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Water Potentials Induced by Growth in Soybean Hypocotyls

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ABSTRACT

Gradients in water potential form the driving force for the movement of water for cell enlargement. In stems, they are oriented radially around the vascular system but should also be present along the stem. To test this possibility, growth, water potential, osmotic potential, and turgor were determined at intervals along the length of dark-grown soybean (*Glycine max* L. Merr., cv. Wayne) hypocotyls. Transpiration was negligible in the dark, humid conditions, so that all water uptake was for growth. Elongation occurred in the terminal 1.5 centimeters of the hypocotyl. Water potential was −3.5 bars in the elongating region but −0.5 bar in the mature region, both in intact plants and detached tissue. There was a gradual transition between these values that was related to the growth profile along the hypocotyl. Tissue osmotic potentials generally paralleled tissue water potentials, so that turgor was the same throughout the length of the hypocotyl. If the elongating zone was excised, growth ceased immediately. If the elongating zone was excised along with mature tissue, however, growth continued, which confirmed the presence of a water-potential gradient that caused longitudinal water movement from the mature zone to the elongating zone. When the plants were grown in vermiculite having low water potentials, tissue water potentials and osmotic potentials both decreased, so that water potential gradients and turgor remained undiminished. It is concluded that growth-induced water potentials reflect the local activity for cell enlargement and are supported by appropriate osmotic potentials.

Water must enter plant cells in order for cell enlargement to occur. Although gradients in water potential necessary to move this water are too small to measure in single cells (15), significant gradients have been predicted in growing tissues (15, 18, 20), because the cells closest to the vascular supply must transport water to a large number of outlying cells (15, 20). They are considered to be induced by growth (15), because they arise from the yielding of the cell walls, which prevents the cells from becoming completely turgid, and by the addition of solutes to the growing cells, which maintains cell osmotic potential.

In soybean stems (hypocotyls), water for enlargement probably moves from the xylem radially along water potential gradients (15). However, the presence of radial gradients implies that there also should be longitudinal differences in water potential that reflect the local activity of growth along the stem (20). Although water potential comparisons have been made between growing and nongrowing tissue (5, 7, 15), the possibility of longitudinal gradients has not been explored. The objective of this work was to determine whether longitudinal gradients exist in water potential, osmotic potential, and turgor and whether a limited water supply alters the gradients.

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MATERIALS AND METHODS

Plant Material. Soybean (*Glycine max* L. Merr., cv. Wayne) seedlings were grown from seed in vermiculite in a dark, humid chamber (29 ± 0.5°C). Prior to planting, the seeds were washed in 1% NaOCl for 3 min and rinsed in distilled H_2O for 1 h. For the first 48 h, the seedlings were grown with adequate water (5 ml of 10^{-4} M $CaCl_2$/g of vermiculite). After 48 h, uniform straight seedlings with 2.5-cm hypocotyls were selected and transplanted into 40 g of vermiculite containing either 200 ml (1.0×) or 25 ml (0.13×) 10^{-4} M $CaCl_2$ solution. The vermiculite had been shaken with the solution to insure uniform mixing. A dim green safelight (spectrum described in Ref. 7) was used during the manipulation of the seedlings.

Growth Measurements. The profile of elongation and diameter growth along the hypocotyl was measured by marking the entire hypocotyl at 3-mm intervals with India ink and by determining the diameter of the hypocotyls at 2-cm intervals along the axis with a micrometer. After 24 h, the measurements were repeated, and length and diameter growth were calculated. When hypocotyl water potential was measured in intact seedlings, elongation rates were determined from the total hypocotyl length before and after the water potential measurement. An India ink mark was made on the hypocotyl 3 cm below the cotyledons in order to compare elongation rates of the basal and elongating regions.

For measuring the rate of elongation for short times, the hypocotyl was connected to a radial displacement transducer by a clip just below the apical hook. The basal end, 2 cm below the transducer attachment, was held in constant position by another clip attached to a rigid bar. The body of the transducer was mounted in the barrel of a microscope that allowed calibration at any time without disturbing the seedling. Seedlings could be attached to the transducer while growing in the vermiculite, with the entire apparatus and seedling in a dark, humid box. When desired, the portion of the hypocotyl above and below the clips could be removed with a razor blade without disturbing the transducer.

Water Potential Measurements. The water potential of various regions of the hypocotyl of intact seedlings was measured by placing four seedlings in a thermocouple psychrometer chamber large enough to hold entire plants (3, 7). Petrolatum was placed in a ring that enclosed a 2-cm length of the hypocotyl to be exposed to the thermocouple. The petrolatum screened the thermocouple sensor from the upper and lower portions of the plant, as previously described (7). Plants were placed in the petrolatum ring, so that only the elongating zone (terminal 2 cm) or mature zone (basal 2 cm) was exposed to the thermocouple. Outside the petrolatum screen, 10^{-4} M $CaCl_2$ bathed the roots. The apparatus was placed so that the seedlings were vertical during the determination. Water potential was measured by the isopiestic technique (6). The thermocouple chamber had been coated with melted and resolidified petrolatum (2), and measurements were corrected for the heat of respiration (1).

For detached segments, hypocotyls were cut in 2-cm intervals

beginning at the cotyledons. Four hypocotyl segments were placed in the bottom of the thermocouple chamber, and water potential was measured by the isopiestic technique with a thermocouple psychrometer for excised tissue (6). Osmotic potential was measured on the same samples after freezing and thawing the tissue in covered psychrometer chambers over Dry Ice. All manipulations for measurement of water potential and osmotic potential were carried out in a humid chamber. Turgor was calculated from the difference between the water potential and the osmotic potential. No correction was made for dilution of cell sap by wall water after freezing and thawing, since the wall occupied only 3% of the cell volume, and dilution would have been negligible (15).

RESULTS

It was necessary to inhibit transpiration in the seedlings, so that water movement in the plant was only associated with growth. The dark, humid environment in which all the measurements were carried out assured that this was the case (transpiration measured by weight loss was 2.5×10^{-3} mg $H_2O \cdot h^{-1} \cdot plant^{-1}$, a negligible amount compared with water movement for rapid growth of 6.2 mg $H_2O \cdot h^{-1} \cdot plant^{-1}$ measured from gain in tissue water content).

It was also necessary to test whether the water potentials determined with detached tissue were accurate measurements of the water potential in intact tissue. Consequently, water potentials were measured in the elongating and mature hypocotyl regions of intact seedlings. The same tissue was then excised and transferred to a psychrometer for excised tissue, and a second measurement was made of the water potential. Table I shows that the water potentials were similar for elongating (−2.8 bar intact and −2.1 bar excised, on average) and basal mature regions (−0.5 bar intact and −0.9 bar excised, on average), although water potentials were slightly less negative in excised than they were in intact tissue of the elongating region. The intact elongating region always grew rapidly during the measurement (1.0 ± 0.3 mm·h^{-1}; Table I). Since the agreement between intact and excised measurements was good, all further determinations were made similarly but with excised tissue.

Elongation of the hypocotyls was restricted to the region 1.5 cm behind the cotyledons, but slow diameter growth extended beyond this region for a distance that increased as the hypocotyls grew in length (Figs. 1 and 2). Hypocotyls growing in water-deficient vermiculite (0.13 of the amount necessary for rapid growth) elongated less rapidly (Fig. 3) than did plants with adequate (1.0×) water (Figs. 1 and 2). In contrast, both the profile and the rate of diameter growth were relatively unaffected by water supply (*cf.*

Table I. *Comparison of ψ_w of Mature and Elongating Zones of Soybean Hypocotyls in Intact Plants and Excised Segments*

Intact measurements were made first; then the tissue was excised and transferred to a psychrometer for detached tissue (6), where the water potential measurement was repeated.

	Intact ψ_w	Detached ψ_w	Growth Rate
		bars	*mm/h*
Mature zone	−0.8	−1.2	0
	−0.4	−0.9	0
	−0.4	−0.5	0
	−0.5	−1.1	0
Mean ± SD	−0.5 ± 0.2	−0.9 ± 0.3	0
Elongating zone	−2.7	−2.1	0.9
	−2.9	−2.0	0.7
	−2.6	−2.0	1.1
	−2.8	−2.3	1.3
Mean ± SD	−2.8 ± 0.1	−2.1 ± 0.1	1.0 ± 0.3

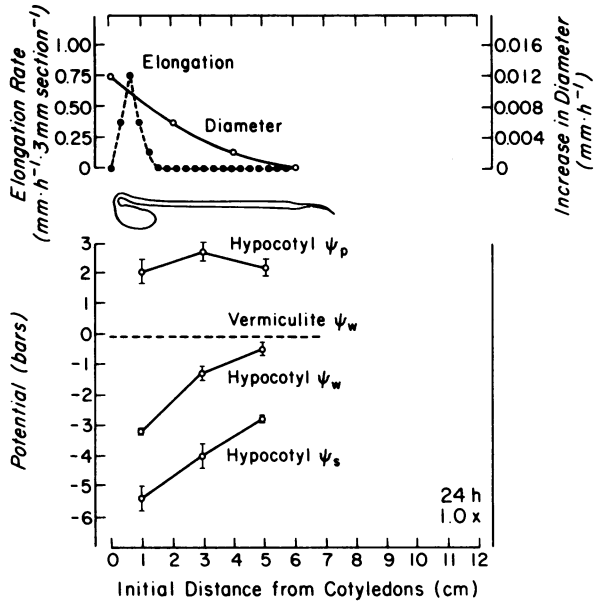

FIG. 1. Water potential (ψ_w), osmotic potential (ψ_s), turgor (ψ_p), and growth profile of soybean hypocotyls 24 h after transplanting to vermiculite having high water content (ψ_w = −0.1 bar). The seedlings had been germinated for 48 h at transplanting. Growth was at 29°C in a dark, humid chamber. Water potentials, osmotic potentials, and turgors are means ± 1 SD.

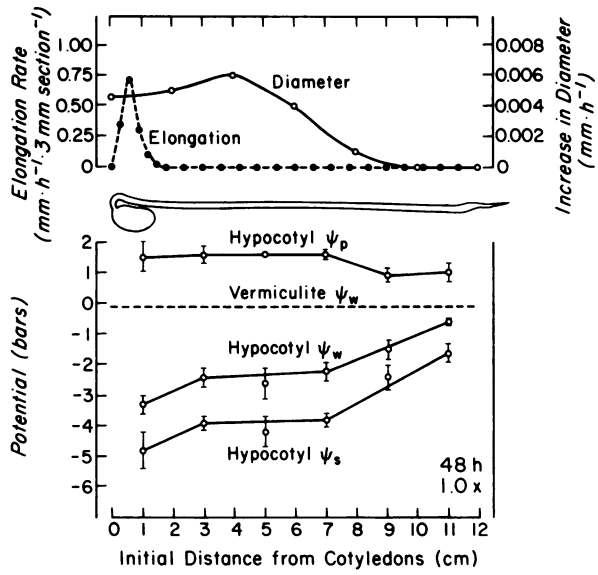

FIG. 2. Water potential (ψ_w), osmotic potential (ψ_s), turgor (ψ_p), and growth profile of soybean hypocotyls 48 h after transplanting to vermiculite having high water content (ψ_w = −0.1 bar). Germination and growth conditions were as in Figure 1. Water potentials, osmotic potentials, and turgors are means ± 1 SD.

Figs. 1, 2, and 3). As a result, the growth profile was constant, even though the rate of elongation had been affected. This result is consistent wth the findings of Meyer and Boyer (12) that the profile of cell lengths was unaltered by low water potentials in the elongating region.

There were large differences in tissue water status along the hypocotyl. In the basal region, where neither elongation nor diameter increase occurred, hypocotyl water potental was similar to that of the vermiculite. Thus, in the plants supplied with adequate water, the water potential was −0.1 bar in the vermiculite

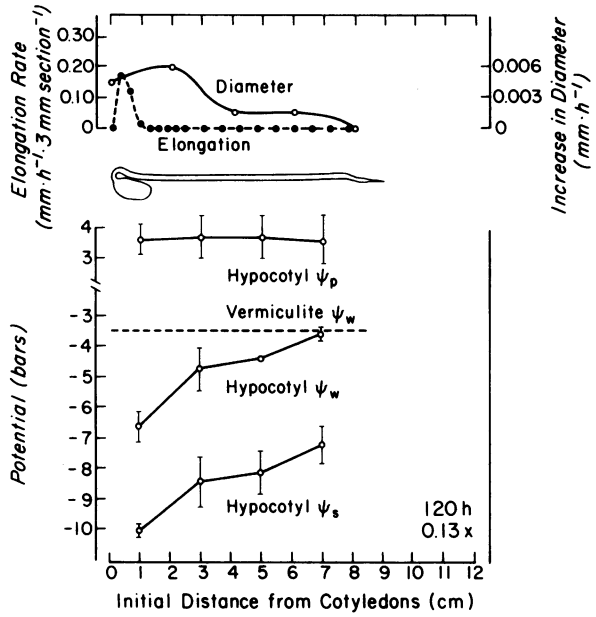

FIG. 3. Water potential (ψ_w), osmotic potential (ψ_s), turgor (ψ_p), and growth profile of soybean hypocotyls 120 h after transplanting to vermiculite having low water content (0.13×, ψ_w = −3.5 bars). Germination and growth conditions were as in Figure 1. Water potentials, osmotic potentials, and turgors are means ± 1 SD.

and −0.5 to −0.6 bar in the basal region (Figs. 1 and 2). In the water deficient plants, the water potential was −3.5 bars in the vermiculite and −3.6 bars in the basal region (Fig. 3). By contrast, the elongating region had a water potential substantially below that of the vermiculite. In plants supplied with adequate water (Figs. 1 and 2), it was −3.2 to −3.4 bars, and, in water-deficient plants, it was −6.6 bars (Fig. 3). Water potentials had intermediate values in the region between the elongating and the basal tissues. Consequently, there was a longitudinal gradient in hypocotyl water potential, the elongating region having a water potential about 3 bars lower than that of the basal region, regardless of the external water potential.

The osmotic potential paralleled the water potential of the hypocotyls (Figs. 1, 2, and 3). When the water potential of the vermiculite decreased 3 bars, osmotic potentials decreased about 4 bars (*cf.* Figs. 1 and 3). Furthermore, the longitudinal gradient in osmotic potential reflected the longitudinal gradient in water potential and, thus, the elongating region had an osmotic potential about 3 bars lower than that of the basal region of the same hypocotyl.

The turgor along the hypocotyl remained essentially constant (Figs. 1, 2, and 3). In hypocotyls of water-deficient plants, turgor was higher than it was in hypocotyls growing with adequate water (*cf.* Figs. 1, 2, and 3). This occurred because the osmotic potential overcompensated for the decrease in water supply (*cf.* Figs. 1, 2, and 3), and the turgor, calculated from the difference between water potential and osmotic potential, necessarily increased.

The presence of a longitudinal gradient in water potential implies that the basal tissue, which had a high water potential, should be capable of supplying water to the elongating tissue, which had a lower water potential. The demonstration of this water transport would provide evidence for a gradient that was completely independent of measurements with a thermocouple psychrometer. The elongating region of an intact hypocotyl was, therefore, attached to a displacement transducer, and the basal and apical portions of the plant were cut away, leaving only the elongating region in a dark, humid environment. If the cuts were made with the base in water, elongation continued (Fig. 4). If the cuts were made in air, elongation ceased immediately (Fig. 4). If the mature region was left attached to the elongating region, however, elongation continued slowly after excision of the rest of the plant in air (Fig. 4). This shows that, in the absence of an external water supply, the elongating region obtained water from the mature tissues and was able to continue growth. This could only have occurred if the water potential of the mature tissue was higher than that of the elongating region. Simultaneous measurements of water potential during this experiment showed that the mature region dehydrated as the elongating region grew (Table II). No such dehydration occurred in the elongating region (Table II).

DISCUSSION

These results show that there is a longitudinal gradient in hypocotyl water potential that reflects the local growth of the tissue when transpiration is negligible. Under these conditions, water movement is for the growth process, and tissue water potentials reflect those involved in growth. The gradient is maintained when water is in short supply and is accompanied by solute accumulation and turgor maintenance throughout the hypocotyl.

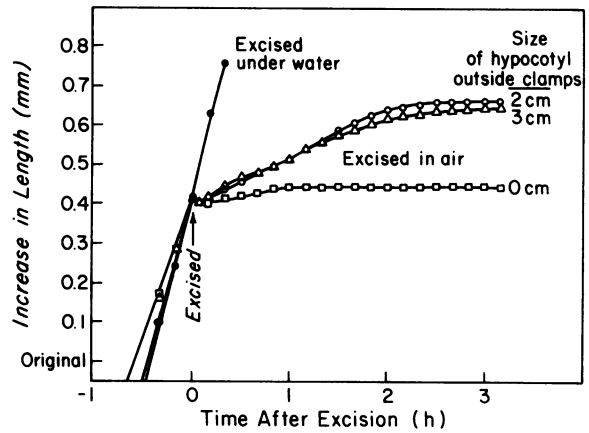

FIG. 4. Increase in length of elongating zone excised above and at varying lengths below the elongating zone. (●), Excised with lower end under water; (□), excised in air; (○), excised in air but with 2 cm basal tissue attached; (△), excised in air but with 3 cm basal tissue attached. Measurements were made in a dark, humid atmosphere that reproduced conditions during growth and in the thermocouple psychrometer chamber. Hypocotyls were from seedlings grown for 24 h after transplanting to vermiculite with high water content. Germination and growth conditions were as in Figure 1. Tissue water potentials are shown in Table II.

Table II. *Water Potentials of Elongating and Basal Regions of Hypocotyls Excised in a Dark, Humid Atmosphere*

A, Water potential of attached basal and elongating regions at time of excision of remainder of plant. B, Water potential of attached basal (2-cm length) and elongating regions 3 h after excision of remainder of plant. C, Water potential of attached basal (3-cm length) and elongating regions 3 h after excision of remainder of plant. D, Water potential of elongating region without attached basal region 3 h after excision. For growth under these conditions, see Figure 4, excision in air.

	Treatment Time	Length of Attached Basal Region	ψ_w of Basal Region	ψ_w of Elongating Region
	h	*cm*	*bars*	
A	0	2	−1.3	−3.2
B	3	2	−2.6	−3.2
C	3	3	−2.5	−3.5
D	3	0		−3.4

As a result, not only does the water potential gradient favor water movement to the sites of growth but solute accumulation assures that turgor is available for the growth process even with a limited water supply.

The longitudinal gradient was demonstrated in intact plants that grew while the water potential was being measured (Table I). It also was observed when excised segments were used for the water potential measurements (Figs. 1, 2 and 3). Therefore, the gradient cannot be an artifact of excision. The similarity of the intact and excised tissue water potentials shows that any change of water potential with excision was too small to detect by our methods. Therefore, if the cell walls continued to extend for a short time after excision, the extension was small and insufficient to alter our conclusions.

The psychrometer chambers for excised tissue were large enough to accommodate only the elongating region of the hypocotyls. Growth of the elongating region would have ceased immediately upon excision, as demonstrated in these experiments. When an excised segment included both elongating and mature regions, however, the tissue continued to grow after excision in a dark, humid atmosphere. In this situation, water was drawn from the wetter mature region, and the water potential of the mature region declined until it approached that of the elongating region. This result indicates that the water potential of growing tissue cannot be measured indiscriminately in excised segments. It is noteworthy, however, that the water potential of the elongating region was unaltered by this growth after excision. Thus, the water potential tended to reflect that of the elongating region, but it was distributed over a larger distance (toward the mature region) than it was in the intact plant (Table II).

The movement of water from nongrowing to growing regions when water is in short supply may illustrate why growing tissue often seems to 'outcompete' other parts of the plant for water. Thus, if rapidly growing tissue retains a lower water potential than does other tissue during a plant water deficit, it may be possible for water to redistribute toward the growing regions within the plant.

Previous attempts to measure growth-induced water potentials have been made (8–10, 16) but have involved incubation of the tissue in solutions of various concentrations. These methods require that the tissue be in osmotic equilibrium with the surrounding medium, in which case growth is prevented during measurement. Furthermore, solute uptake by the tissue must be negligible. To circumvent these problems, Ray and Ruesink (18) determined the kinetics of rehydration by previously dehydrated *Avena* coleoptiles and calculated growth-induced water potentials on the order of −2 bars. The first direct measurements not subject to the problems of solution methods were those of Boyer (3), who determined growth-induced water potentials of attached leaves that were growing during the measurement. A thermocouple psychrometer was used, which permitted water potentials to be determined remotely in the vapor phase, and the method indicated water potentials of −1.5 to −2.5 bars. Subsequently, growth-induced water potentials have been found in growing leaves (4, 5), elongating regions of stems (7, 12, 13, 15), and elongating regions of roots (19). It is possible that these water potentials are present whenever water must travel long distances through cells unmodified for water transport, as in enlarging tissue.

How do the gradients in water potential arise? The central hypocotyl vascular system supplies water to the hypocotyl, and nongrowing cells should equilibrate with the water potential of this supply. In the present study, nongrowing basal tissue had a water potential close to that of the vermiculite, which indicates that the water potential of the vascular system also was close to that of the vermiculite. Therefore, the water potential observed in the elongating region must have arisen in the radial tissue, from the vascular supply outward to the epidermis and inward to the center of the pith (Fig. 5). The psychrometer then must have measured an average water potential for the radial gradient (15). Silk and Wagner (20) have provided a theoretical analysis showing that radial water potential gradients should give rise to longitudinal gradients in cylindrical plant organs. The longitudinal gradient of water potential measured in the present work then represents an array of radial gradients distributed along the hypocotyl and should reflect the intensity of local growth.

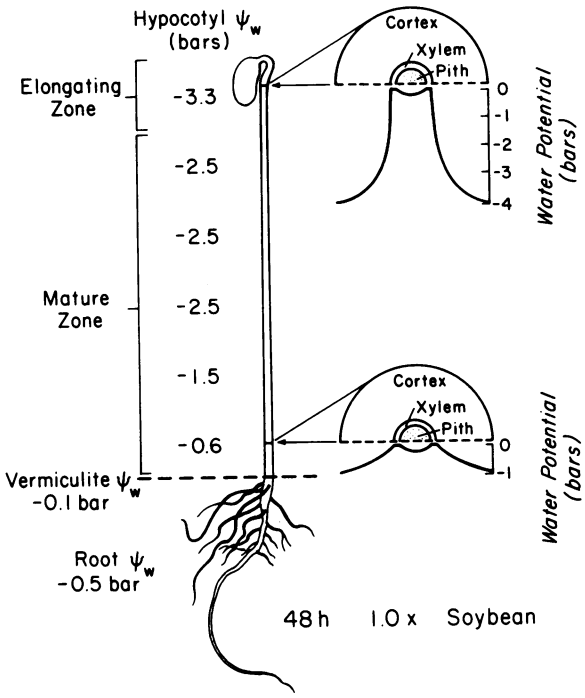

FIG. 5. Summary of the location and extent of growth-induced water potentials in a soybean hypocotyl adequately supplied with water for 48 h after transplanting. Mature zone contains cells slowly increasing in diameter as well as basal cells not enlarging. Radial water potential gradients are shown in sectional views. Longitudinal gradient is shown alongside hypocotyl.

Water potentials lower than those of the vascular supply are induced by growth, because the simultaneous yielding of the cell walls and the high resistance to water movement through the growing tissue prevent full turgidity of the cells. Solute accumulation also must occur continually to maintain the turgor for cell wall extension (14). The size of the radial gradient of water potential depends on the water transmission and wall extensibility properties of the tissue, as suggested by Lockhart (11) and, subsequently, by others (7, 17, 20). Thus, it is not expected that the radial gradient in water potential will be a simple reflection of growth rate, but rather that it will be affected by several factors (7, 11, 15).

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