Comparative Study of Cellulases Associated with Adventitious Root Initiation, Apical Buds, and Leaf, Flower, and Pod Abscission Zones in Soybean

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Cellulase activity was measured in soybean (Glycine max) leaf abscission zones, flower abscission zones, pod abscission zones, apical buds, and adventitious rooting hypocotyls. Immunoprecipitation data showed that a cellulase immunologically similar to the bean abscission cellulase (isoelectric point 9.5) is present in soybean leaf, flower, and pod abscission zones, but is not present in soybean apical buds or rooting hypocotyls. cDNA and genomic clones for two different soybean genes were identified and show sequence similarity with the bean abscission cellulase clone pBAC10. The cDNA clone pSAC1, isolated from a soybean abscission cDNA library, hybridized to transcripts in soybean leaf, flower, and pod abscission zones. Although ethylene has been shown to play a role in the increase in cellulase activity associated with both abscission and adventitious root initiation, no signal was seen for hybridization of the soybean abscission cellulase clone, pSAC1, to RNA from soybean adventitious rooting hypocotyls. In addition, no soybean abscission cellulase transcripts were detected in apical buds. Transcripts for a second soybean cellulase gene (SC2) were not detected in any of the tissues surveyed.

Cell wall hydrolases play an important role in many plant developmental processes. Cellulases (endo-1,4-β-D-glucanases) constitute one class of such cell wall hydrolases. Cellulase activity has been identified in various tissues including leaf abscission zones (Lewis and Koehler, 1979; Tucker et al., 1988), fruit abscission zones (Greenberg et al., 1975), ripening fruit (reviewed by Fischer and Bennett, 1991), senescing cotyledons (Lew and Lewis, 1974), and styles and anthers ( Sexton et al., 1990; del Camplilo and Lewis, 1992), in stems during adventitious root initiation (Linkins et al., 1973), and in tissues undergoing cell division and expansion (Fan and Maclachlan, 1967). Not all of these cellulases have the same pi, suggesting that there are different cellulases with distinct cellular functions.

Several genes encoding cellulases have been studied. Avocado fruit cellulase is expressed in softening avocado fruit (Christoffersen et al., 1984; Tucker et al., 1987; De Francesco et al., 1989; Cass et al., 1990). Bean abscission cellulase is highly expressed in leaf abscission zone cells and adjacent vascular tissue, but is much lower in neighboring petiole and stem tissue (Tucker et al., 1988, 1991). However, other tissues known to have cellulase activity were not screened for expression of these particular genes.

Linkins et al. (1973) reported that a cellulase associated with auxin-induced adventitious rooting in bean had a basic pi similar to that of the pi 9.5 cellulase associated with bean leaf abscission. In addition, they concluded that ethylene synthesis induced by the auxin treatment was essential to the increase in the basic pi cellulase associated with root initiation. One objective of our study was to determine if the same gene expressed during ethylene-induced leaf abscission in soybean (Glycine max) was also expressed during adventitious root initiation. Toward this end we used the bean abscission cellulase antiserum to distinguish between immunologically dissimilar cellulases expressed in soybean. In addition, we identified clones for two different cellulase genes to distinguish among potentially different cellulase transcripts. One of the clones, pSAC1, was identified from a soybean leaf abscission cDNA library and the other, SC2, from a soybean genomic library.

In addition to our study of adventitious rooting material, we wanted to determine if cross-reactive cellulases are also present in extracts from apical buds and three different abscission zones: leaf, flower, and pod. All of the soybean tissues examined contained high levels of cellulase activity. However, only the leaf, flower, and pod abscission zones contained appreciable amounts of cellulase activity that could be immunoprecipitated with the bean abscission cellulase antiserum and contained RNA transcripts hybridizing to the soybean leaf abscission clone pSAC1. The genomic cellulase clone did not show detectable hybridization to any of the RNA extracts from the tissues examined.

MATERIALS AND METHODS

Tissue Samples

The soybean used was Glycine max (L.) Merrill, var Williams. Apical buds were collected from 10- to 14-d-old green-
house-grown soybean plants and immediately frozen in liquid nitrogen. For all other tissue samples explants were prepared as described below, surface-sterilized for 3 min in 10% commercial bleach, and thoroughly rinsed with distilled water. After treatment, tissue samples were collected, placed immediately in liquid nitrogen, and stored at −70°C until analysis.

Explants used for adventitious rooting hypocotyls were prepared from 10- to 14-d-old plants by cutting plants 1 cm above the soil (approximately 3 cm below the cotyledons) and then removing the cotyledons and apical buds. The leaves were left on the explants to increase transpiration rates (see below). Auxin (IBA) was used to initiate an abundance of adventitious roots. PCIB and AVG are inhibitors of auxin action (Law and Davies, 1990) and ethylene synthesis (Yu and Yang, 1979), respectively. One of four treatments was given: (a) 10−6 M IBA for 24 h, then fresh H2O every 24 h for 5 d; (b) 10−6 M IBA + 40 μM AVG for 24 h, then fresh 40 μM AVG every 24 h for 5 d; (c) 10−6 M IBA + 40 μM AVG for 24 h, then fresh 40 μM AVG + 10 μL/L ethylene in air every 24 h for 5 d; or (d) 500 μM PCIB for 24 h, then fresh 500 μM PCIB + 10 μL/L ethylene in air every 24 h for 5 d. For all treatments, explants were placed under a bank of four fluorescent lights (110 WE m−1 s−1) for the first 24 h to increase the transpiration pull of the various treatment solutions into the explant. For the remaining 5 d of the experiment, explants were placed in 9-L bell jars under discontinuous light (16 h at 76 μE m−2 s−1) with either air or 10 μL/L ethylene in air. Tissue was collected at 0, 1, 2, 3, 4, 5, and 6 d.

Explants used for leaf abscission zones were prepared from 10- to 14-d-old soybean plants by cutting 1 cm above the soil and removing the cotyledons, primary leaves, and apical buds. The explants were placed in beakers of water and exposed to 25 μL/L ethylene in air for the time intervals indicated in the figure legends. Treatment of explants with ethylene is necessary to induce and synchronize the onset of abscission in a population of explants. Only the lower (petiolar) leaf abscission zones of explants exposed to ethylene for 48 h. Polysomal RNA was extracted from 2-mm sections of the petiolar leaf abscission zones of explants exposed to ethylene for 48 h. Polysomal RNA was prepared essentially as described by Jackson and Larkins (1976) and then poly(A)+ RNA was separated on oligo(dT) columns (Aviv and Leder, 1972). cDNA was prepared (Tucker et al., 1988) and cloned into XGtl0 as described in the protocol for the Amersham cDNA cloning kit (Amersham, Arlington Heights, IL). The cDNA insert from pBAC10 (Tucker et al., 1991) was used to screen 25,000 plaques from the unamplified soybean cDNA library. Hybridization conditions were 20% formamide, 5× SSPE, 2× Denhardt’s solution, 0.1% SDS, and 150 μg/mL denatured salmon sperm DNA at 42°C. A single plaque, 5SAC1, was selected and the cDNA insert was subcloned into the BamHI endonuclease restriction site in T7/T3α-18 (GIBCO BRL).

The SAC1 cDNA clone was used as a probe to screen a soybean (var Century) genomic library (kindly provided by Greg Wadsworth, Plant Molecular Biology Laboratory, USDA, Beltsville, MD). Moderate stringency conditions were used: hybridization was performed at 42°C in 50% formamide, 5× SSPE, 2× Denhardt’s solution, 150 μg/mL denatured salmon sperm DNA, and the final wash was for 20 min at 42°C in 0.2× SSPE, 0.1% SDS. Positive clones were subcloned into pUC118 or pUC119, restriction mapped, and sequenced according to standard procedures (Sanger et al., 1977; Maniatis et al., 1982). This screening resulted in two overlapping clones for the same putative cellulase gene designated SC2. Sequences were analyzed with the University of Wisconsin Genetics Computer Group program (Devereux et al., 1984).

### Northern Hybridization Analysis

RNA was denatured with glyoxal, separated on 1.2% agarose gels, and transferred to Hybond-N as described by Maniatis et al. (1982). Agarose gels were loaded with either

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**Cellulase Extraction and Activity**

Cellulase was extracted and activity was measured viscometrically essentially as described by Durbin and Lewis (1988) with the following modifications. Cellulase extraction buffer was 20 mM Tris-HCl, 3 mM EDTA, and 0.5 mM NaCl, pH 8.0. Carboxymethylcellulose (purchased from Aqualon2, Wilmington, DE) was dissolved in 100 mM citrate phosphate buffer, pH 6.8, at a concentration of 1.3%. Each sample was assayed twice and averaged, and the average was used to calculate activity on a per gram fresh weight basis.

### Immunoprecipitation

Immunoprecipitation was carried out as described by Durbin et al. (1981). Immunoprecipitin (formalin-fixed Staphylococcus aureus) was purchased from GIBCO BRL (Gaithersburg, MD). The bean strain 9.5 cellulase-specific antiserum and preimmune serum were gifts from Lowell Lewis (Department of Plant Biology, University of California, Berkeley).

### Library Preparation and Screening

Polysomal RNA was extracted from 2-mm sections of the petiolar leaf abscission zones of explants exposed to ethylene for 48 h. Polysomal RNA was prepared essentially as described by Jackson and Larkins (1976) and then poly(A)+ RNA was separated on oligo(dT) columns (Aviv and Leder, 1972). cDNA was prepared (Tucker et al., 1988) and cloned into XGt10 as described in the protocol for the Amersham cDNA cloning kit (Amersham, Arlington Heights, IL). The cDNA insert from pBAC10 (Tucker et al., 1991) was used to screen 25,000 plaques from the unamplified soybean cDNA library. Hybridization conditions were 20% formamide, 5× SSPE, 2× Denhardt’s solution, 0.1% SDS, and 150 μg/mL denatured salmon sperm DNA at 42°C. A single plaque, 5SAC1, was selected and the cDNA insert was subcloned into the BamHI endonuclease restriction site in T7/T3α-18 (GIBCO BRL).

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polysomal RNA or poly(A)$^+$ RNA (see figure legends for specifics). Hybridization conditions are given in the figure legends.

**RESULTS**

**Adventitious Rooting**

Explants from 10- to 14-d-old soybean plants formed adventitious roots when treated with $10^{-6}$ M IBA (a synthetic auxin). After the first 24 h of treatment, the hypocotyls were observed to swell and start to split down the sides. The swelling and splitting continued through d 3, when adventitious root initials were easily visible in the splits. The roots then elongated through d 6, the final day of the experiment. No roots formed on the epicotyl, petiole, or leaf tissues. Hypocotyl, epicotyl, and leaf tissues were collected on each day of the experiment and assayed for cellulase activity (Fig. 1). Cellulase activity increased in hypocotyls through d 3, the day of maximum swelling and splitting, and then decreased as the roots elongated. No significant change in activity over the 6 d of observation was found in epicotyl or leaf tissue.

Since explants will synthesize ethylene in response to exogenous auxin treatment at $10^{-6}$ M (Beyer et al., 1984), the role of ethylene in the induction of adventitious roots was examined. The ethylene synthesis inhibitor AVG (Yu and Yang, 1979) and the auxin action inhibitor PCIB (Law and Davies, 1990) were incorporated into treatments to determine whether the cellulase activity and changes in morphology of the hypocotyls were due to the exogenous auxin treatment, endogenous ethylene synthesis, or both. IBA plus AVG-treated explants did not exhibit the swelling and splitting observed in IBA-treated hypocotyls, and no adventitious roots were initiated. Additionally, no pattern of cellulase activity was observed in these hypocotyls (Fig. 2). When ethylene was added back to this system 24 h after the IBA plus AVG treatment began, the hypocotyls showed delayed swelling and splitting, a few root initials at d 6 of the experiment, and a slight, but not statistically significant, increase in cellulase activity (Fig. 2). Exposure of explants to exogenous ethylene was delayed 24 h to allow time for the IBA and AVG to be taken up by the explants. However, ethylene treatment of IBA plus AVG-treated explants did not fully reproduce the IBA-alone treatment. It is possible that the temporal relationship of inductive concentrations of auxin and ethylene is important to the development of adventitious root initiation and the associated increase in cellulase activity. Modification of this temporal relationship by inhibiting the biosynthesis of ethylene and then adding ethylene at an artificial time may have interfered with the normal development of adventitious roots. Finally, explants treated with ethylene plus PCIB did not form adventitious roots, although hollowing of the pith in the hypocotyls was observed as well as overall senescence of the explants. Cellulase activity remained low in the PCIB plus ethylene-treated hypocotyls until about d 4, when it increased dramatically, correlating with the onset of senescence. Taken all together, these results indicate that both auxin and ethylene are necessary for adventitious root initiation.

**Cellulase Activity Survey; Immunoprecipitation**

A bean abscission cellulase-specific antiserum (Durbin et al., 1981) was used to determine whether the high cellulase activities of several tissues were due to abscission cellulase or an immunologically unrelated cellulase. Tissue extracts were incubated separately with abscission cellulase-specific antiserum and preimmune serum. The antibodies and associated protein were then precipitated with Immunoprecipitin (GIBCO BRL) and the supernatant was assayed for cellulase activity. The percentage of cellulase activity immunoprecipitated with abscission cellulase-specific antiserum is shown in Table I. As expected, the bean abscission cellulase-specific antiserum immunoprecipitated over 90% of the cellulase activity in BLAZ. Over 90% of the cellulase activity in the SLAZ was immunoprecipitated with the bean antiserum, showing that the bean abscission antiserum also recognizes the soybean abscission cellulase. Over 80% of the cellulase activity in soybean flower and pod abscission zones was immunoprecipitated, indicating that most of the cellulase(s) active in these tissues is immunologically related to the bean leaf abscission cellulase. However, less than 10% of the cellulase activity in SABs or adventitious rooting hypocotyls was immunoprecipitated, indicating that cellulases immunologically related to abscission cellulase play a very small, if any, role in the cellulase activity extracted from these tissues.

**Identification of Soybean Cellulase Clones**

The bean abscission cellulase clone pBAClO (Tucker and Milligan, 1991) cross-hybridizes with a 2.0-kb transcript from ethylene-induced SLAZ (Fig. 3). A cDNA library was pre-
pared from poly(A)* RNA extracted from leaf abscission zones of soybean explants exposed to 25 \( \mu \)L/L ethylene for 48 h. The soybean cDNA library was screened using the bean cellulase clone \( \text{pBAC10} \) as a probe. A plaque, \( \text{XSAC1} \), was isolated from a soybean genomic library. These two clones were determined by restriction maps and partial sequences to be overlapping clones for the same gene designated \( \text{SC2} \). One of the plasmid subclones, \( \text{pSC2-3} \), prepared from one of the two phage clones coding for the \( \text{SC2} \) gene, was fully sequenced and the sequence was confirmed. The amino acid sequence for the open reading frame in this genomic clone (\( \text{SC2-3} \)) is also shown in Figure 4. Alignment of the \( \text{SAC1} \) amino acid sequence to the carboxy-terminal half of the sequences for \( \text{BAC10} \) and \( \text{AV363} \) shows 88 and 46% identity, respectively.

The \( \text{pSAC1} \) cDNA insert was then used to probe a northern blot of bean and soybean RNAs to determine expression of complementary RNA (Fig. 3). Transcripts hybridizing to \( \text{pSAC1} \) were not detected in RNA extracts from the abscission zone prior to induction of abscission with ethylene (0 h in Fig. 3) but were easily detected in abscission zones after 48 h of ethylene treatment. Transcripts of a similar size (2.0 kb) were detected in RNA preparations from ethylene-treated petioles but accumulation of transcript in the petiole was severalfold less than in the abscission zone.

Two phage clones that hybridized moderately to \( \text{pSAC1} \) were isolated from a soybean genomic library. These two clones were determined by restriction maps and partial sequences to be overlapping clones for the same gene designated \( \text{SC2} \). One of the plasmid subclones, \( \text{pSC2-3} \), prepared from one of the two phage clones coding for the \( \text{SC2} \) gene, was fully sequenced and the sequence was confirmed. The amino acid sequence for the open reading frame in this genomic clone (\( \text{SC2-3} \)) is also shown in Figure 4. Alignment of the amino acid sequence for the open reading frame in the soybean genomic clone \( \text{SC2-3} \) with each of the other amino acid sequences shown in Figure 4 are in the range of 44 to 55% identity.

### Cellulase Transcripts in Other Tissues

The two unique soybean cellulase clones \( \text{pSAC1} \) and \( \text{pSC2-3} \) were used as probes for northern blots containing RNA from soybean leaf, flower, and pod abscission zones (\( \text{SLAZ} \), \( \text{SFAZ} \), and \( \text{SPAZ} \), respectively) and \( \text{SAB} \) and \( \text{SARH} \). Transcripts hybridizing to the \( \text{SAC1} \) cDNA were found in all the abscission zones (\( \text{SLAZ} \), \( \text{SFAZ} \), and \( \text{SPAZ} \)) but not in \( \text{SAB} \) or \( \text{SARH} \) (Fig. 5), which is consistent with the immunoprecipitation data (Table I).

#### Table I. Percent of cellulase activity in soybean tissues that is immunoprecipitated by bean abscission cellulase-specific antiserum

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Activity</th>
<th>Immunoprecipitation %</th>
</tr>
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<tbody>
<tr>
<td>BLAZ</td>
<td>6800</td>
<td>92</td>
</tr>
<tr>
<td>SLAZ</td>
<td>7650</td>
<td>95</td>
</tr>
<tr>
<td>SFAZ</td>
<td>2080</td>
<td>81</td>
</tr>
<tr>
<td>SPAZ</td>
<td>4830</td>
<td>86</td>
</tr>
<tr>
<td>SAB</td>
<td>1450</td>
<td>4</td>
</tr>
<tr>
<td>SARH</td>
<td>3250</td>
<td>8</td>
</tr>
</tbody>
</table>

Figure 2. Auxin and ethylene effects on cellulase activity in hypocotyls. Auxin (IBA) was given as a 1 \( \mu \)M solution for the first 24 h (d 0–1) and then the explants were transferred to water or a treatment solution that was changed daily for the duration of the experiment. Ethylene (E) was given at 25 \( \mu \)L/L in air starting after the first 24 h (d 1–6). AVG, an ethylene synthesis inhibitor, was given at 40 \( \mu \)M in a solution that was refreshed daily (d 0–6). PCIB, an auxin action inhibitor, was refreshed daily in solution at 50 \( \mu \)M (d 0–6). SE bars are shown only for the IBA and IBA + AVG experiments. Each point is the average of four, four, three, and two experiments for IBA, IBA + AVG, IBA + AVG + E, and PCIB + E, respectively. Cellulase activities are relative units h\(^{-1}\) g\(^{-1}\) fresh weight. The table above the graph indicates the effect of each treatment on swelling and splitting of hypocotyls and the amount of root initiation observed.

Figure 3. Northern blots of poly(A)* RNA (0.5 \( \mu \)g) from bean and soybean tissue extracts. Two identical blots were prepared and each was probed separately with the cDNA inserts from \( \text{pBAC10} \) and \( \text{pSAC1} \). Bean and soybean explants were treated with 25 \( \mu \)L/L ethylene in air for 0 or 48 h. Only the abscission zone tissue (A) was collected at 0 h. From 48-h-treated explants, abscission zones (A), petioles (P), and stems (S) were collected. Hybridization conditions for the \( \text{pBAC10} \)-probed blot were 20% formamide, 5x SSPE, 2 times Denhardt's solution, 150 \( \mu \)g/mL denatured salmon sperm DNA at 42°C, and the final wash was 0.2x SSPE, 0.1% SDS at 37°C for 30 min. Hybridization conditions for the \( \text{pSAC1} \)-probed blot were 60% formamide, 5x SSPE, 2 times Denhardt's solution, 150 \( \mu \)g/mL denatured salmon sperm DNA at 42°C, and the final wash was 0.2x SSPE, 0.1% SDS at 50°C for 30 min.
Cellulase Gene Expression in Soybean

SC2-3 were detected in any of these tissues (data not shown). Therefore, a physiological role for the SC2 gene has not been identified.

**DISCUSSION**

We have surveyed three different abscising tissues in soybean (leaf, flower, and pod) for cellulase activity and specific cellulase gene expression. Those tissues undergoing abscission in response to ethylene contained high levels of cellulase activity that were immunoprecipitated by the bean abscission cellulase antiserum (Table I). Moreover, all the abscission tissues tested contained transcripts hybridizing to the pSAC1 clone (Fig. 5).

The two other tissues examined that also contain high levels of cellulase activity (SAB and SARH) have dissimilar cellulase(s), since abscission cellulase antiserum did not immunoprecipitate the activity (Table I) and no cellulase transcripts hybridizing to pSAC1 were detected (Fig. 5). The identity of the cellulase(s) in these tissues is still unknown.

Cellulase has been shown to be a family of genes in avocado (Tucker et al., 1987; Cass et al., 1990), bean (Tucker and Milligan, 1991), and tomato (Lashbrook and Bennett, 1993). In addition to the identification of a soybean abscission cellulase clone (pSAC1), we have identified a soybean genomic clone for a second putative cellulase gene (SC2). The partial sequence for this gene (SC2-3) has 52% identity with the nucleotide sequence for the abscission cellulase clone pSAC1. No transcript hybridizing to the SC2 gene probe was detected in abscission zones, apical buds, or rooting hypocotyls. Where the SC2 cellulase gene is expressed, or if indeed it is expressed, is unknown. Our tissue survey was by no means an exhaustive one, since all tissues undergoing developmental processes involving changes in cell wall structure are likely to express some cellulase(s).

We routinely see the accumulation of mRNA that hybridizes to abscission-specific cellulase probes in ethylene-treated petioles (Fig. 3); however, accumulation in petioles is always considerably less and of variable abundance compared with accumulation in the abscission zone. In addition, cellulase-specific hybridization is occasionally observed in RNA extracts from ethylene-treated stem samples. This pattern of cellulase RNA accumulation is also frequently observed in bean (Tucker et al., 1988); interestingly, however, the RNA sample from bean shown in Figure 3 did not accumulate detectable amounts of cellulase RNA in the petiole or stem samples. This example further indicates the variability in the transcript accumulation in these tissues. Immunological studies of bean abscission cellulase protein (Sexton et al., 1981; del Campillo et al., 1990) and in situ hybridization with pBAC10 (Tucker et al., 1991) showed accumulation of cellulase protein and mRNA, respectively, to be confined to within approximately 4 mm of the abscission fracture. When

**Figure 4.** Optimal alignment of amino acid sequences for the open reading frames in SAC1 (soybean abscission cellulase); SC2-3 (subclone No. 3 of a 15-kb genomic clone for a second unique soybean cellulase), BAC10 (bean abscission cellulase; Tucker and Milligan, 1991), and AV363 (avocado fruit cellulase; Tucker et al., 1987). Amino acids having position identity with BAC10 are in bold, uppercase letters. Amino acids having position identity with AV363 are in bold, lowercase letters. Dots indicate gaps in the sequences. Numbers above the sequence alignments indicate amino acid position in the BAC10 open reading frame. Underlined amino acids from position 459 to 466 in the BAC10 sequence are corrections in the published sequence for pBAC10 (Tucker and Milligan, 1991).

**Figure 5.** Composite northern blot of polysomal RNA (5 μg) from soybean leaf, flower, and pod abscission zones (LAZ, FAZ, and PAZ, respectively), adventitious rooting hypocotyls (ARH), and apical buds (AB). Blots were probed with cDNA insert from pSAC1. LAZ and FAZ RNA are from explants treated with ethylene for 12 h, and RNA from PAZ is from explants treated with ethylene for 24 h. The AB sample is from 10- to 14-d-old plants and the ARH sample is from hypocotyls of explants from 10- to 14-d-old plants treated with IBA (1 μM) for 24 h followed by water for 48 h. Hybridization conditions were 60% formamide, 5× SSPE, 2× Denhardt's solution, 150 μg/ml denatured salmon sperm DNA at 42°C and final wash was 0.2× SSPE, 0.1% SDS at 50°C for 20 min.
lecting petiole and stem tissue for RNA extraction, we make a special effort to collect tissue that is at least 5 mm away from any abscission fracture. Therefore, the transcript in petioles and stems hybridizing to pBAC10 and pSAC1 appear to be associated with some response other than the formation of the separation layer.

As noted in the introduction, Linkins et al. (1973) reported that a cellulase associated with adventitious rooting in bean had a basic pI similar to that of the pI 9.5 cellulase associated with bean leaf abscission. They observed that auxin stimulated adventitious roots not only in stems but also in petioles. Moreover, they reported that removal of ethylene from their treatment chamber with mercuric perchlorate inhibited root formation and eliminated the increase in the basic pI cellulase while leaving unchanged the increase in the acidic pI cellulase. In our experience with ethylene-induced leaf abscission we have observed that a small amount of adventitious root initiation occurred in the stems of some explants during exposure to ethylene alone without treatment with auxin. It seemed reasonable from the foregoing discussion that some or all of the cellulase transcript we saw in RNA extracts from stem tissue and possibly petioles was associated with adventitious root initiation. However, this does not appear to be correct for soybean. Auxin treatments greatly increase the amount of adventitious roots obtained in soybean explants, and, similar to the conclusions reported by Linkins et al. (1973), our results with the ethylene synthesis inhibitor AVG (Fig. 2) suggest that ethylene synthesis may play a role in adventitious root initiation and the associated increase in cellulase activity. Nevertheless, the cellulase mRNA hybridizing to pSAC1 in soybean abscission zones (Fig. 5) and stems and petioles (Fig. 3) is not linked to the increase in cellulase activity correlating with adventitious root initiation or the associated swelling and splitting of the hypocotyl (Table 1, Fig. 5). The physiological role of the cellulase mRNA that hybridizes to the abscission cellulase probe in ethylene-treated petioles and stems and the cell types involved in this accumulation have yet to be determined.

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The GenBank accession numbers for the sequences of the corrected bean abscission cellulase cDNA pBAC10 and the soybean cellulases pSAC1 and pSC2-3 are M57400, U00730, and U00731, respectively.

LITERATURE CITED