Water Transport Properties of Cortical Cells in Roots of Nitrogen- and Phosphorus-Deficient Cotton Seedlings

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ABSTRACT

Growth-limiting deficiencies of N or P substantially decrease the hydraulic conductance of cotton (Gossypium hirsutum L.) roots. This shift could result from decreased hydraulic conductivity of cells in the radial flow pathway. A pressure microprobe was used to study water relations of cortical cells in roots of cotton seedlings stressed for N or P. During 10 days of seedling growth on a complete nutrient solution, root cell turgor was stable at 0.4 to 0.5 megapascal, the volumetric elastic modulus increased slowly from 5 to 10 megapascals, and the half-time for water exchange increased from 10 to 15 seconds. In seedlings transferred to N-free solution for 10 days, final values for each of those parameters were approximately doubled. Root cell hydraulic conductivity (cell $L_p$) was $1.4 \times 10^{-7}$ meters per second per megapascal at the time of transfer. In the well-nourished controls, cell $L_p$ decreased over 10 days to 38% of the initial value, but in the N-stressed plants it decreased much more sharply, reaching 6% of the initial value after 10 days. Transfer to solutions without P or with an intermediate level of N also decreased cell $L_p$. The changes in root cell $L_p$ were consistent with nutrient effects on intact-root water relations demonstrated earlier. However, cell $L_p$ was about half that of the intact root, implying that substantial water flow may follow an apoplastic pathway, bypassing the cortical cells from which these values were derived.

In recent years evidence has accumulated that nutrient stresses (N and P deficiencies in particular) decrease the hydraulic conductance of intact plants or excised root systems (2, 9, 11–13, 16, 22). The ensuing change in plant water relations can limit leaf growth rates by increasing the water deficit of the expanding leaf blades (11, 12). A developmental study showed that root conductance per unit length changed very early in the progression of nutrient deficiency, before there were any effects on overall root system length or morphology (12). The reasons for this change in root internal properties have not been defined. Root conductance could decline from increased cell numbers or decreased cell $L_p$ in the pathway of water movement, or from structural changes in the root. Here we report the $L_p$ and other water relations properties of cells in the root cortex of nutrient-stressed cotton seedlings.

MATERIALS AND METHODS

Plant Growth

Seeds of cotton (Gossypium hirsutum L. cv Deltapine 70) were germinated in moist vermiculite for 3 d, and the seedlings were then transferred to aerated liquid nutrient solution. The full nutrient solution was a modified half-strength Hoagland solution containing 5 mM N as nitrate and 0.5 mM P as phosphate (14). Nutrient deficiencies were established by substituting chloride salts for the nitrate or phosphate salts (−N and −P solutions, respectively). Plants were also grown on an intermediate-N solution containing 0.2 mM nitrate and 4.8 mM chloride. Day/night growth conditions were as follows: temperature 30/21 ± 2°C, RH 60/90 ± 5%; 550 μmol·m⁻²·s⁻¹ PAR from cool-white fluorescent lamps; and 14-h day-length.

The cotyledons began to unfold immediately after transfer of the plants to nutrient solutions. Area of the larger cotyledon on each seedling was estimated as length × width × 0.86. This coefficient, determined from numerous tracings of cotyledons on paper of known weight per unit area, had a 95% confidence interval of ±0.01. Hourly expansion rates during daytime and at night were calculated by measuring areas at the beginning and end of the light periods over a 48-h interval (3–5 d after transfer). Growth was assumed to be exponential during the period between each pair of sequential measurements.

Cell Size

Roots of seedlings were sectioned by hand with a razor blade approximately 5 cm from the tip, and the cross-sections were mounted on a microscope slide and stained with methylene blue. Diameters of cortical cells were measured with an ocular micrometer under a microscope. Longitudinal free-hand sections were treated similarly to derive cell lengths. All measurements of cell size were made on the taproot. Data from numerous plants were combined because within-plant and between-plant variabilities were equal.

Turgor

Plants in nutrient solution were taken from the growth room to the laboratory and incubated for 20 to 30 min to allow equilibration with their new environment (room temperature, dim light). Intact seedlings were removed from the nutrient solution and their roots were placed on filter paper.

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3 Abbreviations: $L_p$, hydraulic conductivity; $\epsilon$, volumetric modulus of elasticity; $t_w$, half-time for water exchange; $\Psi_w$, water potential; $\Psi_o$, osmotic potential; $\Psi_p$, pressure potential; $\sigma$, reflection coefficient; $\pi^*$, effective external osmotic pressure.
moistened with the same solution. The roots were then covered with another piece of moistened filter paper except for a segment about 1 cm in length located about 5 cm from the tip of the taproot. The entire assembly was supported on a flat platform mounted on a micromanipulator. The exposed segment was aligned with the microcapillary tip of the pressure probe, and the tip was inserted into the cortex of the root. Tip preparation and other procedures were as described by Shackel et al. (17). Because measurements were being made on interior cells, it was normally not possible to view the cell being penetrated. When a cell was penetrated, the pressure was adjusted until the meniscus was just external to the surface of the root, and it was observed for stability (maintenance of constant position for 2 min). Readings from many penetrated cells were unstable, especially in −P roots. Pressure readings were recorded only from those that exhibited stability, indicating the absence of leaks. The pressure was corrected for the average displacement of the meniscus from the impaled cell surface, using Eq. 1 (after determination of $\varepsilon$, see below). The magnitude of the correction varied with $\varepsilon$ from 0.1 to 0.4 MPa. At least three independent measurements of turgor were obtained from cells exhibiting stable readings within the exposed 1-cm segment of each root and on up to 7 plants at any particular age. Readings were combined because within-plant and between-plant variabilities were equal.

**Elastic Modulus**

The volumetric elastic modulus was determined from the relationship

$$
e = V \cdot \Delta \Psi / \Delta V$$

(1)

in which $V$ is mean cell volume (5). Procedures were similar to those described earlier (7, 23). The cell was subjected to a step change in pressure of 0.01 to 0.07 MPa and the resulting change in volume was noted. After a perturbation, volume changed very rapidly for 1 to 2 s, then much more slowly. Returning the pressure to its original value directly after the rapid phase of the response resulted in a return of the meniscus to approximately its original position, indicating that little water had been taken up or lost by the cell. Accordingly, the rapid phase was taken to be due to the elastic properties of the cell wall. Correction for water transport during the perturbation was deemed unnecessary because the readings were obtained in a much shorter interval than the $t_0$, 1 to 2 s versus 10 to 30 s. Readings were repeated 5 to 8 times on each cell (at least 3 per root) and on up to six plants at any particular age. Readings were combined because within-plant and between-plant variabilities were equal. Errors in estimation of $\varepsilon$ were calculated by combining errors in the two components of Eq. 1, i.e. $V$ and $\Delta \Psi / \Delta V$, each of which was determined independently.

**Half-Time of Water Exchange**

After a penetrated cell had displayed a stable turgor, the pressure was changed by 0.05 to 0.1 MPa. The meniscus moved rapidly to a new position in the tip, and was held there by manual adjustment of the pressure. The subsequent time course of change in pressure was recorded on a strip chart recorder. The $t_0$ was determined graphically. Readings were obtained from at least 2 (usually 3) positions within a root and from up to five plants at any particular age. Readings were combined because within-plant and between-plant variabilities were equal.

**Hydraulic Conductivity**

The $L_p$ of cortical cells was calculated from the relationship

$$L_p = V \cdot \ln \frac{2/A \cdot t_{1/2} \cdot (e - \Psi_e)}{(e - \Psi_p)}$$

(2)

in which $V$ = cell volume and $A$ = cell surface area (23). These were calculated from mean cell diameters and lengths assuming that the cells were cylindrical. Because measurements of turgor were made under conditions of very low transpirational water flux, the root $\Psi_e$ was assumed to be zero and $\Psi_p$ was accordingly set equal to $-\Psi_p$. Because $\Psi_p \ll e$, any errors in the approximation of $\Psi_p$ had little influence on calculated values of $L_p$. Errors in estimates of $L_p$ were calculated by combining errors in the independently determined components of Eq. 2, i.e. $V/A$, $t_{1/2}$, $e$, and $\Psi_e$.

**RESULTS**

**Blade Expansion**

Expansion of the cotyledonary blades was markedly affected by the nutrient regime on which the plants were grown. With full nutrients, expansion proceeded rapidly, with slight slowing of growth after about 6 d (Fig. 1). On a nutrient solution either without N or without P, growth began to slow almost immediately after transfer; for the two treatments, the difference in blade area from the control was significant ($P = 0.05$) by 3 and 4 d after transfer, respectively. Growth on an intermediate level of N was less affected than in −N or −P treatments. Essentially all of the growth differences arose during the daytime, with no consistent effects on relative leaf

![Figure 1](attachment:image.png)
expansion rates at night (data not shown). In this respect the seedlings responded to nutrients as reported earlier (11, 12).

**Cell Size**

Cell sizes were determined 5 cm from the tip of the taproot 4, 5, and 8 d after transfer to nutrient solution. In each case the distributions of both diameters and lengths were Gaussian, allowing the use of parametric statistics to assess differences. Diameters were not significantly different over either treatment or age, with a mean ± SE of 55 ± 0.6 μm (n = 468). Cell lengths tended to increase slightly with age, but again differences were small. The mean length was 151 ± 3 μm (n = 720). The volume of a cylindrical cell of these dimensions is 359 ± 8 μL, and this value was used in subsequent calculations. At all times of sampling, the root systems from different treatments were visually indistinguishable (length, diameter, color, etc., except that nutrient deprivation began to inhibit branching after 7 to 10 d); nor were consistent differences noted in the sections examined under the microscope.

**Turgor and Elastic Modulus**

Calculation of \( L_p \) requires knowledge of cell volume and surface area, \( \Psi \) (here assumed equivalent to \( \psi_s \)), \( \epsilon \), and \( t_0 \). These data were measured over the entire period of growth for only the control and the \( -N \) treatments. In both cases turgor increased during development, but much more so in \( -N \) roots than in the controls. In the stressed roots turgor more than doubled from the initial value of 0.4 MPa, whereas in the controls it increased only to 0.5 MPa over 10 d (Fig. 2A). Differences in turgor due to \( N \) stress were apparent by approximately 4 d after transfer (Fig. 2A).

The modulus of elasticity was increased in cells of \( N \)-stressed roots. In the controls, it increased very slowly from 6.2 MPa at 0 to 9.9 MPa 10 d later (Fig. 2B). In the \( -N \) root cells, however, \( \epsilon \) increased more rapidly and to a much greater extent, reaching a value above 20 MPa after 9 d. The timing of increases in \( \epsilon \) approximately coincided with that for increases in turgor. In both cases, effects of \( N \) stress were not firmly established until 4 d after transfer, although there may be small differences before this time (Fig. 2, A and B).

**Half-Time for Water Exchange**

The \( t_0 \) followed a very different pattern from that of turgor or \( \epsilon \). In the controls, \( t_0 \) gradually increased from 10 s to about 15 s with age (Fig. 2C). In the \( -N \) root cells, though, the \( t_0 \) increased very rapidly upon transfer to \( -N \) nutrient solution, reaching its maximum value of 30 s after only 2 to 3 d. This large differential between treatments was then maintained throughout the remainder of the period over which it was monitored. The rapid development of large differences in \( t_0 \) clearly occurred when differences in turgor, \( \epsilon \), and even leaf growth rate, were small (Figs. 1, 2, A and B).

**Hydraulic Conductivity**

The \( L_p \) of cortical cells was calculated from the data of Figure 2, and trends were determined by regression. At the time of transfer to nutrient solutions, \( L_p \) was \( 1.4 \times 10^{-7} \) m·s\(^{-1}\)·MPa\(^{-1}\). In both well-nourished and \( -N \) root cells, the \( L_p \) declined with plant age, but much more rapidly in the latter case (Fig. 3). The trend lines indicate that 10 d after transfer, the \( L_p \) had decreased by 62% in the controls and by 94% in the \( -N \) roots. As little as 2 d of \( N \) deprivation caused more than a twofold difference between the estimated values of \( L_p \) for the two treatments (Fig. 3). Thus, there were large developmental changes in \( L_p \) in both cases, but \( N \) deficiency...
accelerated their appearance. This pattern is very similar to the time courses reported earlier for intact-root conductivity in fully nourished and −P plants (12).

Similar data on cellular water relations were collected for other nutrient-stress treatments, but only at 4 d after transfer. In these experiments, the lowest cell $L_p$ was seen in the −N treatment, followed in increasing order by the −P, intermediate-N, and control treatments (Table I). The effects of stress treatments on cell $L_p$ were all significant (P = 0.05). Notably, the magnitude of these changes closely paralleled the degree of growth limitation by nutrient stress (Fig. 1).

### DISCUSSION

Intact-plant and excised-root experiments revealed earlier that nutrient stress decreases root hydraulic conductivity (11–13). Effects of nutrient stress on root cell $L_p$ reported here may provide a basis for this shift. The alteration of cortical cell $L_p$, and the recent observation that nutrient effects on xylem anatomy are minor (13) both indicate that stress-induced limiting resistances occur in the radial rather than the axial component of the water pathway. This conclusion is consistent with the concept that radial resistance dominates the overall resistance of the root (25).

Earlier work (12) also revealed an age dependency for nutrient stress effects upon root conductivity. The conductivity of excised roots and intact plants decreased as seedlings developed, but more so in −P plants than in well-nourished controls. Similarly, in cortical cells, nutrient deficiency exaggerated the rate of change of $L_p$ but did not otherwise modify developmental patterns. Thus, nutrient effects on $L_p$ may occur partially via some effect on root development. These comparisons among treatments are independent of the age of individual cells, as root extension rates were not different and all measurements were at a fixed distance from the apex. Developmental changes have previously been reported in both cell $\varepsilon$ (20, 21) and $L_p$ (20).

In some species, $\varepsilon$ is dependent upon turgor (18, 19, 23, 24). Such a dependency might account for the changes in $\varepsilon$ of nutrient-stressed cotton root cells, as turgor more than doubled over 10 d of N withdrawal, and changes in turgor and $\varepsilon$ were almost simultaneous (Fig. 2). Measurements with artificially lowered turgor (by displacement of the meniscus) did not reveal any differences in $\varepsilon$ (not shown); however, variability of $\varepsilon$ was such that only large effects could have been demonstrated. N stress also increased $\varepsilon$ of leaf cells of cotton (15) and wheat (8).

In addition to $\varepsilon$, $L_p$ may also be turgor-dependent (21, 23). In cotton root cells this explanation for nutrient effects is discounted, because $L_p$ decreased substantially in nutrient-deficient root cells before any changes in turgor were measurable (Figs. 2 and 3). It is important to note that components of $L_p$ were determined with hydrostatic gradients, yet flow through roots normally is driven by both hydrostatic and osmotic gradients. However, Tyerman and Steudle (24) showed that cell $L_p$ determined with a pressure probe was the same whether flow was osmotically or hydrostatically driven. Therefore, the conclusions from our studies may be independent of the experimental methods.

Comparisons of hydraulic properties of cells with those of intact roots can often support deductions about pathways of water flow (1, 7, 18, 19, 25). Table II presents comparisons between hydraulic conductivities of cells and of intact roots of cotton. The intact-root conductivities were generated earlier by pressure-flux methods and were validated against intact transpiring plants (12). In both the control and the −P treatment, the intact-root conductivity was about twice the $L_p$ of individual cells. These data provide no support for cell-to-cell (vacuolar) flow of water, in which case each cortical cell in the pathway acts as a resistor in series (1, 25). In cotton roots with approximately five cell layers outside the stele, the cell-to-cell model predicts a root conductivity about 10% of cell $L_p$. Evidence from barley (18) and wheat (7) roots did support a cell-to-cell pathway, but a reevaluation led Jones et al. (6) to assign the pathway a minor role in wheat. A cell-to-cell pathway was also discounted in maize roots (19).

The criteria that preclude existence of a vacuolar pathway also preclude operation of a significant symplastic pathway (i.e., through plasmodesmata). Relaxation of cells after a perturbation could occur by water movement through either the plasmalemma or the plasmodesmata. Thus, the cell $L_p$ combines the conductivity to water of both sites.

The alternative to vacuolar or symplast flow is an apoplastic pathway (1, 25). In this model, water flow is usually envisioned to bypass cortical cells, with the Casparian strip forcing water to enter the symplast at the endodermis. As water enters and exits the symplast, it must cross two membranes, and the maximum root $L_p$ therefore is half that of individual cells (25). Our data are inconsistent with this model as well (Table II), although the discrepancy is smaller than with the other models above. However, this comparison may be flawed because $L_p$ was determined at only one site along the length of the root, and it was not determined specifically on the endodermal cells.

A third possibility is that a large portion of the radial water flow bypasses cell membranes entirely. As a test of this hypothesis, root reflection coefficients and effective external osmotic pressures were calculated from earlier data (12) ac-
According to Fiscus (3), values of \( \sigma \) were 0.90 and 0.76 for control and \( -P \) roots, respectively, and values of \( \sigma' \) were 0.247 and 0.138 MPa for the same roots. Both these parameters are consistent with increased shunting of water around cell membranes with nutrient stress. The values of \( \sigma \) represent more or less the limits of the normal range cited in the literature for roots (see "Discussion" in 19). Even when roots have a low \( \sigma \), cortical 'sleeves' from those roots retain a very high \( \sigma' \), showing that the loss of semipermeability is not a property of the cells per se (19). This evidence implies that a low \( \sigma \) for roots may be associated with some apoplastic flux of water through a bypass, and that such a pathway may occur rather frequently. Hanson et al. (4) reported a dominant apoplastic bypass in roots of red pine during \( O_2 \) deprivation. Possible locations for a bypass include sites of lateral root emergence, where the Caspian strip is discontinuous (10).

Finally, we note that decreases in root conductivity (12) and root cell \( L_p \) (Fig. 2) are among the earliest plant responses to nutrient stress, preceding even the changes in leaf expansion rate. Earlier studies (11, 12) strongly imply that root hydraulic conductivity is a primary limitation to leaf expansion in nutrient-stressed seedlings. Presuming that properties of root cell membranes contribute to the stress-induced changes, then these studies have specified a site to search for the initial metabolic events leading to stress injury.

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LITERATURE CITED