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ORIGINAL ARTICLE

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Change in XET activities, cell wall extensibility and hypocotyl elongation of soybean seedlings at low water potential

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Abstract In dark-grown soybean (*Glycine max* [L.] Merr.) seedlings, exposing the roots to water-deficient vermiculite ($\psi_w = -0.36$ MPa) inhibited hypocotyl (stem) elongation. The inhibition was associated with decreased extensibility of the cell walls in the elongation zone. A detailed spatial analysis showed xyloglucan endotransglucosylase (XET; EC 2.4.1.207) activity on the basis of unit cell wall dry weight was decreased in the elongation region after seedlings were transplanted to low ψ_w. The decrease in XET activity was at least partially due to an accumulation of cell wall mass. Since cell number was only slightly altered, wall mass had increased per cell and probably led to increased wall thickness and decreased cell wall extensibility. Alternatively, an increase in cell wall mass may represent a mechanism for regulating enzyme activity in cell walls, XET in this case, and therefore cell wall extensibility. Hypocotyl elongation was partially recovered after seedlings were grown in low-ψ_w vermiculate for about 80 h. The partial recovery of hypocotyl elongation was associated with a partial recovery of cell wall extensibility and an enhancement of XET activity in the hypocotyl elongation zone. Our results indicate XTH proteins may play an important role in regulating cell wall extensibility and thus cell elongation in soybean hypocotyls. Our results also showed an imperfect correlation of spatial elongation and XET activity along the hypocotyls. Other potential functions of XTH and their regulation in soybean hypocotyl growth are discussed.

Keywords Cell elongation · Cell wall extensibility · *Glycine* · Water potential · Xyloglucan endotransglucosylase

Abbreviations ψ_w: Water potential (MPa) · XET: Xyloglucan endotransglucosylase · XTH: Xyloglucan endotransglucosylase/hydrolase · XXXGol: Reduced xyloglucan heptasaccharide

Introduction

Cell elongation and expansion in plants is a complex but well-coordinated process. During elongation, biochemical processes keep the cell walls extensible and turgor pressure extends the walls irreversibly, making the wall compartment larger (Cleland 1971; Taiz 1984; Cosgrove 1993, 1999). At the same time, the expansion of the walls keeps the turgor pressure from reaching its maximum, resulting in a lower water potential (ψ_w) in the elongating cells than in the non-elongating cells (Boyer 1968, 1985, 1988, 2001). The lower ψ_w derived from cell expansion, i.e., the growth-induced ψ_w, draws water from the source such as the xylem or non-elongating cells and into the elongating cells. Theoretically, anything that can change the turgor or the growth-induced ψ_w or the extensibility (ability of the wall to be irreversibly extended by turgor pressure) may in turn affect the elongation rate.

Water deficit inhibits cell elongation in plants by affecting turgor, cell wall extensibility or the growth-induced ψ_w. Turgor pressure was often fully maintained in stem and leaf growing regions at low ψ_w when elongation was inhibited, indicating that the inhibition of stem and leaf elongation by low ψ_w was due, at least

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partially, to a decrease in cell wall extensibility (Michelena and Boyer 1982; Van Volkenburgh and Boyer 1985; Westgate and Boyer 1985; Nonami and Boyer 1990a; Chazen and Neumann 1994; Cramer and Bowman 1991). Root elongation was also inhibited by low ψ_w but to a lesser extent. A correlation between decreased cell wall extensibility and cell elongation was also reported in roots at low ψ_w (Pritchard et al. 1993). One study with maize primary roots showed that cell wall extensibility in the very apical region was actually increased at low ψ_w and maintained cell elongation when turgor pressure was greatly reduced (Spollen and Sharp 1991; Wu et al. 1996). An increase in wall extensibility in roots at low ψ_w was also suggested in several other studies (Kuzmanoff and Evans 1981; Hsiao and Jing 1987; Itoh et al. 1987; Frensch and Hsiao 1994, 1995; Triboulot et al. 1995; Pritchard et al. 1993).

The biochemical basis of changes in wall extensibility is still not clear. Wall metabolism will affect the components in the wall, and the ratio of different polymers and activity of different wall loosening and tightening agents may play important roles in regulating wall extensibility (Carpita and Gibeaut 1993; Carpita et al. 1996; Cosgrove 1999). Studies with cultured tobacco cells (Iraki et al. 1989a, 1989b), grape leaves (Sweet et al. 1990), wheat coleoptiles (Wakabayashi et al. 1997), cucumber hypocotyls (Sakurai et al. 1987) and roots (Zhong and Lauchli 1993) showed that wall composition was greatly affected by dehydration or exposure to high concentrations of salts or polyethylene glycol. In general, wall material accumulated at lower rates at low ψ_w, and associated with this reduction was a decrease in [^{14}C]glucose incorporation to less than 50% of the control rate in expanding grape leaf tissue, with the greatest inhibition of cellulose synthesis (Sweet et al. 1990). This was also true in NaCl-treated cotton roots (Zhong and Lauchli 1988). In chick-pea, osmotic stress caused an increase in α-galactosidase, an autolytic activity (Muñoz et al. 1993).

Proteins in cell walls are also thought to be involved in controlling cell wall extensibility under water deficit. Xyloglucan endotransglucosylase/hydrolases (XTHs) are wall proteins that exhibit two distinct enzymatic activities: xyloglucan endotransglucosylase (XET) and/or xyloglucan endohydrolase (XEH) (Rose et al. 2002). Since the XET activity of XTHs can cleave and graft xyloglucan molecules, XTHs may play a role in cell wall loosening and metabolism (Smith and Fry 1991; Nishitani and Tominaga 1991; Fry et al. 1992; Nishitani 1995; Fry 2004). However, XTH failed to cause cucumber cell walls to extend in an in vitro assay (McQueen-Mason et al. 1993). It was believed that XTH might be involved in elongation growth indirectly. A recent study showed that a tomato XTH (tXET) was able to cause creep of cell wall analogues (bio-composite materials based on *Acetobacter xylinus* cellulose and xyloglucan), supporting the notion that XTH may indeed play important roles in regulating wall mechanical properties and thus the growth process in plants (Chanliaud et al. 2004). Expansins are the only proteins so far capable of causing plant cell wall extension in vitro and are believed to play a critical role in controlling cell wall extensibility and thus cell elongation (McQueen-Mason et al. 1992). In primary roots of maize, dehydration caused an increase in XET activity specifically in the root elongation zone (Wu et al. 1994). In the same region of maize primary roots, the activity and amount of expansin proteins were increased under low ψ_w (Wu et al. 1996). The enhancement of XET and expansin activities is closely associated with an increase in cell wall extensibility (Wu et al. 1996), suggesting these cell wall proteins are playing important roles in controlling cell wall extensibility and thus elongation of the roots at low ψ_w.

It is not known how these cell wall proteins are regulated in the stems when cell elongation is inhibited at low ψ_w. The objective of this study was to examine XET activity in soybean hypocotyls (later to become stems) whose elongation was inhibited by low ψ_w in association with a reduction in cell wall extensibility.

Materials and methods

Soybean seedling culture and harvest

Soybean (*Glycine max* [L.] Merr. cv. Williams) seeds were disinfected in 1% NaOCl for 8 min, rinsed in water for 1 h, and germinated in vermiculite in saturating humidity in the dark at 29°C. The vermiculite was hydrated with a 5 mM calcium solution (2.5 mM $CaCl_2$ and 2.5 mM $Ca(NO_3)_2$, Ψ_w of −0.06 MPa). After 48–50 h, seedlings with uniform hypocotyl and root lengths were transplanted into vermiculite pre-adjusted to −0.06 MPa or −0.36 MPa using various amounts of 5 mM Ca^{2+} solution. Vermiculite Ψ_w was measured by isopiestic thermocouple psychrometry (Boyer and Knipling 1965). When necessary for experiments, illumination was provided by a green safelight (Nonami and Boyer 1989). This seedling culture method differs slightly from our previous methods (Meyer and Boyer 1972; Cavalieri and Boyer 1982; Nonami et al. 1997) because the vermiculite we could obtain recently adsorbs large amounts of Ca^{2+}, releasing K^+ in exchange and causing Ca^{2+}-deficiency symptoms. With 2.5 mM $CaCl_2$ and 2.5 mM $Ca(NO_3)_2$, the exchange sites were saturated and sufficient Ca^{2+} was available in the bulk solution to be equivalent to past experiments and to sustain fast growth for several days.

Hypocotyl elongation was determined from the length increase measured with a ruler at various times. Elongation zones of hypocotyls were determined from the separation of ink marks applied with a paintbrush (Higgins, Eberhard Faber, Lewisburg, TN, USA). Marking with a paintbrush did not inhibit hypocotyl elongation. The space between marks and the diameter of the hypocotyls were determined with a dissecting microscope having a calibrated ocular scale.

Cell wall extensibility measurement

Cell wall extensibility was measured as a relative plastic deformability. The elongation zone of the hypocotyl was first stretched with a constant force (20 g) for 9–12 min to measure total tissue extension. Removal of the force caused a contraction of the tissue (elastic extension). A net plastic extension was calculated by subtracting elastic extension from total tissue extension (Nonami and Boyer 1990a). To calculate relative plastic deformability, the net plastic extension was divided by the length of the elongation zone and then multiplied by the average cross-sectional area of the elongation zone, then divided by the force (20 g). The unit will be equivalent to MPa^{-1} (Nonami and Boyer 1990a).

Assay for XET activity

In order to analyze the spatial distribution of XET activity, the whole hypocotyls were cut into serial segments (4–20 mm long, depending on the length of the elongating regions). At each serial position, segments were pooled and immediately frozen in liquid nitrogen. Samples were weighed and stored at −76°C until XET activity was assayed.

Hypocotyl (0.3–1.7 g fresh weight, equivalent to 14–16 hypocotyl segments) tissues were homogenized with a mortar and pestle in ice-cold 50 mM Mes (pH 6.0), 10 mM sodium ascorbate, and 10 mM $CaCl_2$. The homogenate was centrifuged for 10 min at 8,800 *g* and 4°C, and the supernatant was used for the XET assay. The activity was assayed according to Fry et al. (1992) using 0.075% xyloglucan (from *Tropaeolum* seeds), 5 mM sodium ascorbate, 5 mM $CaCl_2$, 50 mM Mes (pH 6.0), 0.2 μM (400 Bq) of reduced xyloglucan heptasaccharide ([^{3}H]XXXGol; purified from digested Tamarind xyloglucan, labeled at carbon 1 of the glucitol), and 10 μl of extract in a final reaction volume of 40 μl. The assay mixture was incubated at 25°C in a water bath for 1 h, during which the rate of the reaction was linear. During the enzyme reaction, XET transfers unlabeled large xyloglucan molecules to ^{3}H-labeled oligosaccharides ([^{3}H]XXXGol) and the product (labeled large xyloglucan molecules) is adsorbed by hydrogen bonding to the cellulose in the filter paper (Whatman #3 MM). The unreacted oligosaccharide label did not bind and was rinsed away. XET activity was calculated from the counts bound to the filter paper. Background XET activity was determined by deleting xyloglucan from the assay, in which case the assay depended on endogenous xyloglucans in the tissue extract. Subtracting background activity from total activity did not affect the results, and the data are presented for total XET activity.

XET activities were expressed on the basis of total soluble protein or cell wall dry weight. Soluble protein content was measured (Bradford 1976) in the samples used for XET assay but cell wall dry weight was measured in parallel samples because a large amount of tissue was required. Hypocotyl (1.8–4 g fresh weight) tissues were homogenized with mortar and pestle in 1.5% SDS. After centrifuging at 11,000 *g* for 10 min, the pellets were resuspended and walls were purified by the method of Wu et al. (1994) based on procedures in Fry (2000). This method removes proteins with phenol/glacial acetic acid (5:2) and starch with dimethyl sulfoxide (DMSO; 90%). After cell walls were washed three times with 70% ethanol, the wall pellets were dried at 80°C for 24 h.

In order to calculate the rate of cell wall deposition along the hypocotyls, the relative rate of cell expansion at a particular position was first estimated from the curve fitting the spatial elongation pattern. The rate of cell wall deposition was then calculated by multiplying the estimated relative elongation rate by the cell wall dry weight at each particular position along the hypocotyls.

Cell length measurement

Hypocotyls from the seedlings that had been transplanted into vermiculite having high or low Ψ_w for 24 h were cut longitudinally using a razor blade. The lengths of the cells in the middle four layers of cortical tissue were measured under the microscope. Three cells were measured at each position per hypocotyl. Three hypocotyls were used for measurement in each experiment of total four experiments.

Results

Elongation growth and cell wall extensibility

After transplanting to vermiculite at high ψ_w, hypocotyls continued to grow rapidly (Fig. 1a). Hypocotyl elongation was about 2 mm h^{-1} right after transplanting and accelerated to a rate of 3 mm h^{-1} before dropping sharply when the hypocotyl above the cotyledons (epicotyl) emerged at about 60–70 h (Fig. 1b). Low ψ_w caused an immediate near cessation of hypocotyl elongation for 25 h (Fig. 1a). The hypocotyls elongated at about 10% of the rate at high ψ_w initially, then more rapidly until they reached about 50% of the maximum rate at high ψ_w (Fig. 1b). The elongating region became longer as the hypocotyls lengthened (Fig. 1c). The lengthening of the hypocotyl elongation zone was delayed by low ψ_w. The delay was caused mostly by the decreased rate of hypocotyl growth because at the same hypocotyl length the elongation zone had a similar length regardless of ψ_w (Fig. 1c, inset).

Associated with the inhibition of hypocotyl elongation at low ψ_w was a decrease in cell wall extensibility. Relative plastic deformability was constant with time at high ψ_w. Low ψ_w caused a 35% reduction in cell wall extensibility. When hypocotyl elongation was partially recovered after 80 h in low ψ_w, cell wall extensibility was

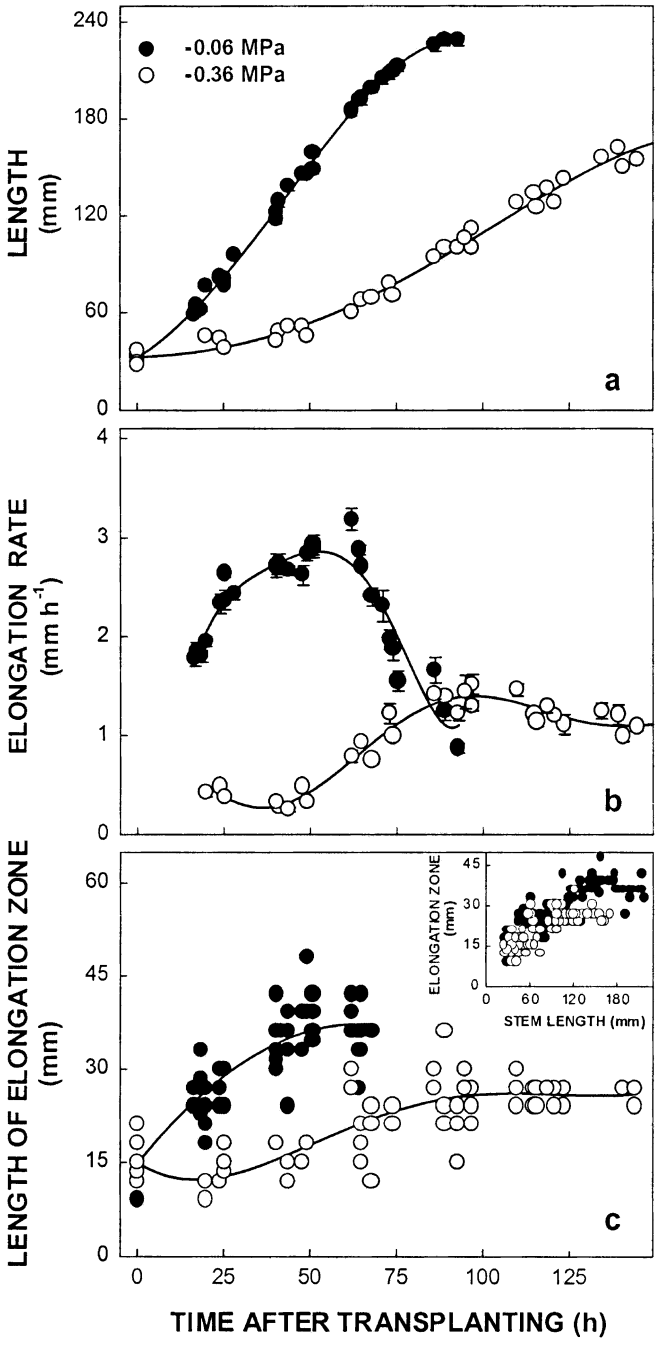

Fig. 1a–c Elongation (**a**), elongation rate (**b**), and length of the elongation zone (**c**) of soybean (*Glycine max*) hypocotyls at various times in soybean seedlings transplanted to vermiculite having high ψ_w (−0.06 MPa) or low ψ_w (−0.36 MPa). Total length of hypocotyls (**a**) was measured at various times and the elongation rates (**b**) were the slopes of **a**. Data were combined from three experiments for **a** and **b**. Each data point represents the mean of 9–12 seedlings. The error bars (SE) of some data points are small enough to be incorporated into the symbols. The length of the elongation zone (**c**) was based on the separation of ink marks at 24 h after marking on hypocotyls. Data were combined from three experiments. Eight to 12 plants were used for elongation zone determination in each experiment. The *inset* shows the length of the elongation zone for hypocotyls of various lengths. Each data point is a single measurement. The data were combined from five experiments. The lines in the figure were generated using linear regression curve fitting in SigmaPlot. At a 95% confidence interval, the correlation coefficients (r^2) are 1.0 and 0.99 for −0.06 MPa and −0.36 MPa treatments in **a**, respectively; 0.88 and 0.95 for −0.06 MPa and −0.36 MPa treatments in **b**, respectively; and 0.80 and 0.71 for −0.06 MPa and −0.36 MPa treatments in **c**, respectively

also partially recovered, showing only 19% reduction (Table 1).

Hypocotyl elongation and cell wall extensibility were reported in our previous studies (Nonami and Boyer 1990a). Due to the change in growth medium, we re-examined these parameters. The results showed little difference from our previous studies.

Spatial distribution of XET activity along the hypocotyl

To study if XET activity was associated with the decrease in cell wall extensibility, we examined the spatial distribution of XET activity along the hypocotyls. XET activity (on a total soluble protein and unit length basis) was unaffected or even increased in certain regions of the hypocotyl elongation zone after 48 h at low ψ_w compared to high ψ_w (Fig. 2a,b). On the basis of unit cell wall dry weight, however, XET activity was greatly reduced at low ψ_w in the growing zone of the hypocotyl (Fig. 2c). Beyond the 20-mm region of rapid elongation, XET activity remained high and extended into the mature region. Only in the most mature basal part of the hypocotyl did the activity begin to decline. When hypocotyl elongation had partially recovered after 96 h at low ψ_w, XET activity had more than fully recovered in the elongation zone on the basis of cell wall dry weight or unit length (Fig. 2a,c). The activity peaked in the non-elongating region between the 20- and 60-mm positions 96 h after low-ψ_w treatment regardless of the basis of expression.

Table 1 Relative plastic deformability of elongation zones in soybean (*Glycine max*) hypocotyls. Relative plastic deformability was measured in vivo after soybean seedlings were grown in different Ψ_w for various times. The elongation zone of the hypocotyl was first stretched with a constant force (20 g) for 9–12 min to measure total tissue extension. Removal of the force caused a contraction of the tissue (elastic extension). A net plastic extension was calculated by subtracting elastic extension from total tissue extension. To calculate relative plastic deformability, the net plastic extension was divided by the length of the elongation zone and then multiplied by the average cross-sectional area of the elongation zone, then divided by the force (20 g); Nonami and Boyer 1990a). The experiments were repeated four times. Three to 5 hypocotyls were measured in each experiment. Data in the table are means ± SE

Hours after transplanting	Relative plastic deformability (MPa^{-1}) × 10^{-2}	
	−0.06 MPa	−0.36 MPa
24	9.41 ± 1.16	6.11 ± 0.96
48	9.66 ± 0.66	6.06 ± 0.97
96	–	7.66 ± 0.90

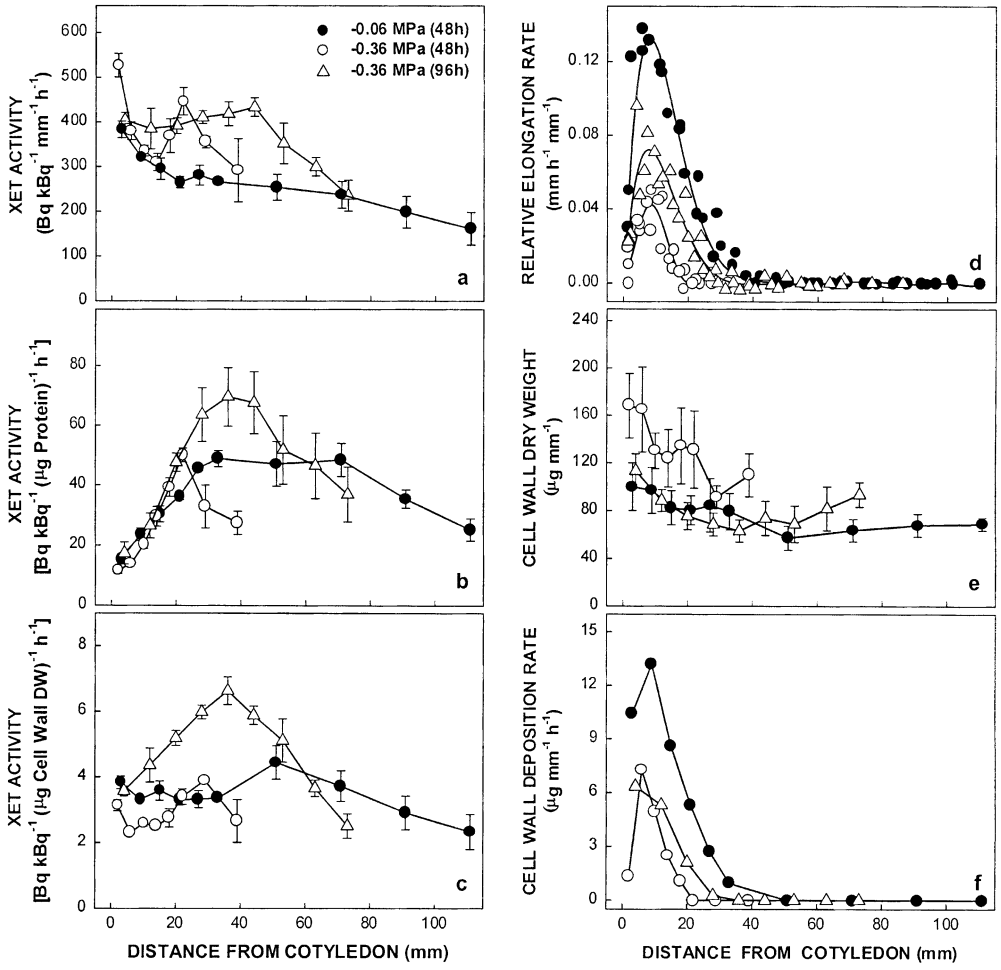

Fig. 2a–f Spatial distributions of XET activity in soybean hypocotyls expressed on unit length basis (**a**), on soluble protein basis (**b**) and on cell wall dry weight basis (**c**); and spatial distributions of relative elongation rates (**d**), cell wall dry weight (**e**), and rates of wall deposition (**f**) along hypocotyls of soybean seedlings grown at high ψ_w (−0.06 MPa) or low ψ_w (−0.36 MPa). XET activity is the mean ± SE of three experiments. Fourteen to 16 hypocotyl segments per position were used for assay in each experiment. For cell wall dry weight measurement, more hypocotyls were harvested in each experiment. The data were the mean ± SE of three experiments. Data shown in **d** are from one of three experiments and show three seedlings in each treatment. Rates of wall deposition were calculated from the data for relative elongation rates and cell wall dry weight as described in Materials and methods. Data were individual measurements (**d**), means ± SE (**a–c,e**), and the product (**d**) x (**e**) of the smoothed data in **d** for three experiments. The lines in **d** were generated using linear regression curve fitting in SigmaPlot. At a 95% confidence interval, the correlation coefficients (r^2) are 1.0, 0.90 and 0.82 for −0.06 MPa (48 h), −0.36 MPa (48 h) and −0.36 MPa (96 h) treatments, respectively

Changes in cell wall dry weights

The discrepancy in XET activity between per unit cell wall dry weight and per unit length indicated an accumulation of cell wall mass in hypocotyls at low ψ_w. Cell wall dry weights were uniformly greater along the hypocotyls after 48 h at low ψ_w (Fig. 2e). The increases were apparent by 24 h (data not shown). When hypocotyl elongation returned to an intermediate rate in the seedlings exposed to low ψ_w (96 h, Fig. 1b, 2d), wall dry weights became the same as in the high-ψ_w control at 48 h (Fig. 2e). Multiplying the relative rate of elongation (Fig. 2d) by the wall dry weight (Fig. 2e) gave the deposition rates for new wall dry weight, i.e., the rate of wall biosynthesis at various positions along the hypocotyl (Fig. 2f). In the hypocotyls, biosynthesis was diminished after 48 h at low ψ_w and remained somewhat less after 96 h compared to the high-ψ_w control (Fig. 2f).

In order to determine whether the increased dry weight of the cell walls was caused by an increase in cell number at low ψ_w (the cells would be shorter), cell length was measured along the hypocotyls in the first 25 mm at 24 h after transplanting. Cell length in the first 15 mm was the same at high and low ψ_w (Fig. 3). The first 15 mm encompassed nearly the whole elongation region at low ψ_w, but the final cell length was shorter at low ψ_w than at high ψ_w (Fig. 3) because low ψ_w caused an earlier cessation of cell expansion.

In order to address the possibility that other forms of cell expansion might occur outside of the elongation zone of the hypocotyls and roots, we examined the spatial distribution of radial expansion. However, little radial expansion occurred outside of the zone of elon-

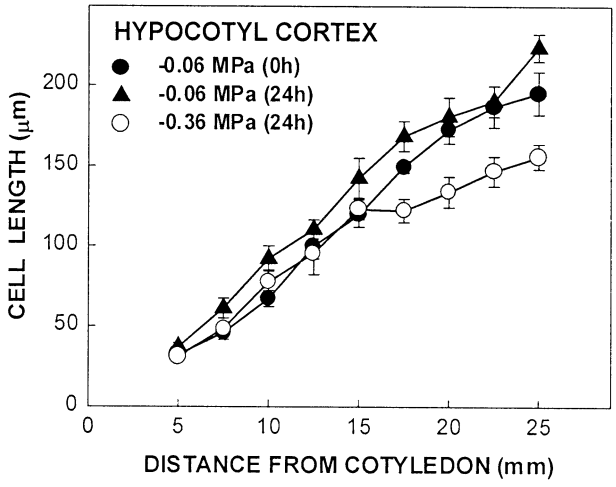

Fig. 3 Lengths of hypocotyl cortical cells of soybean seedlings grown at high ψ_w (−0.06 MPa) or low ψ_w (−0.36 MPa). Cell length was measured from the middle four layers of cortical cells of the hypocotyls 24 h after seedlings were transplanted to vermiculite having high or low ψ_w. Data are means ± SE of four experiments. In each experiment, three hypocotyls were used for determining cell length. Three cells were measured at each position per hypocotyl

gation in the hypocotyl (Fig. 4). Hypocotyl diameters averaged 2 mm (Fig. 4 inset).

Discussion

XTHs have been proposed to be important for regulating the polymer length and insertion of xyloglucans into the cell wall, which could alter the extensibility of the cell wall (Nishitani and Tominaga 1991; Smith and Fry 1991; Thompson and Fry 2001; Fry et al. 1992). The decrease in XET activity in soybean hypocotyls on the basis of cell wall dry weight and low extensibility after

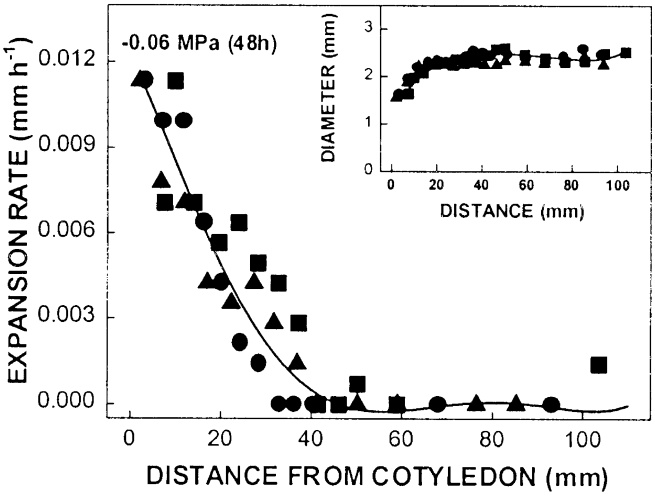

Fig. 4 Spatial distribution of radial expansion rate of soybean hypocotyls grown at high ψ_w (−0.06 MPa) for 48 h after transplanting. Data are from one of three experiments. Different symbols represent individual seedlings

48 h at low ψ_w supports this concept, and the surge in XET activity and partial recovery in extensibility by 96 h in the hypocotyls adds further evidence favoring the concept. A decrease in XET activity was also reported in the basal 5–10 mm of maize primary roots treated with polyethylene glycol solution ($\psi_w = -0.96$ MPa) for 24 h, which was correlated with a decrease in cell wall extensibility and cell elongation in that region (Pritchard et al. 1993). In the apical 5–6 mm of maize primary roots at low ψ_w (−1.6 MPa), however, Wu et al. (1994) found an enhanced XET activity, which was correlated with an increase in cell wall extensibility (Wu et al. 1996). These results strongly support a potential role of XTH associated with cell wall extensibility and thus cell elongation.

Low ψ_w induced by osmoticum typically allows wall synthesis to continue (Bonner 1934; Loescher and Nevins 1972; Iraki et al. 1989a; Wakabayashi et al. 1997; Sakurai et al. 1987; Zhong and Lauchli 1993). In soils having low ψ_w, Sweet et al. (1990) found continued wall synthesis but at a lower rate than at high ψ_w, consistent with our results. In soybean hypocotyls, rates of elongation were even more affected than the rate of cell wall synthesis. As a result, despite the lower rates of biosynthesis, the wall dry weight increased. Because the overall effect on cell number (4% in 25 mm) was too small to account for the increased wall dry weight in the hypocotyls at low ψ_w, i.e. the dimensions were unchanged in most of the cells of the elongating region, the accumulation of cell wall dry weight led to more wall per cell, suggesting that greater wall mass might have thickened the walls. Bret-Harte et al. (1991) found thicker walls in tissues whose growth had been inhibited by osmoticum. The increase in cell wall thickness in soybean hypocotyl at low ψ_w may cause a decrease in cell wall extensibility directly or/and through a dilution of wall-loosening proteins in cell walls, i.e. a decrease in XET activity in unit of cell wall mass.

Under conditions similar to ours, Nonami and Boyer (1990a, 1990b) found low extensibility in hypocotyls of soybean seedlings grown for 24–48 h at low ψ_w. In the present work (96 h), the extensibility recovered to an intermediate level afterward, when wall synthesis recovered slightly. The wall mass returned to control levels after this recovery. Therefore, a correlation existed at low ψ_w between wall extensibility, biosynthesis, and dry mass. The lower rates of synthesis may have contributed to the decreased extensibility of the walls in the hypocotyl tissues. It also is possible that the composition of the walls changed and might have contributed to the loss in extensibility. Iraki et al. (1989b) and Sweet et al. (1990) found less cellulose forming in walls at low ψ_w than at high ψ_w, and Iraki et al. (1989a) showed that the cellulose-deficient walls had a lower tensile strength. This supports the concept that cellulose reinforces the walls against the bursting tendencies of turgor pressure, but Iraki et al. (1989b) point out that extensibility probably depends more on the hemicelluloses than celluloses.

Unlike roots, whose spatial distribution of XET activity is closely correlated with cell elongation (Pritchard et al. 1993; Wu et al. 1994), the highest XET activity in the soybean hypocotyls is located beyond the elongation zone (Fig. 2a–c). A similar pattern was found in asparagus spears with the highest XET activity at the base where elongation had ceased (O'Donoghue et al. 2001). Since radial expansion of the soybean hypocotyl stops as cell elongation ceases at the end of the elongation zone, the highest XET activity may be associated with other processes in the hypocotyls. XTH is comprised of a multigene family (de Silva et al. 1994; Okamoto et al. 1994; Schünmann et al. 1997; Campbell and Braam 1999; Uozu et al. 2000) associated with many physiological processes (reviewed by de Silva et al. 1994; Nishitani 1995, 1998; Campbell and Braam 1999). We may have detected activity of different XTH isozymes along the length of the hypocotyls. Different XTH isozymes may exist in the elongation zone and non-elongation zone, which may explain why the total XET activity imperfectly matches the spatial cell elongation in the hypocotyls. In support of this notion, Catalá et al. (2001) reported that two tomato XTH genes, LeEXT and LeEXT2 were differentially expressed in the etiolated hypocotyls. LeEXT was abundantly expressed in the rapid elongation region, while LeXET2 was expressed more abundantly in the mature/non-elongating region. In soybean, there are at least four XTHs that have been studied. They are involved in different processes, such as cell expansion and response to elicitor from bacteria (Okazawa et al. 1993; Zurek and Clouse 1994; Hagihara et al. 2004). Additional ESTs for soybean XTH can be found in the TIGR soybean gene database (http://www.tigr.org).

It needs to be pointed out that XET activity assayed in vitro may not necessarily represent the action in vivo (Fry 2004). In maize primary roots, a higher expansin activity was found in the region near maturation of cell elongation (basal 5 mm) than in the region of fastest elongation (apical 5 mm; Wu et al. 1996). However, the basal 5 mm of the roots only showed very limited capability of responding to expansins in an in vitro reconstitution assay, indicating that factors other than expansins are controlling cell wall extensibility in the basal region. One of the possible explanations is that the modification of cell wall structure in the basal region of the roots makes cell walls less susceptible to expansin action (Wu et al. 1996). This could be applied to the XET activity in the soybean hypocotyl also, i.e. a higher XET activity in the non-elongating region assayed in vitro may not function in vivo due to limited access to its substrates. XET activity in vivo will also depend on the type of substrate. A recent study indicated that XTH might be capable of promoting or suppressing cell elongation, depending on the substrates used. Integration of long xyloglucan molecules into pea hypocotyl without epidermis could suppress cell elongation, but addition of xyloglucan oligosaccharide promoted cell elongation of pea hypocotyl (Takeda et al. 2002). Thus, caution must be taken when interpreting any results based on total XET activity from an in vitro assay without knowing in vivo substrates.

It is interesting that the amount of cell wall mass accumulated in the hypocotyl elongation zone during early low-ψ_w treatment (48 h) disappeared after hypocotyl elongation had partially recovered (Fig. 2a–e). The enhancement of XET activity in the first 40 mm of the soybean hypocotyl at 96 h after low-ψ_w treatment is closely correlated with the disappearance of the accumulated cell wall mass. Although the disappearance could have been caused by the displacement of the growing cells into the mature region as new cells were added to the elongating region, the cells in the mature region showed little evidence of greater wall dry weight. Therefore, it is likely that wall dry weight was mobilized by the enlarging cells during their recovery to intermediate rates of growth. One of the processes that XTH is known to be involved in is seed xyloglucan mobilization (de Silva et al. 1993; Fanutti et al. 1993). It also is possible that some XTHs in the soybean hypocotyls are performing a similar function and are involved in mobilization of xyloglucan at low ψ_w.

In summary, our studies demonstrated a close correlation between change in XET activity and cell wall extensibility. Both the increase in cell wall thickness and a decrease in XET activity in cell walls may contribute to the decrease in cell wall extensibility and thus inhibition of cell elongation in soybean hypocotyls at an early stage of low-ψ_w treatment. Our studies may also indicate other functions of XTH in soybean hypocotyl growth.

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