Water Deficit and Abscisic Acid Cause Differential Inhibition of Shoot *versus* Root Growth in Soybean Seedlings[1]

Analysis of Growth, Sugar Accumulation, and Gene Expression

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ABSTRACT

Roots often continue to elongate while shoot growth is inhibited in plants subjected to low-water potentials. The cause of this differential response to water deficit was investigated. We examined hypocotyl and root growth, polysome status and mRNA populations, and abscisic acid (ABA) content in etiolated soybean (*Glycine max* [L.] Merr. cv Williams) seedlings whose growth was inhibited by transfer to low-water potential vermiculite or exogenous ABA. Both treatments affected growth and dry weight in a similar fashion. Maximum inhibition of hypocotyl growth occurred when internal ABA levels (modulated by ABA application) reached the endogenous level found in the elongating zone of seedlings grown in water-deficient vermiculite. Conversely, root growth was affected to only a slight extent in low-water potential seedlings and by most ABA treatments (in some, growth was promoted). In every seedling section examined, transfer of seedlings into low-water potential vermiculite caused ABA levels to increase approximately 5- to 10-fold over that found in well-watered seedlings. Changes in soluble sugar content, polysome status, and polysome mRNA translation products seen in low-water potential seedlings did not occur with ABA treatments sufficient to cause significant inhibition of hypocotyl elongation. These data suggest that both variation in endogenous ABA levels, and differing sensitivity to ABA in hypocotyls and roots can modulate root/shoot growth ratios. However, exogenous ABA did not induce changes in sugar accumulation, polysome status, and mRNA populations seen after transfer into low-water potential vermiculite.

In higher plants, both shoot growth rate and tissue water potential decline as water deficit develops. However, at some tissue water potentials where shoot growth is inhibited, roots continue to elongate, resulting in increased root/shoot ratios (16, 19, 33, 36). While the adaptive value of the differential response to water deficit is clear (8), the molecular basis for this response is unknown. Since water deficit causes a similar decline in water potential in the growing zones of roots and hypocotyls (24), the differential growth response of these regions may be due to genetic or metabolic control.

Altered levels of plant growth substances may be involved in modulating these responses to water deficits (see refs. 6 and 10 for reviews). For example, the similarity between the effects of water deficit and ABA treatment suggests that the influence of water deficit could be, in part, mediated by changing endogenous ABA levels. Increases in the root/shoot growth ratio have been induced by water deficit (which results in high endogenous ABA levels) and exogenous ABA (4, 35). These observations suggest the possible involvement of ABA in the differential response of roots and shoots to water deficit. Thus, ABA may act as a signal for the initiation of regulatory processes involved in adaptation during growth at low-water potential (6, 10).

Here, we continue to investigate the biochemical and metabolic events which occur during water deficit using etiolated soybean seedlings grown at 100% RH (2, 9, 22, 24–26). This system avoids water deficit induced changes in photosynthesis and transpiration which complicates analysis of growth inhibition in field-grown plants. When well-watered soybean seedlings are transferred to low-water potential vermiculite ($\psi = -0.30$ MPa), hypocotyl growth rates decline within 30 min. Low growth rates persist for 48 to 60 h. Only a part of this growth rate inhibition can be accounted for by reduced turgor in cells of the hypocotyl zone of cell elongation (26). Root elongation is also inhibited by water deficit, but growth recovered within 8 h and eventually exceeds the growth rate before imposition of stress (24). Water deficit reduces protein synthesis, as measured by disaggregation of polysomes (20), and results in the accumulation of several polysomal mRNAs in the zone of cell elongation in the hypocotyl. Furthermore, decreased hypocotyl elongation rates caused by water deficit are accompanied by increased ABA levels (2). In this study we examine root and shoot growth, protein synthesis, soluble sugar levels, polysomal mRNA populations, and ABA contents in roots and shoots of etiolated soybean seedlings whose hypocotyl growth is inhibited by water deficit or ABA treatment.

MATERIALS AND METHODS

Plant Material

Soybean (*Glycine max* [L.] Merr. cv Williams; Illinois Foundation Seed, Champaign, IL) seedlings were grown in

the dark as previously described (2, 24–26). All seedling manipulations were performed at 100% RH under a green safelight (transmission 475–575 nm). The following seedling sections were defined: hook and the zone of elongation the first 5 mm and next 15 mm along the hypocotyl below the cotyledon with the mature region of the hypocotyl the remaining portion of the hypocotyl. The root tip was defined as the terminal 15 mm of the primary root. This section contained a number of structures, including the elongating zone of the root. The remaining portion of the root was defined as the mature root. Seedlings were transplanted 48 h after imbibition into either a similarly saturated vermiculite (ψ = −0.01 MPa) or low-water potential vermiculite (ψ = −0.30 MPa). For one experiment, five different vermiculite water contents were used. Vermiculite was prepared by adding either 200 mL (ψ = −0.01 MPa), 100 mL (ψ = −0.06 MPa), 50 mL (ψ = −0.09 MPa), 25 mL (ψ = −0.30 MPa), or 12.5 mL (ψ = −0.37 MPa) of 0.1 mM $CaCl_2$ to 40 g of dry vermiculite. Hypocotyl and root growth were determined by direct measurement accurate to the nearest mm. Vermiculite water potentials were measured by the isopiestic technique (5).

ABA Application

($\pm$)-ABA (10^{-3} M) was sprayed onto seedlings every 6 h for a 24 h period as previously described (termed spray treatment; 2) or was applied to roots by saturating vermiculite with an appropriate concentration (ranging from 10^{-3} to 10^{-7} M [$\pm$]-ABA) in 0.1 mM $CaCl_2$ for 24 h. If roots were to have their internal ABA contents measured, after removal of the seedling from the vermiculite they were washed three times in distilled water before sectioning and freezing in liquid N_2.

ABA Determination

ABA was extracted as previously described (2) and quantified using gas chromatography-electron capture detection (2). Quantification and confirmation of ABA in some samples was also performed using a Hewlett-Packard 5970 Series Mass Selective Detector coupled to a Hewlett-Packard 5890A Gas Chromatograph (Hewlett-Packard, Avondale, PA). Column and temperature conditions for GLC were: HP-1 fused silica capillary column (12 m × 0.2 mm i.d., 0.33 μm methyl silicon film thickness; Hewlett-Packard) held isothermally at 80°C for 1 min, then temperature programmed from 80°C to 180°C at 25°C/min. At 180°C the rate of temperature increase was changed to 10°/min. ABA was quantified in samples and standards by monitoring m/z 190, m/z 162, m/z 134, m/z 125 (ABA), and m/z 263 (base peak of enderin). Dwell time for all ions was 100 ms.

ABA Accumulation in Soybean Sections

Seedlings (2 d old) were transplanted into well-watered, well-watered plus appropriate concentrations of ($\pm$)-ABA, or low-water potential vermiculite. Seedlings were harvested at 0, 12, or 24 h after transfer. Root and hypocotyl length was measured to the nearest mm, the tissue was sectioned, frozen,

and used for polysome analysis (see below) or lyophilized for ABA analysis.

Sugar Content Determination

Total soluble sugars were extracted from lyophilized seedling segments by grinding in 80% aqueous ethanol followed by boiling for 5 min. This was repeated twice. Supernatants were pooled and evaporated to dryness with a stream of N_2. The residue was dissolved in a known volume of water, and sugar content was determined by the phenol-sulfuric acid method (11).

Polysome Preparation, Two-dimensional Electrophoresis, and Fluorography

Polysome profiles and analysis were performed as previously described (20). Polysome RNA isolation, *in vitro* translation, electrophoresis, and fluorography were performed as previously detailed (20).

RESULTS

Water Deficit and Growth

Transfer of well-watered seedlings to vermiculite with increasingly negative water potentials resulted in hypocotyl growth to be progressively inhibited while root elongation was unaffected, increasing the root/shoot growth ratio for these plants (Fig. 1). Similar observations were described when soybean seedlings were transplanted into a single low water potential vermiculite (−0.3 MPa; 24, 26). With the exception of Figure 1, low-water potential vermiculite is defined to have a water potential of −0.3 MPa.

The dissimilar response of roots and shoots to water deficit could result from roots being closer than shoots to an external supply of water. To test this possibility, 2 d old soybean seedlings were removed from well-watered vermiculite and placed horizontally at 100% RH. Within 3 to 4 h, hypocotyl elongation was completely inhibited while root elongation was inhibited approximately 25% (Fig. 2). In addition, root growth continued for 24 h while hypocotyl growth was com-
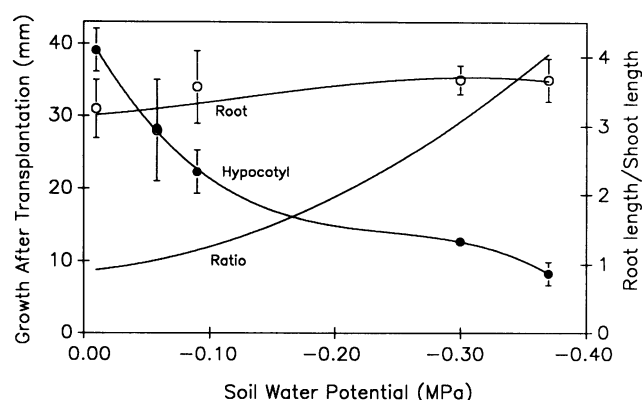
Figure 1. Elongation of etiolated soybean hypocotyls and primary roots 24 h after transfer into vermiculite of different water potentials. Values shown are the means ±SE (for a 24 h period) between four sets of 16 seedlings each for hypocotyls (●) and roots (O).
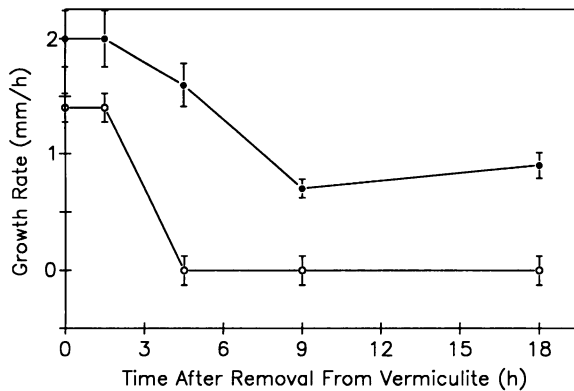
Figure 2. Growth rate of etiolated soybean seedlings removed from vermiculite. Two d old seedlings were removed from well-watered vermiculite and placed horizontally on styrofoam such that no vermiculite was in contact with the root surface. Root (●) and shoot (○) elongation were measured at 3, 6, 12, and 24 h after transfer. Values shown are the mean ± SD of 10 seedlings per treatment. The initial growth rates for hypocotyls and roots were 1.4 and 2 mm/h, respectively.

Table I. *Abscisic Acid Content in Various Sections of Etiolated Soybean Seedlings*

Two d old seedlings were transplanted into well-watered or low-water content vermiculite. After growth for 24 h, seedlings were cut into sections and analyzed for ABA content. Data shown are the mean ± SD for 10 seedlings of two replicates per treatment.

Section	ABA Content	
	+24 h Well-watered	+24 h Water Deficit
	µg/g dry weight	
Cotyledon	0.08 ± 0.01	0.91 ± 0.14
Hook	0.63 ± 0.11	4.30 ± 0.71
Elongating	0.60 ± 0.02	3.71 ± 0.33
Mature	0.91 ± 0.02	5.01 ± 0.71
Mature root	0.45 ± 0.10	4.53 ± 0.08
Root tip	3.52 ± 0.20	29.72 ± 2.39

pletely inhibited. By 24 h, the mature portion of the root had turned a light brownish color, and the hypocotyl had visibly decreased in diameter. The appearance of the root tip (terminal 10–15 mm), on the other hand, was indistinguishable from a well-watered root tip.

Abscisic Acid Levels

All sections of etiolated soybean seedlings transplanted to low-water potential vermiculite accumulated ABA (Table I). At 24 h after transfer to low-water potential vermiculite, there was a 5- to 10-fold difference in ABA content compared with well-watered seedlings. Levels of ABA found in plants exposed to low-water potential vermiculites for 24 h may not be equivalent to levels of ABA found at other times posttransfer. For example, in the elongating region of the hypocotyl, ABA levels reached their maximum accumulation 24 h posttransfer. ABA content then slowly declined such that double the

amount of ABA present in well-watered seedlings was present at 60 h posttransfer (2).

Exogenous ABA and Growth

Exogenous ABA (spray treatment) affected elongation and dry weight accumulation of hypocotyls and roots in a similar fashion as water deficit (Fig. 3). In roots (Fig. 3, B and D), growth was enhanced to a small extent, while elongation was inhibited in hypocotyls (Fig. 3, A and C). Hypocotyl growth began to recover by 48 h posttransfer in seedlings growing in low-water potential vermiculite.

In hypocotyls, growth was progressively inhibited by increasing concentrations of ABA (spray treatments; Table II). For the most part, root elongation was unaffected by any ABA concentration sprayed. At some concentrations, a slight
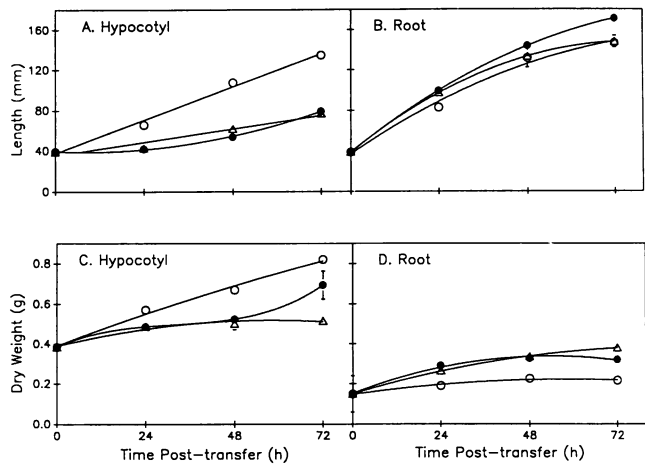
Figure 3. Hypocotyl and root elongation (A and B) and dry weight accumulation (C and D) of etiolated soybean seedlings grown in well-watered (○), well-watered plus applied 10^{-3} (±)-ABA (△; spray treatment), or low-water potential (●) vermiculite. Seedlings grown under standard growth conditions for 48 h were transplanted to well-watered or low-water potential vermiculite. Some of the well-watered seedlings were sprayed with 10^{-3} M (±)-ABA. Values shown represent means ± SE between four sets of 32 seedlings per treatment. Some SE marks reside within symbols.

Table II. *Effect of Exogenous Abscisic Acid (Spray Treatment) on Elongation Growth and Dry Weight Accumulation of Hypocotyls and Roots of Etiolated Soybean Seedlings*

Data shown are the increase in hypocotyl length and dry weight that occur in the 24 h period beginning 48 h postimbibition. Data shown are means ± SE for 4 sets of 16 seedlings each. Length is expressed as increase per seedling. Dry weight is expressed as the total increase for 16 seedlings.

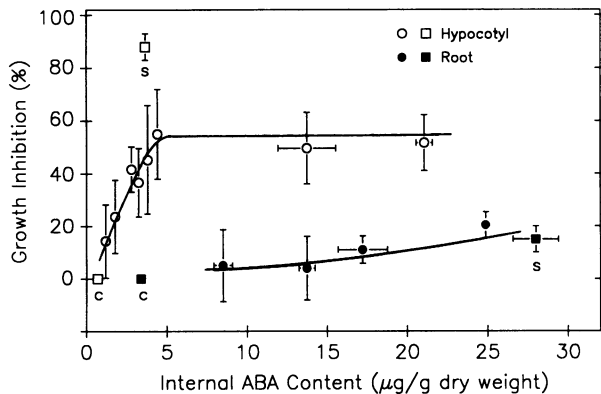| [ABA]	Hypocotyl		Root	
	Δ in Length	Dry weight accumulation	Δ in Length	Dry weight accumulation
M	*mm*	*g*	*mm*	*g*
10^{-9}	34.0 ± 4.2	0.12 ± 0.03	31 ± 7	0.050 ± 0.009
10^{-6}	36.1 ± 4.2	0.13 ± 0.0	33 ± 7	0.067 ± 0.018
10^{-4}	28.5 ± 1.5	0.09 ± 0.01	43 ± 8	0.061 ± 0.005
10^{-3}	21.2 ± 0.7	0.09 ± 0.01	34 ± 9	0.068 ± 0.002

increase in dry weight and elongation was observed. The concentration of ABA in seedlings sprayed with 10^{-3} M ABA was 3- to 4-fold over that found in the zones of elongation (hypocotyl and root) of seedlings transplanted into water-deficient vermiculite.

Using vermiculite saturated with ABA ranging from 10^{-3} M to 10^{-7} M, it was possible to vary the internal concentration of ABA between the levels found in the elongating region of the soybean hypocotyl in seedlings grown in well-watered or water-deficient vermiculite (Fig. 4). Maximum inhibition of elongation occurred at internal concentrations at or greater than those found during water deficit. In roots, growth was inhibited less than in hypocotyls at all ABA concentrations tested (Fig. 4).

Changes in Sugar Levels, Polysomes, and Translatable mRNA
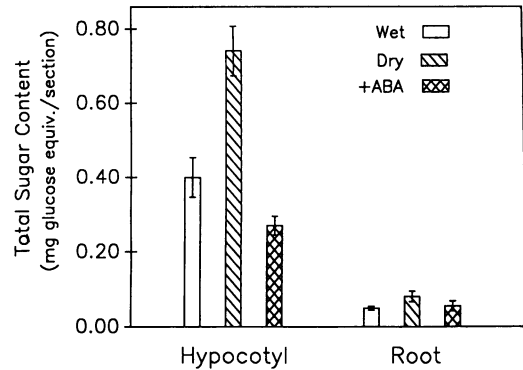
Soluble sugars accumulated in the elongating region of the soybean hypocotyl when water for growth was limiting (Fig. 5; 22). Water deficit approximately doubled the total sugar content in hypocotyls and roots compared with well-watered seedlings (Fig. 5). Sugar levels were not increased by exogenous ABA (spray treatment) in either hypocotyls or roots. In fact, exogenous ABA caused a slight decrease in sugar content in hypocotyls.

Water deficit inhibited elongation (89%) and caused a large decrease in polysome content (LP/P[3] and P/T) in soybean

Figure 5. Total soluble sugar content in the elongating regions of seedlings grown in well-watered (wet), well-watered plus applied 10^{-3} M (±)-ABA (spray treatment), or low-water potential vermiculite (dry). The data are expressed in mean ± SD for four groups of 4 sections for hypocotyls or four groups of 16 sections for root tips.

hypocotyls (Table III; 20). ABA treatment (plants grown in well-watered vermiculite containing 5×10^{-6} M ABA), which gave an internal concentration in the elongating region of the hypocotyl similar to that seen during water deficit, inhibited hypocotyl growth 26% but did not affect LP/P or P/T (Table III). Higher internal ABA levels which caused 70% hypocotyl growth inhibition also did not cause a significant change in polysome status (Table III).

Transfer of soybean seedlings to low-water potential vermiculite inhibited root growth only to a small extent. Polysome status was unaffected by this treatment (Table III). Polysome aggregation was not decreased by any ABA treatments. In fact, 5×10^{-6} M ABA caused a slight increase in LP/P and P/T paralleled with a stimulation of root growth.

Translatable mRNA populations of hypocotyls and roots from seedlings exposed to water deficit or ABA were examined (Figs. 6 and 7). Previously, it has been reported that exposure of plants to low-water potential vermiculite altered the polysomal mRNA population in hypocotyl cells from the zone of elongation (20). This result was reproduced (Fig. 6, A and B; induced translation products are numbered 1-7 [circles]; products which are downregulated are bracketed and labeled E [20] and compared to changes induced by exogenous ABA; Fig. 6, C and D). ABA treatments which gave internal ABA concentrations similar to those which occur in low-water potential seedlings did not induce translation products 1 to 7 or decrease products in region E. At high internal ABA levels, a new set of polysomal translation products was induced (Fig. 6, C and D; identified by boxes) with little effect on the water deficit responsive translation products (numbered 1-7). However, most of the mRNAs induced by high internal ABA were not found in either water-deficient seedlings or when the internal ABA level was similar to that found during water deficit.

The translatable mRNA populations of mature roots and root tips (terminal 15 mm) in well-watered and low-water potential seedlings were compared next (Fig 7, A-D). The translatable mRNA populations from these two regions of the root differed significantly. For example, root tips were enriched in translation products in region E in contrast to the

Figure 4. Inhibition of elongation in hypocotyls (upper curve) and roots (lower curve) occurring after transfer of 2 d old well-watered seedlings into vermiculite containing different ABA concentrations. Individual data points were obtained for growth inhibition occurring between 48 to 60 h and between 60 to 72 h as a function of the internal ABA content in the respective zone of elongation at 60 and 72 h, respectively. The same growth data was also plotted against the average ABA content between 48 to 60 h and 60 to 72 h. Individual members from each cohort (every 0.5 μg/g dry weight for hypocotyls [n = 28] and 5.0 μg/g dry weight for roots [n = 15]) were pooled to obtain a mean ± SD for each group. Also plotted are the endogenous ABA levels found in hypocotyls (□) and roots (■) in seedlings grown in well-watered (C) or water-deficient (S) vermiculite.

[3] Abbreviations: LP/P, ratio of large (≥hexamer) polysomes to total polysomes, a measure of the rate of ribosome binding to mRNA; P/T, ratio of polysomes to total ribosomes, a measure of the capacity for protein synthesis.

Table III. *Growth, ABA Content, and Size Classes of Polysomes from the Elongating Regions of Soybean Seedlings*

Tissue was harvested 24 h after transfer of plants to well-watered [($\pm$)-ABA application to roots] or low-water potential vermiculite. P/T represents the proportion of total ribosomes aggregated into polysomes and LP/P represents the proportion of polysomes having six or more ribosomes. The ratios P/T and LP/P were determined by comparing the appropriate areas from UV traces of the polysome sucrose gradient profile. Determinations of ABA in the zone of elongation in the hypocotyl or root are the mean $\pm$ SD from three replicates in one experiment. For the non-ABA treatments, polysome status measurements are the mean $\pm$ SD for two measurements from one experiment. Otherwise, the values are mean $\pm$ SD for six measurements (two separate experiments with three replicates each). Lengths of the hypocotyl and root at 48 h were 35.3 $\pm$ 0.5 and 53.8 $\pm$ 2.2 mm, respectively.

Treatment	Δ in Length	ABA	LP/P	P/T
	mm	*$\mu g/g$ dry wt*		
Hypocotyl				
48 h		0.85 $\pm$ 0.07	0.76 $\pm$ 0.01	0.41 $\pm$ 0.04
+24 h well-watered	40.0 $\pm$ 2.7	0.87 $\pm$ 0.22	0.77 $\pm$ 0.06	0.43 $\pm$ 0.02
+24 h water deficit	4.4 $\pm$ 3.8	3.60 $\pm$ 0.34	0.58 $\pm$ 0.03	0.22 $\pm$ 0.01
+24 h 5 $\times$ 10^{-6} M ($\pm$)-ABA	29.2 $\pm$ 1.9	3.30 $\pm$ 0.17	0.82 $\pm$ 0.02	0.48 $\pm$ 0.02
+24 h 10^{-3} M ($\pm$)-ABA	11.5 $\pm$ 4.2	256 $\pm$ 40	0.77 $\pm$ 0.03	0.38 $\pm$ 0.04
Root				
48 h		2.23 $\pm$ 0.45	0.75 $\pm$ 0.01	0.49 $\pm$ 0.05
+24 h well-watered	52.5 $\pm$ 2.7	2.66 $\pm$ 0.81	0.81 $\pm$ 0.06	0.51 $\pm$ 0.01
+24 h water deficit	44.7 $\pm$ 6.2	27.56 $\pm$ 3.21	0.76 $\pm$ 0.01	0.49 $\pm$ 0.01
+24 h 5 $\times$ 10^{-6} M ($\pm$)-ABA	61.5 $\pm$ 2.4	44.35 $\pm$ 3.32	0.82 $\pm$ 0.06	0.55 $\pm$ 0.02
+24 h 10^{-3} M ($\pm$)-ABA	23.5 $\pm$ 6.4	1648 $\pm$ 200	0.75 $\pm$ 0.02	0.41 $\pm$ 0.04

nongrowing mature root (*cf.* Fig. 7, A and B with C and D). Interestingly, this same group of mRNAs (region E) is found in the hypocotyl zone of cell elongation but not in nonelongating regions of the hypocotyl (dividing and mature) (Fig. 6 and ref. 20). Transfer of seedlings to low-water potential vermiculite caused only a few translation products to change in mature root sections (Fig. 7, A and B). Polysomal mRNA translation products from root tips were also altered minimally by water deficit (Fig. 7, C and D). However, translation products in region E and others (marked by diamonds) decreased to some extent in abundance. Treatment with 5 $\times$ 10^{-6} M ABA caused very few changes in root tip mRNA populations (Fig. 7E). However, a higher concentration of ABA induced a fairly large set of RNAs (Fig. 7F; boxes). Many of these same translation products were also induced in the elongation region of the hypocotyl (*cf.* Fig. 7F with Fig. 6D; products identified with boxes and arrows).

DISCUSSION

Soybean Hypocotyls and Roots Respond Differently to Water Deficit

Generally, plants show increased root/shoot growth ratios during conditions where water is limiting (19, 33, 36), including etiolated soybean seedlings grown in vermiculite with water potentials $<$−0.2 MPa (Fig. 1; 22, 24). This response to water deficit is advantageous to plants growing under conditions of limited water availability. A reduction in stem (or leaf) growth, coupled with continued root growth, will result in improved plant water status. In particular, the continued growth of roots in drying soil must occur if water uptake is to be maintained (8). For example, in soybean seedlings transferred to low water potential vermiculite for 48 h, the root/

shoot length ratio increases from 1.1 to 3.0 (Figs. 1 and 3). At this time, water status improves and hypocotyl growth recovers (24, 26).

### Exogenous Abscisic Acid and Water Deficit Cause Similar Changes in Growth

Transfer of soybean seedlings to low-water potential vermiculite induces a transient decrease in cell turgor in the inner cortical cells of the elongating region of the hypocotyl and causes disruption of water potential gradients (24, 26). These changes in water status are correlated with the inhibition of shoot and root growth (24, 26). With time, the water potential of the growing zones declines to a similar extent and turgor pressure is reestablished in these regions (24, 26). However, shoot growth remains inhibited while root growth recovers (24). Thus, changes in water status alone cannot explain the patterns of growth observed in plants exposed to low-water potential, in this case −0.3 MPa. In soybeans, the dissimilar response of roots and shoots does not result from the fact that roots are nearer than hypocotyls to water in the soil (Fig. 2). When seedlings are removed from soil at 100% RH, increases in the root/shoot growth ratio are still observed. These data suggest that biochemical differences exist which contribute to the differential response to water deficit.

Plant growth regulators, such as ABA, could modulate differential inhibition of shoot *versus* root growth (8, 10). Soybean seedlings transferred to low-water potential vermiculite accumulate 5- to 10-fold higher levels of ABA than are found in well-watered seedlings. Similar increases in ABA occurred in all sections of the seedling, indicating that differential accumulation of ABA does not contribute to the observed differences in root *versus* shoot growth. However, note that on a dry weight basis root tips had the highest and
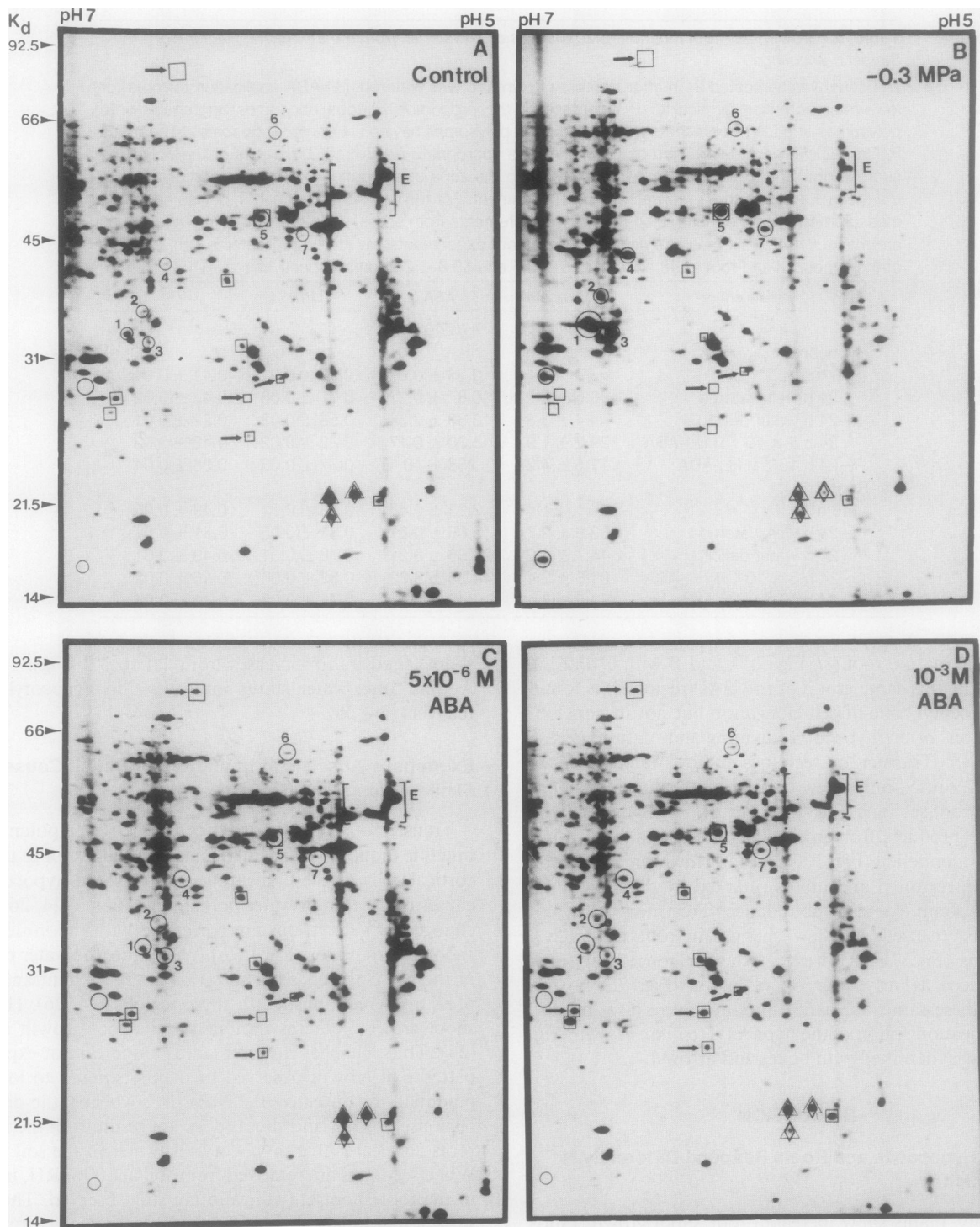
Figure 6. Two-dimensional isoelectric focusing/SDS-PAGE and fluorography of *in vitro* translation products of polysomal mRNA from the elongating region of the hypocotyl. The elongating regions of hypocotyls were harvested 24 h after transfer of 48 h-old seedlings to well-watered vermiculite (A), low-water potential vermiculite (B), well-watered plus 5×10^{-6} M (C), or 10^{-3} M ($\pm$)-ABA (D). The numbers above each flurograph indicate the pH range of the first dimension and the numbers at left indicate the M_r of protein standards (Bio-Rad) in the second dimension. Circles denote areas where proteins in the elongating region of the soybean hypocotyl were induced by transplantation in low-water potential vermiculite (circles with numbers identical to those found in ref. 20). Diamonds denote translation products which decreased in seedlings transplantated into low-water potential vermiculite. Squares indicate translation products induced by treatment with ABA, while triangles indicate translation products reduced by ABA (arrows indicate identical translation products in hypocotyls and root tips). Polysomal mRNA was obtained from the same polysomes analyzed in Table III.
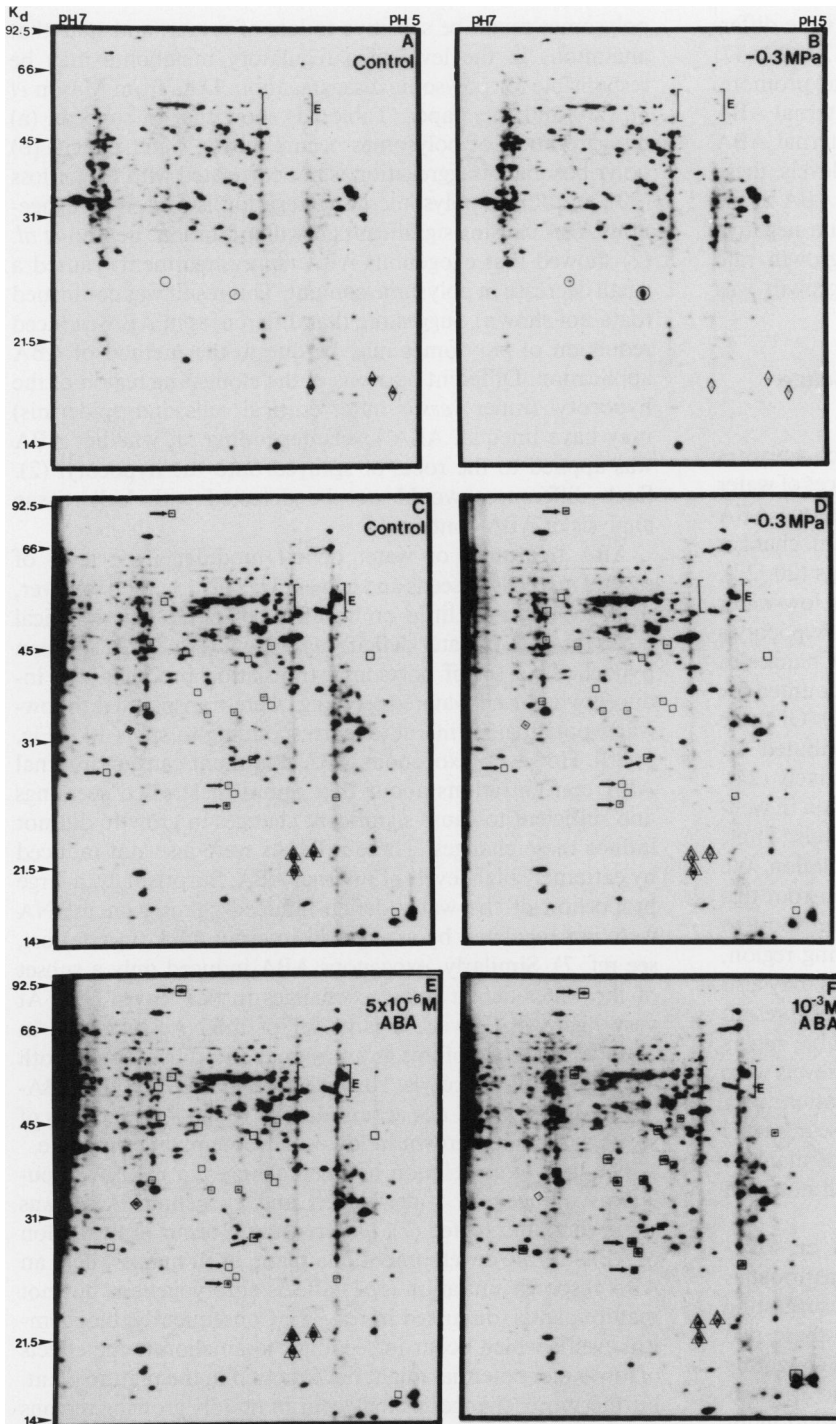
Figure 7. Two-dimensional isoelectric focusing/ SDS-PAGE and fluorography of *in vitro* translation products of polysomal mRNA from the mature root and root tip of soybean roots. These regions were harvested 24 h after transplanting 48-h-old seedlings to well-watered vermiculite (A and C) or low-water potential vermiculite (B and D). Root tips were harvested 24 h after transplanting 48-h-old seedlings to well-watered vermiculite containing 5×10^{-6} M (E) or 10^{-3} M ($\pm$)-ABA (F). Symbols as described in Figure 6.

cotyledons the lowest ABA content. Variation in ABA content could result from differences in dry weight per cell volume and rates of ABA transport and catabolism.

In general, exogenous ABA inhibits shoot growth (2, 4, 35) while not affecting, stimulating, or inhibiting root growth (1, 23, 27, 37). Growth and dry weight accumulation of hypocotyls of soybean seedlings treated with ABA was reduced, but not to the same degree as seedlings grown in low-water potential vermiculite (Table II; Figs. 3 and 4). In this paper

we demonstrate that modulation of the internal ABA content between the limits found in well-watered and low-water potential seedlings can cause significant changes in the rate of hypocotyl elongation (Fig. 4).

Root growth, on the other hand, was not significantly inhibited by internal ABA levels between the endogenous ABA concentrations found in roots of seedlings grown in well-watered or water-deficient vermiculite (Fig. 4). Both the amount of ABA needed for half-maximal inhibition and the

maximum amount of inhibition which occurred were different in roots and hypocotyls. Other workers (1, 23, 27, 37) noted that exogenous ABA may have no effect, may promote, or may inhibit root growth, depending on the external ABA content. However, without knowledge of the internal ABA concentration and the normal endogenous ABA levels, these observations are hard to evaluate. By quantifying ABA in the elongating zone of maize roots, it was shown that a negative correlation existed between ABA content and growth rate (31). However, below a certain concentration, growth was independent of ABA content (31).

Exogenous ABA, Assimilate Partitioning, and Solute Accumulation

Increased ABA content may act as a signal which activates mechanisms designed to counteract the consequences of water deficit. Hence, it was of interest to see if exogenous ABA could cause the previously described biochemical changes occurring during water deficit in soybean seedlings (20, 22). When well-watered seedlings are transferred to low-water potential vermiculite, the zones of elongation in hypocotyls and roots osmotically adjust (Fig. 5; 22, 24, 26). Changes in free amino acids, glucose, fructose, and sucrose accounted for most of the dry matter increase in hypocotyls (22). Of these solutes, soluble sugars and free amino acids contributed 4.4 and 3 μmol/hypocotyl zone of elongation, respectively (24). However, at levels which inhibit hypocotyl elongation in well-watered seedlings, ABA had no effect on sugar levels. Thus, ABA is not implicated as a signal for sugar accumulation. We were able to detect an increase in sugar content in root tips with water deficit, suggesting that soluble sugars contribute to the osmotic adjustment of root tips. In this growing region, other osmotic agents such as organic or amino acids may also be used (24) for osmotic adjustment. Jones et al. (17) present data indicating that wheat roots accumulated soluble sugars when incubated in 5×10^{-6} M ABA. Internal ABA levels were not measured, but at this external ABA concentration, root growth was significantly inhibited. Thus, the increase in sugars observed (17) may result from an accumulation of material which would have been used for growth had growth not been inhibited by ABA.

Continued root growth in low-water potential or ABA-treated seedlings suggests that continued import of assimilates into the root occurred. In fact, dry weight increases, suggesting that more material is transported to roots under these two treatments. Since dry weight accumulation in the elongating region of hypocotyls was reduced over that found in well-watered plants (Fig. 3C), ABA causes a redistribution of assimilates from hypocotyls to roots (18).

Exogenous ABA and Protein Synthesis

Plants exposed to water deficit show decreases in polysome status (LP/P and P/T), especially in the hypocotyl growing region (20). For this reason, the effect of ABA on polysome levels was of special interest. It has been hypothesized that the reductions in polysome status observed during water deficit are a result of reductions in growth, rather than a direct effect of water deficit (15, 32). Scott et al. (32) suggest that

polysomes might be sensitive to loss of turgor, and state that alterations in the level of a regulatory metabolite may be responsible for polysome disaggregation. Data from Mason et al. (20) and this paper (Table III) show that in soybean (a) disaggregation of polysomes occurs during water deficit, (b) polyribosome disaggregation is not correlated with turgor loss (20), and (c) no polysome disaggregation is caused by exogenous ABA causing significant growth inhibition. Bensen et al. (2) showed that exogenous ABA (spray treatment) caused a small decrease in polysome content. This result was confirmed (data not shown), suggesting that differences in ABA-induced reduction of polysomes may be due to the method of ABA application. Different portions of the elongating region of the hypocotyl (inner versus outer cortical cells and epidermis) may have unequal ABA levels depending on whether ABA was applied to the roots or sprayed onto the hypocotyl (2). Such differences would not be detected with bulk tissue analysis of ABA content.

ABA treatment or water deficit modulates the level of several mRNAs in seeds and leaves (3, 7, 12, 13, 34). However, in soybean roots, little or no effect of either physiological levels of ABA or water deficit was evident (Fig. 7). In soybean hypocotyls, a set of polysomal translation products was induced when well-watered seedlings were transplanted to low-water potential vermiculite (corresponding to spots 1–7, Fig. 6; 20). However, exogenous ABA treatment causing internal ABA concentrations about that found in stressed seedlings and sufficient to cause significant changes in growth, did not induce these changes. These mRNAs were also not induced by extremely high levels of internal ABA. Surprisingly, a large proportion of the water deficit-induced changes in mRNA were not regulated by changes in internal ABA (in contrast, see ref. 7). Similarly, exogenous ABA induced only a subset of the water deficit induced changes in pea leaves (12). At very high ABA levels, a large set of mRNAs increased in abundance. Some of this group were induced by ABA in both root tips and hypocotyls. However, the majority of the ABA-induced mRNs did not accumulate in roots or hypocotyls of soybean seedlings grown in low-water potential vermiculite.

The lack of correlation between changes in mRNA populations induced by water deficit and exogenous ABA was somewhat unexpected (7). Differences do occur in the action of ABA during development of a plant, as in maize where an ABA response mutation (vp1) affects embryogenesis but not mature plants (discussed in ref. 38). Consequently, biochemical events which occur in seedlings to ameliorate the effects of low-water potential might not function in the mature plant. Furthermore, changes which occur in rapidly growing regions (such as the growing region of a leaf) may not occur in the mature tissue of that organ. Finally, transfer of soybean seedlings into low-water potential vermiculite may not induce the same responses as a severe dehydration (7, 12).

Speculation on the Mode of ABA Action during Water Deficit

The ability of ABA to cause stomatal closure is well established (28, 38). The action of ABA is rapid, and works by altering H^+/K^+ transport in guard cells (38). However, the large water-deficit-induced accumulation of ABA (levels can

rise 10- to 50-fold) does not appear to be directly involved in stomatal closure. Only a small portion of the ABA which accumulates is needed for closure to occur (38) and closure occurs prior to the large buildup of ABA (14). No function for this 'excess' ABA has been described, but one possibility may be to ameliorate some of the deleterious effects of water deficit. Contrary to the situation seen with stomatal closure, hypocotyl growth rate was incrementally inhibited by ABA concentrations between levels found in well-watered and water-deficient seedlings. In addition, there was no effect on root growth at ABA levels where shoot growth was inhibited. Based on these observations, we propose that one function of water-deficit-induced ABA is to differentially inhibit shoot growth relative to root growth. ABA-induced inhibition of shoot growth does not involve changes in polysome status nor large changes in mRNA populations. Other studies have shown that ABA can act rapidly (within minutes) to inhibit auxin-induced proton secretion and growth (29, 30). ABA may inhibit hypocotyl elongation in water-deficient seedlings in a similar manner. At extremely high internal ABA levels (higher than those observed in water-deficient seedlings), changes in mRNA populations were seen (Figs. 6 and 7). It is possible that this group of ABA-responsive mRNAs are induced under more severe conditions of water deficit, such as that during seed desiccation.

CONCLUSIONS

Rapid changes in water status which lead to reduced cell turgor (16) or loss of water potential gradients (26) will inhibit growth. However, in soybean and barley, localized growing regions are capable of rapid osmotic adjustment and water status alone cannot explain the observed pattern of growth inhibition (21, 26). In the soybean seedling system, increased ABA levels could account for a significant portion of the inhibition observed in hypocotyl elongation. Because ABA can play a significant role in modulating growth, perhaps both water deficit and ABA treatment alter wall properties. Since changes in sugar content, polysome aggregation, and many of the changes in mRNA populations which occur during water deficit are not modulated by ABA, other mechanisms must operate in low-water potential seedlings to modulate these changes. Different mechanisms affecting growth could result from the evolution of modern growth modulating processes (i.e. multicellular plants; ABA responses) in the presence of ancient ones (i.e. unicellular; turgor responses).

LITERATURE CITED

1. **Abou-Mandour AA, Hartung W** (1980) The effect of abscisic acid on growth and development of intact seedlings, root and callus cultures and stem and root segments of *Phaseolus coccineus*. Z Pflanzenphysiol **100**: 25–33
2. **Bensen RJ, Boyer JS, Mullet JE** (1988) Water-deficit-induced changes in abscisic acid, growth, polysomes, and translatable RNA in soybean hypocotyls. Plant Physiol **88**: 289–294
3. **Bewley JD, Larsen KM, Papp JT** (1983) Water-stress-induced changes in the pattern of protein synthesis in maize seedling mesocotyls: a comparison with the effects of heat shock. J Exp Bot **34**: 1126–1133
4. **Biddington NL, Dearman AS** (1982) The effect of abscisic acid on root and shoot growth of cauliflower plants. Plant Growth Regul **1**: 15–24
5. **Boyer JS, Knipling EB** (1965) Isopiestic technique for measuring leaf water potentials with a thermocouple psychrometer. Proc Natl Acad Sci USA **54**: 1044–1051
6. **Bradford KC, Hsiao TC** (1982) Physiological responses to moderate water stress. *In* OL Lange, PS Nobel, CB Osmond, H Ziegler, eds, Encyclopedia of Plant Physiology (New Series), Vol 12B. Physiological Plant Ecology II. Springer-Verlag, Berlin, pp 263–324
7. **Bray EA** (1988) Drought- and ABA-induced changes in polypeptide and mRNA accumulation in tomato leaves. Plant Physiol **88**: 1210–1214
8. **Caldwell MM** (1976) Root extension and water absorption. *In* OL Lange, L Kappen, E-D Schulze, eds, Water and Plant Life. Ecological Studies, Vol 19. Springer-Verlag, Berlin, pp 63–85
9. **Cavalieri AJ, Boyer JS** (1982) Water potentials induced by growth in soybean hypocotyls. Plant Physiol **69**: 492–496
10. **Davies WJ, Metcalfe J, Lodge TA, da Costa AR** (1986) Plant growth substances and the regulation of growth under drought. Aust J Plant Physiol **13**: 105–125
11. **Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F** (1956) Colorimetric method for determination of sugars and related substances. Anal Chem **28**: 350–356
12. **Guerrero FD, Mullet JE** (1988) Reduction of turgor induces rapid changes in leaf translatable RNA. Plant Physiol **88**: 401–408
13. **Heikkila JJ, Papp JET, Schultz GA, Bewley JD** (1984) Induction of heat shock protein messenger RNA in maize mesocotyls by water stress, abscisic acid and wounding. Plant Physiol **76**: 270–274
14. **Henson IE** (1981) Changes in abscisic acid content during stomatal closure in pearl millet (*Pennisetum americanum* [L.]) Leeke. Plant Sci Lett **21**: 121–127
15. **Hsiao TC** (1970) Rapid changes in levels of polyribosomes in *Zea mays* in response to water stress. Plant Physiol **46**: 281–285
16. **Hsiao TC, Jing J** (1987) Leaf and root expansive growth in response to water deficits. *In* DJ Cosgrove, DP Knievel, eds, Physiology of Cell Expansion During Plant Growth. American Society of Plant Physiologists, Rockville, MD, pp 180–192
17. **Jones H, Leigh RA, Tomas AD, Wyn Jones RG** (1987) The effect of abscisic acid on cell turgor pressures, solute content and growth of wheat roots. Planta **170**: 257–262
18. **Karmoker JL, van Steveninck RFM** (1979) The effect of abscisic acid on sugar levels of *Phaseolus vulgaris* L. cv Redland Pioneer. Planta **146**: 25–30
19. **Kramer PJ** (1983) Water relations of plants. Academic Press, New York
20. **Mason HS, Mullet JE, Boyer JS** (1988) Polysomes, messenger RNA, and growth in soybean stems during development and water deficit. Plant Physiol **86**: 725–733
21. **Matsuda K, Riazi A** (1981) Stress-induced osmotic adjustment in the growing regions of barley leaves. Plant Physiol **68**: 571–576
22. **Meyer RF, Boyer JS** (1981) Osmoregulation, solute distribution, and growth in soybean seedlings having low-water potentials. Planta **151**: 482–489
23. **Mulkey TJ, Evans ML, Kuzmanoff KM** (1983) The kinetics of abscisic acid action on root growth and gravitropism. Planta **157**: 150–157
24. **Nonami H** (1986) Mechanism of cell enlargement at low-water potentials. PhD thesis, University of Illinois, Urbana-Champaign
25. **Nonami H, Boyer JS, Steudle E** (1987) Pressure probe and isopiestic psychrometer measure similar turgor. Plant Physiol **83**: 592–595
26. **Nonami H, Boyer JS** (1989) Turgor and growth at low-water potentials. Plant Physiol **89**: 798–804
27. **Pilet PE, Rebeaud JE** (1983) Effect of abscisic acid on growth and indolyl-3-acetic acid levels in maize roots. Plant Sci Lett **31**: 117–122
28. **Raschke K** (1987) Action of abscisic acid on guard cells. *In* E

Zeiger, GD Farquhar, IR Cowan, eds, Stomatal Function. Stanford University Press, Palo Alto, CA, pp 253–279

29. **Reed NR, Bonner BA** (1974) The effect of abscisic acid on the uptake of potassium and chloride into *Avena* coleoptile sections. Planta **116:** 173–185

30. **Rehm MM, Cline MG** (1973) Rapid growth inhibition of *Avena* segments by abscisic acid. Plant Physiol **51:** 93–96

31. **Reymond F, Saugy M, Pilet PE** (1987) Quantification of abscisic acid in a single maize root. Plant Physiol **85:** 8–9

32. **Scott NS, Munns R, Barlow EWR** (1979) Polyribosome content in young and aged wheat leaves subjected to drought. J Exp Bot **30:** 905–911

33. **Sharp RE, Davies WJ** (1979) Solute regulation and growth by roots and shoots of water-stressed maize plants. Planta **147:** 43–49

34. **Singh NK, Larosa PC, Handa AK, Hasegawa FM, Bressan R** (1987) Hormonal regulation of protein synthesis associated with salt tolerance in plant cells. Proc Natl Acad Sci USA **84:** 739–743

35. **Watts S, Rodriguez JL, Evans SE, Davies WJ** (1981) Root and shoot growth of plants treated with abscisic acid. Ann Bot **47:** 595–602

36. **Westgate ME, Boyer JS** (1985) Osmotic adjustment and the inhibition of leaf, root, stem and silk growth at low-water potentials in maize. Planta **164:** 540–549

37. **Yamaguchi T, Street HE** (1977) Stimulation of the growth of excised cultured roots of soya bean by abscisic acid. Ann Bot **41:** 1129–1133

38. **Zeevaart JAD, Creelman RA** (1988) Metabolism and physiology of abscisic acid. Annu Rev Plant Physiol Plant Mol Biol **39:** 439–473