significant pressure gradient can occur across the tonoplast and since its high hydraulic conductivity will keep the activity of water similar on either side, the bulk π gradients within the sequential cytoplasms across the cortex must mirror those measured here in the vacuoles. Again, these are directed opposite to those required for bulk inward diffusion, but this does not rule out inwardly directed concentration steps of some individual solutes (such as K^+, NO_3^-, etc). Preliminary data (not shown) indicate that, at least under nontranspiring conditions, no measurable gradients in K^+, Na^+, Cl^-, Ca^{2+}, P, or S occur across the root vacuoles, but this does not rule them out from the sequential cytoplasms. If inwardly directed gradients of some solutes do occur in the cytoplasm, however, the π gradient in the cytoplasm must be made up by a solute, emanating from the stele, that is either lost into the rhizosphere or is metabolically removed within the cortex. Stationary gradients of this solute on the order of 120 to 160 mM (equivalent to 0.3–0.4 MPa π) would be required across the outer five cell layers in maize roots (Fig. 4). Since we know that the gradient can collapse within 20 min, this would require a considerable sink for the material. Although organic solutes, such as sugars and amino acids, might behave in this way in expanding tissue, it is unlikely in mature tissue.

It would appear, then, that the bulk symplasmic flow will be centrifugal (i.e. outwardly directed) unless the plasmodesmata are closed. Therefore, there must exist opposing flows of both water and solutes. The plasmodesmatal path would appear to be a likely pathway for the outward movement of solutes when the gradients collapse because of cessation of transpiration. However, the location of the inward pathway under transpiring conditions remains unclear. As noted above, it can be either via the cell-to-cell pathway driven by solvent drag or through the apoplast and into the innermost cortex by flow (and/or) pressure-dependent active uptake.

The situation under nontranspiring conditions, as well as following excision, may well follow the conventional pathway, although it is worth noting that many, if not most, of the data on membrane transport would appear to have been obtained on excised tissue.

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