Journal of Experimental Botany, Vol. 51, No. 350
WD Special Issue, pp. 1543–1553, September 2000

Journal of Experimental Botany

Adaptation of roots to low water potentials by changes in cell wall extensibility and cell wall proteins

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Received 15 September 1999; Accepted 4 April 2000

Abstract

It is common for the root/shoot ratio of plants to increase when water availability is limiting. This ratio increases because roots are less sensitive than shoots to growth inhibition by low water potentials. The physiological and molecular mechanisms that assist root growth under drought conditions are reviewed, with a focus on changes in cell walls. Maize seedlings adapt to low water potential by making the walls in the apical part of the root more extensible. In part, this is accomplished by increases in expansin activity and in part by other, more complex changes in the wall. The role of xyloglucan endotransglycosylase, peroxidase and other wall enzymes in root adaptation to low water potential is evaluated and some of the complications in the field of study are listed.

Key words: Drought, expansin, peroxidase, water deficits, wall loosening, xyloglucan endotransglycosylase.

Introduction

Low water potential (ψ_w) caused by soil water deficit is one of the major natural limitations to the productivity of natural ecosystems and agriculture, resulting in large economic losses in many regions (Boyer, 1982). In the past, irrigation has been a key agricultural solution to this problem, but suitable water supplies are facing increasing societal demands and come at increasingly high financial and environmental costs. Hence, it is important to consider alternative strategies for ameliorating the detrimental effects of water deficit, for example, by increasing the drought resistance or salt tolerance of crop plants (Apse *et al*., 1999). For this, a detailed understanding of how plants respond to low water potential is of great significance. This review will focus on the growth responses of roots to water deficits.

When plants are subjected to low ψ_w, the growth of leaves and stems is rapidly inhibited (Acevedo *et al*., 1971; Nonami and Boyer, 1990; Van Volkenburgh and Boyer, 1985; Chazen and Neumann, 1994). In contrast, roots may continue to elongate at low values of ψ_w which completely inhibit shoot growth (Westgate and Boyer, 1985; Sharp *et al*., 1988; Spollen *et al*., 1993). This differential response of roots and shoots to low ψ_w is considered to be an adaptation of plants to dry conditions since continued root elongation facilitates water uptake from the soil (Sharp and Davies, 1989; Spollen *et al*., 1993; Sharp *et al*., 1997).

In the above studies, growth is largely a matter of cell expansion, which requires co-ordinated water uptake and irreversible cell wall enlargement (Cosgrove, 1993*a*). Numerous possibilities have been put forward to account for the high sensitivity of growing cells to low ψ_w, including a collapse of the water potential gradient that drives water movement (Nonami and Boyer, 1990), a reduction in cell turgor pressure that provides the expansive force necessary for cell wall extension (Frensch and Hsiao, 1994), a reduction in cell wall yielding properties (Chazen and Neumann, 1994; Neumann, 1995; Cramer and Schmidt, 1995; Cramer and Bowman, 1991), as well as complex and indirect mechanisms (Munns, 1993). Physiologists have come to appreciate that the underlying causes for growth inhibition by water deficits are not simple and depend on the time scale of the response, the particular tissue and species in question, and the particular means used to lower ψ_w.

In a similar way, the basis for the relative resistance of root growth, in comparison with shoot growth, to water deficits may be complicated and probably involves both osmotic adjustment of cell turgor pressure and adjustment

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Abbreviations: PEG, polyethylene glycol; ψ_w, water potential; XET, xyloglucan endotransglycosylase.

of cell wall yielding properties (Neumann, 1995; Frensch and Hsiao, 1994, 1995; Spollen *et al.*, 1993). In a detailed study of maize primary roots grown in vermiculite kept at high ψ_w (−0.02 MPa) or low ψ_w (−1.6 MPa), it was found that roots were able to continue to elongate, although at a reduced rate, at low ψ_w, whereas shoot elongation was completely inhibited (Sharp *et al.*, 1988). A major and surprising finding of this study was that root cell elongation at low ψ_w was completely maintained in the apical 2–3 mm of the root (Fig. 1A). This region is also called the 'distal elongation zone' and plays an important role in root gravitropism (Evans and Ishikawa, 1997; Ishikawa and Evans, 1995). Although substantial osmotic adjustment occurred in this region at low ψ_w, the decrease in osmotic potential was insufficient to compensate for the decrease in ψ_w, suggesting that turgor was greatly reduced (Sharp *et al.*, 1990). Direct turgor measurements throughout the elongation zone with a pressure probe confirmed that turgor pressure was indeed reduced from 0.7 MPa in roots at high ψ_w to approximately 0.3 MPa at low ψ_w (Fig. 1B) (Spollen and Sharp, 1991). These results suggested that cell wall yielding properties had increased in the apical 2–3 mm of roots at low ψ_w, such that cells could maintain their elongation rate even at a reduced turgor. Consistent results were also found in other studies where low ψ_w was imposed on roots by osmotic agents (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). At present, the molecular basis for such adjustment of cell wall yielding in plants at low ψ_w is poorly understood and is the major topic of this review.

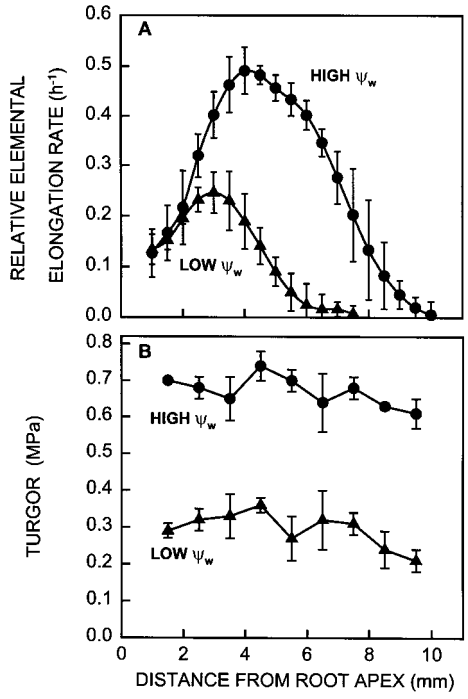

Fig. 1. Spatial distribution of (A) relative elemental elongation rate, and (B) turgor in the apical 10 mm of maize primary roots grown at high (−0.02 MPa) or low (−1.6 MPa) ψ_w in vermiculite. (Modified from Spollen and Sharp, 1991.)

According to current models of primary cell walls (McCann and Roberts, 1991; Carpita and Gibeaut, 1993; Darvill *et al.*, 1980), cellulose microfibrils, which are the major tensile elements of the wall, interact with matrix components such as hemicelluloses, forming a complex network. The interactions between the polymers endow the wall with strength or stiffness. However, the primary cell wall is also capable of expanding, indicating that the interactions between wall polymers can be modified to make walls extensible for elongation. Cellulose microfibrils are neither extensible nor degradable during cell elongation, they can only move apart (Fry, 1989). This means that the network between microfibrils is key in determining cell wall yielding behaviour. Viscoelasticity analyses similarly point to the matrix components as the key determinants of wall viscoelastic behaviour (Probine and Barber, 1966; Cleland, 1971). It should be noted, however, that the extension of the wall of living cells is not simply a viscoelastic extension; it seems that continuous action by the cell or by wall enzymes, as well as synthesis and integration of new materials into the cell wall, is an essential component of normal cell wall expansion (Cosgrove, 1993*b*). Several cell wall enzymes are believed to play important roles in modifying the wall network and thus possibly in modifying the wall's ability to extend (Taiz, 1984; Fry, 1995; Cosgrove, 1999; Ito and Nishitani, 1999). In this review, the focus will be on several recent studies of cell wall modifying enzymes in roots grown at low ψ_w, to evaluate possible mechanisms for cell wall adjustments in response to water deficits.

Expansin

Expansins are cell wall proteins uniquely able to induce cell wall extension *in vitro* (Cosgrove, 1997). They are thought to be the primary mediators of 'acid growth' (Rayle and Cleland, 1992) because they have an appropriate pH dependence and because they can fully restore pH-dependent extension in heat-denatured cell walls. Moreover, expansin activity is stimulated and inhibited by the same chemical agents that affect endogenous pH-dependent wall extension (McQueen-Mason *et al.*, 1992). Tests to date indicate that expansins do not possess significant hydrolytic or transglycosylase activity against the major constituents of the cell wall. Based on the fact that expansins can cause extension of pure cellulose paper, it has been proposed that expansins may weaken the non-covalent bonding between glucans (McQueen-Mason and Cosgrove, 1994). The current model for expansin action is shown in Fig. 2.

Several studies have demonstrated that expansins can

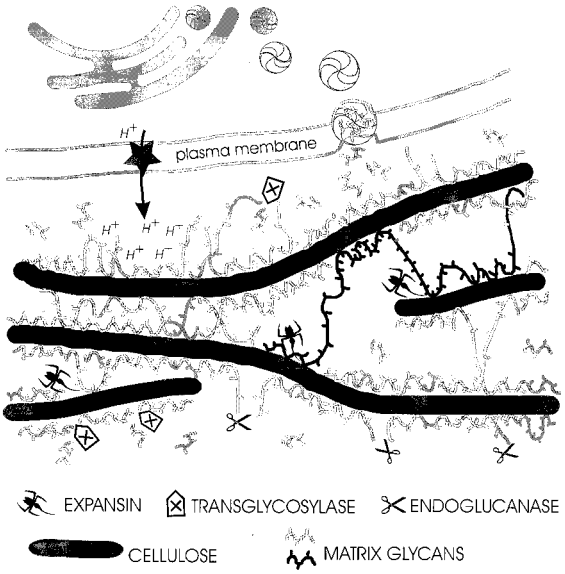

Fig. 2. Model of the cell wall and some of the activities that may alter cell wall extensibility. Cellulose microfibrils are coated with matrix glycans such as xyloglucan and embedded in a pectin/hemicellulose matrix. Expansins are thought to loosen the adhesion of matrix to the microfibrils, allowing slippage of the microfibrils and extension of the cell wall in response to the tensile forces generated by cell turgor pressure. H^+-ATPases (star) in the plasma membrane can lower wall pH and thereby activate expansins. Transglycosylases, such as XET, can cut and ligate matrix glycans to one another, while endoglucanases can cut the xyloglucan backbone, making the wall more sensitive to expansin-induced wall extension. Cross-linking enzymes (not shown) could have the opposite effect. (Modified from Cosgrove, 1997.)

also induce cell wall elongation *in vivo*, as well as *in vitro*. Application of exogenous expansin proteins to excised *Arabidopsis* hypocotyls, cucumber root hairs or cultured tobacco cells can stimulate their elongation or expansion (Link and Cosgrove, 1998; Moore *et al.*, 1995). Recently, it has been reported that exogenous application of partially purified expansins to the tomato shoot apical meristem could change the pattern of phyllotaxy (Fleming *et al.*, 1997, 1999). The distortion of phyllotaxy may have been the result of a local alteration of the physical stress pattern in the meristem caused by expansin-induced cell wall loosening (Green, 1997).

Studies with maize primary roots grown at low ψ_w vermiculite (-1.6 MPa) indicated that expansins probably play a role in growth maintenance by making the cell walls more extensible (Wu *et al.*, 1996). The first indication of this was from measurements of acid-induced extension of isolated wall specimens. The apical region of roots grown at low ψ_w had significantly higher acid-induced extension compared with control roots grown at high ψ_w (Fig. 3A). This region included the root cells whose growth was resistant to low ψ_w (Sharp *et al.*, 1988). These extensometer results provided direct support for the hypothesis that the cells walls in the apical part of the maize root become more extensible in response to low ψ_w (Spollen and Sharp, 1991).

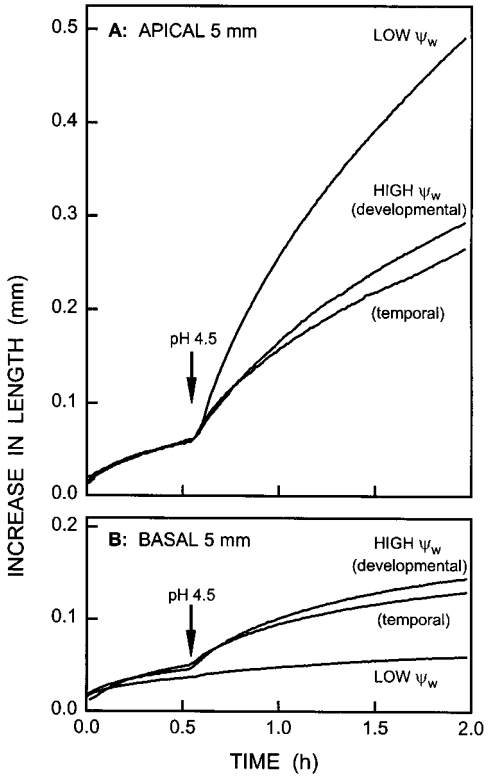

Fig. 3. Acid-induced extension of maize primary roots grown at high (-0.03 MPa) or low (-1.6 MPa) ψ_w. Roots grown at low ψ_w were harvested 48 h after treatment. The developmental control: roots were grown in high ψ_w vermiculite until roots reached the same length (~ 5 cm) as those grown at low ψ_w. The temporal control: roots were harvested at the same time as roots grown at low ψ_w (48 h). The apical 10 mm section of each roots was cut into two equal 5 mm sections, labelled as apical 5 mm or basal 5 mm. Root sections were then clamped in extensometers and bathed in a neutral pH buffer (pH 6.8). Acid-induced extension occurred when the bathing buffer was changed from pH 6.8 to 4.5 (Wu *et al.*, 1996).

Since acid-induced extension is a signature of expansin activity, cell walls were extracted for comparative assays of expansin activity from roots grown at low and high ψ_w. Expansin activities in cell wall protein extracts were assayed in a reconstitution system by adding extracted cell wall proteins from maize roots to heat-inactivated walls clamped in an extensometer, to test for wall extension activity. By this assay, roots grown at low ψ_w had higher expansin activity than well-watered controls (Fig. 4). Consistent with these activity assays, Western blots also indicated that roots grown at low ψ_w had more expansin protein than controls. Thus, the increase in expansin activity in roots at low ψ_w could be attributed at least in part to the presence of a larger amount of expansin (Wu *et al.*, 1996). Currently, the possibility is being investigated that the expression of expansin genes is up-regulated in roots in response to low ψ_w. Results from this laboratory (in preparation) indicate that expression of at least three expansin genes is specifically up-regulated in the apical region of the roots after growth

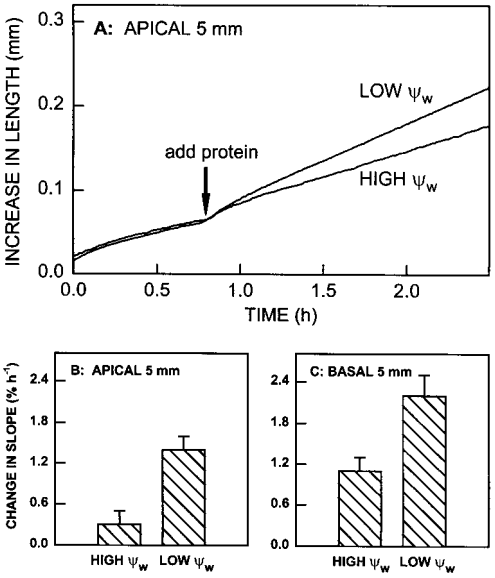

Fig. 4. Extractable expansin activity from cell walls of maize primary roots grown at high or low ψ_w. Activity was assayed by adding cell wall proteins extracted from maize root tips to induce extension of heat-inactivated cucumber hypocotyls clamped in an extensometer. Change in slope (%): the difference in slopes before and after adding expansin proteins (indicated by arrow) was normalized with the initial tissue segment length between clamps (modified from Wu *et al.*, 1996).

at low ψ_w. This is also the region of the maize root reported to have maximal H^+-pumping activity (Versel and Mayor, 1985; Peters and Felle, 1999), which would enhance expansin activity by lowering cell wall pH.

Another related finding from the study by Wu *et al.* is that the apical cell walls of roots grown at low ψ_w were more responsive to exogenous expansin (Wu *et al.*, 1996). This difference in responsiveness to expansin was interpreted to mean that a modification of cell wall structure had occurred at low ψ_w, perhaps increasing the accessibility of expansin to its site of action within the wall or perhaps increasing the ease of polymer movement that occurs for each loosening action by expansin. The possible mechanism for the change in expansin responsiveness will be discussed further below.

Whereas the apical region of the elongation zone became more extensible after low ψ_w treatment, the opposite reaction occurred in the basal part of the elongation zone (5–10 mm from the apex). These walls became less extensible after low ψ_w treatment (Fig. 3B) and they became essentially unresponsive to exogenous expansin. Even in roots grown at high ψ_w, the cell walls from this region of the growth zone showed slower acid-induced extensions and expansin-induced extensions, compared with walls in the more apical part of the root. Assays of endogenous expansin in this region indicated high levels even after low ψ_w treatment (Fig. 4B, C). It has been suggested that this region of decelerating cell elongation is a region of cell wall 'stiffening' (Tomos and Pritchard, 1994). The extensometer results show that low ψ_w caused the cell walls in this region of the maize root to become significantly stiffer, at least in the sense that they are less responsive to expansin and to acidic pH. In accordance with these findings, a recent paper reported that acid-induced elongation in intact, growing maize roots was largely confined to the apical 6 mm of the elongation zone (Winch and Pritchard, 1999).

To summarize this section, experimental studies indicate that up-regulation of expansins make cell walls more extensible when maize roots adapt to low ψ_w treatment, particularly in the apical part of the root known as the distal elongation zone. The response is more complex than this, however, because the apical region also becomes more responsive to expansins, whereas the basal, or decelerating, part of the elongation zone undergoes some kind of apparent stiffening reaction, making the walls inextensible and unresponsive to expansin and pH in extensometer assays.

Xyloglucan endotransglycosylase (XET)

Xyloglucan is the major hemicellulose in the primary cell walls of most land plants, with the notable except of grasses, where other polymers are thought to function in coating cellulose microfibrils. It is hypothesized that xyloglucans can form tethers between microfibrils, thereby contributing to the strength of cell walls (Hayashi, 1989; Fry, 1989; McCann *et al.*, 1990). The enzyme XET is able to cut and graft xyloglucan molecules to one another *in vitro* and *in vivo* (Thompson *et al.*, 1997; Ito and Nishitani, 1999). The cutting and rejoining mechanism by XET fits well into the cell wall model (Fig. 2) and has been proposed as a potential mechanism for cell wall extension (Fry *et al.*, 1992).

Some studies show a suggestive correlation of XET activity with elongation rate (Fry *et al.*, 1992; Pritchard *et al.*, 1993; Wu *et al.*, 1994; Smith *et al.*, 1996), but in other studies the XET pattern does not match elongation very well. In maize leaves the peak in XET activity preceded the peak in elongation rate (Palmer and Davies, 1996), while in pea epicotyls the XET activity peaked after the region of highest growth and extended well into the non-elongating region (Fry *et al.*, 1992). One complication in these activity studies is that plants have multiple XET genes which differ in their expression pattern and may possess distinct enzymatic activities (Rose *et al.*, 1996; Nishitani, 1997; Campbell and Braam, 1999). There has been an attempt to relate elongation of barley leaves with the spatial pattern of XET gene expression, but methodological difficulties in the kinetic analysis were later reported by Peters *et al.* (Peters *et al.*, 1999), casting doubt on the earlier conclusions (Schuenmann *et al.*, 1997). Thus, the correlation between XET activity and growth has not proved a convincing way to under-

stand XET function. Genetic alteration of XET activity by antisense methods may prove more informative (Ito and Nishitani, 1999).

Another approach to test for wall loosening activity by XET is to test for its ability to modify wall rheological properties *in vitro*. In one such study, assays of the ability of XET to induce wall extension, in the manner that expansin acts, failed to give a positive result (McQueen-Mason *et al*., 1993). There do not appear to be any other published studies testing the concept that XET directly loosens cell walls.

Based on the weight of the published evidence, a more likely role for XET is in the incorporation of newly secreted xyloglucan into the wall or perhaps rearrangement or disassembly of already bound xyloglucans (Thompson *et al*., 1997; Nishitani, 1998; Nishitani and Tominaga, 1992; Rose and Bennett, 1999). Such action might be expected to alter the rheological properties of cell walls, particularly if xyloglucans really serve as a binding agent between microfibrils; by such a mechanism, XET might under some circumstances act as a 'stiffening' or 'tightening' agent, making the walls less extensible by knitting a tighter weave around the cellulose microfibril. However, this prediction does not appear to have been tested yet by appropriate experiments.

In addition to the biochemical studies referenced above, several physiological studies lend circumstantial evidence to support the notion that XET is involved in cell wall remodelling or disassembly (Nishitani, 1997; Campbell and Braam, 1999). It was reported that up-regulation of XET gene expression was closely associated with cell breakdown and aerenchyma formation in the roots of flood-treated maize seedlings (Saab and Sachs, 1996). The pattern of expression of a touch-induced XET (*TCH4*) and the localization of the corresponding protein suggest a role for XET in morphogenesis and mechanical strengthening of wind-stimulated *Arabidopsis* plants (Antosiewicz *et al*., 1997). XET is also associated with cell wall metabolism and softening during fruit ripening (Redgwell and Fry, 1993; Rose and Bennett, 1999). Some enzymes in this family have both transglycosylase and hydrolase activity, depending on assay conditions, and hydrolytic activity is probably required for mobilization of storage xyloglucan in nasturtium seeds (Fanutti *et al*., 1993; Rose *et al*., 1996).

Two studies have examined the possible involvement of XET in maize root growth at low ψ_w (Pritchard *et al*., 1993; Wu *et al*., 1994). Both studies showed that XET activity is correlated with the spatial growth pattern. However, the two studies showed different responses in XET activity when roots were subjected to low ψ_w. For the roots grown in vermiculite of low ψ_w (-1.6 MPa) for 48 h, XET activity was greatly enhanced in the apical 5 mm region (Fig. 5). This pattern was held to be consistent with a role for XET in making walls more extensible

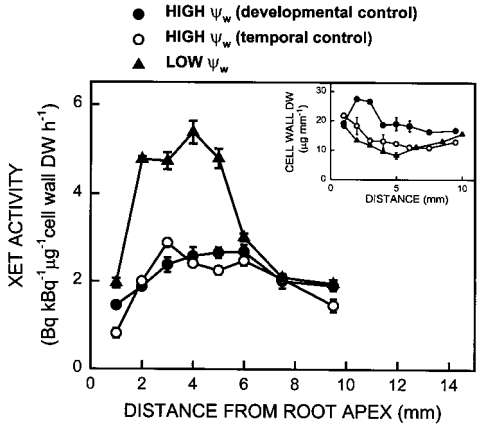

Fig. 5. Distribution of XET activity in elongation zones of maize primary roots grown at high or low ψ_w. XET activity was expressed on the basis of cell wall dry weight. Inset shows the spatial distribution of cell wall dry weight along the root tip (modified from Wu *et al*., 1994).

after water deficit, so that roots are able to elongate despite reduced turgor (Wu *et al*., 1994). In the second study, maize roots were grown in polyethylene glycol (PEG) solution of low ψ_w (-0.96 MPa) and such roots did not show an enhancement of XET activity in the tip region (Pritchard *et al*., 1993). As discussed previously by Wu *et al*., the difference in results might be caused by differences between the two experimental systems, such as severity and time of the low ψ_w treatment (Wu *et al*., 1994). An increase in XET activity was indeed found in the apical region of maize primary roots grown in the PEG system when the growth conditions were more similar to the ones used in the vermiculate study (Y Wu, SC Fry, RE Sharp, unpublished data). At a ψ_w of -1.9 MPa, the elongation rate and the turgor of roots grown in PEG solution were very similar to that of roots grown in vermiculite of -1.6 MPa (Fig. 6; Table 1). XET activity was increased in the roots grown at low ψ_w, whether XET activity was expressed on the basis of fresh weight or soluble protein (Table 1). However, the absolute activity of XET was much lower in roots grown in PEG than in vermiculite, possibly because XET is a soluble protein (Hetherington and Fry, 1993) and could easily leach out of the root and into the hydroponics system.

A recent study showed that preincubation of pea segments in xyloglucan oligosaccharides in a neutral pH buffer could enhance acid-induced extension in pea shoot segments (Cutillas-Iturralde and Lorences, 1997). The authors proposed that this enhancement arises from participation of the xyloglucan oligosaccharides in transglycosylation reactions, consequently reducing the size of xyloglucans, which might reduce microfibril-microfibril bonding and facilitate wall extension by expansins. If this is true, then an increase in XET activity in the roots at low ψ_w may serve a similar purpose. This might explain

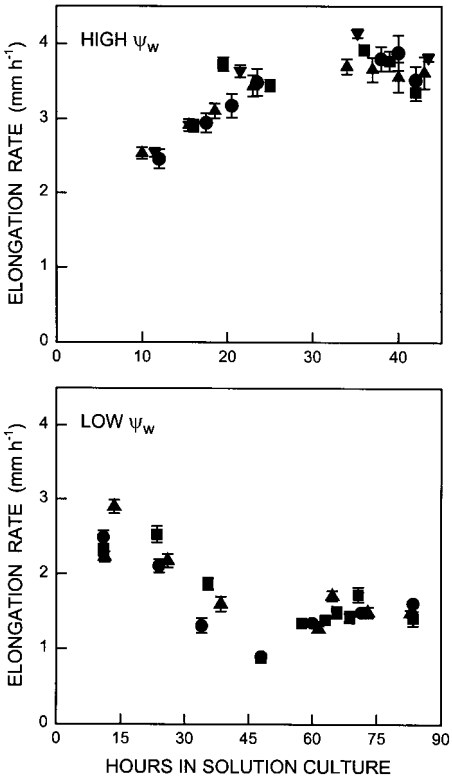

Fig. 6. Elongation rate of primary roots growing at high (−0.02 MPa) or low (−1.9 MPa) ψ_w in PEG solution. Seedlings, germinated in germination paper, with radicles ~3.5 cm long were transplanted to specially designed root culture channels (0.7 × 0.7 × 23 cm, about 15 ml in volume including tubing) containing aerated nutrient solution (5 mM MES, 0.5 mM $CaSO_4$, 6 μM H_3BO_4, pH 6.0). The kernels and shoots were not immersed in the solution. To impose the low ψ_w treatment gradually, the high ψ_w nutrient solution in the root culture channels was mixed at 0 h after transplanting with 4 ml of PEG 8000 solution ($\psi_w = -1.9$ MPa), followed by an additional 8 ml at 12 h and 11 ml at 24 h. At 36 h after transplanting, the solution was drained completely and replaced with the −1.9 MPa PEG solution. Aeration was made by bubbling solution with pure O_2, giving a solution concentration of 40–45 kPa, which was found to give optimal root growth at either low or high ψ_w. Different symbols in the figure represent means of different experiments (Y Wu, SC Fry and RE Sharp, unpublished data).

why roots grown at low ψ_w vermiculite are more responsive to expansins in the wall extension assay mentioned in the section above. This concept might be tested by *in vitro* treatment of walls with XET, followed by expansin assays. It should be noted, however, that grass cell walls are relatively deficit in xyloglucan, and so the structural significance of this polymer for cell wall mechanics is uncertain (Carpita, 1996).

It has been reported that micromolar concentrations of xyloglucan oligosaccharides promote elongation of pea stem segments (McDougall and Fry, 1990). To explain the phenomenon, it was hypothesized that the oligosaccharides might be involved in XET-mediated cell wall loosening (Smith and Fry, 1991). To test if this hypothesized mechanism exists in roots, xyloglucan oligosaccharides were added to the roots grown at low ψ_w, since roots grown at low ψ_w showed a higher XET activity and thus a more pronounced enhancement of elongation by oligosaccharides would be expected. Due to the inefficiency of uptake by roots in dry vermiculite, the experiment was conducted with roots grown in PEG solution (−1.9 MPa). When the PEG solution was bubbled with oxygen (Verslues *et al.*, 1998), roots grew at a comparable rate as those in low ψ_w vermiculite (−1.6 MPa). More importantly, the turgor reduction and XET increase were reproduced as in the vermiculite system (Table 1). Contrary to the hypothesis, however, addition of xyloglucan oligosaccharides (range from 10^{-6}–10^{-10} M) did not affect root elongation (Fig. 7). These results indicate that xyloglucan oligosaccharides do not alter maize root growth. However, some caveats and limitations must be noted. First, roots may require a higher concentration of xyloglucan oligosaccharide to promote cell elongation. In pea epicotyls, lower concentrations did not promote elongation, but instead had an inhibitory effect on elongation (York *et al.*, 1984; Augur *et al.*, 1992). Second, uptake of xyloglucan oligosaccharides might be difficult in the viscous PEG solution, or xyloglucan oligosaccharides might not penetrate deep enough to reach the important site of action. In shoots, epidermal tissue is said to be rate-limiting for organ elongation (Kutschera, 1992), whereas in roots the stele or other inner cell layers may be the growth-limiting tissue (Pritchard, 1994). Finally, although root extracts have relatively high XET activity, much of the enzyme might be compartmented intracellularly, and so not present in the cell wall compartment.

Further studies are needed at the gene expression level and at the protein level in roots grown at low ψ_w to

Table 1. *Turgor pressure and XET activity of roots grown in high or low ψ_w PEG solution*

Maximum turgor pressure was estimated from the ψ_w of the solution culture medium and ψ_s of root tissue. The apical 10 mm of the roots were equally divided into apical and basal 5 mm regions for ψ_s and XET activity measurements. Data are means ±SE of single measurements from three or four experiments (turgor pressure) and two experiments (XET activity) (Y Wu, SC Fry and RE Sharp, unpublished data).

ψ_w (MPa)	Turgor pressure (MPa)		XET activity			
			(Bq kBq^{-1} mg^{-1} FW h^{-1})		(Bq kBq^{-1} μg^{-1} soluble protein h^{-1})	
	Apical 5 mm	Basal 5 mm	Apical 5 mm	Basal 5 mm	Apical 5 mm	Basal 5 mm
−0.02 ± 0.00	0.92 ± 0.03	0.83 ± 0.02	13.88 ± 0.15	9.29 ± 0.66	2.54 ± 0.11	11.53 ± 3.04
−1.91 ± 0.04	0.40 ± 0.08	0.40 ± 0.07	29.26 ± 1.73	11.61 ± 0.66	4.46 ± 0.81	6.88 ± 0.05

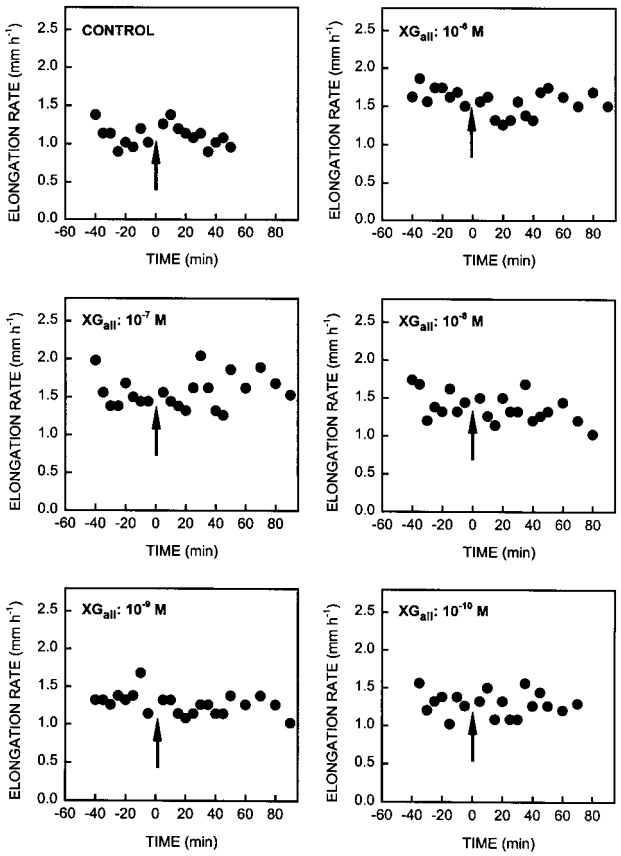

Fig. 7. Effect of adding different concentrations of xyloglucan oligosaccharides (a mixture of XXFG, XXLG and XXXG) on maize primary root elongation at a ψ_w of -1.9 MPa. After roots had reached a steady elongation (see Fig. 6), xyloglucan oligosaccharides solutions were injected into the culture medium (indicated by arrow). To measure root elongation at high resolution, a video camcorder equipped with a macro lens was used to increase the magnification approximately 100 times. For long-term root elongation, root length was marked on the root culture channel (Y Wu, SC Fry and RE Sharp, unpublished data).

confirm the XET activity results as well as to determine how XET is regulated. Preliminary studies showed that the increase in XET activity in the apical region was not due to enhanced gene expression. A slight increase in mRNA level was found in the more basal region of the roots grown at low ψ_w, however (Saab, 1999). It is possible that the enhanced XET activity in the roots at low ψ_w is regulated at the post-translational level.

Glucanase

In studies of auxin-induced growth of grass coleoptiles, a role for glucanases in cell wall loosening and growth induction has long been suspected (Hoson, 1993), but direct evidence that glucanases can induce cell wall extension *in vitro* is lacking. An indirect role for glucanases, as a synergist for expansin action, has been proposed (Cosgrove, 1997). This idea is supported by results showing that pretreatment of walls with 'cellulases' makes the walls more responsive to expansin action (Cosgrove and Durachko, 1994). Relatively little work on this topic has been done with roots, however, so it remains an open question as to whether these enzymes are specifically involved in root responses to water stress.

Agents for cell wall stiffening

By wall stiffening, it is meant that the wall structure is modified so that it is less extensible in response to expansin or other cell wall loosening agents. Wall stiffening is hypothesized to occur in the decelerating or basal part of the root elongation zone, as mentioned above (Tomos and Pritchard, 1994). The biochemical basis of wall stiffening is not well defined at this stage, but might be caused by cross-linking of wall polymers, by increases in non-covalent binding of polymers to each other, or by increases in the viscosity of the matrix polymers (Cosgrove, 1997).

In the case of maize primary roots adapted to low ψ_w, an increase in cell wall stiffening may occur in basal elongation region. The evidence for this suggestion is indirect and is based primarily on two observations. First, cells in the basal region ($\sim$6–11 mm from the apex) cease growth prematurely (Fig. 1A). This results in a shortened elongation zone. The growth cessation in this region cannot simply be due to a loss of turgor because pressure probe measurements show turgor to be approximately constant throughout the elongation zone (Spollen and Sharp, 1991; Pritchard *et al.*, 1990, 1993). Therefore, gradients in cell wall yielding properties are hypothesized as the primary basis for variations in elongation rate along the root. Second, extensometer assays of walls from this region show them to be capable of less acid-induced extension and less expansin-induced extension (Wu *et al.*, 1996). Presumably these walls are less extensible because they have undergone a change in structure, making the polymers less mobile; however, there is little experimental evidence that addresses the nature of this change in wall structure and it is probably quite complex (Cosgrove, 1997).

As described above, it is conceivable that XET might be involved in such changes in wall structure. However, XET activity, when expressed per unit cell wall dry mass, was not changed in the basal region of water-stressed maize roots, indicating that changes in XET are not responsible for the shortening of elongation zone (Wu *et al.*, 1994).

Another candidate wall stiffening agent is peroxidase. This enzyme can catalyse oxidative cross-linking of phenolic groups in the cell wall, and such action might make the wall less extensible (Fry, 1986; Schopfer, 1996). An extreme instance of this is the formation of lignin, which is usually thought to occur well after cell elongation has ceased. However, a recent study of the maize coleoptile

concludes that lignification may be an important process for growth cessation (Muesel *et al*., 1997). Peroxidase may also catalyse the insolubilization and possible cross-linking of wall structural proteins, such as hydroxyproline-rich glycoproteins (Showalter, 1993; Otte and Barz, 1996). Such insolubilization has been hypothesized to make the wall stiffer, but this point has not been rigorously demonstrated.

Several studies have presented circumstantial evidence that peroxidase is involved in the normal cessation of cell elongation by wall stiffening. For instance, wall peroxidase activity was closely associated with growth cessation in fescue leaves (Macadam *et al*., 1992), and drought-induced inhibition of leaf growth in *Lolium* was correlated with an increase in peroxidase activity in the leaf elongation zone (Bacon *et al*., 1997). Growth inhibition of pea epicotyls by xyloglucan nonasaccharide was correlated with higher peroxidase activity (Warneck *et al*., 1996). Stimulation of root elongation by ascorbate was likewise attributed, in part, to an inhibition of peroxidase activity (Cordoba-Pedregosa *et al*., 1996) and in a similar vein inhibition of peroxidase gene expression in tobacco using antisense methods led to a modest ($\sim$10%) increase in plant height (Lagrimini *et al*., 1997). Other studies have also reported changes in peroxidase activity or gene expression that correlate in some way with wall mechanical properties or with growth cessation (Sancho *et al*., 1996; Sanchez *et al*., 1995; Schunmann *et al*., 1994).

While these results are suggestive of a role for peroxidase in cell wall stiffening and growth cessation, some caution is needed because the connection between the two is open to various interpretations. For example, the growth stimulation observed in antisense tobacco plants with reduced peroxidase expression (Lagrimini *et al*., 1997) was attributed not to changes in cell wall linkages, but to possible oxidation of auxin by peroxidase. Indeed, the promiscuity (non-selectivity) of these enzymes makes it difficult to attribute a particular physiological response in living plants to a specific reaction because it can be involved in many reaction pathways simultaneously. A second problem is that robust measurements of wall 'stiffening' are rarely used in these studies. Despite the long prevalence of the idea that phenolic cross-linking as a possible mechanism for making walls less extensible, the relationship between the degree of phenolic cross-linking of the wall and extensibility of the wall has received little in the way of detailed study. One serious difficulty is that many simultaneous structural changes in the wall occur as cells mature, making it difficult to attribute stiffening to one specific mechanism. *In vitro* systems are needed, where a single attribute of the wall can be changed and the consequential effects on cell wall behaviour tested. Unfortunately, the complexity and heterogeneity of cell wall structure makes such a test system very challenging to manipulate in any but relatively simple ways.

In maize roots, an increase in peroxidase activity was detected when roots were subjected to low ψ_w (using PEG) when dianisidine was used as a substrate. However, the results were reversed when another substrate was used for the enzyme assay (Tomos and Pritchard, 1994; Pritchard, 1994). The difficulty of accurate assays of specific peroxidases in the cell wall and the uncertainty of natural substrates for these enzymes makes this field fraught with many technical difficulties.

Conclusions and further ideas

Growing roots adapt to water deficits by a combination of osmotic and cell wall changes. The changes in the wall are complex and opposite in the apical region versus the basal region of the elongation zone. This differential response adds another level of complexity to these studies because whole-root growth responses are a summation of these opposing responses. Thus, estimates of cell wall yielding properties based on the elongation of whole roots represent numerical averages that miss this important spatial detail. At the molecular level, increases in expansin activity help to make the cell walls of the apical region of the root more extensible; the cell wall also changes its responsiveness to expansin, but the molecular nature of such change is still poorly understood.

In addition to the major themes outlined above, it should be noted that water deficits may induce many other changes in plant cells, including changes in cell wall polysaccharide composition and gene expression (Iraki *et al*., 1989; Zhong and Lauchli, 1993; Creelman and Mullet, 1991). However, there is still a major gap in our ability to relate such changes to the growth behaviour of cells and to cell wall properties. More difficult to measure are ephemeral conditions in the cell wall established by transmembrane proteins, such as plasma membrane electron transport systems, ion channels, and H^+-ATPases. These activities can control wall pH, ion concentrations, and redox potential, which in turn may modulate both the activity of wall enzymes and the physical properties of the wall matrix. There is some evidence that water deficits can affect these processes (Van Volkenburgh and Boyer, 1985; Surowy and Boyer, 1991), and so it is possible that they also play a role in the integrated response of plant roots to water stress, but technical limitations of the measurements poses serious obstacles to detailed assessment of their role.

Acknowledgement

This work is supported by a grant from the US Department of Agriculture.

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