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Effect of Inhibition of Abscisic Acid Accumulation on the Spatial Distribution of Elongation in the Primary Root and Mesocotyl of Maize at Low Water Potentials[1]

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ABSTRACT

Previous work showed that accumulation of endogenous abscisic acid (ABA) acts both to maintain primary root growth and inhibit shoot growth in maize seedlings at low water potentials (ψ_w) (IN Saab, RE Sharp, J Pritchard, GS Voetberg [1990] Plant Physiol 93: 1329–1336). In this study, we have characterized the growth responses of the primary root and mesocotyl of maize (*Zea mays* L. cv FR27 × FRMo17) to manipulation of ABA levels at low ψ_w with a high degree of spatial resolution to provide the basis for studies of the mechanism(s) of ABA action. In seedlings growing at low ψ_w and treated with fluridone to inhibit carotenoid (and ABA) biosynthesis, ABA levels were decreased in all locations of the root and mesocotyl growing zones compared with untreated seedlings growing at the same ψ_w. In the root, low ψ_w (−1.6 megapascals) caused a shortening of the growing zone, as reported previously. The fluridone treatment was associated with severe inhibition of root elongation rate, which resulted from further shortening of the growing zone. In the mesocotyl, low ψ_w (−0.3 megapascal) also resulted in a shortened growing zone. In contrast with the primary root, however, fluridone treatment prevented most of the inhibition of elongation and the shortening of the growing zone. Final cell length measurements indicated that the responses of both root and mesocotyl elongation to ABA manipulation at low ψ_w involve large effects on cell expansion. Measurements of the relative changes in root and shoot water contents and dry weights after transplanting to a ψ_w of −0.3 megapascal showed that the maintenance of shoot elongation in fluridone-treated seedlings was not attributable to increased water or seed-reserve availability resulting from inhibition of root growth. The results suggest a developmental gradient in tissue responsiveness to endogenous ABA in both the root and mesocotyl growing zones. In the root, the capacity for ABA to protect cell expansion at low ψ_w appears to decrease with increasing distance from the apex. In the mesocotyl, in contrast, the accumulation of ABA at low ψ_w appears to become increasingly inhibitory to expansion as cells are displaced away from the meristematic region.

The primary root of maize continues to grow at low ψ_w[3] that completely inhibit shoot growth (28). We recently provided evidence that endogenous ABA, which accumulates to high concentrations in maize seedlings at low ψ_w, functions both to maintain primary root growth and inhibit shoot growth under these conditions (24). Our approach was to inhibit ABA accumulation at low ψ_w using two methods: a mutant deficient in carotenoid (and ABA) biosynthesis (vp 5), and FLU, an inhibitor of carotenoid (and ABA) biosynthesis. Results obtained with the two methods were very similar, and showed that inhibition of ABA accumulation at low ψ_w was associated with severe inhibition of primary root elongation and promotion of shoot elongation compared with untreated seedlings growing at the same ψ_w.

It is becoming increasingly evident that there can be large differences in the growth response to low ψ_w at different positions within the growing zone of an organ. In the maize primary root, low ψ_w that cause complete inhibition of elongation in the basal region of the growing zone have no effect on elongation near the apex, resulting in a shortened growth zone (28, 30). Differential growth sensitivity to low ψ_w treatments has also been observed within growing zones of wheat seminal roots (17), soybean hypocotyls (16), and grape shoots (26). It has also been demonstrated that the growth response to applied hormones can vary greatly at different positions within growing zones (7, 10–13).

Therefore, to understand further the role of ABA in regulating root and shoot growth responses to low ψ_w, spatial gradients in ABA concentration as well as in the cells' capacity to respond to the hormone must be considered. Developmental variations in responsiveness that may occur as cells are displaced away from the meristems will obviously be missed if the organs or growing zones are examined as a whole. Our objectives in this paper were to determine (a) the spatial distribution of ABA in the growing zones of the primary root and mesocotyl of maize at low ψ_w with and without treatment with FLU, and (b) the spatial distribution of elongation rate within the organs to identify locations that exhibit a growth response to decreased ABA content at low ψ_w.

MATERIALS AND METHODS

Seeds of maize (*Zea mays* L. cv FR27 × FRMo17) were germinated in moist vermiculite, transplanted into vermiculite of either high or low water content contained in Plexiglas boxes, and grown in the dark at 29°C and near-saturation humidity, as previously described (28). For the FLU treat-

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[3] Abbreviations: ψ_w, water potential(s); FLU, fluridone.

ments, FLU was added at a concentration of 10 μM to the water mixed with the vermiculite at planting and at transplanting (24). Experiments were performed on the primary root and mesocotyl. For each organ, there were four treatments: high ψ_w; high ψ_w +FLU; low ψ_w; and low ψ_w +FLU. Transplanting times and vermiculite ψ_w were different for the two organs, as detailed below.

Primary Root Experiments

Thirty-six hours after planting, seedlings were transplanted into vermiculite having a ψ_w of approximately −0.03 MPa (high ψ_w) or −1.6 MPa (low ψ_w). For the low ψ_w treatment, experimental measurements were made 48 h after transplanting, at which time the primary root was approximately 50 mm long. Previous studies showed that root elongation rate (24, 28), osmotic potential (27), and turgor (30) were constant by this time. For the high ψ_w treatments, measurements were made 20 h after transplanting, when the primary root was also approximately 50 mm long.

The low ψ_w +FLU treatment was performed using two protocols. In the first, termed low ψ_w +FLU [time], seedlings were transplanted and experimental measurements were made at the same times as in the low ψ_w treatment. This protocol was used in our previous paper on the effects of FLU on root growth at low ψ_w (24). Because the FLU treatment caused roots to grow much more slowly, however, root lengths at 48 h after transplanting were considerably less than in the low ψ_w treatment. Therefore, we added another protocol, termed low ψ_w +FLU [development], in which seedlings were transplanted to low ψ_w vermiculite at 48 h instead of 36 h after transplanting. In this protocol, the roots were longer at transplanting (31 mm compared with 5 mm), but grew to the same length as in the other treatments (approximately 50 mm) by 48 h after transplanting. The two protocols were essential for this treatment because the root growth response to ABA manipulation at low ψ_w might vary with stage of development as well as with the duration of low ψ_w exposure.

Mesocotyl Experiments

Sixty hours after planting, seedlings were transplanted into vermiculite having a ψ_w of approximately −0.03 MPa (high ψ_w) or −0.3 MPa (low ψ_w). Experimental measurements were made 5 h after transplanting, until which time the FLU treatment prevented most of the inhibition of shoot elongation caused by transplanting to low ψ_w (24).

Spatial Distribution of Elongation

For the primary root, the spatial distribution of elongation was determined by time-lapse photographic analysis of marked roots using slight modifications of the procedures described by Silk *et al.* (29) and Sharp *et al.* (28). In brief, the Plexiglas face against which the roots were growing was removed, and the apical 20 mm of all the visible roots were marked at approximately 0.5-mm intervals with an ultra-fine ballpoint pen under a dim blue-green light (maximum transmission: 512 nm, range: 460–560 nm). After 1 h, a series of five photographs was taken at 15-min intervals using a blue-green flash (optical characteristics as above). Enlarged prints were made of the apical 15 mm of roots that (a) elongated within ±15% of the mean rate for that treatment, and (b) showed minimal inhibition of elongation rate by the marking procedure (28). Computer-assisted digitization of mark displacement on each series of prints provided longitudinal displacement velocities that were interpolated to equally spaced positions, 0.5-mm apart, using cubic splines. Erickson's five-point differentiating formula was applied to the interpolated velocity values to provide the spatial distribution of relative elemental elongation rate (6). For the low ψ_w +FLU treatment, Erickson's three-point differentiating formula (6) was also applied because the displacement velocity changed considerably over small distances near the root apex.

For the mesocotyl, procedures were similar to those used for the primary root. Because mesocotyl elongation is very sensitive to inhibition by dim light, however (15, 25), a relatively fast film (ASA 100) and wide lens-aperture combination was used to reduce flash intensity to a minimum. As a result, mesocotyl elongation rate was unaffected and no bending of the shoots was observed during the photographic procedure.

Cell Length Profiles

Cell lengths were measured in the growing zones of the primary root and mesocotyl to evaluate the effects of the low ψ_w and low ψ_w +FLU treatments on longitudinal cell expansion. For the primary root, fresh longitudinal sections, consisting of the outer few cortical layers and the epidermis, were cut by hand using a razor blade and examined under a microscope. For each root, lengths of 6 to 20 cells were measured at various distances from the root apex using a graticule. (No distinction was made between cortical and epidermal cells, although the samples were dominated by cortical cells.) For the mesocotyl, the final length of cortical cells in each treatment was determined by measuring cell lengths at the end of the growing zone (determined from the spatial distribution of relative elemental elongation rate). For each mesocotyl, lengths of 10 to 12 cells were measured in the third to seventh file from the epidermis. Values are presented in the text as means ± SD of five mesocotyls.

Spatial Distribution of ABA Content

ABA content was determined in 1-mm serial segments of the primary root and mesocotyl growing zones by radioimmunoassay (18). Roots and shoots were selected for uniformity of elongation rate (within ±20% of the mean rate for that treatment), and were sectioned using a cutter block containing razor blades spaced 1 mm apart. Roots were sectioned beginning 0.5 mm from the apex, and mesocotyls were sectioned beginning at the coleoptilar node. Sample size (roots and mesocotyls) ranged from 20 (low ψ_w) to 40 segments (high ψ_w). Root cap samples comprised 20 0.5-mm apical segments. Sectioning was conducted under a blue-green safelight at near-saturation humidity, and samples were immediately weighed, frozen in liquid nitrogen, and freeze-dried. Extraction procedures and assay validation were described in Saab *et al.* (24).

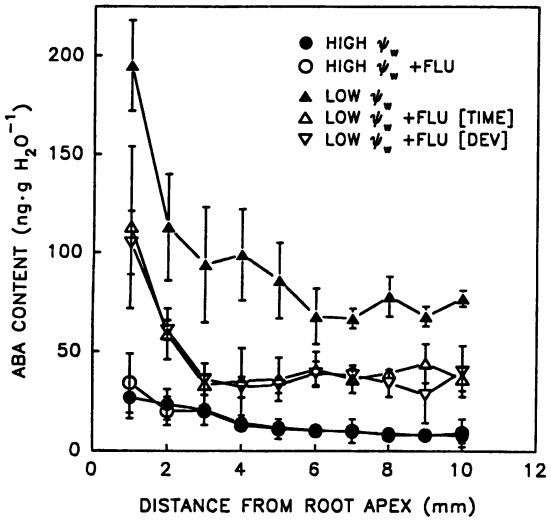

Figure 1. Effect of FLU on the spatial distribution of ABA content in the apical 10 mm of primary roots growing at high ψ_w (−0.03 MPa) or low ψ_w (−1.6 MPa) in vermiculite. The low ψ_w +FLU treatment was performed using time ([TIME]) and development ([DEV]) protocols (see "Materials and Methods"). Data are means ± SD of three to four measurements, each comprising 20 to 40 1-mm segments.

Changes in Root and Shoot Water and Dry Weights after Transplanting

Seedlings were treated as described above for mesocotyl experiments (transplanted at 60 h into vermiculite at a ψ_w of either −0.03 or −0.3 MPa). Immediately before and 5 h after transplanting, five to eight samples of five to eight seedlings each were harvested per treatment. Roots and shoots were excised at the seed junction and weighed before and after oven-drying at 70°C to constant weight. At the same times, additional seedlings were sampled for measurements of mesocotyl ψ_w by isopiestic thermocouple psychrometry (4). The ψ_w was measured in 8-mm sections of the mature region adjacent to the growing zone (starting at 12 mm from the coleoptilar node). The experiment was repeated twice.

RESULTS

Effect of Low ψ_w and FLU on the Spatial Distribution of ABA Content in the Primary Root

At high ψ_w, ABA was present in all locations of the root tip (apical 10 mm), with somewhat higher contents in the apical 3 mm (Fig. 1). FLU had little effect on ABA contents at high ψ_w. The low ψ_w treatment caused a rise in ABA content in all locations, especially toward the apex. The apical 1-mm segment (0.5–1.5 mm from the apex) contained the highest content of ABA (195 ng g^{-1} H_2O); however, this segment may have included a portion of the root cap, which contained an even higher ABA content. (Root cap ABA contents were: high ψ_w, 36 ± 10 ng g^{-1} H_2O; low ψ_w, 349 ± 195 ng g^{-1} H_2O; values are means ± SD of three to four measurements).

In the low ψ_w +FLU treatment (both time and development protocols), ABA content was decreased by approximately 50% in all locations of the apical 10 mm compared with the low ψ_w treatment. The whole profile was very similar in the two protocols.

Effect of Low ψ_w and FLU on the Spatial Distribution of Elongation in the Primary Root

The low ψ_w treatment decreased root elongation rate by 53% compared with the high ψ_w treatment. The low ψ_w +FLU treatment decreased root elongation rate by 86 and 84% for the time and development protocols, respectively, compared with the high ψ_w +FLU treatment. Treatment with FLU at high ψ_w caused only a 13% decrease in root elongation rate. (Root elongation rates are given in the legend to Fig. 2.) These results are consistent with those reported previously (24).

At high ψ_w, the primary root growing zone comprised the apical 11.5 mm, with the maximum relative elemental elongation rate occurring at 4 to 5 mm from the apex (Fig. 2). Treatment with FLU caused a small decrease in elongation rates beyond 3 mm. The low ψ_w treatment caused complete inhibition of elongation beyond 6.5 mm from the apex, but had no effect in the apical 2.5 mm (Fig. 2); these data are similar to those reported previously (28, 30). In the low ψ_w +FLU treatment (both time and development protocols), elongation rates were further inhibited in basal regions, so that the growing zone comprised only the apical 4 mm. In the apical 1 mm, however, elongation rates appeared to be unaffected by the FLU treatment. Because of the potential errors in quantifying the distribution of elongation rates over such a small region, we attempted to verify this result using two approaches.

First, the distribution of relative elemental elongation rate

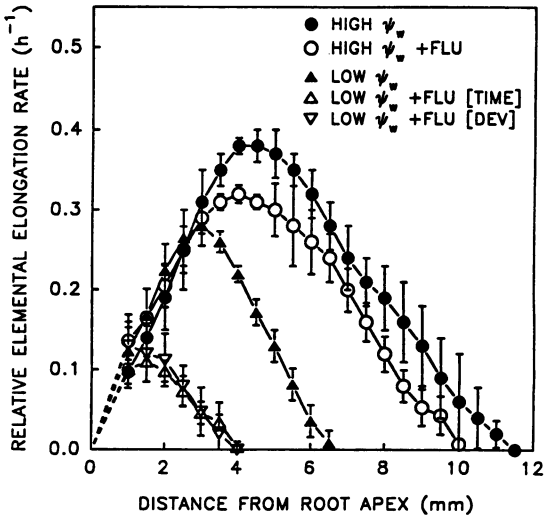

Figure 2. Effect of FLU on the spatial distribution of relative elemental elongation rate in the apical 12 mm of primary roots growing at high ψ_w (−0.03 MPa) or low ψ_w (−1.6 MPa) in vermiculite. The low ψ_w +FLU treatment was performed using time ([TIME]) and development ([DEV]) protocols (see "Materials and Methods"). Data are means ± SD (n = 3–4). Root elongation rates during the measurements were (± SD): high ψ_w, 2.35 ± 0.17 mm h^{-1}; high ψ_w +FLU, 2.05 ± 0.15 mm h^{-1}; low ψ_w, 1.10 ± 0.10 mm h^{-1}; low ψ_w +FLU [time], 0.29 ± 0.01 mm h^{-1}, low ψ_w +FLU [development], 0.32 ± 0.06 mm h^{-1}.

in the low ψ_w +FLU treatment (both protocols) was recalculated using a three-point differentiating formula (6) instead of the five-point formula used for the profiles shown in Figure 2. Because these formulae were applied to values of displacement velocity interpolated to 0.5-mm intervals, the three-point formula allowed elongation rates to be derived over 1-mm instead of 2-mm distances, which is potentially more accurate when elongation rates change over small distances. Results of the two methods were very similar, however, and are shown for the five-point formula only.

Second, we have provided the displacement velocity profiles from which the elongation rate distributions were derived (Fig. 3). Values are shown only from 0 to 6.5 mm from the apex to increase the resolution in the apical region. These data show that the increase in displacement velocity with distance was very similar between treatments up to 1.5 mm from the apex. Taken together, the results of Figures 1 through 3 indicate that longitudinal expansion close to the root apex is unaffected by low ψ_w and by the added effect of decreased ABA content.

We examined the profiles of cortical cell lengths in the primary root to determine the extent to which effects of the low ψ_w and low ψ_w +FLU treatments on root elongation were associated with inhibited cell expansion. Final cell length was achieved 12 mm from the apex in the high ψ_w treatment, 6 mm in the low ψ_w treatment, and 4 mm in the low ψ_w +FLU [time] treatment (Fig. 4), in agreement with the results of photographic determination of length of the growing zone (Fig. 2). In this experiment, the low ψ_w treatment decreased root elongation rate by 67% compared with the high ψ_w treatment (Fig. 4, elongation rates in legend). Final cell length was reduced by 54%, indicating that much of the inhibition

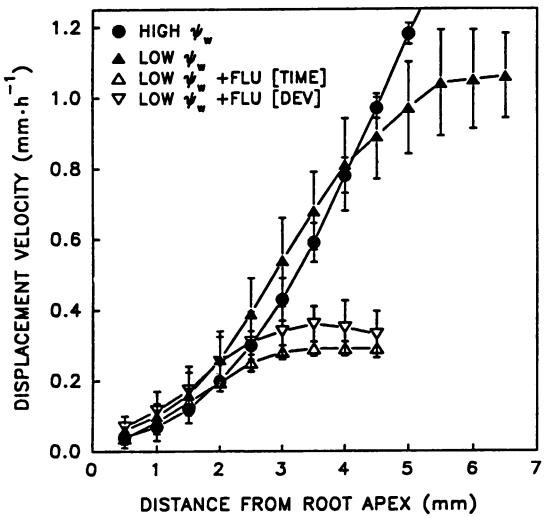

Figure 3. Effect of low ψ_w (−1.6 MPa) and FLU on the spatial distribution of displacement velocity in the apical 7 mm of primary roots, compared with untreated roots at high ψ_w (−0.03 MPa). The low ψ_w +FLU treatment was performed using time ([TIME]) and development ([DEV]) protocols (see "Materials and Methods"). Data are means ± SD from three to four roots. Differentiation of displacement velocity with respect to position for each root gave the relative elemental elongation rate distributions shown in Figure 2.

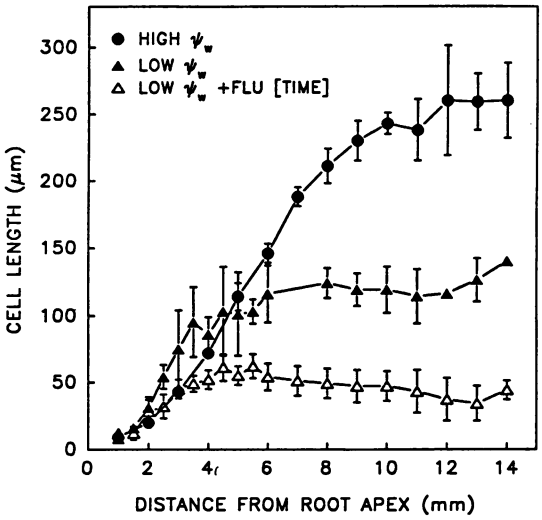

Figure 4. Effect of low ψ_w (−1.6 MPa) and FLU on the spatial distribution of cortical cell length in the apical 14 mm of primary roots, compared with untreated roots at high ψ_w (−0.03 MPa). The low ψ_w +FLU treatment was performed using the time ([TIME]) protocol (see "Materials and Methods"). Data are means ± SD of 6 to 12 roots. For each root, the lengths of 6 to 20 cells were averaged at each position. Root elongation rates over the 10 h prior to sampling were (± SD): high ψ_w, 2.81 ± 0.56 mm h^{-1}; low ψ_w, 0.92 ± 0.26 mm h^{-1}; low ψ_w +FLU [time], 0.27 ± 0.04 mm h^{-1}.

of root elongation rate at low ψ_w was accounted for by inhibition of cell expansion. In the low ψ_w +FLU [time] treatment, root elongation rate and final cell length were decreased by 71 and 41%, respectively, compared with the low ψ_w treatment, indicating that inhibition of ABA accumulation was associated with further inhibition of cell expansion, and probably also inhibition of rate of cell production.

Because the roots in the high ψ_w and low ψ_w treatments were growing under steady-state (time-invariant) conditions (27, 28, 30), the effect of low ψ_w on cell production rate could be estimated by dividing the root elongation rate by final cell length (9). The rate of cell production at low ψ_w was reduced by only 30% (7.6 cells h^{-1} at low ψ_w *versus* 10.8 cells h^{-1} at high ψ_w). This result confirms that, under our conditions, cell expansion in the maize primary root was comparatively more sensitive to low ψ_w than the rate of cell production. This differs from the findings of Fraser *et al.* (9), who observed a greater inhibition of the rate of cell production than cell expansion in a similar experiment with a different variety.

Cell production rate could not be estimated accurately for the low ψ_w +FLU treatment because of the gradual increase in root elongation rate (24) and final cell length (Fig. 4) after transplanting. Under conditions of nonsteady growth rate, treatment effects on cell production are not necessarily reflected in the concurrent final cell length.

Effect of Low ψ_w and FLU on the Spatial Distribution of ABA Content in the Mesocotyl

In the high ψ_w and high ψ_w +FLU treatments, ABA was present in all locations of the apical 10 mm, with slightly

higher contents found near the coleoptilar node (Fig. 5). The low ψ_w treatment induced a rise in ABA content in all locations, with a trend of increasing content toward the coleoptilar node. In the low ψ_w +FLU treatment, the rise in ABA content was largely prevented in all locations.

Effect of Low ψ_w and FLU on the Spatial Distribution of Elongation in the Mesocotyl

The low ψ_w treatment decreased mesocotyl elongation rate by 50% compared with the high ψ_w treatment (Fig. 6, legend). Treatment with FLU at low ψ_w increased elongation rate to that of the high ψ_w +FLU treatment. The mesocotyl growing zone at high ψ_w comprised the uppermost 11.5 mm of the mesocotyl, with maximum elongation rates occurring at 3 to 6 mm from the coleoptilar node. Treatment with FLU at high ψ_w had little effect on this profile (Fig. 6). In the low ψ_w treatment, elongation was completely inhibited in the basal 3.5 mm, resulting in a shortened growing zone of about 8 mm in length. Unlike the case with the primary root, however, elongation rates were reduced in the apical as well as basal regions. In the low ψ_w +FLU treatment, elongation rates were similar to those of the high ψ_w +FLU treatment in all locations. Thus, FLU prevented most of the shortening of the growing zone caused by the low ψ_w treatment.

Much of the inhibition of mesocotyl elongation rate at low ψ_w was accounted for by inhibition of cell expansion. The low ψ_w treatment decreased the final length of cortical cells by 36% compared with the high ψ_w treatment (low ψ_w, 80 ± 7 μm; high ψ_w, 125 ± 7 μm). In the low ψ_w +FLU treatment, the inhibition of final cell length was prevented (low ψ_w +FLU, 122 ± 6 μm; high ψ_w +FLU, 129 ± 9 μm). Thus, inhibition of ABA accumulation at low ψ_w was associated with maintenance of mesocotyl cell expansion.

Effects of low ψ_w and FLU on the rate of cell production in the mesocotyl could not be estimated. Because the entire mesocotyl was elongating at 0.5 to 1 mm h^{-1}, depending on treatment, any effects on cell production rate (*i.e.* while the cells are in the meristematic region adjacent to the coleoptilar node) will not be reflected in the cells present at the end of the growing zone (8–12 mm from the coleoptilar node) after only 5 h.

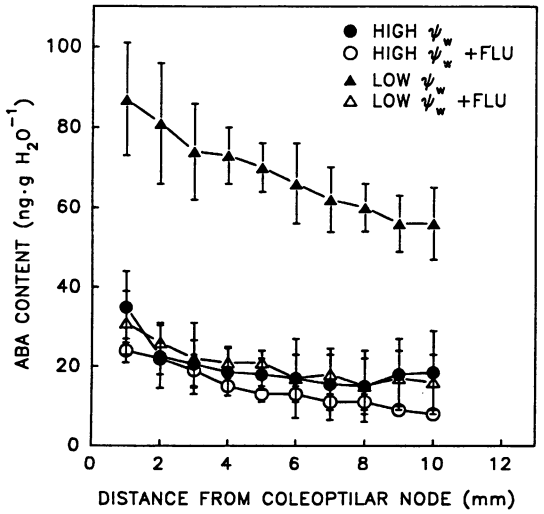

Figure 5. Effect of FLU on the spatial distribution of ABA content in the apical 10 mm of the mesocotyl of seedlings growing at high ψ_w (−0.03 MPa) or low ψ_w (−0.3 MPa) in vermiculite. Data are means ± SD of three measurements, each comprising 20 to 40 1-mm segments.

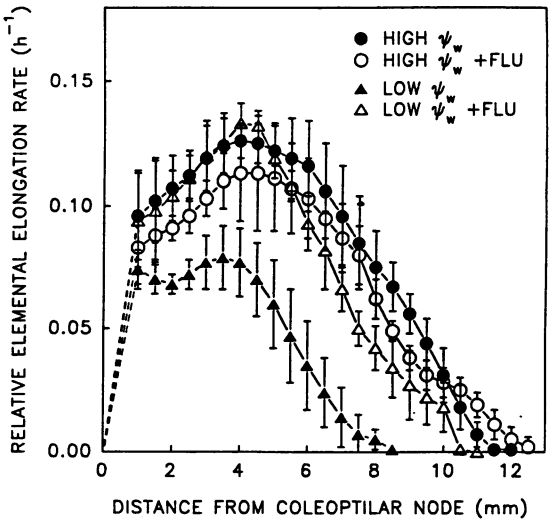

Figure 6. Effect of FLU on the spatial distribution of relative elemental elongation rate in the apical 13 mm of the mesocotyl of seedlings growing at high ψ_w (−0.03 MPa) or low ψ_w (−0.3 MPa) in vermiculite. Data are means ± SD (n = 3–4). Mesocotyl elongation rates during the measurements were (± SD): high ψ_w, 1.00 ± 0.10 mm h^{-1}; high ψ_w +FLU, 0.90 ± 0.09 mm h^{-1}, low ψ_w, 0.50 ± 0.10 mm h^{-1}; low ψ_w +FLU, 0.90 ± 0.10 mm h^{-1}.

Effect of Low ψ_w and FLU on the Competition for Water and Dry Weight between Roots and Shoot

It is important for this and future studies to document that the observed growth responses in the primary root and mesocotyl are direct effects of the low ψ_w and FLU treatments on the organ in question, rather than indirect responses resulting from growth inhibition or promotion elsewhere in the seedling. For the primary root, this condition is met: experiments were performed 48 h after transplanting to a low ψ_w of −1.6 MPa, at which shoot growth is completely inhibited (28). Thus, observed responses of root elongation to the low ψ_w and low ψ_w + FLU treatments are clearly independent of effects on shoot growth (24).

Mesocotyl experiments were performed 5 h after transplanting to a low ψ_w of −0.3 MPa. This time was chosen because treatment with FLU prevented nearly all the growth inhibition caused by low ψ_w until this time (24), suggesting that increased levels of endogenous ABA were responsible for most of the inhibition of shoot growth. Results of longer-term experiments, however, indicated that FLU treatment at a ψ_w of −0.3 MPa also caused a decrease in primary root growth concomitant with the maintenance of shoot growth (data not shown). It could be argued, therefore, that the maintenance of mesocotyl elongation by FLU at 5 h after transplanting to low ψ_w

might be caused indirectly by increased availability of water and/or seed reserves due to the inhibition of root growth. To test this possibility, we measured the relative changes in water content and dry weight of the roots and shoot during the first 5 h after transplanting to a ψ_w of −0.3 MPa, with or without FLU (Table I). Changes in seed water content and dry weight were also measured, but these data lacked adequate resolution due to the large variability in seed size and the short duration of the experiments.

The roots lost water during the first 5 h after transplanting to low ψ_w, while shoot water content was unchanged. In the low ψ_w +FLU treatment, the roots lost about the same amount of water, whereas the shoot, in contrast, gained considerable water during the same period. Water uptake by the shoot was approximately three times the amount lost by the roots. Dry weight analyses in the same experiments showed that the low ψ_w +FLU treatment doubled the increase in shoot dry weight while having little effect on the gain in root dry weight compared with the low ψ_w treatment. The results indicate that the promotion of shoot growth at low ψ_w by FLU cannot be explained by increased availability of water or seed reserves due to the inhibition of root growth.

The difference in shoot water uptake between the low ψ_w and low ψ_w +FLU treatments during the first 5 h after transplanting raises the possibility that the rate of development of water deficit in the shoot may have been different in the two treatments. Ideally, comparisons of shoot growth responses to low ψ_w with and without treatment with FLU should be made at the same tissue water status. Thus, we measured the ψ_w of mature tissues adjacent to the mesocotyl growing zone immediately before and 5 h after transplanting in the two treatments (mature tissues were chosen to avoid possible errors associated with psychrometric measurements of ψ_w in excised, growing tissues [5]). These measurements showed unexpectedly low ψ_w values (around −0.3 MPa) before transplanting and, curiously, no decrease in ψ_w in either treatment 5 h after transplanting to vermiculite at −0.3 MPa. The low ψ_w values before transplanting may be a result of high concentrations of apoplastic solutes (5); however, explanation of these results requires further investigation.

Summary of Results

Results of experiments with the primary root indicate that (a) low ψ_w caused a rise in ABA content in all locations of the growing zone while elongation was maintained selectively in apical regions, (b) inhibition of ABA accumulation in the growing zone following treatment with FLU at low ψ_w was associated with further inhibition of elongation in basal regions, and (c) low ψ_w and decreased ABA content affected both cell expansion and the rate of cell production.

For the mesocotyl, results indicate that (a) low ψ_w caused a rise in ABA content and an inhibition of elongation in all regions of the growing zone, (b) inhibition of ABA accumulation following treatment with FLU at low ψ_w was associated with maintenance of elongation in all regions and thereby prevention of shortening of the growing zone, and (c) the effects of low ψ_w and decreased ABA content were exerted largely on cell expansion.

Table I. *Effect of FLU on the Changes in Water Content and Dry Weight of the Roots and Shoot of Maize Seedlings during the First 5 h after Transplanting to Low ψ_w (−0.3 MPa)*

Values are per seedling and are means ± SE from three experiments.

	Change in Water Content		Change in Dry Weight	
	−FLU	+FLU	−FLU	+FLU
	mg			
Roots	−6.9 ± 4.1	−6.7 ± 3.9	+3.1 ± 0.2	+3.3 ± 0.1
Shoot	+0.6 ± 1.4	+17.6 ± 3.4	+1.5 ± 0.3	+2.8 ± 0.5

DISCUSSION

We have examined with a high degree of spatial resolution the involvement of increased levels of endogenous ABA in the maintenance of primary root growth and inhibition of mesocotyl growth in maize seedlings at low ψ_w. By comparing the spatial distributions of elongation rate and ABA content with or without treatment with FLU to inhibit ABA accumulation, we have identified those locations that exhibit a growth response to decreased ABA content at low ψ_w. The results provide evidence for gradients in tissue responsiveness to endogenous ABA within both growth zones.

In the primary root, the analysis indicates the existence of four regions within the growing zone in terms of response to low ψ_w and the accumulation of endogenous ABA: (a) in the basal 6.5 to 11.5 mm, elongation was completely inhibited by low ψ_w despite the presence of high concentrations of ABA; (b) in the basal 4 to 6 mm, some elongation was maintained at low ψ_w but was entirely dependent on the accumulation of ABA; (c) in the apical 2 to 3 mm, elongation was fully maintained at low ψ_w, but only in the presence of high levels of ABA; and (d) in the apical 1.5 mm, elongation was unaffected by low ψ_w and the added effect of decreased ABA content. These results point to a developmental gradient in the response of cell expansion to endogenous ABA at low ψ_w, whereby the capacity for ABA to protect cell expansion declines as the cells are displaced away from the root meristem. The results do not rule out a role for endogenous ABA in the protection of elongation in the apical 1.5 mm; although the FLU treatment reduced ABA content in that region by about one-half without affecting elongation rate, a substantial ABA content did remain in the treated tissue, possibly due to its proximity to the cap that contained the largest ABA content in the root tip.

In the mesocotyl, elongation in the basal region of the growing zone was also completely inhibited at low ψ_w. In contrast with the primary root, however, this inhibition was dependent on the accumulation of high levels of ABA. In the apical 8 mm of the mesocotyl, some elongation continued at low ψ_w despite the presence of higher levels of ABA than in the basal region. Thus, the results suggest that the capacity for ABA to inhibit mesocotyl expansion increases as cells are displaced away from the meristematic region. It should be noted, however, that conclusions on gradients in responsiveness to ABA in both the root and mesocotyl are not definitive without data on the active-site concentration of ABA in the tissues. It is possible that ABA accumulates in different com-

partments in responsive and nonresponsive locations, even though the overall tissue content of the hormone may be similar.

Other studies have demonstrated differential responsiveness within growing regions to applied hormones (7, 10–13). In the maize primary root, for example, applications of IAA can promote elongation near the apex while inhibiting elongation in basal regions (7). In view of such results, the spatial resolution of this study was essential to confirm that the maintenance of shoot growth at low ψ_w in FLU-treated seedlings was indeed due to prevention of the growth inhibition induced by low ψ_w. Without spatial growth data, it was conceivable that FLU promoted rapid elongation in some locations while having no effect (or even causing growth inhibition) in others. Our results show that this was not the case; instead, the distribution of elongation in the mesocotyl was very similar in the low ψ_w +FLU treatment to that observed at high ψ_w.

Longitudinal distributions of ABA content have previously been reported for roots and shoots at high ψ_w (8, 14, 20, 21) and for roots at low ψ_w (19), although not with the degree of spatial resolution reported here. In agreement with the earlier reports, we observed a trend of increasing ABA content toward the apical meristematic regions, which was more pronounced at low ψ_w. This may reflect the increased ratio of cytoplasm to vacuole in younger cells, and the tendency for ABA to accumulate preferentially in the cytoplasmic compartment (1, 3). The relationship of the spatial distribution of ABA content to the growth pattern has not been investigated previously in either roots or shoots.

Our results indicate that the action of ABA in both the maintenance of primary root growth and the inhibition of mesocotyl growth at low ψ_w involves large effects on cell expansion. In addition to protecting cell expansion in the root, the results suggest that accumulation of endogenous ABA may play a role in maintaining cell production at low ψ_w. This needs to be confirmed by direct examination of meristematic activity, however, because cell production rate could not be calculated accurately in the low ψ_w +FLU treatment. Barlow and Pilet (2) showed that both cell expansion and division were inhibited by ABA application to maize primary roots, and Robertson *et al.* (22, 23) showed that ABA application reduced the rate of cell production in sunflower roots by inhibiting cell division and reducing the size of the meristematic zone. These results, however, were achieved using seedlings growing at high ψ_w. We have previously argued that tissues are likely to respond differently to ABA depending on their water status (24). Indeed, recent results indicate that ABA applications to roots at high ψ_w that raised the internal ABA level to that found endogenously in roots at low ψ_w resulted in growth inhibition. On the other hand, when the same ABA level was achieved by ABA application to FLU-treated roots at low ψ_w, the growth rate was promoted (R.E. Sharp, G.S. Voetberg, I.N. Saab, unpublished data).

In conclusion, the spatial resolution of the experiments reported here underscores the complexity of the responses of root and shoot elongation to low ψ_w and endogenous ABA. This detailed characterization is an essential step toward understanding the mechanisms involved. Future work should also take into account radial patterns of responsiveness to ABA to address the involvement of the various tissues of roots and shoots in regulating the growth responses.

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