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Root Growth Maintenance at Low Water Potentials[1]

Increased Activity of Xyloglucan Endotransglycosylase and Its Possible Regulation by Abscisic Acid

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Previous work suggested that an increase in cell wall-loosening contributes to the maintenance of maize (*Zea mays* L.) primary root elongation at low water potentials (ψ_w). It was also shown that root elongation at low ψ_w requires increased levels of abscisic acid (ABA). In this study we investigated the effects of low ψ_w and ABA status on xyloglucan endotransglycosylase (XET) activity in the root elongation zone. XET is believed to contribute to wall-loosening by reversibly cleaving xyloglucan molecules that tether cellulose microfibrils. The activity of XET per unit fresh weight in the apical 10 mm (encompassing the elongation zone) was constant at high ψ_w but increased by more than 2-fold at a ψ_w of −1.6 MPa. Treatment with fluridone to decrease ABA accumulation greatly delayed the increase in activity at low ψ_w. This effect was largely overcome when internal ABA levels were restored by exogenous application. Spatial distribution studies showed that XET activity was increased in the apical 6 mm at low ψ_w whether expressed per unit fresh weight, total soluble protein, or cell wall dry weight, corresponding to the region of continued elongation. Treatment with fluridone progressively inhibited the increase in activity with distance from the apex, correlating with the pattern of inhibition of elongation. Added ABA partly restored activity at all positions. The increase in XET activity at low ψ_w was due to maintenance of the rate of deposition of activity despite decreased deposition of wall material. The loss of activity associated with decreased ABA was due to inhibition of the deposition of activity. The results demonstrate that increased XET activity is associated with maintenance of root elongation at low ψ_w and that this response requires increased ABA.

Root elongation is often less inhibited than shoot growth at low ψ_w, and this is considered a mechanism of plant adaptation to water-limited conditions (Sharp and Davies, 1989; Spollen et al., 1993). The primary root of maize (*Zea mays* L.) continues to elongate in vermiculite at low ψ_w that are completely inhibitory to shoot growth (Sharp et al., 1988). Studies of the spatial distribution of longitudinal expansion revealed that elongation was completely maintained in the apical few millimeters at ψ_w as low as −1.6 MPa but was inhibited progressively as cells were displaced further from the apex, resulting in a shortened elongation zone (Sharp et

al., 1988). Direct turgor measurements in the cortex and stele with a cell pressure probe showed that turgor was approximately 0.7 MPa throughout the elongation zone at high ψ_w but was decreased to approximately 0.3 MPa at all locations at a ψ_w of −1.6 MPa (Spollen and Sharp, 1991). The complete maintenance of elongation in the apical few millimeters despite the large decrease in turgor indicated that longitudinal cell wall-loosening may have increased in this region in response to the low ψ_w treatment. Similar results were obtained by Pritchard et al. (1993) following exposure of maize primary roots to solutions of low ψ_w. Several earlier reports also suggested that wall-loosening increased in roots at low ψ_w, although these studies lacked spatial resolution to resolve the location of such adjustment (Kuzmanoff and Evans, 1981; Hsiao and Jing, 1987; Itoh et al., 1987).

Nothing is known about the metabolic basis of increases in cell wall-loosening in roots at low ψ_w. As a first step, we have examined the hypothesis that low ψ_w increases XET activity in the maize primary root elongation zone. XET has been proposed to cause cell wall-loosening by cleaving xyloglucan molecules that tether adjacent cellulose microfibrils and to rejoin the cut ends to other xyloglucan chains to maintain some tethers (Smith and Fry, 1989; Nishitani and Tominaga, 1991; Fry et al., 1992). The net effect would be to relieve tension in the wall and promote expansion by allowing microfibril separation. XET could also assemble and elongate xyloglucan chains from smaller units and thereby contribute to wall synthesis.

XET activity was shown to correlate with the spatial distribution of elongation rate at high ψ_w in pea (*Pisum sativum* L.) stems (Fry et al., 1992) and maize primary roots (Pritchard et al., 1993). In the latter study total extractable XET activity did not increase in the root apical region at low ψ_w at the time enhanced wall-loosening was believed to occur. However, the short duration of low ψ_w exposure in these experiments (15 min) may have been insufficient to allow for increases in bulk tissue XET activity, as measured. As noted

Abbreviations: FLU, fluridone; GPC, gel permeation chromatography; PC, paper chromatography; ψ_w, water potential(s); XET, xyloglucan endotransglycosylase; XXXG, xyloglucan heptasaccharide; XXFG, xyloglucan nonasaccharide; XXXGol, reduced xyloglucan heptasaccharide.

by the authors, a role for increased XET activity in increasing wall-loosening could not be excluded because of the possibility of rapid redistribution of XET from intraprotoplasmic stores. In this study we have examined the spatial distribution of XET activity in the elongation zone of roots growing steadily during prolonged exposure to low ψ_w in vermiculite. The results show that XET activity is substantially increased in the apical few millimeters of roots at low compared to high ψ_w.

Previous work showed that the maintenance of maize primary root elongation at low ψ_w requires accumulation of endogenous ABA (Saab et al., 1990). This was demonstrated using FLU and the $vp5$ mutant to inhibit carotenoid (and ABA) synthesis. In both cases root elongation at low ψ_w was severely inhibited but recovered when ABA levels were restored by exogenous application (Sharp et al., 1993b, 1994). The inhibition of root elongation was associated with further shortening of the elongation zone toward the apex (Saab et al., 1992). How ABA acts to maintain root elongation at low ψ_w is not known, although several studies suggest that ABA might affect cell wall-yielding properties (reviewed by Spollen et al., 1993). Therefore, the second objective of this study was to examine the effect of decreased ABA accumulation at low ψ_w on the relationship of XET activity with the spatial distribution of root elongation. The results show that the enhancement of XET activity at low ψ_w is greatly decreased by FLU treatment (and restored by ABA) in close correlation with the inhibition of elongation rate.

MATERIALS AND METHODS

Maize Seedling Culture and Harvest

After germinating in moist vermiculite, seedlings of maize (*Zea mays* L. cv FR27 × FRMo17) with radicles approximately 1 cm long were transplanted to Plexiglas boxes containing vermiculite at either high ψ_w (−0.03 MPa) or low ψ_w (−1.6 MPa) and grown in the dark at 29°C and near-saturation humidity (Sharp et al., 1988). Vermiculite ψ_w were measured by isopiestic thermocouple psychrometry (Boyer and Knipling, 1965). When necessary for measurements of root elongation and for harvesting, illumination was provided by a green safelight (Saab et al., 1990). To examine the effect of decreased ABA accumulation at low ψ_w, in some experiments FLU (Dow Chemical Co., Midland, MI) was added at a concentration of 10 μM to the water mixed with the vermiculite in which seeds were germinated and into which seedlings were transplanted (Saab et al., 1990; Ober and Sharp, 1994). To test whether the effects of FLU could be overcome with exogenous ABA, (±)ABA (Sigma) was added at various concentrations together with FLU to the vermiculite into which seedlings were transplanted. (ABA was not supplied during imbibition because it inhibited germination.)

At various times after transplanting, primary roots were selected for uniformity of elongation rate (±20% of the mean for the treatment). The apical 1 cm, which encompassed the elongation zone, was harvested either as a whole or in serial 1- or 2-mm segments (after excising the apical 0.5 mm to remove the major portion of the root cap). Samples were immediately frozen in liquid nitrogen and weighed prior to measurements of XET activity and soluble protein content.

At low ψ_w, the spatial distribution of XET activity was measured 48 h after transplanting, when the roots were approximately 5 cm long. At high ψ_w, measurements were made at both 20 h (developmental control, roots 5 cm long) and 48 h (temporal control, roots 10–12 cm long). In the low ψ_w +FLU treatment (without and with added ABA), measurements were made at 48 h. The harvest times were the same as those used previously to obtain spatial distributions of root elongation rate (Saab et al., 1992). In all treatments, root elongation was constant at the time of sampling (Saab et al., 1990).

Culture of Other Species

Seedlings of four other species were cultured at high ψ_w as described for maize and assayed for XET activity in the root elongation zone. Pea (*Pisum sativum* L. cv Wando), bean (*Phaseolus vulgaris* L. cv Blue Lake Bush 274), soybean (*Glycine max* [L.] Merr. cv Williams), and squash (*Cucurbita maxima* Duch. cv Sunbar) were grown until the primary roots were approximately 5 cm long (46, 38, 42, and 30 h after transplanting, respectively). Root elongation rates were constant at these times. The elongation zone was harvested from roots selected for uniformity of elongation rate; the length of the elongation zone was determined for each species in preliminary experiments by time-lapse photography of marked roots (Sharp et al., 1988).

XET Activity

Fifteen to 30 mg fresh weight of root tissue (three 1-cm segments or 30–40 1-mm segments) were homogenized in a microcentrifuge tube using a stainless steel rod after adding 35 μL of 50 mM Mes buffer (pH 6.0) containing 10 mM sodium ascorbate and 10 mM CaCl$_2$. The rod was rinsed with 35 μL of buffer five times, and the tubes were then centrifuged for 10 min at 8800g. These procedures were conducted in a cold (4°C) room or on ice. XET activity in the supernatant was assayed according to the procedure of Fry et al. (1992). The final reaction mixture (40 μL) was 0.125% xyloglucan (from *Tropaeolum* seeds), 5 mM sodium ascorbate, 5 mM CaCl$_2$, 50 mM Mes (pH 6.0), and 0.2 μM (400 Bq) [^{3}H]XXXGol (Fry et al., 1993) and contained 10 μL of root extract. The mixture was incubated at 25°C in a shaking water bath for 1 h, and then the reaction was stopped by adding 10 μL of 90% formic acid. The entire reaction mixture was dried on Whatman 3 MM filter paper (4 × 4 cm), which was then rinsed with running tap water for 45 min to remove unreacted oligosaccharides. After the filter paper was dried in an oven (60°C), the remaining ^{3}H was quantified by scintillation counting. XET activity was calculated from the fraction of total label incorporated into product (xyloglucan, which bound to the filter paper). Background XET activity was measured in assays without added xyloglucan but was always less than 1% of total activity; therefore, data were calculated using total activity.

XXXG (Glc$_4$-Xyl$_3$) was prepared from xyloglucan from *Rosa* cell-suspension cultures as described by McDougall and Fry (1988). [1-^{3}H]XXXGol was derived by reducing XXXG with NaB^{3}H$_4$. XXXG consists of a cellotetraose backbone

[β-(1→4)-linked glucan] with one terminal xyloside linked α-(1→6) to each of the three nonreducing Glc units (Fry et al., 1992).

Preliminary experiments showed that freezing in liquid nitrogen and rapid thawing did not alter XET activity. Activity was constant in the pH range 5.5 to 6.5. Reaction rate was linearly proportional to the amount of enzyme extract present in the reaction mixture and to reaction time up to 2 h.

XET activities were expressed per unit fresh weight, total soluble protein, or cell wall dry weight. Fresh weight and soluble protein content (Bradford, 1976) were measured for the samples used for XET assay. To determine the spatial distribution of cell wall dry weight, additional experiments were conducted because of the large amount of tissue required. Seedlings were grown and harvested as for measurement of XET activity, except that all roots were sampled (40 per box) to collect 150 segments per position for each of two to three replicates per treatment, which were then extracted for cell wall dry weight determination as described below. Measurements of XET activity per millimeter length were then divided by the mean local cell wall dry weight content for the particular treatment.

The method of cell wall extraction was modified from procedures described by Fry (1988). The root segments were ground with 1 mL of 0.5% SDS solution and centrifuged for 10 min at 8800g, and then pellets were washed three times with distilled water, resuspended in 80% phenol:glacial acetic acid (5:2), and left overnight on a shaking table. After the samples were centrifuged, residues were resuspended in 80% phenol:glacial acetic acid:distilled water (5:2:1). After these procedures, precipitation of protein from the phenol solution using 10% ammonium formate and acetone (1:50) was undetectable. Residues were then centrifuged, washed three times with 70% ethanol, resuspended in 90% DMSO overnight to remove starch, centrifuged, and washed three times with 70% ethanol and once with distilled water. Cell wall dry weight was obtained after drying at 60°C for 24 h.

Substrate Specificity and Degradation Assays

Substrate specificity of XET activity was examined using samples of the apical 10 mm of roots grown at high ψ_w (20 h after transplanting). Cellotetraose and mixed-linkage β-glucan (Sigma) were tested as competing and alternate substrates, respectively. Mixed-linkage glucan was first dissolved at 80°C.

The possible degradation of [^{3}H]XXXGol during the XET assays by α-xylosidase and β-glucosidase was examined using PC (Whatman No. 1, descending method, 16 h in ethyl acetate:acetic acid:water [10:5:6]) and GPC (Bio-Gel P2, 200-mL bed volume, equilibrated and eluted with pyridine:acetic acid:water [1:1:23]). Both procedures could separate [^{3}H]-XXXGol from [^{3}H]XXGol, a likely product if degradation occurred. In addition, the GPC could separate XXXGol from GXXGol, the product of α-xylosidase action alone. Samples of 1-mm segments were taken at 3 mm from the apex of roots grown at high ψ_w, low ψ_w, or low ψ_w +FLU (20, 48, and 48 h after transplanting, respectively). Extracts were reacted for 1 h according to the XET assay procedure described above.

Deposition Rates of XET Activity and Cell Wall Dry Weight

Net local deposition rates (net rate of addition per millimeter of root length) of XET activity and cell wall dry weight were calculated using the continuity equation (Silk et al., 1984) as described by Sharp et al. (1990). Briefly, this calculation combines the spatial distribution of density (content per millimeter length of XET activity or cell wall dry weight) with that of displacement velocity (mm h^{-1}; from Saab et al. [1992] for the same treatments) to determine the net deposition rate profile that must occur to maintain the observed distribution of density in the face of tissue expansion and displacement from the root apex. Deposition rates were not corrected for local rates of change, which were estimated to be negligible based on the results of time-course experiments. Division of XET activity deposition rates per millimeter length by the local cell wall dry weight content (μg mm^{-1}) gave deposition rates per microgram cell wall dry weight.

RESULTS

Species Comparison of XET Activity

The content of xyloglucan in the cell walls of Gramineae is less than in other species (reviewed by Carpita and Gibeaut, 1993). Therefore, XET activity was measured in the primary root elongation zone of a range of species at high ψ_w to assess how the activity in maize compares with that in other species. Maize had the highest XET activity per unit fresh weight or soluble protein of the five species examined (Table I). Activities were substantially lower in bean and squash and least in soybean and pea.

Substrate Specificity of XET Activity in Maize

To examine the substrate specificity of XET activity in the maize primary root tip (apical 10 mm), cellotetraose and mixed-linkage β-glucan (a major hemicellulose in maize primary cell walls) were tested as competing and alternate substrates, respectively (Table II). Cellotetraose, at a 1000-fold molar excess to [^{3}H]XXXGol, did not inhibit XET activity; thus, the side chains on the xyloglucan oligosaccharide are required for activity. There was no activity when mixed-linkage β-glucan was used in place of xyloglucan. These

Table I. *XET activity in the primary root elongation zone of several species at high ψ_w*

Length of the elongation zone ranged from 8 to 11 mm, depending on species. Samples were harvested when roots were 5 cm long. Data are means ± SE (n = 3–4). FW, Fresh weight.

Species	XET Activity	
	$Bq\ kBq^{-1}\ mg^{-1}\ FW\ h^{-1}$	$Bq\ kBq^{-1}\ \mu g^{-1}\ soluble\ protein\ h^{-1}$
Maize	42.9 ± 1.4	5.1 ± 1.3
Bean	35.5 ± 2.9	3.5 ± 0.2
Squash	20.4 ± 0.8	2.4 ± 0.4
Soybean	12.1 ± 0.8	1.6 ± 0.1
Pea	7.8 ± 0.9	1.0 ± 0.1

Table II. *Substrate specificity of XET activity*

Measurements are of the apical 10 mm of maize primary roots grown at high ψ_w. Final substrate concentrations in the reaction mixtures are indicated in parentheses. Data are means of two experiments (except where noted), in each of which duplicate measurements were made.

Substrates in Addition to [³H]XXXGol	Relative XET Activity
	%
Xyloglucan (0.125%)	100
Xyloglucan (0.125%) plus cellotetraose (200 µM)	105
Mixed-linkage glucan (0.125%)	4
Mixed-linkage glucan (0.625%)	−4[a]

[a] Single experiment.

results show that the activity in maize root tips should be specific to xyloglucans.

Analysis of substrate by PC indicated that about 33% of the radiolabel was not in [³H]XXXGol. The impurity was an as-yet-unidentified compound that eluted with [³H]XXXGol when analyzed by GPC. In XET assays of extracts of 1-mm root segments taken 3 mm from the apex of roots grown at high ψ_w, low ψ_w, or low ψ_w +FLU, no other radiolabeled low mol wt compounds were found by either PC or GPC. The impurity became a slightly greater fraction of the sample, probably because it did not react as well as [³H]XXXGol, if it did at all. Whatever α-xylosidase and β-glucosidase activities were present in the extracts, the absence of radiolabeled oligosaccharides of lower mol wt after the reaction shows that these activities did not degrade [³H]XXXGol during the XET assays in any treatment. XET assays were not corrected for the presence of impurity.

Effect of Low ψ_w and ABA Status on the Time Course of XET Activity in the Maize Primary Root

A time-course experiment was conducted as an initial examination of the effects of low ψ_w and ABA status on XET activity in the maize primary root tip (apical 10 mm, encompassing the elongation zone). Figure 1 shows that at high ψ_w XET activity per unit fresh weight was almost constant for the 100-h duration of the experiment. In contrast, activity increased steadily for the first 46 h after transplanting to a ψ_w of −1.6 MPa and then remained at more than twice the level at high ψ_w for the remainder of the experiment. Previous work showed that it takes approximately 35 h after transplanting to this ψ_w for the root tip osmotic potential to reach a constant level (Sharp et al., 1990), which is probably indicative of the duration of tissue ψ_w decline. Thus, XET activity in the root elongation zone increased progressively as the severity of water stress increased. The contribution of dehydration to the increase in XET activity at low ψ_w was minor, since previous work showed that the relative water content of the apical 10 mm had decreased by only 17% 48 h after transplanting to −1.6 MPa (Sharp et al., 1990).

Treatment with FLU prevented the increase in XET activity at low ψ_w for the first 36 h after transplanting (Fig. 1). This effect accompanied the decrease in ABA accumulation and

severe inhibition of root elongation rate reported previously (Saab et al., 1990). Activity gradually recovered for the remainder of the experiment, eventually reaching almost the level in −FLU roots. The gradual recovery of XET activity in FLU-treated roots is consistent with results of previous experiments under the same conditions that showed a slow increase in both root elongation rate and tip ABA content after 50 h, such that both parameters recovered to approximately the values in −FLU roots by 120 h (Saab et al., 1990). This probably reflects progressive dilution and/or metabolism of the FLU absorbed during imbibition, combined with low rates of FLU uptake after transplanting.

The inhibitory effect of FLU on the rate of increase in XET activity at low ψ_w was largely overcome when 0.7 mM ABA was mixed with the vermiculite; the increase in activity was completely restored by 60 h after transplanting. In other experiments at this ψ_w, 0.7 mM ABA fully restored the root tip ABA content and the root elongation rate of FLU-treated roots by approximately 50 and 70 h after transplanting, respectively (Sharp et al., 1994). The requirement for such a high applied ABA concentration to restore the internal ABA content was due to limited uptake from the dry vermiculite.

The results in Figure 1 show that exposure to low ψ_w results in a substantial increase in XET activity in the root elongation zone and that this response requires the accumulation of ABA.

Effect of Low ψ_w on the Spatial Distribution of XET Activity in the Maize Primary Root

Figure 2 reproduces previous results from Saab et al. (1992), showing that at low ψ_w elongation was maintained prefer-
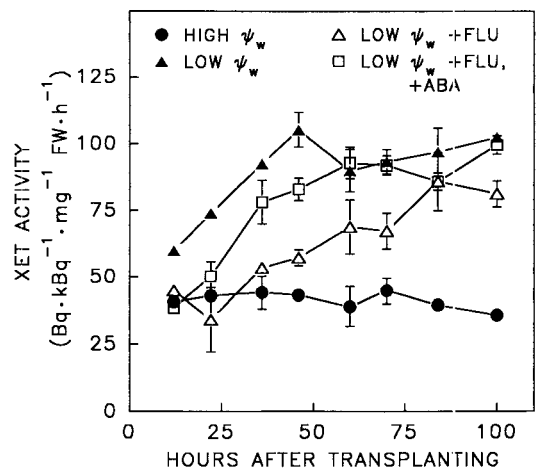

Figure 1. Time course of XET activity per milligram fresh weight (FW) in the apical 10 mm of maize primary roots after transplanting to vermiculite at high ψ_w (●; −0.03 MPa) or low ψ_w (▲; −1.6 MPa). Treatment with FLU (△) to decrease ABA accumulation greatly delayed the increase in XET activity at low ψ_w. The effect of FLU was largely overcome when 0.7 mM ABA (□) was mixed with the vermiculite; this treatment was shown to restore the internal ABA level in the apical 10 mm to that of −FLU roots by 50 h after transplanting (Sharp et al., 1994). Data are means ± SE of single measurements from three experiments.
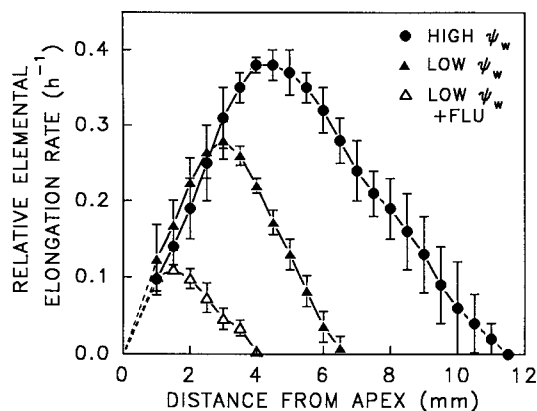
Figure 2. Spatial distribution of relative elemental elongation rate in the apical 12 mm of maize primary roots at high ψ_w (−0.03 MPa, 20 h after transplanting), low ψ_w (−1.6 MPa), or low ψ_w +FLU. Low-ψ_w measurements were made 48 h after transplanting. Data are means ± SD ($n = 3$–4). Modified from Saab et al. (1992), which also shows accompanying profiles of ABA content.

entially toward the root apex. This resulted in a shortening of the elongation zone from 11.5 mm at high ψ_w to 6.5 mm at a ψ_w of −1.6 MPa. To examine the relationship of the increase in XET activity at low ψ_w (Fig. 1) to the maintenance of elongation rate in the apical region, the spatial distribution of XET activity was determined at high and low ψ_w at the same times as the growth distribution was measured (20 h [developmental control] and 48 h after transplanting, respectively). Activities were constant in both treatments at these times (Fig. 1). The results are shown in Figure 3, expressed on the basis of fresh weight, total soluble protein, and cell wall dry weight.

At high ψ_w, the profile of XET activity varied with the basis of expression. Activity per unit fresh weight was highest 2 to 3 mm from the apex (Fig. 3A), whereas activity per unit soluble protein was lowest close to the apex (Fig. 3B) because of the large protein content in this region (Fig. 3B, inset). On a cell wall dry weight basis (Fig. 3C), the activity profile corresponded closely to the distribution of elongation rate (Fig. 2).

The increase in XET activity at low ψ_w was greatest in the apical 6 mm on all bases of expression (Fig. 3), correlating with the region of continued elongation (Fig. 2). In the 6.5- to 10-mm region in which elongation was completely inhibited, activity remained increased on the basis of fresh weight but was similar at high and low ψ_w when expressed per unit protein and cell wall dry weight.

The roots at low ψ_w were thinner than at high ψ_w when compared at the same length (Sharp et al., 1988). Consequently, contents of fresh weight, soluble protein, and cell wall dry weight per unit root length were each considerably less at low ψ_w than in the high ψ_w developmental control (Fig. 3, insets). The roots at high ψ_w grew thinner as they grew longer, however, and in the temporal control (48 h after transplanting), fresh weight, protein, and cell wall dry weight contents were much closer to those of the low ψ_w treatment. Despite these changes, the spatial distribution of XET activity
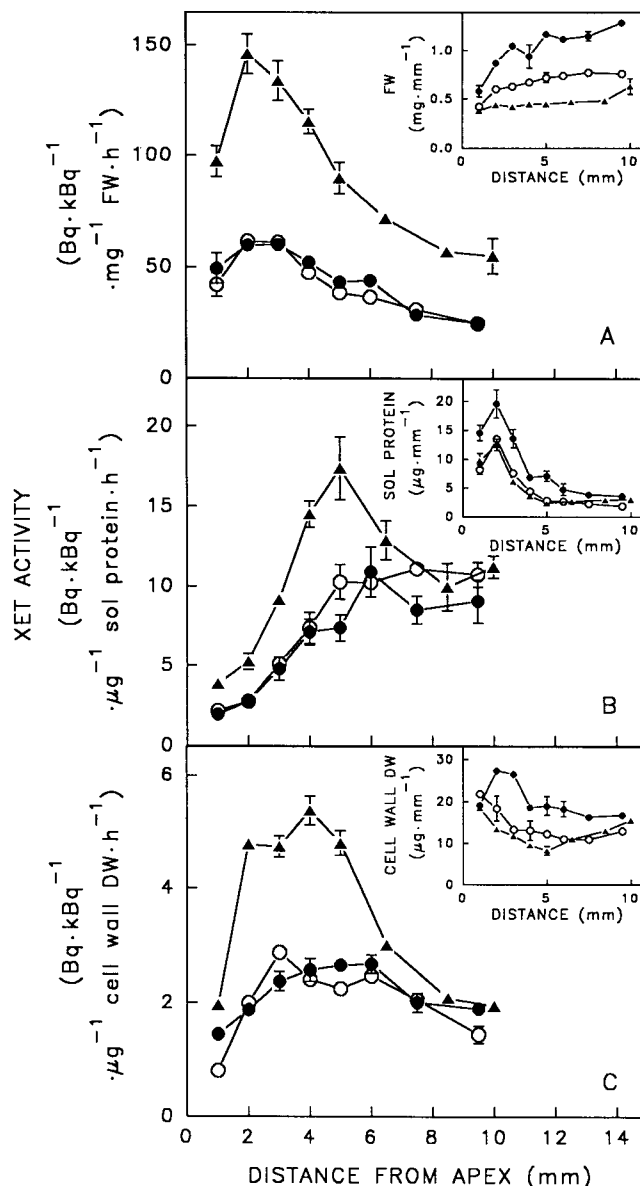

Figure 3. Spatial distribution of XET activity in the apical 10 mm of maize primary roots at high ψ_w (●, O; −0.03 MPa) or low ψ_w (▲; −1.6 MPa, 48 h after transplanting). High ψ_w measurements were made either 20 h (●; developmental control) or 48 h (O; temporal control) after transplanting. XET activities are expressed on the basis of fresh weight (FW; A), total soluble protein (B), and cell wall dry weight (DW; C). Data are means ± SE ($n = 3$ [high ψ_w] or 4 [low ψ_w]). Insets, spatial distributions of the contents per millimeter length of fresh weight (A), total soluble protein (B), and cell wall dry weight (C) (means ± SE). Fresh weight and soluble protein contents were determined for the samples measured for XET activity. Cell wall dry weight distributions were determined using two sets of 150 roots per treatment.

was very similar in the two high ψ_w controls on all bases of expression. Thus, the increase in activity at low ψ_w cannot be explained simply as the result of the effect on root morphology. This question is addressed in more detail below.

Effect of Decreased ABA Accumulation on the Spatial Distribution of XET Activity at Low ψ_w

Also shown in Figure 2 is the effect of FLU on the spatial distribution of elongation rate at a ψ_w of -1.6 MPa, as previously published (Saab et al., 1992). The severe inhibition of root elongation in FLU-treated roots was caused by progressive inhibition of longitudinal expansion as cells were displaced beyond the apical millimeter, such that the elongation zone was further shortened to 4 mm in length. To assess the relationship between this pattern and the inhibition of XET activity that resulted from FLU treatment (Fig. 1), the spatial distribution of activity was measured 48 h after transplanting (when the growth distribution was determined), without and with the addition of 0.7 mM ABA to restore internal ABA levels.

The results are shown in Figure 4. In the apical millimeter, XET activity was higher (fresh weight and cell wall dry weight bases) or unaffected (soluble protein basis) in FLU-treated compared to untreated roots. With increasing distance from the apex, however, the increase in activity at low ψ_w was progressively inhibited by FLU treatment on all bases of expression. On both the protein and cell wall dry weight bases, the activity in low ψ_w +FLU roots decreased below that of the roots at high ψ_w beyond 4 mm from the apex. Thus, the effect of FLU on the profile of XET activity at low ψ_w corresponded closely to the pattern of inhibition of elongation rate, regardless of the basis of expression.

Addition of ABA fully or partly restored XET activity (depending on position and basis of expression) over the whole of the elongation zone; the overall incomplete restoration at this time was consistent with the results of the time-course experiment (Fig. 1).

Deposition Rates

As noted above, roots were thinner when grown under water-limited conditions (Sharp et al., 1988). Therefore, the increase in XET activity at low ψ_w could have resulted from decreased deposition rates of fresh weight, protein, and cell wall dry weight rather than an increase in the net rate of synthesis or activation of XET.

In contrast, whereas root elongation is inhibited in FLU-treated roots at low ψ_w, root diameter is *increased* compared to $-$FLU roots beyond 2 mm from the apex (Sharp et al., 1993b). Accordingly, fresh weight, protein, and cell wall dry weight contents were each considerably greater in the 2- to 10-mm region in +FLU than in $-$FLU roots (Fig. 4, insets). In each case, the effect was partially reversed by addition of ABA. Thus, the inhibition of XET activity associated with decreased ABA accumulation at low ψ_w may have been due to increased deposition rates of fresh weight, protein, and cell wall dry weight rather than decreased rates of XET synthesis or activation. To assess these questions in quantitative terms, the information in Figures 2 through 4 was
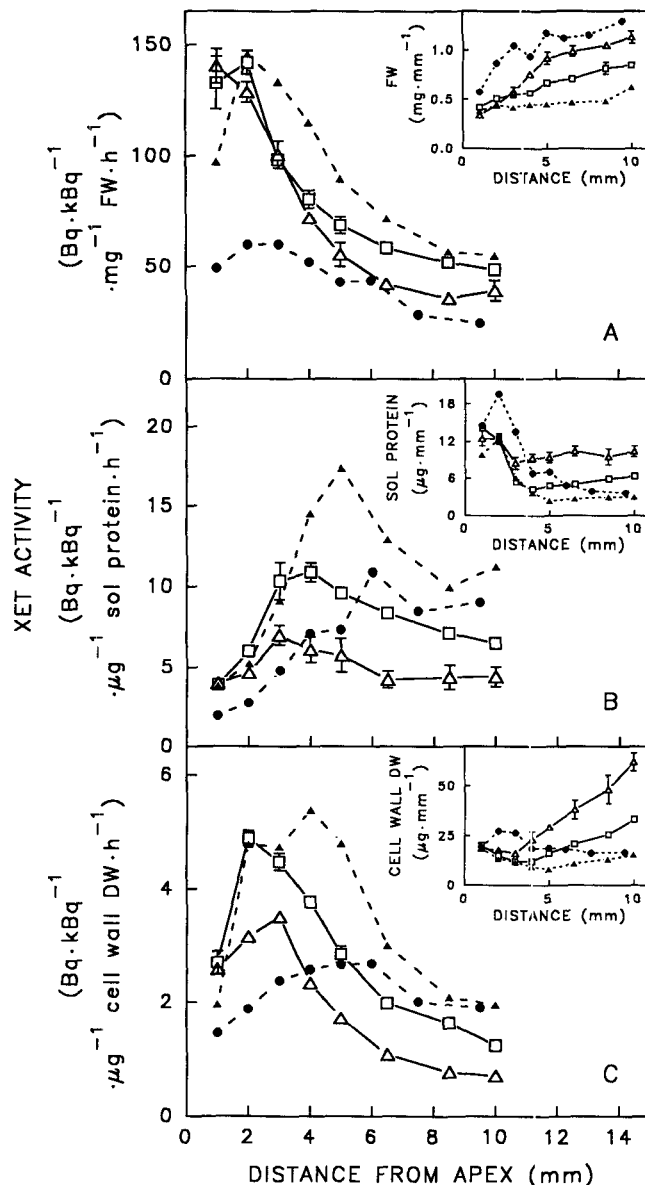

Figure 4. Effect of FLU without ($\triangle$) or with ($\square$) 0.7 mM ABA on the spatial distribution of XET activity in the apical 10 mm of maize primary roots 48 h after transplanting to a ψ_w of -1.6 MPa. Data are means $\pm$ SE ($n = 6$ [+FLU] or 4 [+FLU, +ABA]). Mean XET activities at high ψ_w (●; 20 h) and at low ψ_w without FLU (▲) are reproduced from Figure 3. XET activities are expressed on the basis of fresh weight (FW; A), total soluble protein (B), and cell wall dry weight (DW; C). Insets, Spatial distributions of the contents per millimeter length of fresh weight (A), total soluble protein (B), and cell wall dry weight (C) (means $\pm$ SE). Fresh weight and soluble protein contents were determined for the samples measured for XET activity. Cell wall dry weight distributions were determined using two ($-$FLU) or three (+FLU) sets of 150 roots per treatment. Mean values for the distributions of fresh weight, soluble protein, and cell wall dry weight at high ψ_w (●; 20 h) and at low ψ_w without FLU (▲) are reproduced from Figure 3.

combined in the continuity equation (Silk et al., 1984) to compute the spatial distribution of deposition rates of XET activity and cell wall dry weight in the high ψ_w, low ψ_w, and low ψ_w +FLU treatments. Cell wall dry weight was chosen because XET activities on this basis most closely corresponded to the spatial patterns of elongation rate (Figs. 2–4) and because the effect of ABA status at low ψ_w was more pronounced on the content of cell wall dry weight than on fresh weight or soluble protein (Fig. 4, insets).
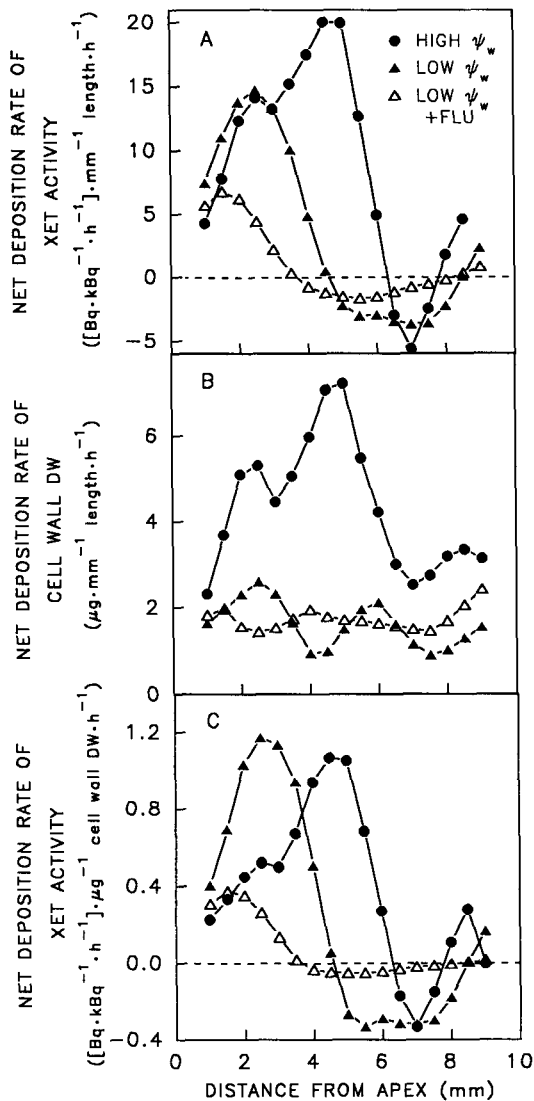
The results are shown in Figure 5. Relative to the high ψ_w

Figure 5. Spatial distribution of the net deposition rates of XET activity per millimeter length (A), cell wall dry weight (ψ_w) per millimeter length (B), and XET activity per microgram cell wall dry weight (C) in the apical 10 mm of maize primary roots at high ψ_w (●; −0.03 MPa, 20 h), low ψ_w (▲; −1.6 MPa), or low ψ_w +FLU (△). Data were calculated using the continuity equation (Silk et al., 1984) from mean values of XET activity and cell wall dry weight per millimeter length (resolved at 0.5-mm intervals by linear interpolation; from the experiments in Figs. 3 and 4), and growth velocity distributions (from Saab et al., 1992).

developmental control, the net deposition rate of XET activity per unit root length was not increased in the low ψ_w treatment at any location; rates were similar in the two treatments in the apical 3 mm and were decreased at low ψ_w in more basal locations (Fig. 5A). Deposition of XET activity decreased to zero at 4.5 mm from the apex at low ψ_w, whereas rates were maximal in this region at high ψ_w. In contrast, deposition of cell wall dry weight was decreased throughout the apical 10 mm at low compared to high ψ_w (Fig. 5B). Therefore, the increase in XET activity in the apical region at low ψ_w was due to the maintenance of deposition of XET activity despite decreased deposition of cell wall dry weight. As a consequence, deposition of activity per unit cell wall dry weight was greatly increased in the apical 3 mm at low ψ_w (Fig. 5C), correlating with the complete maintenance of elongation in this region (Fig. 2).

Deposition of XET activity per unit root length was decreased progressively with distance from the apex in FLU-treated compared to untreated roots at low ψ_w (Fig. 5A). In contrast, cell wall dry weight deposition was similar for these two treatments throughout the apical 10 mm (Fig. 5B). Thus, deposition of XET activity per unit cell wall dry weight was not maintained in FLU-treated roots beyond the apical millimeter (Fig. 5C). These results indicate that the inhibition of XET activity associated with decreased ABA accumulation at low ψ_w was due to inhibition of XET synthesis and/or activation and not to the accompanying changes in root morphology.

DISCUSSION

Our results show that the maintenance of root elongation at low ψ_w is associated with increased activity of XET. Regardless of the basis of expression, activity was increased by approximately 2-fold in the apical region of roots growing in vermiculite at a ψ_w of −1.6 MPa compared to both developmental and temporal controls at high ψ_w. This response is consistent with a role in increasing wall-loosening to allow continued elongation in the apical region at low ψ_w despite incomplete turgor maintenance (Spollen and Sharp, 1991).

These findings are in contrast to those of Pritchard et al. (1993), which showed no increase in extractable XET activity within the apical 10 mm of maize primary roots following either short-term (15 min) or long-term (24 h) exposures to mannitol solutions of low ψ_w. As noted in the introduction, it is possible that, in the short-term experiments (when the spatial growth-turgor relationship suggested an increase in wall-loosening in the apical few millimeters), there was insufficient time for an increase in bulk tissue XET activity to occur. In the long-term studies, turgor had fully recovered and, therefore, increases in wall-loosening were not anticipated. It should be noted that, although Pritchard et al. conducted experiments at a higher ψ_w (−0.96 MPa) than in our studies, in a preliminary experiment we measured an increase in XET activity in the root apical 10 mm after 48 h at a ψ_w of only −0.3 MPa (data not shown).

Our results also indicate that the shortening of the elongation zone at low ψ_w was not caused by a decrease in XET activity, since activities were not decreased in the basal region at low compared to high ψ_w on any basis of expression. These

findings also differ from those of Pritchard et al. (1993), which showed no change or a small decrease in activity in the basal region on the bases of fresh weight or total dry weight, respectively, following 24 h of osmotic stress.

The deposition rate analysis revealed that the increase in XET activity in the apical region at low ψ_w resulted from sustained synthesis and/or activation of XET despite decreased deposition of cell wall material. This, together with the increase in specific activity on a total soluble protein basis, indicates that the increase in activity reflects a metabolic response to the low ψ_w condition. Other possible explanations for the increase in activity were excluded, as follows. First, if degradation of substrate during the enzyme assays by α-xylosidase and β-glucosidase activities had been greater at high ψ_w, this could have led to an apparent increase in XET activity at low ψ_w. Results of the substrate degradation assays showed that this was not the case. Second, the fact that the roots at low ψ_w had decreased contents of fresh weight, protein, and cell wall dry weight could not in itself explain their higher XET activity, since this was also the case for the high ψ_w temporal control. The possibility that differential XET extractability between the high and low ψ_w treatments could explain our results seems unlikely, since a study of pea stems at high ψ_w showed that there was negligible residual activity following the extraction procedures we used (Fry et al., 1992).

It is very unlikely that the XET activity measured reflects an enzyme that in vivo would favor another substrate. The failure of cellotetraose and mixed-linkage β-glucan to act as substrates indicates that the enzyme acts on xyloglucan specifically. XET from pea also was very specific for xyloglucan (Fry et al., 1992). Furthermore, the exo- and endo-β-glucanases that act on mixed-linkage β-glucan to cause loosening are not readily extracted from the cell wall with the procedure used for XET extraction (Nevins, 1992). Consequently, if these enzymes also possessed β-glucan transglycosylase activity, it would not be in the extracts used for XET assay.

In reports of preliminary experiments from this study a different xyloglucan oligosaccharide (XXFG) was used as substrate for the XET assay, with comparable results (Sharp et al., 1993a; Spollen et al., 1993). XXFG has the same structure as XXXG but with α-(1→2)-L-fucosyl-β-D-Gal linked glycosidically to O-2 of the Xyl side chain nearest the reducing terminus, and is a structure found in xyloglucan from vegetative tissues, including, in small amounts, those of the Gramineae (McDougall and Fry, 1994). The enzyme in maize roots may thus be similar to that in pea, in which both substrates also react with the enzyme (Fry et al., 1992).

Although it remains to be conclusively demonstrated that XET contributes to cell wall-loosening in vivo, our results provide strong evidence for an association of XET activity with cell elongation rate in the maize primary root. First, activity correlated with the spatial growth pattern at high and low ψ_w particularly on the basis of cell wall dry weight. Second, XET activity decreased in close correspondence with the spatial distribution of the inhibition of elongation rate when ABA levels at low ψ_w were decreased with FLU. We cannot exclude the possibility that XET activity functions in wall synthesis or metabolism without playing the proposed role in wall-loosening, although if this were the case a reason for the increase in activity at low ψ_w is not clear.

The relatively small content of xyloglucan in maize and other Gramineae does not preclude an important role for this polysaccharide in wall yielding in these species. Covalent cross-linkage of xyloglucan to other wall polysaccharides by phenolics could make it part of a much larger structure. Such cross-linking is suggested in analyses of maize coleoptile wall structure: extraction of xyloglucan with mild alkali is greatly improved by conditions that cleave etherified and esterified phenolics (Carpita, 1986), and xyloglucan chromatographs with other matrix components under conditions that do not cleave phenolics (Kato and Nevins, 1991). Interestingly, XET activities were higher in Gramineae than in other species both in the present study (Table I) and in a previous report in which the growing regions of leaves and stems were examined (Fry et al., 1992).

The results obtained with FLU demonstrate that the increase in XET activity at low ψ_w requires the accumulation of ABA. When ABA levels were restored by exogenous ABA application, recovery of XET activity (Fig. 1) followed the restoration of ABA and preceded the recovery of root elongation rate (Sharp et al., 1994). Therefore, restoration of XET activity was not a consequence of growth recovery but may have been a cause. The mechanism by which ABA accumulation is required for the increase in XET activity is unknown. ABA may be directly involved in regulation of XET synthesis, activation, or metabolism, or the interaction may be indirect. For example, recent studies indicate that ABA plays a role in restricting ethylene synthesis or sensitivity in roots at low ψ_w (Spollen and Sharp, 1994), and it is possible that increased ethylene action in FLU-treated roots may somehow impair XET activity.

Applied ABA has generally been associated with *decreases* in cell wall yielding in both roots and shoots at high ψ_w (Van Volkenburgh and Davies, 1983; Jones et al., 1987; Kutschera et al., 1987; Wakabayashi et al., 1989). However, other work from this laboratory showed that root growth responds differently to ABA depending on the water status; ABA applications at high ψ_w that raised the internal level to that required for growth maintenance at low ψ_w resulted in growth inhibition (Sharp et al., 1993b, 1994). Also, ABA accumulation acts differentially to inhibit shoot growth while maintaining root growth at low ψ_w (Saab et al., 1990).

Although our results demonstrate that XET activity increases substantially in the maize primary root elongation zone at low ψ_w, it remains to be shown how important this response is for the maintenance of root elongation. This will require specifically altering XET activity in vivo. Recent experiments have shown that expansin-like activity is also increased in the root apical region at low ψ_w (Y. Wu., D.J. Cosgrove, R.E. Sharp, unpublished data), and the involvement of other putative wall-loosening enzymes needs to be assessed.

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