Modification of Expansin Transcript Levels in the Maize Primary Root at Low Water Potentials

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We previously demonstrated that maintenance of cell elongation in the apical region of maize primary roots at low water potentials ($\psi_w$) was associated with an increase in expansin activity and extractable expansin protein. Here, we characterized the spatial pattern of expansin gene expression along the growing maize root and studied the effect of low $\psi_w$ on expansin gene expression. Roots were divided into three segments: apical 0 to 5 mm, subapical 5 to 10 mm, and non-growing 10 to 20 mm. Of the five expansin genes expressed in control roots, two $\alpha$-expansins ($Exp1$ and $Exp5$) and two $\beta$-expansins ($ExpB2$ and $ExpB8$) are expressed specifically in the growing region, whereas expression of $\beta$-expansin $ExpB6$ is shifted basipetally. After seedlings were transplanted to vermiculite with a $\psi_w$ of $-1.6$ MPa, transcripts for $Exp1$, $Exp5$, and $ExpB8$ rapidly accumulated in the apical region of the root. These mRNA changes correlated with the maintenance of root elongation and increases in wall extensibility found previously. The $\beta$-expansins $ExpB2$ and $ExpB6$ showed distinctive patterns of expression and responses to low $\psi_w$, indicative of distinctive functions. Inhibition of abscisic acid (ABA) accumulation at low $\psi_w$ (by fluridone treatment) had no effect on expansin expression, except that $ExpB2$ transcript level showed a minor dependence on ABA. Gene-specific regulation of $\alpha$- and $\beta$-expansin mRNA pools likely contributes to growth alterations of the maize (Zea mays) root as it adapts to a low $\psi_w$, but these changes do not appear to be mediated by changes in ABA content.

Root elongation is often less inhibited at low water potentials ($\psi_w$) than are shoot and leaf elongation (Westgate and Beyer, 1985; Sharp and Davies, 1989; Spollen et al., 1993). The difference in sensitivity between roots and shoots to low $\psi_w$ is considered an adaptation of plants to soil drying since maintenance of root elongation allows plants to pursue a receding water source into deeper soil (Sharp and Davies, 1989; Spollen et al., 1993). Thus, understanding the mechanisms of root growth adaptation to low $\psi_w$ may provide important information for improving plant performance under water-limited conditions.

The rate of cell expansion depends on cell wall-yielding properties and turgor pressure inside the cell (Lockhart, 1965; Cosgrove, 1993; Proseus et al., 2000). Studies have shown that adjustment of wall-yielding properties plays an important role in root growth maintenance at low $\psi_w$. In many cases where roots were subjected to low $\psi_w$ treatments, root elongation rate resumed before turgor pressure recovered, suggesting an increase in cell wall-yielding properties (Kuzmanoff and Evans, 1981; Hsiao and Jing, 1987; Itoh et al., 1987; Pritchard et al., 1993; Frensch and Hsiao, 1994, 1995; Triboulot et al., 1995). In primary roots of maize (Zea mays) seedlings grown in vermiculite at a $\psi_w$ of $-1.6$ MPa, cell elongation in the apical few millimeters was fully maintained despite a decrease in turgor of 60%, indicating that cell wall loosening increased preferentially toward the root apex (Spollen and Sharp, 1991). Direct assessment of cell wall extension properties in the apical region of water-stressed (WS) maize primary roots revealed a large increase in acid-induced extensibility compared with control roots grown at high $\psi_w$ (Wu et al., 1996).

Since cell wall-loosening proteins are believed to play key roles in controlling cell wall extension (Taiz, 1984; Fry, 1995; Ito and Nishitani, 1999; Cosgrove, 2000), activities of expansins and xyloglucan endotransglycosylase (XET) were examined to see if they correlated with the increase in cell wall extensibility in the apical region of WS maize roots. XET activity was enhanced in the apical region of maize roots at low $\psi_w$ (Wu et al., 1994); however, the hypothesized role for XET in cell loosening could not be confirmed by in vitro assays (McQueen-Mason et al., 1993). In contrast, expansins are capable of inducing cell wall extension in vitro and in vivo (for review, see Cosgrove, 1999, 2000). Expansin activity was found to be enhanced in the apical region of maize roots at low $\psi_w$, and this response was associated with a higher abundance of $\alpha$-expansin proteins (Wu et al., 1996).
At the time this work was carried out, the existence of a second family of expansins (β-expansins) was not recognized, but it appears likely that β-expansins may be significant for wall loosening in the grasses (for review, see Cosgrove, 2000).

To further understand the regulation of expansin activity in WS roots, in this study we have analyzed the spatial pattern of transcript levels of expansin genes expressed in the apical region of maize primary roots grown at high and low \( \psi_w \). In a previous study (Wu et al., 2001), we identified the major \( \alpha \)- and β-expansins genes expressed in different parts of the maize plant, and here we have focused on the five expansin genes known to be expressed in the root. Since abscisic acid (ABA) accumulation is required for the maintenance of root elongation at low \( \psi_w \) in maize seedlings (Saab et al., 1990; Sharp et al., 1994; Spollen et al., 2000), we also examined the dependence of low \( \psi_w \)-induced changes in expansin transcript levels on ABA accumulation.

RESULTS

Spatial Pattern of Expansin Gene Expression

Previous work identified five expansin genes expressed in the maize root, namely the two \( \alpha \)-expansins, Exp1 and Exp5, and the three β-expansins, ExpB2, ExpB6, and ExpB8 (Wu et al., 2001). An initial experiment was conducted to evaluate the spatial pattern of expansin gene expression along the root and to compare expansin gene expression of an inbred line (FR697) and a hybrid line (cv FR27 × FRMo17) to see if they responded to low \( \psi_w \) similarly. This comparison was made because the initial mRNA probe design and gene expression analyses were done with the inbred line (Wu et al., 2001), whereas the previous physiological work was done with the hybrid line (Wu et al., 1996). The northern-blot comparisons (Fig. 1) showed that the two lines had similar patterns of expansin gene expression, but the hybrid gave a stronger signal in many cases.

In control roots (grown at high \( \psi_w \)), two \( \alpha \)-expansins (Exp1 and Exp5) and two \( \beta \)-expansins (ExpB2 and ExpB8) were specifically expressed in the elongation zone, with nearly equal signals in the apical region A (0–5 mm) and the subapical region B (5–10 mm), and with little expression in the nonelongating region C (10–20 mm). This pattern of expression correlates with the distribution of elongation growth along the maize root (see Sharp et al., 1988). Contrary to this pattern, ExpB6 was expressed mainly in the subapical (B) and nonelongating (C) regions of well-watered (WW) roots. Thus, this expansin is unlikely to be directly involved in root elongation.

Effect of Low \( \psi_w \) on Expansin Gene Expression

Ten hours of low \( \psi_w \) treatment appeared to cause an increase in transcript levels of all the expansin genes in the apical region A (Fig. 1). The increase in ExpB2 expression was small and reproducible, but was not significantly greater than the control expression, when a statistical evaluation was made (Table 1). In the subapical region B, low \( \psi_w \) treatment resulted in a significant reduction in Exp5 transcripts and an increase in ExpB6 transcripts. Modest changes in transcript abundance for Exp1, ExpB2, and ExpB8 in region B were also observed in this particular experiment, but a statistical analysis of three experiments indicates these changes were not significant at the 0.05 probability level (Table 1).

Compared with roots harvested 10 h after transplanting, roots harvested at a later time showed a reduced response to low \( \psi_w \). For the later harvest, tissues were collected when the roots attained the same length (50 mm), that is, at 20 h for the high \( \psi_w \) treatment and 48 h for the low \( \psi_w \) treatment. Although the response was attenuated at this later time, the effect of low \( \psi_w \) on expansin transcript levels was still apparent. The root growth response to water stress in this and subsequent experiments was confirmed to be similar to that reported previously (Sharp et al., 1988). For example, the WW control roots shown in Figure 1 grew at an average elongation rate of 2.7 mm h\(^{-1}\) for inbred seedlings and 2.6 mm h\(^{-1}\) for hybrid seedlings during the interval between the 10 and 20 h time points, and low \( \psi_w \)
reduced elongation rates to 1.2 mm h$^{-1}$ (inbred) and 0.76 mm h$^{-1}$ (hybrid). Because inbred and hybrid lines showed a similar pattern of response to low $\psi_w$ and since transcript levels were higher in the hybrid line for most of the genes examined, the rest of the experiments were conducted with the hybrid line.

A time course was carried out to study the changes in transcript levels in more detail (Fig. 2). For Exp1, Exp5, and ExpB8, a rapid and sustained increase in transcript levels was found for the apical 0 to 5 mm region after transplanting to WS conditions. In the 5 to 10 mm region, in contrast, Exp 5 showed a rapid decrease in expression. For Exp5, transcript levels for control roots also decreased substantially with time in both regions. A similar, but less pronounced decrease was apparent in the expression of some of the other expansin genes in the control tissues. This decrease might be developmental, or might be related to alterations in gene expression associated with transplanting (e.g. touch-induced responses). The expression of ExpB2 in the control roots showed a complicated pattern, including a peak 15 h after transplant, but low $\psi_w$ did not result in a clear change in expression. In contrast to the other expansins, Exp B6 exhibited a rapid increase in expression in the subapical 5 to 10 mm region, in addition to a smaller and delayed increase in the 0 to 5 mm region.

We conclude that the low $\psi_w$ condition elicits an enhanced expression of selected expansin genes in the apical region, whereas the basal region responds in a more complicated pattern. The distinctive response patterns observed for these five expansin genes indicates that these changes are active responses, not simply passive consequences of the changes in root growth kinematics (see “Discussion”).

Because we do not yet have a full census of all expansin genes in maize, it is possible that expansin genes other than those studied here may also be expressed in roots and be regulated by low $\psi_w$. Therefore, to test whether the expression patterns seen in Figures 1 and 2 were representative of the overall pattern for expansin gene expression we probed the northern blots using several expansin cDNAs containing conserved coding regions as probes. The sequence conservatism among expansins is sufficiently high to allow cross hybridization between sequences within a family (M. Shieh, Y. Wu, and D.J. Cosgrove, unpublished data), so this test should give an estimate of the summed expression of each expansin family. The probe mixture for $\alpha$-expansins was composed of five $\alpha$-expansin cDNAs and the probe mixture for $\beta$-expansins was composed of seven $\beta$-expansin cDNAs. Similar to the patterns seen in Figures 1 and 2, the transcript level for “all” $\alpha$- or $\beta$-expansin genes showed an enhancement in region A (Fig. 3). Thus, these results confirmed to the expectation for the general expression pattern observed above and make it unlikely that an unidentified expansin gene might be expressed at high levels in the root in a pattern substantially different from that seen in Figures 1 and 2.

### Is ABA an Intermediary in the Expansin Response?

Since accumulation of ABA enhances the maintenance of root elongation in WS maize seedlings (Saab et al., 1990; Sharp et al., 1994; Spollen et al., 2000), we explored the possibility that the alteration of expansin gene expression was regulated by ABA. Seedlings were therefore treated with fluridone (FLU), an inhibitor of ABA biosynthesis. Figure 4B shows that treatment with FLU prevented about 70% of the accumulation of ABA in the 0 to 5 and 5 to 10 mm regions of the root tip 15 h after transplanting to low $\psi_w$. Compared with previous studies that encompassed longer time periods, FLU treatment in these experiments caused a smaller reduction (22%) in elongation of the WS roots (Fig. 4A). We also attempted to restore ABA levels in FLU-treated roots by treatment with exogenous ABA (0.5 mm). This treatment was shown previously to restore root elongation of FLU-treated seedlings at a $\psi_w$ of −1.6 MPa (Spollen et al., 2000), as was also the case in the present experiments (Fig. 4A). However, the addition of ABA only partially restored bulk ABA levels in the root tip (Fig. 4B), probably because of the short duration of the treatment.

The same root samples from the experiment shown in Figure 4 were used for gene expression analyses. Exp1, Exp5, and ExpB8 showed similar changes in transcript levels in response to low $\psi_w$ treatment in FLU-treated roots as found in roots not treated with FLU (Fig. 5; Table II). Transcript levels increased in region A and decreased in region B. Therefore, we conclude that decreased ABA accumulation (FLU treatment) did not have a substantial effect on the low $\psi_w$-induced changes in expression of these genes. FLU treatment did cause a modest, but statistically significant, reduction in expression of ExpB2, and this effect was reversed by addition of ABA (however, the reversal did not attain a statistical level of $P < 0.05$; see Table II). The addition of ABA to FLU-treated roots did not significantly affect transcript levels of any of these expansin genes (Table II). In the
longer roots used for this experiment (see “Materials and Methods”), ExpB6 transcript was less responsive to low $\psi_w$ (Figs. 1 and 2), but again showed enhanced expression in region B. These results do not support the hypothesis that ABA mediates the effect of low $\psi_w$ on expansin gene expression.

**DISCUSSION**

Our results show that four expansins (Exp1, Exp5, ExpB2, and ExpB8) are expressed specifically in the elongating region of maize primary roots and these are thus good candidates for expansin genes involved in maize root elongation, whereas ExpB6 expression is basipetal to the peak of the elongation zone. Low $\psi_w$ treatment differentially affects expression of these genes in the apical and subapical regions of the root elongation zone. In the apical region, low $\psi_w$ increases expansin transcript levels for Exp1, Exp5, ExpB6, and ExpB8. These changes in the apical region correlate closely with the enhancement of wall extension properties, as well as the enhance-
and for 10 and 48 h under WS conditions. Roots grown in low
after seedlings were grown for 10 and 20 h under WW conditions
zone of roots experiencing low
This coincides with a shortening of the elongation
came localized to the apical 5-mm region (Fig. 2A).
explore new soil volume for water (Sharp et al., 1990;
of resources and can elongate at minimal cost to
limited water supply roots can concentrate their use
response is believed to be adaptive, such that under
the growth pattern of the root tip. This root growth
regulation is part of the mechanism used to regulate
the maize root, and we propose that expansin gene
expression in the root tip.

Figure 3. Overall expansin gene expression at high or low \( \psi_w \) ass-
essed by northern-blot analysis. Root tips were cut into three sec-
tions from the apex, A (0–5 mm), B (5–10 mm), and C (10–20 mm),
after seedlings were grown for 10 and 20 h under WW conditions
and for 10 and 48 h under WS conditions. Roots grown in low \( \psi_w \)
vermiculite for 48 h reached the same length as those grown in high
\( \psi_w \) vermiculite for 20 h. \( \alpha \)-Expansins, RNA blot was probed with a
mixture of five \( \alpha \)-expansin cDNAs; \( \beta \)-expansins, RNA blot was probed with a mixture of seven \( \beta \)-expansin cDNAs.

Figure 4. Root length increase (A) and ABA content (B) of the 0- to
5-mm (A) and 5- to 10-mm (B) regions of the root tip 15 h after
transplanting to WW or WS conditions. ABA accumulation under
water stress was decreased by treatment with FLU, and ABA was
added to FLU-treated roots to restore root elongation. Data are
means ± se (root length increases, \( n = 102; \) ABA contents, \( n = 3 \)).
The experiment was repeated with similar results.

ment of expansin activity and protein abundance
found in a previous study (Wu et al., 1996). These
results are consistent with the hypothesis that the
adaptive wall loosening and growth maintenance in
the apical region of maize roots, in response to low
\( \psi_w \), are the result, at least in part, of altered expansin
gene expression in the root tip.

With increasing time after transplanting to low \( \psi_w \),
the expression of \( \text{Exp1, Exp5, ExpB2,} \) and \( \text{ExpB8} \)
became localized to the apical 5-mm region (Fig. 2A).
This coincides with a shortening of the elongation
zone of roots experiencing low \( \psi_w \). In maize primary
roots growing at high \( \psi_w \) the elongation zone
comprises the apical 11 mm, whereas in roots at a \( \psi_w \) of
\(-1.6 \) MPa the elongation zone is confined to approxi-
ately the apical 6 mm (Sharp et al., 1988). Thus, the
spatial pattern of expression of these expansin genes
 correlates with the position of the elongation zone in
the maize root, and we propose that expansin gene
regulation is part of the mechanism used to regulate
the growth pattern of the root tip. This root growth
response is believed to be adaptive, such that under
limited water supply roots can concentrate their use
of resources and can elongate at minimal cost to
explore new soil volume for water (Sharp et al., 1990;
Voetberg and Sharp, 1991; Liang et al., 1997).

From previous studies there is ample evidence that
\( \alpha \)-expansins function as wall-loosening agents for
control of plant cell growth and for other processes
(for summary, see Cosgrove, 2000) and may be redis-
tributed during maize root gravitropism (Zhang and
Hasenstein, 2000). The expression patterns found in
this study are consistent with a role for the
\( \alpha \)-expansins \( \text{Exp1} \) and \( \text{Exp5} \) in maize root growth.

In contrast to \( \alpha \)-expansins, much less is known
about the functions and properties of \( \beta \)-expansins.
The possible biological roles of only two \( \beta \)-expansins
have been studied up to now. A \( \beta \)-expansin secreted
by grass pollen is thought to aid in pollen tube pen-
etration of the stigma and style (Cosgrove et al., 1997),
whereas in soybean cultures the expression of a
\( \beta \)-expansin gene (\( \text{CIM1} \)) is linked to cytokin-
induced cell proliferation (Downes and Crowell,
1998). Our results with the maize root show that the
\( \beta \)-expansins \( \text{ExpB2} \) and \( \text{ExpB8} \) are expressed in a
pattern consistent with a role in wall-loosening for
root cell elongation, very similar to the \( \alpha \)-expansins
\( \text{Exp1} \) and \( \text{Exp5} \).

\( \text{ExpB2} \) and \( \text{ExpB8} \) are notable because they are un-
usually similar in sequence, with 96% identity at the
nucleotide level in the coding region for the mature
protein (Wu et al., 2001). We should note that the
gene-specific probes used to assess the expression of
these two genes cross react to a small extent, and so
it is possible that the signals on the northern blots
contain a small amount of “blending” of the signals
for these two genes. However, because the temporal
patterns of expression differed substantially for these
two genes (Fig. 2), this is unlikely to be a serious
problem in these experiments; the differences in re-
response to low \( \psi_w \) also suggest that the promoters for
the two genes have functionally diverged with re-
spect to the root response to water stress.

Compared with \( \text{ExpB2} \) and \( \text{ExpB8} \), the distinctive expression pattern for the \( \beta \)-expansin \( \text{ExpB6} \) points to
ABA accumulation contributes to the maintenance of maize primary root elongation at low $\psi_w$ (Saab et al., 1990; Sharp et al., 1994; Spollen et al., 2000), and it is possible that the alterations of expansin gene expression induced by low $\psi_w$ were mediated by changes in root ABA levels. However, our results give little support for this hypothesis. Treatment with FLU largely blocked accumulation of ABA in the WS roots (Fig. 4), yet most of the expansin genes were still expressed in a pattern typical of roots at low $\psi_w$. In our experiments FLU treatment had only a small effect on root elongation, and so the ameliorating effect of ABA on the elongation of WS roots reported in other studies (Saab et al., 1990; Sharp et al., 1994; Spollen et al., 2000) was not so apparent here, probably because of the shorter duration of the experiment. At least in the time frame studied here, the regulation of expansin gene expression does not appear to be ABA dependent. This conclusion, however, is compromised by the fact that FLU did not completely block ABA accumulation during water stress, so it is possible that the slightly elevated ABA levels in the roots were sufficient for inducing the observed changes of expansin transcripts. Nevertheless, our results with FLU and ABA additions make this an unlikely scenario, except for ExpB2, whose expression was partly modulated by ABA.

In contrast, various hormones are reported to modify transcript levels of $\alpha$-expansin genes in other systems. For example, auxin increases $\alpha$-expansin transcript levels in tomato hypocotyls (Caderas et al., 2000; Catala et al., 2000), gibberellin stimulates $\alpha$-expansin gene expression in rice internodes (Cho and Kende, 1997), and ethylene causes accumulation of $\alpha$-expansin transcripts in Rumex palustris and Rumex acetosa leaves (Vriezen et al., 2000) and in ripening tomato fruit (Rose et al., 1997). Expansins are encoded by a large gene family and it appears that each gene has a distinctive promoter with sensitivities to particular developmental states and environmental conditions (Cosgrove, 2000).

In summary, our study showed that low $\psi_w$ induced an increase in transcripts of $\alpha$- and $\beta$-expansins a biological role different from that of the other expansin genes studied here. Its high expression in the subapical and non-growing regions hints at a possible role in cell differentiation or vascular formation. Previous in situ localization studies indicate that some expansin genes are closely associated with xylem and vascular tissue development (Cho and Kende, 1998; Im et al., 2000). The enhanced expression of ExpB6 in region B during water stress is mostly likely the result of compression of the elongation zone; region B in WS roots thus becomes more like region C in WW controls.

There is one apparent discrepancy between our current results and those previously published: namely, in the subapical 5- to 10-mm region, the abundance of extractable $\alpha$-expansin protein was increased after water stress (Wu et al., 1996), whereas we found that the abundance of $\alpha$-expansin mRNAs is reduced in this region after water stress. This difference probably arises because $\alpha$-expansins are relatively stable proteins that bind avidly to the cell wall. Plant cells are not known to have mechanisms for turning over such extracellular proteins. Thus, the $\alpha$-expansins found in the subapical 5- to 10-mm region were probably synthesized when the cells were in the apical 0- to 5-mm region, where they had elevated mRNA levels for Exp1 and Exp5. These $\alpha$-expansin proteins may be immobilized onto older wall layers formed in the apical region and thus may not be located in an appropriate site for influencing cell growth by the time the cells are displaced into the subapical region (Cosgrove, 1999). It is also notable that in WS roots, the cell walls in this subapical region lose their sensitivity to expansin-induced wall loosening (Wu et al., 1996). This is likely to be an independent effect of low $\psi_w$ on cell wall properties and an additional basis for reduced growth of the subapical region at low $\psi_w$.  

### Table II. Relative mRNA levels in the apical 0- to 5-mm region of WS roots 15 h after transplanting, normalized to mRNA in the apical 0- to 5-mm region of control (WW) roots

<table>
<thead>
<tr>
<th>Expansin</th>
<th>Treatment</th>
<th>WS</th>
<th>WS + FLU</th>
<th>WS + FLU + ABA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp1</td>
<td></td>
<td>3.9 (1.1)</td>
<td>3.3 (0.9)ns</td>
<td>4.5 (1.1)ns</td>
</tr>
<tr>
<td>Exp5</td>
<td></td>
<td>1.7 (0.3)</td>
<td>2.1 (0.2)ns</td>
<td>2.2 (0.2)ns</td>
</tr>
<tr>
<td>ExpB2</td>
<td></td>
<td>1.4 (0.05)</td>
<td>1.0 (0.06)*</td>
<td>1.5 (0.25)ns</td>
</tr>
<tr>
<td>ExpB6*</td>
<td></td>
<td>3.6</td>
<td>2.9</td>
<td>3.0</td>
</tr>
<tr>
<td>ExpB8</td>
<td></td>
<td>2.6 (0.5)</td>
<td>1.8 (0.3)ns</td>
<td>2.9 (0.9)ns</td>
</tr>
</tbody>
</table>

* Only one data set was available, so no s is given.
genes in the apical 5 mm of maize primary roots in association with an increase in expansin activity in this region. The increase in expansin activity in this region likely contributes to enhanced cell wall extensibility and thus helps root cells maintain elongation at reduced turgor pressure.

MATERIALS AND METHODS

Seeding Culture and Growth

Maize (Zea mays cv FR27 × FRMo17 or FR697 inbred, from Pioneer) seeds were imbibed for 24 h in 1 mM CaSO₄ and were germinated for 17 h in vermiculite well moistened with 1 mM CaSO₄ (Spollen et al., 2000). Seedlings with primary roots about 10 mm in length were transferred into Plexiglas boxes containing vermiculite at a ψw of −0.03 MPa (WW) or −1.59 ± 0.18 MPa (WS; means ± sd of all experiments). The different ψw values were obtained by thorough mixing with different amounts of 1 mM CaSO₄ and were measured by isopiestic thermocouple psychrometry (Boyer and Knipling, 1965). Seedlings were then grown in the dark at 29°C and near-saturation humidity (Sharp et al., 1988). At various times after transplanting, the apical 20 mm of the primary roots were harvested into three sections: region A (0–5 mm), region B (5–10 mm), and region C (10–20 mm). The elongation zone constitutes the apical 11 mm in WW roots and is shortened to approximately 6 mm at a ψw of −1.6 MPa (Sharp et al., 1988; Spollen and Sharp, 1991). Harvesting was carried out under a green safelight (Saab et al., 1990) in the same chamber used for seeding growth. Root samples were immediately frozen in liquid nitrogen.

To manipulate root ABA content after transplanting to low ψw, seeds were germinated for 22 h in vermiculite containing 1.5 μM FLU (SePRO, Carmel, IN), and seedlings with roots approximately 20 mm long were transferred to low ψw (−1.6 MPa) vermiculite containing 1.5 μM FLU, or FLU plus 0.5 mM ± ABA (Sigma-Aldrich, St. Louis). Longer roots at transplanting were used for this experiment to facilitate recovery of elongation upon addition of ABA to FLU-treated roots (Spollen et al., 2000). Details of FLU preparation were described in Ober and Sharp (1994); ethanol and Tween 20 (final concentrations of 0.006% and 0.002% [v/v], respectively) were added to the control treatment. An exogenous ABA concentration of 0.5 mM was used because previous work showed that this concentration almost completely restored the root elongation rate of seedlings treated with 1.5 μM FLU (Spollen et al., 2000). The requirement for such a high applied ABA concentration was due to limited uptake from the dry vermiculite (Sharp et al., 1994). ABA was not added prior to transplanting because it inhibits germination. At 15 h after transplanting, the apical 10 mm of the roots were harvested into two sections, region A (0–5 mm) and region B (5–10 mm). ABA content was measured by radiolmmunoassay (Quarrorie et al., 1988); harvesting and extraction procedures and assay validation were described in Saab et al. (1990) and Sharp et al. (1994). Root elongation was recorded by measuring root length at transplanting and again at harvesting.

RNA Analysis

Total RNA was extracted from plant tissues with TRIZol reagent (Gibco-BRL, Rockville, MD) according to the manufacturer’s instructions. The RNA pellet was dissolved in RNase-free water plus RNasesecure (Ambion, Austin, TX). Twenty micrograms of total RNA was separated on a 1% (w/v) denatured agarose gel (6.5% [v/v] formaldehyde) and was vacuum-transferred to nylon membrane (Amer- sham, Piscataway, NJ). The blot was then hybridized to 32P-labeled gene probes specific for expansins previously found to be expressed in the root. The design and test of gene-specific probes were described by Wu et al. (2001). The mixed probe for α-expansin expression was composed of Exp1, Exp2, Exp3, Exp4, and Exp5 cDNAs. The mixed probe for β-expansin expression was composed of ExpB1, ExpB2, ExpB3, ExpB5, ExpB6, and ExpB8 cDNAs. GenBank accession numbers for these genes are: AF332169 through AF332173 for Exp1 through 5 and AF332174 through AF332181 for ExpB1 through 8.

Northern blots were pre-hybridized in UltraHyb solution (Ambion) at 60°C overnight and were hybridized to the probe in the same solution at 60°C for 20 h. The blots were washed at 65°C for 20 min twice in 5% SEN (5% [w/v] SDS, 1 mM EDTA, and 40 mM Na2HPO4) and for 20 min once in 1% SEN (1% [v/v] SDS, 1 mM EDTA, and 40 mM Na2HPO4). The blots were exposed to phosphor screens (Molecular Dynamics, Sunnyvale, CA) overnight at room temperature. The image was scanned with Storm Machine (Molecular Dynamics) and was quantitatively analyzed with ImageQuant software (Molecular Dynamics) by integrating the signal over the area of the band and correcting for background.

In all experiments, 20 μg of total RNA per sample was analyzed by northern blot, and uniform loading was confirmed by the ethidium bromide fluorescence of the rRNA bands (e.g. Fig. 1). This in effect normalizes the signal based on rRNA. Although other methods of normalization are possible in principle (e.g. per cell or per dry mass of cell wall or per amount of transcript of a “housekeeping” gene), the variations in efficiency of RNA extraction between samples makes this procedure for normalization the most practical and the least subject to artifacts.

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