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Direct Demonstration of a Growth-Induced Water Potential Gradient[1]

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When transpiration is negligible, water potentials in growing tissues are less than those in mature tissues and have been predicted to form gradients that move water into the enlarging cells. To determine directly whether the gradients exist, we measured water potentials along the radius of stems of intact soybean (*Glycine max* [L.] Merr.) seedlings growing in vermiculite in a water-saturated atmosphere. The measurements were made in individual cells by first determining the turgor with a miniature pressure probe, then determining the osmotic potential of solution from the same cell, and finally summing the two potentials. The osmotic potentials were corrected for sample mixing in the probe. The measurements were checked with a thermocouple psychrometer that gave average tissue water potentials. In the elongating region, the water potential was highest near the xylem and lowest near the epidermis and in the center of the pith. In the basal, more mature region of the same stems, water potentials were near zero next to the xylem and throughout the tissue. These basal potentials reflected mostly the potential of the xylem, which extended into the elongating tissues. Thus, the high basal potential confirmed the high potential near the xylem in the elongating tissues. The psychrometer measurements for each tissue gave average potentials that agreed with the average of the cell potentials from the pressure probe. We conclude that a radial gradient was present in the elongating region that formed a water potential field in three dimensions around the xylem and that confirmed the predictions of Molz and Boyer (F.J. Molz and J.S. Boyer [1978] Plant Physiol 62: 423–429).

This work was undertaken to determine whether gradients in Ψ_w extend from the xylem into growing tissues of plants. Such gradients have been proposed to be necessary for the uptake of water from the xylem by cells in growing tissues (Ray and Ruesink, 1963; Boyer, 1968; Molz and Boyer, 1978; Silk and Wagner, 1980; Boyer, 1985, 1988). Limited water supplies can markedly inhibit growth, and there is evidence that altered gradients might be a cause (Westgate and Boyer, 1985; Nonami and Boyer, 1989a, 1990a). Internal water can be mobilized to support growth when external supplies are limited (Matyssek et al., 1991b) and gradients in Ψ_w have been proposed to supply the force (Matyssek et al., 1991a). Negative pressures (tensions) have been detected in the apoplast of enlarging tissues and might form the basis for gradients (Nonami and Boyer, 1987). However, a first report of gradients between enlarging cells in soybean stems (Nonami and Boyer, 1989b) was not confirmed in castor bean (Meshcheryakov et al., 1992) or pea (Malone and Tomos, 1992) when measurements were made with a pressure probe. Therefore, to determine whether the gradients exist, we used a pressure probe to make detailed measurements of the gradients and checked them with a thermocouple psychrometer.

MATERIALS AND METHODS

Plant Material

Soybean (*Glycine max* [L.] Merr. cv Williams) seedlings were grown from seeds disinfected for 3 min in a 1% solution of NaOCl and rinsed with flowing water for 1 h. Each seed was sown in a 200-mL beaker containing vermiculite with adequate water (5.0 mL of 0.1 mM $CaCl_2$ g^{-1} of vermiculite; Ψ_w of −0.01 MPa measured with an isopiestic thermocouple psychrometer). The seedlings were grown at 29 ± 0.5°C in saturating humidity in the dark. Seedlings were 61 to 70 h old when used for the experiments. All seedling manipulations were done under a green safelight in saturating humidities under the same conditions as those in which the seedlings were grown (green fluorescent bulb wrapped in green plastic sheet having maximum transmission at 525 nm and negligible transmission below 475 nm and above 575 nm).

Growth and Cell Size Measurement

The stems were marked at 5-mm intervals with India ink and grown for 13 h, after which the distance between marks was determined. The rate of elongation was calculated for each interval from the length gained during the growth period. Growth was measured during the pressure probe and psychrometer determinations by marking the basal, nonelongating zone of the stem with India ink near the surface of the vermiculite before the water status measurements. The stem length between the India ink mark and the base of the cotyledons was measured before and immediately after the pressure probe or psychrometer measurements. The growth

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Abbreviations: A, surface area of cells (m^2); Lp, hydraulic conductivity for water ($m\ s^{-1}\ MPa^{-1}$); Ψ_w, water potential (MPa); Ψ_s, osmotic potential; Ψ_p, turgor; Ψ_s^s, osmotic potential of sampled cell; Ψ_s^p, osmotic potential of previously penetrated cells; Ψ_s^m, osmotic potential of mixture from sampled cell and previously penetrated cells; V^s, volume of solution from sampled cell (m^3); V^p, volume of solution from previously penetrated cells.

rate of the stem was calculated by dividing the difference of the two lengths by the time interval between the length determinations.

Immediately after the growth rate determination in the pressure probe experiments, the stem was excised horizontally 13 mm behind the cotyledons in the zone of elongation (Fig. 1). The diameter of individual cells was measured using a microscope and may be regarded as similar for a distance of 5 mm above and below the excision. Subsequently, the stem was sectioned vertically, and the cell lengths were measured within 1 mm of the horizontal cut. A similar set of measurements was performed in the basal stem (Fig. 1). The volume of individual cells was calculated by assuming that each cell was a cylinder.

Tissue Water Status Measurement

The Ψ_w of the stem elongating zone (Fig. 1) was measured continuously over long times while the intact seedlings were growing in a guillotine thermocouple psychrometer (Boyer et al., 1985). The measurement involved placing four whole seedlings into a thermally stable chamber and sealing a small vapor chamber around the elongating portion of the stems with petrolatum (Vaseline). A thermocouple bearing a Suc solution of known Ψ_w was inserted into the vapor chamber to measure the Ψ_w. The measurement was isopiestic, i.e. the vapor pressure of the solution was the same as that of the tissue, and no net vapor exchange took place. This prevented errors caused by the diffusive resistance of the tissue to water vapor and assured that the tissue neither hydrated nor dehydrated significantly during the course of the measurement (Boyer and Knipling, 1965).

To determine the Ψ_s of the elongating stem tissue, four hypocotyl segments about 1.5 cm long were excised from the elongation zone of similarly grown seedlings of the same age,

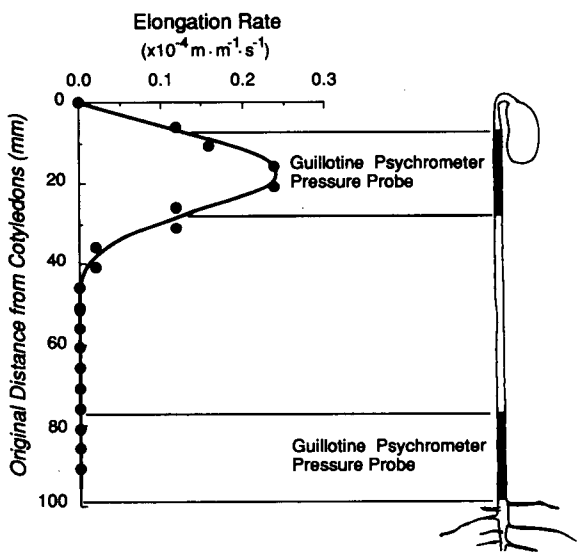

Figure 1. Profile of elongation rate along stems of dark-grown soybean seedlings 70 h after germination. The regions used for measurements are shown as blackened areas on the stem.

and the Ψ_s of the tissue was measured isopiestically after freezing at −70°C and thawing. Because the volume of the apoplast was 3.9% of the total water volume of the tissue (Molz and Boyer, 1978), the effects of dilution by the solution in the apoplast were neglected, and the Ψ_p was calculated from the difference between the Ψ_w and the Ψ_s. The Ψ_w, Ψ_s, and Ψ_p of the basal region (Fig. 1) were measured similarly.

Cell Water Status Determination

The seedling stems were coated with petrolatum and further covered with wet tissue paper to minimize water loss during the pressure probe measurements. This precaution was essential because the small green safelight used to illuminate the probe caused slight local heating that resulted in water loss and turgor loss despite the saturated atmosphere in which the experiments were conducted.

Cell Ψ_p was measured in stems of the intact plants with a pressure probe (Hüsken et al., 1978) according to the procedures of Nonami et al. (1987). Elongating and basal cells were sampled in the same stems at approximately the same position where the cell length measurements were made (Fig. 1). The capillary tip was sharpened at an angle of about 40° until oil was lost at a rate that depressurized the probe within 1 to 2 s (Narashige model EG-4; Optical Apparatus, Ardmore, PA). Continuous tests were made of hydraulic contact with the cell and any possible leakage (Nonami et al., 1987). Although the tip of the probe could not be seen, its entry into a cell could be detected by the rapid movement of cell solution into the capillary of the probe. The Ψ_p was determined by returning the solution/oil boundary (meniscus) to the position it had before entering the cell. After the Ψ_p was measured, cell solution was rapidly removed from the same cell with the probe and transferred to a nanoliter freezing point osmometer (Clifton Technical Physics, Hartford, NY) to determine cell Ψ_s according to the method of Nonami and Schulze (1989). Cell Ψ_w was obtained by algebraically summing the Ψ_p and Ψ_s in the same cell (Nonami and Schulze, 1989).

When solution was extracted from cells deep within the stem, the microcapillary contained some solution from intervening cells because the meniscus of the cell solution/oil boundary had to be kept visible as deeper cells were penetrated. Visibility was achieved by progressively filling the microcapillary with cell solution as the probe tip was pushed into the stem. Thus, it was necessary to account for sample mixing in the capillary during the sample removal. To minimize mixing, only 10 to 100 pL of solution in the tip of the micropipette were placed in the sample holder of the osmometer. The volume of the sample could be easily observed under the microscope during delivery to the osmometer. Samples exceeding 100 pL were discarded.

Malone et al. (1989) indicated the importance of avoiding dilution of the sample resulting from water entering the cell when Ψ_p decreased during the removal procedure. The effect can be important in cells having rapid half-times for water exchange. The cortical cells of soybean stems had half-times on the order of 1 to 3 s, and the other cells had half-times of 4 to 6 s. Sampling times needed to be 1 to 3 s or less (Malone

et al., 1989) and we minimized the problem by extracting solution within 1 to 2 s.

Size of Pressure Probe Microcapillaries

The mixing effects could be calculated if the sample volume and the volume of solution already in the microcapillary were known. These volumes were determined from the inner volume of the microcapillary. Capillaries (Thin-Wall Capillary, 1.0/0.75 mm [o.d./i.d], TW100F-4; World Precision Instruments, Inc., Sarasota, FL) were pulled with a needle/pipette puller (model 730; David Kopf Instruments, Tujunga, CA) that allowed similarly sized tips to be formed repeatedly. The cumulative inner volume of the capillary was calculated from the inner radius of the capillary as a function of distance from the tip by rotating the integrated function around the x axis. The required volumes were obtained by noting the positions of the oil/solution meniscus in the microcapillary before and after sampling for Ψ_s.

RESULTS

Figure 1 shows that elongation was restricted to the apical 46 mm of the stem. The 46-mm length compares well with previous measurements made during much shorter times (Nonami and Boyer, 1990b). The center of this region was used for water status measurements (Fig. 1). Elongation rates for the whole stems were rapid during measurements, averaging 0.54 μm s^{-1} with the pressure probe and 0.45 μm s^{-1} inside the psychrometer, and indicated that experimental manipulation did not inhibit elongation significantly. There was no elongation in the basal stem (Fig. 1). However, a very slow diameter growth was observed (Cavalieri and Boyer, 1982).

The average tissue Ψ_w was initially −0.23 MPa in the stem elongating region and gradually increased to −0.19 MPa at the end of the experiment (Fig. 2C) when measured with the psychrometer in intact plants (Boyer et al., 1985). In the basal tissues, Ψ_w values ranged from −0.06 to −0.04 MPa (Fig. 2F) and were almost equilibrated with the water potential of the vermiculite (−0.01 MPa). The measurements were made in seedlings of the same age as used for the pressure probe (62–70 h old) and indicated that the Ψ_w was virtually stable in both the elongating and basal regions during this time.

The water potential difference between the two regions (Fig. 2, C and F) was mostly attributable to a difference in Ψ_s, which was about −0.6 MPa in the elongating region but −0.45 MPa in the basal region (Fig. 2, B and E). The Ψ_p was approximately the same in the two regions (0.4 MPa, Fig. 2, A and D).

We explored the Ψ_w of individual cells at various positions inside the intact stems using the pressure probe. In agreement with the psychrometer, the Ψ_p was similar in the elongating and basal regions (about 0.45 MPa in both, Fig. 3, A and D). A small Ψ_p gradient is suggested in the basal tissue. The Ψ_s of the cells was more negative in the elongating region than in the basal region (Fig. 3, B and E), and the cells closest to the epidermis were the most negative (−0.7 MPa in the elongating region, −0.55 MPa in the basal region).

Because the Ψ_p and Ψ_s were measured in each cell, the Ψ_w could be determined directly in each cell without concern for the variability in Ψ_p and Ψ_s between cells. Figure 3C shows that in the elongating region, Ψ_w was least negative in the small cells close to the xylem (−0.13 MPa) and most negative at the epidermis (−0.28 MPa). The Ψ_w indicated that a gradient extended from the xylem outwardly to the epidermis and inwardly to the pith. In contrast, Ψ_w in the basal region of the same stems was higher than in the elongating region, ranging from −0.02 to −0.08 MPa (Fig. 3F). There were no gradients having statistical significance.

The accuracy of the pressure probe measurements has been tested and appears satisfactory for determinations of Ψ_p in these tissues (Nonami et al., 1987). The osmometer also has been shown to give valid determinations of the Ψ_s (Malone et al., 1989; Nonami and Schulze, 1989). However, the sampling for Ψ_s could have been a source of error, and therefore, we explored this possibility. In particular, there could have been mixing of the sample with the solution obtained from other cells because the microcapillary of the pressure probe had to be filled progressively with solution

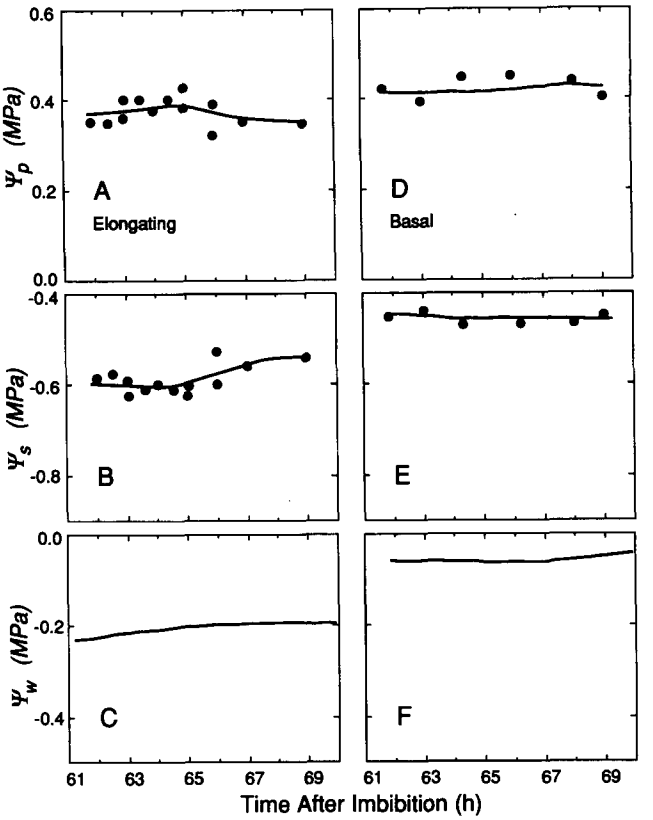

Figure 2. Ψ_p, Ψ_s, and Ψ_w of the elongating region (A, B, and C, respectively) and the basal region (D, E, and F, respectively) of stems of soybean seedlings measured with the guillotine psychrometer at various times after germination. A and D, Ψ_p determined from measurements of Ψ_s and Ψ_w shown in B, C, E, and F. B and E, Ψ_s measured in stem tissue excised from four seedlings. C and F, Ψ_w measured in stem tissue of four intact seedlings. The latter data are shown as continuous recorder tracings. Stems grew about 0.45 μm s^{-1} during the measurement.

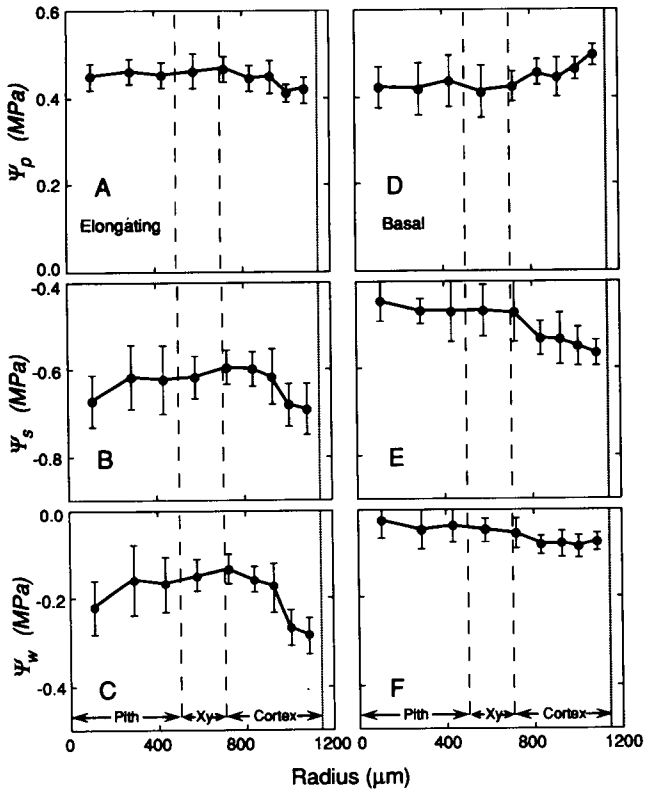

Figure 3. Ψ_p, Ψ_s, and Ψ_w in the elongating region (A, B, and C, respectively) and basal region (D, E, and F, respectively) of the same stems of intact soybean seedlings measured with the pressure probe and nanoliter osmometer. Potentials were measured at various positions along the radius of the stem. Vertical bars indicate 95% confidence intervals calculated from Student's percentage t distribution for six to 18 cells at each data point except the center of the pith in the basal tissue where four cells were measured. Pith, Xy, and Cortex indicate pith region, xylem region including small undifferentiated cells that will become xylem and phloem cells, and cortical cell region, respectively. Vertical dotted line at 1125 μm of the radius indicates the surface of the epidermis. Growth rate of the seedlings was determined during cell Ψ_w measurements and was 0.54 μm s^{-1}.

from other cells penetrated on the way into the tissue. It was not possible to expel this solution without adding large amounts of solute to the cell to be sampled and changing its Ψ_s (e.g. Zhu and Boyer, 1992). Therefore, it was necessary to retain the solution from the intervening cells in the microcapillary and correct for the mixing effect.

The observed Ψ_s of the sample was corrected for complete mixing according to the equation $\Psi_s^p \cdot V^p + \Psi_s^s \cdot V^s = \Psi_s^m \cdot (V^p + V^s)$, where V is the volume of the solution, and the superscripts p, s, and m indicate the sample from the previously penetrated cells, sampled cells, and mixture of the two in the microcapillary, respectively. When rearranged, this relationship gives:

$$\Psi_s^s = \Psi_s^m + \frac{V^p}{V^s}(\Psi_s^m - \Psi_s^p) \quad (1)$$

which indicates that the osmotic potential of the mixed sample, Ψ_s^m, would be between the true Ψ_s^s of the sampled cells and the Ψ_s^p of the previously penetrated cells. In other words, the sampling procedure would tend to minimize any osmotic potential gradient.

We corrected for this effect by determining the volume of cell solution in the microcapillary just before sampling and noting the volume of the subsequent sample. In the elongating region, no correction was necessary in the epidermal cells because no solution was present from previously penetrated cells. In the cortical cells, the Ψ_s of the intervening cells was so close to the Ψ_s of the cortical cells that mixing had a negligible effect. At deeper positions, the cells became small and undifferentiated around the xylem (radial position of 550 to 800 μm, Fig. 4B). The microcapillary had to penetrate 400 μm to reach these cells and contained about 60 pL of solution (V^p) from the intervening cortical cells (Fig. 5B), which had an average Ψ_s^p of −0.64 MPa (Fig. 3B). The small cells generally gave a sample volume (V^s) of about 30 pL, which may have mixed with the 60 pL already in the microcapillary. The Ψ_s^m of the mix was −0.60 MPa (Fig. 3B for xylem small cells). Equation 1 indicates that the true Ψ_s^s then should have been −0.52 MPa.

In the pith region, however, the cells were much larger. At 1000 μm from the epidermal surface, pith cells had a volume of about 1200 pL (Fig. 4B). About 400 pL from the intervening cells filled the microcapillary (Fig. 5B). We removed large samples of more than 100 pL. By measuring the Ψ_s of only 10 pL of this solution in the tip 200 μm of the microcapillary, the sample was restricted to the narrowest part of the microcapillary and was far from the solution collected from intervening cells. Thus, the pith samples were unlikely to be affected by mixing. In the basal region, similar dimensions and corrections applied except that cell volumes were larger than in Figure 4B. Accordingly, Figure 6 shows that correcting the Ψ_s for mixing altered the Ψ_w only for the small cells around the xylem. In the elongating region, these corrections caused Ψ_w to be about 0.08 MPa higher (Fig. 6A) and in the basal region about 0.02 to 0.04 MPa higher (Fig. 6B).

Another error in sampling for Ψ_s could have been caused by dilution from water entering the cell (Malone et al., 1989). The cell Ψ_p and Ψ_w decreased as the sample was withdrawn, and water would have entered the cell in response. The effect cannot be estimated accurately because the pressure in the microcapillary was not the same as in the cell when the sample was being withdrawn. However, the hydraulic properties have been determined for the cortical cells of similar stems (Nonami and Boyer, 1990b), and these allow an approximation. If we assume that a cortical cell typical of the growing region had a surface area (A) of 4×10^{-8} m^2 and a hydraulic conductivity (Lp) of 2×10^{-6} m s^{-1} MPa^{-1} (Nonami and Boyer, 1990b), an extreme decrease in Ψ_p of 0.4 MPa would completely eliminate the Ψ_p and cause water to enter at a rate $ALp\Delta\Psi_w$ = 32 pL or 8% of the average volume of the cell (400 pL) in 1 s. Thus, in cortical cells, the microcapillary would initially receive undiluted cell solution but after 1 s would receive solution diluted by 8%. If we assume complete mixing in the microcapillary, this would cause the sample to be diluted 4% on average. This error would cause

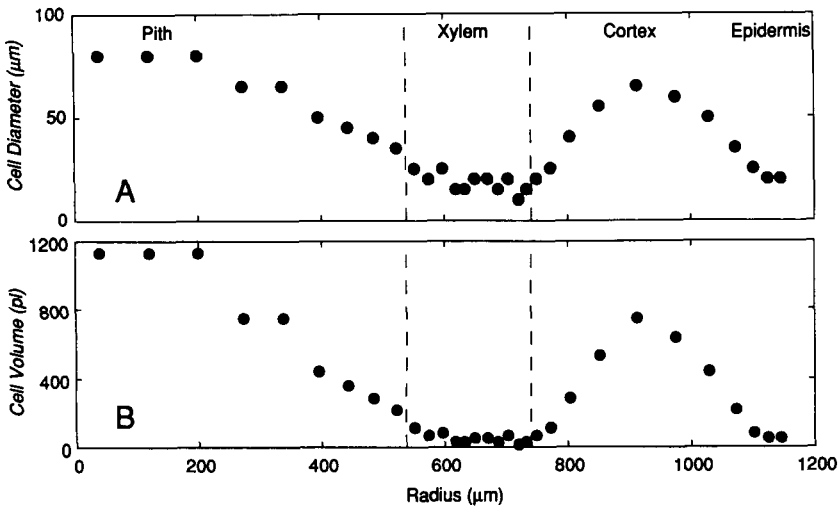

Figure 4. Diameter (A) and volume (B) of cells in the elongating region of soybean stems plotted along the radius of the stem. This figure gives dimensional details of cells used in Figure 3. Volumes in B were calculated from diameters in A and lengths of cells (data not shown). Each point represents an individual cell.

Ψ_s to be about 0.025 MPa lower than reported for the cortical cells, which would steepen the gradient a small amount.

A similar analysis is not possible for the other cells in the stem but, for the small cells around the xylem, we were able to measure a half-time of 4 to 6 s for water exchange with the pressure probe by monitoring the Ψ_p relaxation after a turgor pulse. This relaxation was sufficiently slow compared to the time of sampling (1–2 s) that dilution would be undetectable. In view of the small dilution in the cortical cells and undetectable dilution in the small cells around the xylem, no correction was made for dilution.

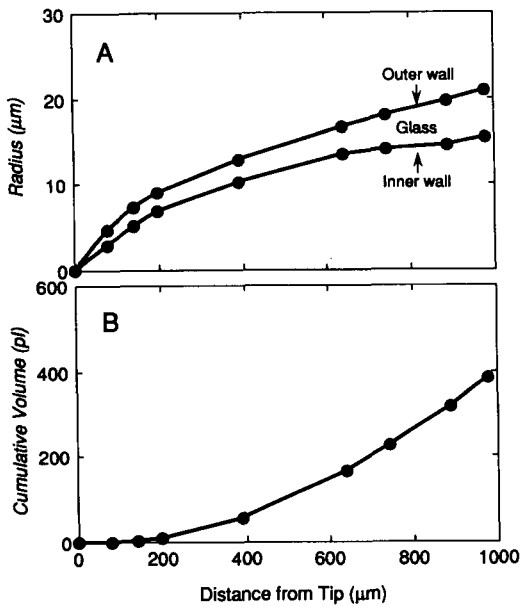

Figure 5. Radius (A) and cumulative inner volume (B) of microcapillaries used in the pressure probe. A, Radii of the inner and outer walls of microcapillary at various distances from the tip. The region between the two walls was glass. B, The cumulative inner volume at various distances from the tip calculated by volume-integration of the inner wall profile in A.

DISCUSSION

The presence of radial gradients in Ψ_w in elongating tissue but absence of significant gradients in slowly growing or mature tissue is consistent with the concept that water flows radially out of the xylem into the enlarging cells that surround the vascular system. The gradients extended outwardly into the cortex and inwardly into the pith, both of which were undergoing elongation. The gradient was larger for the cortex than for the pith as expected from the larger volume of water that had to be transported to the cortex because of the cylindrical geometry of the stem.

The tissue shown as xylem in Figure 3 consisted mostly of small undifferentiated cells that eventually would become vascular tissue as the plant developed (for micrograph, see Steudle and Boyer, 1985). Except for the xylem itself, these cells had the highest Ψ_w whether corrected for mixing or not. The number of differentiated xylem cells was small in the elongating region (10–20 protoxylem vessels), and the pressure potential in the xylem could not be measured directly

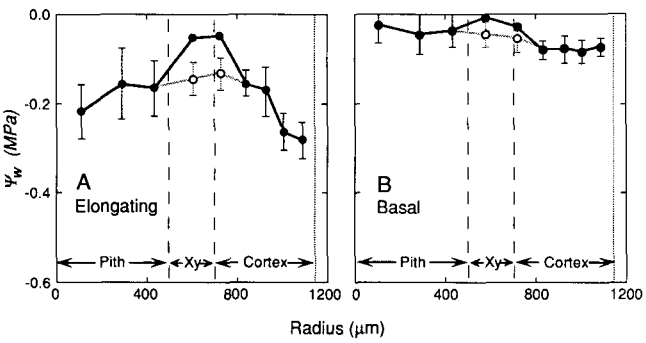

Figure 6. Ψ_w at various radial positions corrected for sample mixing effects in the osmotic potential determinations. A, Stem elongating region; B, stem basal region. The corrected Ψ_w values are shown as dark points and the uncorrected Ψ_w values as open points. When the correction was negligible, dark and open points were superimposed and were the same as in Figure 3.

with the pressure probe. Instead, the xylem water potential was inferred from the water potential of equivalent small cells in the basal stem because there was negligible water transport in this tissue (growth was very slow and transpiration was negligible). In this region, the water potential was close to that of the vermiculite (−0.01 MPa). Because the xylem connected the basal tissue to the vermiculite hydraulically, the xylem must have had a similar water potential. Xylem water potentials measured with a pressure chamber confirm this concept and indicate that the high xylem potential extended into the elongating region (Nonami and Boyer, 1990a). Therefore, in the elongating region, the xylem must have had a water potential of approximately −0.01 MPa.

This indicates that, in the elongating region, the Ψ_w gradient was particularly steep next to the xylem. Thus, previous predictions of the shape of the gradients were essentially correct (Ray and Ruesink, 1963; Molz and Boyer, 1978; Silk and Wagner, 1980). The volume-weighted average of these gradients (Tyree and Hammel, 1972; Boyer et al., 1985) measured with the psychrometer was consistent with the potentials observed with the pressure probe. Also, the potential differences between the elongating and basal tissues were confirmed. Thus, Ψ_w gradients clearly were associated with the growth process.

This conclusion differs from the recent one of Meshcheryakov et al. (1992), who observed Ψ_w close to zero in the cortex of the elongating tissues of castor bean seedlings using a pressure probe. Because water clearly was being absorbed by the tissue, Meshcheryakov et al. (1992) concluded that gradients probably were present but were too small to detect. We cannot explain the small magnitude of the gradients in castor bean. Malone and Tomos (1992) observed Ψ_w below zero in the elongating epidermis and cortex tissues of pea in agreement with the present work. However, they observed a Ψ_w significantly below zero in the mature tissues, which is counter to the present observations. They carefully kept humidities high around the plant but did not coat the seedlings with Vaseline. When we attempted similar measurements without Vaseline, Ψ_p was depressed because transpiration occurred under the illumination necessary for the pressure probe despite high humidity (Nonami and Boyer, 1987). This could lead to lowered Ψ_w in the mature tissue. By coating with Vaseline, we could avoid this problem in the present work. The psychrometer gave similar data with uncoated seedlings. The isopiestic determinations were based on an atmosphere demonstrated to be in vapor equilibrium with the tissue so that no evaporation could occur. The close correspondence between the psychrometer and pressure probe data indicates that Ψ_s and Ψ_p were measured with a high accuracy.

Evidence for Ψ_w gradients associated with growth also has been found in other species such as sunflower (Boyer, 1968, 1970, 1974) and maize (Sharp and Davies, 1979; Westgate and Boyer, 1984, 1985). The gradients have several consequences. First, because measurements of the apoplast solution indicate that it is dilute (Scholander et al., 1965; Klepper and Kaufmann, 1966; Boyer, 1967; Nonami and Boyer, 1987), most of the gradient in Ψ_w must consist of a tension in the apoplast solution. The tension has been detected directly with a pressure chamber (Nonami and Boyer, 1987). Second, the tension implies that there is a significant frictional resistance to water movement out of the xylem and into the enlarging tissues. This probably occurs because all of the cells in the enlarging tissue must be supplied with water from only a few xylem vessels (Molz and Boyer, 1978). Third, the Ψ_w gradients form mostly because of differences in Ψ_s between cells, not differences in Ψ_p. This implies that patterns of solute transport and use are major contributors to the creation of Ψ_w gradients (Schmalstig and Cosgrove, 1990; Meshcheryakov et al., 1992). Fourth, the Ψ_p in the enlarging cells does not balance the Ψ_s, and the gradient is ultimately generated by this lack of balance inside the enlarging cells. There is evidence that the Ψ_p is kept from balance by the enlargement of the cell walls (Boyer, 1968; Nonami and Boyer, 1987), and the Ψ_w of the cells is transmitted to the apoplast as a tension.

The gradients in Ψ_w are most accurately visualized in three dimensions in a soybean stem (see cover). Because the stem is cylindrical, the stem radius shown in Figure 6 can be rotated around the long axis of the stem so that the gradient describes a surface having its maximum at the xylem, a minimum in the center of the pith, and a minimum at the epidermis. The surface constitutes a potential field arrayed around the xylem tissues. Transport to cells outside of the xylem will occur when the surface is oriented consistently downward outside of the xylem.

Because the xylem water potential represents the initial part of the field, it is a particularly important determinant of water entry and growth rate. The solution in the xylem is subjected to pressures ranging from positive to negative depending on root pressures and transpiration, and changes in the xylem potential can occur rapidly and alter the shape of the field. If the xylem potential decreases, the field next to the xylem can invert and block all outward water movement to the enlarging tissues. The block would occur because the field is the driving force for water movement out of the xylem and would be in the wrong direction next to the xylem. Cell enlargement would cease immediately until the field again assumed its consistent downward shape outside the xylem.

Nonami and Boyer (1989a) obtained evidence for the inversion of the field when they transplanted soybean seedlings to vermiculite of low water content and observed a rapidly declining growth rate. The Ψ_p decreased in the cells adjacent to the xylem but not in outlying cells. Nonami and Boyer suggested that this would invert the Ψ_w field and block outward water flow, thus starving the elongating cells for water. After a few hours, the field recovered.

Matyssek et al. (1991a, 1991b) also observed growth effects attributable to the potential field. Stem growth decreased within 1 min after removing the external water supply. There was a rapid decrease in the xylem water potential in the enlarging region (Matyssek et al., 1991a) that immediately inhibited growth.

It must be emphasized that the field is most reliably detected only when transpiration is virtually zero. If significant transpiration takes place, growth-induced Ψ_ws are obscured by transpiration-induced potentials (Boyer, 1974; Westgate and Boyer, 1984). In the present work, transpiration was prevented by growing and measuring the seedlings in a water-saturated atmosphere and further protecting the pres-

sure probe measurements by coating the seedlings with Vaseline.

Growth-induced Ψ_ws have been observed in all growing regions of higher plants tested so far (Boyer, 1985, 1988) except castor bean (Meshcheryakov et al., 1992). Their prevalence indicates that frictional resistances to radial water flow may be an important feature of the growth process in complex tissues. In tissues exhibiting gradients, dehydrated tissues from growing regions rehydrate only slowly (Molz and Boyer, 1978; Boyer et al., 1985; Steudle and Boyer, 1985). The slow kinetics predict gradients in potential similar to those found here (Ray and Ruesink, 1963; Molz and Boyer, 1978). The hydraulic conductivities of stem cortical cells are on the order of 10^{-6} m s^{-1} MPa^{-1}, which would not constitute a particularly large resistance to water flow (Cosgrove and Cleland, 1983; Nonami and Boyer, 1990b). Therefore, the frictional resistance must be located between the xylem and the cortical cells, perhaps in the small cells around the xylem. This is supported by the long half-times for water exchange in these cells (4–6 s) compared to the cortical cells (1–3 s). The small cells are tightly packed and, because the xylem vessels are few in number and contact only a few of the small cells (Steudle and Boyer, 1985), a substantial frictional resistance might result. This anatomical characteristic is common and may explain why a growth-induced potential field can be present in higher plants but negligible in single cells such as *Chara corallina* (Molz and Boyer, 1978; Zhu and Boyer, 1992).

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