Effects of Low Water Potential on Cortical Cell Length in Growing Regions of Maize Roots¹

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ABSTRACT

Roots growing under low water potential commonly exhibit a marked decrease in growth rate and in diameter. Using median longitudinal sections of fixed maize (Zea mays L. cv WF9 × Mo 17) seedling roots, we investigated the cellular basis for these effects. Cortical cells in the shorted elongation zone of water stressed roots were longer than cortical cells in the comparable location of well-watered roots. Nearly twofold differences in cell length were seen in the region 2 to 4 millimeters behind the root apex. The shortened growth zone, however, leads to a final mean cortical cell length approximately 30% shorter in the stressed roots. These differences were present regardless of the age of the control roots. These data, and the slower growth rate seen in water-stressed roots, suggest that the water deficit causes a significant reduction in the rate of cell supply to the cortical cell files.

We have investigated the anatomical basis for the altered growth patterns seen in response to water stress. It is well known that corn roots growing under conditions of severe water stress exhibit a much slower root extension rate than that seen in well-watered corn (6, 13, 17). A significantly shorter growth zone has also been seen in these slowly growing roots (17). The cellular growth patterns underlying these phenomena have not been well understood to date. It is not known whether the cell length profile over the growth zone of water-stressed roots resembles that of well-watered roots, but cells merely stop growing sooner. If water deficit changes cell division rates as well as growth rates, then a more complicated pattern of cell lengths would occur.

This work focused on the root cortex because of the clearly defined files of cells found there. The middle five files of cortical cells were used. These files are characterized by larger cells (due to a shorter meristematic zone in this region of the cortex), and by the uniform size of cells in adjacent files (14).

Cells were measured at 0.25 mm increments to provide a profile of cell lengths occurring along the elongation-only zone of the corn root. The spatial distribution of cell lengths collected in this study provides a quantitative characterization of effects of water deficit. The cell length data were also used in conjunction with a simple continuum model (8, 11, 18) to assess the effects of water stress on the rate of cell supply.

MATERIALS AND METHODS

Seeds of Zea mays L. (cv WF9 × Mo 17) were germinated at 25°C in the dark in vermiculite moistened with 10⁻⁴ M CaCl₂. Forty-five hours after plantings, seedlings with radicles approximately 10 mm long were transplanted into plexiglass boxes containing saturated vermiculite (3.7 g 10⁻⁴ M CaCl₂ per 1 g vermiculite), or vermiculite moistened to only 2% (w/w) of this level. This corresponded to soil water potential values of approximately ~0.03 MPa and ~1.60 MPa, respectively. The seedlings were grown at 25°C in the dark until the roots attained an overall length of 60 mm. For the well-watered roots this was a period of 20 h, and for the water-stressed roots 50 h of growth were required to reach this length. A second set of well-watered roots was grown for 50 h to provide a temporal control.

Upon harvesting, the overall length of the roots was measured, and the apical 15 mm was excised and placed in 2.5% glutaraldehyde in 0.1 M potassium phosphate buffer (pH 7.0) overnight at room temperature. After three 15 min washes in buffer, the root apices were dehydrated in ethanol (10%, 25%, 50%, 70%; 30 min each) and stored in 70% ethanol at 4°C. The roots were dehydrated further in 95% ethanol before infiltrating. Infiltrated roots were cut and embedded as 7.5 mm segments in glycol methacrylate (Historesin, LKB-Produkter). Serial longisections were cut at 3 μm intervals on a JB-4 microtome, and stained with toluidine blue O at 60°C (15). Median sections were photographed with Kodak Panatomic-X film.

For transverse sections, only the apical 10 mm of the root was originally excised prior to fixation. Later, thin slices of tissue were cut 2.5 and 5.5 mm behind root apices for use in cutting transverse sections. All other procedures were the same as for the longisections.

Root extension rates were calculated from initial and final root lengths over the growth period.

Cell Size Data

Cortical cell lengths were measured on prints made from 35 mm negatives. Every 0.25 mm along the root, cell size measurements were made of the five "middle" cortical cell files on each of five to seven roots. The same five files were used for all the measurements whenever possible, but occasionally one or more of the cell measurements at a given point needed to be taken from the corresponding region on the opposite side of the stele. Cell size at each point was taken as an average of these 25 to 35 cells. Overall cell diameter was also measured at each point.

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Transverse section measurements were also taken on prints made from 35 mm negatives. Measurements for overall root diameter, stelar diameter, and number of cortical cell files were made in orthogonal directions from each of 15 roots at 2.5 mm, and each of five roots at 5.5 mm from the root tip.

RESULTS

Cell lengths obtained from each treatment were quite variable between adjacent cortical cell files, and among comparable files in different roots. This was not surprising because an extra mitotic division often occurs in a given cell file, but not in the adjacent files (14, 18). The variability is clearly seen in the representative standard deviations of Figure 1A.

The time and length controls exhibited similar cell length profiles. However, the cell length profile over the growth zone of water-stressed roots was considerably different from cells in the corresponding location of well-watered roots. Using z-tests, it was found that the cell lengths in the roots grown at low water potential were significantly different from cells of the control roots from 1.5 to 5.25 mm behind the root cap junction. A comparison of the control groups of roots with the water-stressed roots showed that the cells of the latter were nearly twice as long in the region 2 to 4 mm behind the root apex (Fig. 1B). A comparable cell length was not seen among all three sets of roots until near the end of the growth zone of the water-stressed roots. Similar distributions of cell lengths were seen by Paolillo (16) in well-watered and stressed soybean hypocotyls.

The final cell length attained in these roots was found to be smaller under water stress, consistent with earlier reports in the literature (4, 9, 16). In the well-watered roots (both time and length controls) a final cell length of about 270 to 280 μm was seen. The final cell length in osmotically stressed roots was, on average, 195 μm; and, as mentioned above, this final length occurred at a point much closer to the root tip. It is important to note that the point at which cell length became invariant with longitudinal position was 6 mm in stressed roots, but was not until 11 mm in controls. The end of the growth zone seen by this method corresponded with growth zone length data obtained in marking experiments (17).

Cell flux is the number of cells passing a given point per unit time. The data collected in this study were used to calculate cell flux for each set of roots. Continuity considerations indicate that if growth and cell production are steady (time invariant), then cell flux is uniform throughout the cell file. So, cell flux can be evaluated at the base of the elongation zone as the ratio of the overall root extension rate, \( V \), to the mature cell length, \( L \) (8, 11, 18). The results indicated that water stress caused a decrease in the cell flux as shown in Table I. The time for successive cells to pass a given point has been termed the "cellochron" (18). If growth and cell division rates are steady, then the cellochron, evaluated as \( L/V \), is the time needed to add a cell to the "elongation-only" zone and also to displace a cell from the elongation zone. Water stress caused the cellochron to increase from 0.1 to 0.2 h.

Measurements of overall root diameter taken from photographs of root median longissections and root transverse sec-

Table I. Cell Flux and Cellochron Calculation

<table>
<thead>
<tr>
<th>Calculation</th>
<th>Water Stress</th>
<th>Control-Length</th>
<th>Control-Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature cell length, ( L ) (mm/cell)</td>
<td>0.20</td>
<td>0.27</td>
<td>0.28</td>
</tr>
<tr>
<td>Root extension rate, ( V ) (mm/h)</td>
<td>1.00</td>
<td>2.60</td>
<td>2.60</td>
</tr>
<tr>
<td>Cell flux (cells/h)</td>
<td>5.13</td>
<td>9.63</td>
<td>9.29</td>
</tr>
<tr>
<td>Cellochron (h/cell)</td>
<td>0.20</td>
<td>0.10</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Figure 1. (A) Average cortical cell length as a function of position for water stressed and well-watered roots. Each point is the average of 25 to 35 measured values. Dashed lines represent the mature cortical cell length of water stressed roots. One control set was grown to the same length as the stressed roots (60 mm), and the other control set was grown for the same amount of time (50 h). Statistically, cell lengths of stressed roots were found to be significantly different from 1.5 mm to 5.25 mm behind the root cap junction. (B) Ratio of stressed cell length to control cell length. This figure gave a clearer picture of the region where cell lengths differ. Cell lengths of the water stressed roots were significantly larger than the controls until about 6 mm behind the tip. Control cells were larger than stressed cells after 8 mm.
tions showed similar trends for the three sets of growth conditions (Figs. 2 and 3). The thinning of roots due to water stress was clearly seen. The thinning of roots that occurred over time was also seen in the set of control roots grown for 50 h.

Stelar diameter data were obtained from photographs of transverse sections at two positions behind the root apex (Fig. 3). The trends seen in overall root diameter among the three sets of growth conditions were mirrored in the stelar measurements. Within each set of roots the stelar diameter was the same at points 2.5 and 5.5 mm behind the root apex. In terms of overall diameter, the stelar occupied a lesser percentage of the root at 5.5 mm than it did at 2.5 mm (Table II). These data suggest that the thinning of the stelar (through a decrease in number of files and/or size) played no greater role in overall root thinning under water stress than did decreased radial growth in cortical cell files. Thus, thinning due to water stress appears to differ from thinning due to mechanical impedance. Root thickening is produced primarily by increased stelar diameter (19). By growing the corn seedlings in vermiculite, the soil hardening that often accompanies water loss was virtually eliminated. This enabled our study to focus on the overall thinning of water stressed roots as well as other effects caused primarily by water deficit.

The data on number of cortical cell files suggested that there was a one file decrease (on each side of the stelar) in the control roots as they aged (Table III). The data from the water stressed roots were intermediate to the two sets of controls.

**DISCUSSION**

When investigating the growth of plant organs at the cellular level it is necessary to look at more than cell size profiles. Smaller cells found in tissue growing under adverse conditions do not always imply a slower growth rate. The overall growth and/or cell division rate of the organ in question needs to be measured in addition to the cell size profile, in order to completely understand the growth processes occurring. The best papers dealing with growth from an anatomical perspective have made use of the interrelationships between these processes. Good discussions of the theoretical aspects of the problem have been provided by Goodwin and Stepka (10), Green (12), and Gandar and colleagues (2, 3, 7, 8). Experimental work recognizing the relationship between anatomical structure and growth includes studies by Barlow et al. (1).

In characterizing effects of water stress, investigators have tended to ignore the anatomical theory. Brustrom (4) studied cell elongation patterns occurring under different levels of short-term osmotic stress. We note, however, that our work is not truly comparable to his, because the water potential of our growth medium was lower. He found that hydroponically grown wheat seedlings treated with 0.2 M mannitol showed a shorter growth zone, and shorter mature cell lengths. Burström plotted cell length against "cell number," i.e. the serial number of the cell. The interpretation of the trends included the assumption that cell number was proportional to time. However, in steady growth, the proportionality constant is given by the cellochron which may vary with the treatment. Thus, Burström discounted the possibility that change in the rate of cell supply occurred in the roots grown in hypertonic solutions. It would have been more informative to plot cell length against position. If a time course is desired it should

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**Table II. Stele as Percentage of Overall Diameter**

The ratio of the stelar diameter to the overall root diameter was derived from the data shown in Figure 3.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Distance from Root Cap Junction (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>Water stress</td>
<td>43.6</td>
</tr>
<tr>
<td>Length control</td>
<td>47.8</td>
</tr>
<tr>
<td>Time control</td>
<td>45.9</td>
</tr>
</tbody>
</table>

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Figure 2. Overall root diameters measured from the root longisection photographs used for cell length measurements. Thinning was seen when the roots aged, and when they were grown at low water potential. Data are means ± 1 SD (n = 5–7).

Figure 3. Root and stelar diameter data measured on the photographs of root transverse sections. Overall root thinning seen with both age and water stress was consistent with trends shown in Figure 2. For each growth condition, the magnitude of the stelar diameter was relatively uniform at 2.5 and 5.5 mm behind the root cap junction.

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be approximated by plotting against \([\text{cell number} \times (L/V)]\) rather than cell number alone. This would show any variation in the cell profile existing under the two different growth regimes. In our study of water stressed roots we saw a reduction in mature cell lengths, but we also saw an increase in cell length close to the apex.

González-Bernáldez et al. (9) directly measured cell-cycle rates to verify the relationships among overall root growth rate, cell-cycle rates and mature cell length for roots growing under varying osmotic pressures. They found that in roots growing under high osmotic pressures the major factor contributing to the decrease in root growth was the reduction in cell elongation, but altered cell-cycle rates were also involved. This conclusion was different from that reached by Burström (4), who assumed no change in cell division rates was occurring in roots grown under osmotic stress. Both groups focussed on the mature epidermal cell lengths, and thus did not directly measure any variation in the length of younger cells that may have existed.

In our work, the overall root extension rate combined with the final cell length data allowed a calculation of the cell flux for these roots (Table I). The cell flux values showed that new cells were added to a file in water stressed roots at about half the rate they were added in well-watered roots. The rate at which cells are supplied to a growing file depends on the local rates of transverse cell division, as well as on the time during which cell divisions occur in the mitotic zone of the particular file cell. The data collected here did not distinguish duration effects from effects on the rate of local cell division. However, we can conclude unambiguously that water stress caused a reduction in the rate of cell supply in the cortex of corn roots.

It has been suggested that cells in root apices have a certain limiting biomass at which they are unable to proliferate (5). If there was a greater reduction in the rate of mitotic activity in these stressed roots, compared to the reduction in the rate of cell elongation (over the zone of mitotic activity), this putative limiting biomass would be reached at a point closer to the root apex. This would mean that the extent of the meristematic zone in the water stressed roots was reduced in size. This spatially earlier start to exponential growth could be enough to provide the longer cell lengths in water stressed roots that were seen in this study.

The shorter growth zone seen in the water stressed roots does not mean that the cells reached the end of the growth zone sooner as suggested by some (6). In fact, a temporal analysis of the growth patterns occurring here suggested that cells reached the end of the growth zone at about the same time under both growth regimes (17).

In our study, we combined the overall growth rate of the corn roots with the cell length profiles, to assess the developmental processes underlying the smaller cells seen in the water stressed roots. We were able to conclude that shorter cells were not only a result of slower growth, but were also due to a reduction in the rate of cell supply.

**LITERATURE CITED**


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**Table III. Number of Cortical Files**

The number of cortical cell files was counted on photos taken of root transverse sections.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Distance from Root Cap Junction (mm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>Water stress</td>
<td>9.3 ± 0.3</td>
</tr>
<tr>
<td>Length control</td>
<td>10.0 ± 0.2</td>
</tr>
<tr>
<td>Time control</td>
<td>9.0 ± 0.4</td>
</tr>
</tbody>
</table>

* The data at 2.5 mm and 5.5 mm are averages of 15 and 5 values, respectively.