Characterization of the Expression of the Petunia Glycine-Rich Protein-1 Gene Product

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ABSTRACT

We have examined the expression of the petunia (Petunia hybrida) glycine-rich protein-1 (ptGRP1) gene product using an antibody raised against a synthetic peptide comprising amino acids 22 through 36 of the mature ptGRP1 protein. This antibody recognizes a single protein of 23 kilodaltons. Cell fractionation studies showed that, as predicted (CM Condit, RB Meagher [1986] Nature 323: 178–181), ptGRP1 is most likely localized in the cell wall. In addition, it was found that (extractable) ptGRP1 is present in much higher abundance in unexpanded than in fully expanded tissue, with highest levels of accumulation in the bud. This same developmentally regulated pattern of protein expression was found in all varieties of petunia tested. In addition, tissue blots of petunia stem sections showed that ptGRP1 is localized to within the vascular tissue (at least the phloem or cambium) and to either the epidermal cells or to a layer of collenchyma cells directly below the epidermis. Localization of ptGRP1 antigen in these cell types is shown to occur at different times in the overall development of the plant and at different quantitative levels.

The isolation and sequencing of the ptGRP1 gene provided the first published indirect evidence that structural proteins other than extensin might be present in the cell walls of dicotyledonous plants (4). At the time of the discovery of the ptGRP1 gene, it was a novel idea that structural proteins other than extensin might be present in the higher plant cell wall and hinted at a previously unsuspected complexity in the organization of this structure. This idea is no longer novel. A glycine-rich protein has been isolated from strawberry fruits (12) and two GRP genes from French bean (fb) have been isolated and sequenced (8), one of which, the fbGRP1.8 gene, has been shown by immunocytochemistry to code for a cell wall protein specific to protoxylem cells (9). In addition a third class of cell wall structural proteins, the proline-rich proteins has been described (1, 14).

Genes from all three classes of cell wall proteins have been shown to be members of multigene families and, whenever studied, expression of these genes on either the mRNA and/or protein product level has been shown to exhibit developmental and/or cell specificity. Immunocytochemistry studies of soybean seed coat extensin have shown that this protein is primarily localized in the walls of palisade epidermal cells and hourglass cells and was detected only during the maturation phase of growth of these cell types (3). In addition, fbGRP1.8 was localized in the walls of protoxylem cells and it has been suggested that the appearance of this protein is associated with lignin deposition in this cell type (9). Further, a protein immunologically related to carrot p33 (a proline-rich protein) was localized solely to the apical hook of soybean seedlings (14). At the mRNA level, the four members of the ptGRP family have been shown to exhibit distinctly different developmental and organ specific patterns of message accumulation (5) while the two described soybean proline-rich genes exhibit differing developmental patterns of mRNA accumulation (1). These data indicate that individual genes of all three classes of cell wall proteins may have specific roles to play in the overall development of the plant.

In this paper, further support is given to this theory as we report that the expression of the ptGRP1 gene product, like its message (5), appears to be developmentally regulated and shows a remarkable cell specificity, ptGRP1 antigen being localized to, at least, either the vascular cambium or phloem and to either the epidermis or to a layer of cells directly beneath the epidermis. These data also indicates that it is unlikely that the ptGRP1 and fbGRP1.8 genes are homologues because the two genes appear to be expressed in different cell types.

MATERIALS AND METHODS

Plant Tissue

Greenhouse-grown Petunia hybrida cv Mitchell, White 69 (W69), Red 51 × Violet 23 (R X V), and Violet 23 (V23) plants were used as a source of tissue for these studies. (The W62, Vxr and V23 strains were a kind gift of Dr. T. Gerats, Free University of Amsterdam, The Netherlands.) Appropriate tissue was collected in liquid N₂ and stored at −70 °C until use.

Protein Extracts

Unless otherwise stated, tissue was ground in liquid N₂ and 1 mL of 2X Laemmli sample buffer (10) per g of tissue was
added. This mixture was allowed to thaw until a slurry formed and was then immediately heated at 100 °C for 3 min and stored at −20 °C until use. Protein content of samples was determined by the method of Peterson (11).

**Preparation of Anti-ptGRP1 Antiserum**

The predicted structure of the ptGRP1 gene (isolated from the petunia Mitchell variety) is essentially composed of three parts: a 27 amino terminal amino acid signal sequence, followed by a hydrophilic 42 amino acid region in which 13 of the 20 charged residues of this protein are located. The remaining 316 carboxy-terminal amino acids of which 75% are glycyl-residues has been predicted to form an eight-stranded β-pleated sheet (4). To prepare an anti-ptGRP1 antibody a peptide comprising amino acids 22 thru 36 of the mature ptGRP1 protein was synthesized (a generous gift of the Monsanto Corp.). This peptide was derived from the unique non-glycyl hydrophilic amino-terminal portion of the mature ptGRP1 protein and was thus most likely to be the most variable between gene family members. The synthetic peptide was coupled via the cysteine residue of the peptide to the lysine residues of the carrier protein, KLH by the method of Green et al. (7). In short, 0.7 mg of m-maleimido-benzyloxy-N-hydroxysuccinimide ester dissolved in 50 μl of dimethylformamide was added dropwise to 4 mg of KLH dissolved in 10 mM sodium phosphate buffer (pH 7.2) and allowed to react for 30 min at room temperature. The reaction product, KLH-MB, was then passed through a Sephadex G-25 column equilibrated with 50 mM sodium phosphate (pH 6.0) to remove free m-maleimido-benzyloxy-N-hydroxysuccinimide ester. KLH-MB was then reacted with 5 mg of the peptide dissolved in PBS (8 g NaCl, 0.2 g KCl, 1.44 g Na2HPO4 and 0.24 g KH2PO4/L H2O) for 3 h at room temperature. Using this method, it was estimated that 10 to 20 peptides were coupled to each KLH molecule. Rabbits were injected once with sufficient peptide-KLH conjugate to contain 100 to 200 μg of coupled peptide emulsified in Freund’s complete adjuvant. Rabbits were injected 14 and 28 d after the initial injection and every 30 to 45 d thereafter with 100 μg coupled peptide mixed with incomplete Freund’s adjuvant. Ten days after the second and subsequent injections rabbits were bled and the IgG fraction from the crude sera was purified by HPLC protein A affinity chromatography (Bio-Rad). All studies in this report were performed using IgG isolated from the third bleed.

**Gel Electrophoresis and Western Analysis**

SDS-PAGE was performed by the method of Laemmli (10). Equal amounts of protein were loaded in each gel lane. After electrophoresis proteins were transferred electrophoretically to Immobilon transfer membranes (Millipore) as per the manufacturers directions. After transfer, the membrane blots were stained for 5 min in a solution of 0.2% (w/v) Ponceau S in 3% (w/v) TCA. Blots were destained in distilled H2O and checked to determine if equal transfer of protein in all gel lanes had occurred. If equal transfer had occurred, the membranes were then washed for 30 min in TBS (50 mM Tris-HCl, pH 7.4, 200 mM NaCl) to remove all traces of stain. Blocking was performed for 3 h in a solution of TBS, 0.2% Tween (v/v), 3% (w/v) BSA, and 5% (v/v) goat serum (Sigma). Blots were then reacted with either rabbit preimmune IgG or anti-ptGRP1 IgG at a dilution of 1:200 in the same solution for 12 h at room temperature. After reaction with primary antibody, blots were washed three times for 10 min each in a solution of TBS, 0.2% Tween 20, 0.1% SDS, with a final wash of 5 min in TBS, 0.2% Tween. Blots were then incubated at room temperature with goat anti-rabbit-IgG alkaline phosphatase conjugate (Sigma) at a dilution of 1:2500 (v/v) in TBS, 0.2% Tween 20 (v/v), 3% BSA (w/v), 5% goat serum (v/v), and 0.1% SDS (w/v). After 60 min, the blots were washed as after the primary antibody reaction with the exception that the final wash was in TBS. Alkaline phosphatase color development was performed by placing the blots on a piece of parafilm and pipeting onto the blots a solution of 100 mM Tris-HCl pH 9.5, 100 mM NaCl, 5 mM MgCl2, containing 0.33 μg/ml 5-bromo-4-chloro-3-indolyl phosphate, and 16.5 μg/mL nitroblue tetrazolium.

**Tissue Blot Localization of ptGRP1**

To localize ptGRP1, tissue blots (3) were performed on cross-sections of stems at sites approximately 2, 6, 10, 14, and 18 mm beneath the apical bud of a lateral stem. These stem sections (all from the same stem) were cut and lightly pressed for 30 s to a piece of cellulose nitrate which had been equilibrated in 0.5 M NaOH as this concentration of NaOH was found to solubilize ptGRP1 in high amounts in an undegraded form (see “Results”). These blots were then neutralized by placing them on a sheet of Whatman 3MM paper saturated with TBS for 5 min × 3. Western analysis was then performed on these blots as indicated above. Blots were photographed on T Max, 400 ASA film (Kodak) using a Zeiss Photomicroscope 2.

Free-hand sections of petunia stems were photographed as above.

**RESULTS**

**Specificity of anti-GRP1 IgG**

To prepare an anti-ptGRP1 antibody, rabbits were injected with a synthetic peptide comprising amino acids 22 through 36 of the mature ptGRP1 protein coupled to KLH. IgG was isolated and the specificity of our anti-ptGRP1 IgG was determined by western blot analysis of Mitchell petunia tissue which had been ground in Laemmli buffer. After electrophoresis and transfer to Immobilon Western analysis was performed using either preimmune IgG or anti-ptGRP1 IgG. The results of this experiment (Fig. 1) show that anti-ptGRP1 IgG recognizes a single protein of approximately 23 kD (lane 2) not recognized by the pre-immune IgG (lane 1). The 23 kD protein which reacts with anti-ptGRP1 IgG is close to the predicted size of 25.6 kD of the mature ptGRP1 protein. In *vitro* translation of ptGRP1 RNA (synthesized off a T7 RNA polymerase promoter) also results in the production of a protein of lower apparent molecular weight than predicted (26 kD rather than 28.6 kD, the predicted size of the pre-
ptGRP1 protein [data not shown]). Thus, it is likely that the glycine rich portion of the molecule is responsible for this aberrant migration in SDS gels.

**Cell Fractionation Studies**

Cell fractionation studies to determine the cellular location of the ptGRP1 gene product were first performed according to Cassab et al. (2). This procedure involves the grinding of leaves in a low salt buffer to extract soluble proteins, followed by low speed centrifugation and the extraction of the insoluble material with detergent to solubilize membrane proteins. The remaining insoluble material is then extracted with high salt. Proteins solubilized by this latter step are considered to originate in the cell wall. Using this procedure, we found that the 23 kD protein was not present in any fraction, including the salt insoluble pellet. Since the salt insoluble pellet was resuspended in Lamelli (10) buffer and heated at 100 °C for 3 min, conditions which we have shown (Fig. 1) are capable of solubilizing ptGRP1, the absence of detectable amounts of ptGRP1 in all four fractions, indicates that at some point in the extraction the amino-terminal hydrophilic portion (at least) of the ptGRP1 gene product, was degraded (data not shown). Use of protease inhibitors benzamidine, PMSF, and ?-amino caproic acid throughout the extraction procedure did not stabilize the protein.

Since we had found that the 23 kD protein was protected in the presence of SDS (in Laemmli sample buffer), we hypothesized that high amounts of nonionic detergent might also stabilize the protein. Leaves were therefore ground in 10 mM Tris (pH 7.4), 4 mM Na2S2O5, 1% PVP (w/v), 4% (v/v) NP-40, 5 mM ?-amino caproic acid, 1 mM benzamidine, and 25 ?g/mL PMSF. Insoluble material was pelleted (33K rpm, 30 min in a Beckmann Ti 50 rotor), resuspended in Laemmli buffer, while an equal volume of 2X Laemmli buffer was added to the soluble fraction. Samples were then heated, electrophoresed, and Western analysis performed as stated in

**Figure 1.** Western analysis of total protein extracted from Mitchell petunia leaves. Lane 1, Preimmune serum (P); lane 2, anti-ptGRP1 serum (I). Numbers to the left indicate size of protein standards run in parallel.

**Figure 2.** Cellular fractionation studies. (A) Leaves were ground in a buffer containing 10 mM Tris (pH 7.4), 4 mM Na2S2O5, 1% PVP (w/v), 4% NP-40 (v/v), 5 mM ?-amino caproic acid, 1 mM benzamidine, and 25 ?g/mL PMSF. Material was then spun at 33K rpm for 30 min in a Ti 50 rotor. An equal volume of 2X Laemmli sample buffer was added to the supernate, while the pellet was resuspended in 1X Laemmli sample buffer. Gel electrophoresis, transfer and reaction with antibodies was performed as stated in “Materials and Methods.” Lane 1, supernate (S); lane 2, pellet (P). Numbers to the left indicate sizes of protein standards run in parallel. (B) Leaves were ground in 0.4 M NaOH. Material was centrifuged and treated as above with the exception that the supernate was neutralized with HCl before addition of Laemmli sample buffer. Electrophoresis, transfer, and reaction with antibodies was performed as stated in “Materials and Methods.” Lane 1, supernate (S); lane 2, pellet (P). Numbers to the left indicate sizes of protein standards run in parallel.
“Materials and Methods.” As can be seen in Figure 2A, anti-pGRP1 serum reacted with a 23 kD protein that is not solubilized by low salt plus nonionic detergent. This indicates that the 23 kD protein is neither a soluble nor an extrinsic membrane protein. (Repeated washing of the NP40 pellet with the above buffer to insure the removal of all solubilized proteins resulted in the loss of detectability of the 23 kD peptide [data not shown].) To determine whether the 23 kD protein is an intrinsic membrane protein, leaves were ground in 0.5 M NaOH. (High alkali solubilizes all cell proteins except those that are intrinsic to the membrane.) The resultant grindate was spun at 33K rpm, 30 min in a Ti 50 rotor. The pellet and supernate fractions were treated as above with the exception that the supernate fraction was first neutralized with HCl before addition of 2 X Laemmli Buffer. Western analysis of these fractions (Fig. 2B) shows that the 23 kD protein is solubilized by 0.5 M NaOH indicating that it is also not an intrinsic membrane protein. From these data we have therefore concluded that the 23 kD protein is most likely a cell wall protein.

Developmental Expression of ptGRP1

We had previously shown that the expression of the pt-GRP1 gene appeared to be developmentally regulated, in that the steady state levels of ptGRP1 message are higher in young tissue than in old (5). To determine whether the levels of ptGRP1 also exhibited this same type of developmental expression, total protein extracts were made from the apical bud and first apical leaf (Fig. 3, lane 1) from six separate stems (bud + leaf number 1) and also from each of the next six leaves (leaves numbers 2 to 7, numbering from the apex) from one of these stems (Fig. 3, lanes 2 thru 7) and Western analysis was performed. (The weights of leaves 2 thru 7 were as follows: 0.07 g, 0.11 g, 0.16 g, 0.18 g, 0.22 g, 0.21 g. Thus only leaves 6 and 7 were fully expanded.) As can be seen in Figure 3, the highest amounts of ptGRP1 protein are present in extracts of the bud plus the first apical most leaf (Fig. 3, lane 1). Slightly lower amounts are present in the next lower leaf (Fig. 3, lane 2), while still lower amounts are present in the third leaf beneath the bud (Fig. 3, lane 3), etc. As equal amounts of protein from each sample were electrophoresed, these results indicate that as leaf position increases in distance from the apex, decreasing amounts of ptGRP1 are present per unit weight of protein. From these data, it appears that both ptGRP1 mRNA and protein product are developmentally regulated.

Densiotometer tracings of each of the ptGRP1 peaks of the above blot were made. Since we determined the total amount of protein extracted from each leaf, it was possible to calculate the percentage of total leaf protein from each sample that was applied to the gel. Using this latter calculation and having determined the area of each peak, it is then possible to calculate the relative amount of ptGRP1 present in each leaf as a percent of total protein. When this calculation was performed we found that leaf number 2 contains approximately three times as much extractable ptGRP1 protein on a percent protein basis as does leaf number 7 or 8. (The value of 3.25 is likely to be an underestimation of the relative amounts of ptGRP1 in these leaves as this blot was overdeveloped to show the presence of ptGRP1 antigen in all leaves and was not performed in the linear range of the reaction.)

The expression of the ptGRP1 gene product in three other varieties of petunia (W62, VxR and V23) was compared with that of the Mitchell strain (Mit.). As can be seen in Figure 4 the ptGRP1 protein in all four strains is present in higher amounts in unexpanded leaves (U) than in expanded leaves (E), indicating that the developmental expression of this gene is controlled in a similar manner in all strains. This results indicate that the ptGRP1 gene is present and is likely to serve the same function in all four strains.

Although the developmental expression of this protein appeared to be the same in all four varieties, the extractable levels of this protein in each strain were not equivalent. In particular, the V23 strain contains much lower levels of ptGRP1 than do the other three strains. As the steady-state level of ptGRP1 RNA also appears to be depressed in the V23 variety, this low level of expression may be due to the fact that there is an ~100 bp deletion located between −1300 and −379 of the 5′ flanking region of this gene in the V23 strain (CM Condit, in preparation).

Localization of ptGRP1

To localize ptGRP1, tissue blots (3) were performed on cross-sections of stems at sites approximately 2, 6, 10, 14, and 18 mm beneath the apical bud of a lateral stem. Figure 5 shows the results of this experiment. In stem cross-sections...
located 2 mm beneath the bud (Fig. 5A) there is a great deal of soluble ptGRP1; however, higher quantities of antigen can be seen to be localized in the vascular tissue. Proceeding down the stem, there is a lower concentration of soluble antigen present. It is unclear whether this is due to a dilution of antigen caused by cell growth or to insolubilization or degradation of the antigen. However, in cross-sections located 6, 10, 14, and 18 mm beneath the bud (Fig. 5B, C, D, and E, respectively), antigen can be seen localized in discrete points within the vascular system, arguing that ptGRP1 most likely has some type of cell specificity within this tissue. In addition, in sections 14 and 18 mm beneath the bud there is localization of ptGRP1 to the epidermal cells or to a layer of cells directly below the epidermis. The quantity of antigen present in these latter cells never equals that present in the vascular tissue. There is also a generalized light staining of the cortex not present when blots are reacted with pre-immune serum (Fig. 5F).

Based on the pattern of staining in the vascular system seen in the tissue prints and on the particular anatomical characteristics of developing petunia stems, it is most likely that this protein is localized to cells of the phloem or cambium rather than, or in addition to those of the xylem. As can be seen in Figure 6, the vascular system of petunia is arranged as a vascular cylinder surrounding the pith rather than as discrete vascular bundles. In addition, petunia contains both an internal and external phloem again arranged as cylinders. The xylem in this species is located between the internal and external phloem. As can be seen in a cross-section of a young

**Figure 4.** Comparison of the expression of the ptGRP1 gene in four varieties of petunia by Western analysis: Mitchell (Mit.), White 62 (W62), Violet 23 X Red 51 (VxR), and Violet 23 (V23). Samples were prepared, electrophoresed, transferred, and reacted with antibodies as in “Materials and Methods.” Lane 1, Mitchell petunia, unexpanded leaves (U); lane 2, Mitchell petunia, fully expanded leaves (E); lane 3, W62, unexpanded leaves; lane 4, W62, fully expanded leaves; lane 5, VxR, unexpanded leaves; lane 6, VxR, fully expanded leaves; lane 7, V23, unexpanded leaves; lane 8, V23 fully expanded leaves. Numbers to the left indicate sizes of protein standards run in parallel.

**Figure 5.** Developmental expression of ptGRP1 in petunia stems. Cross-sections of a lateral stem of petunia were blotted to cellulose nitrate and Western analysis performed as stated in “Materials and Methods.” Cross sections of a stem 2 mm (A), 6 mm (B), 10 mm (C), 14 mm (D), 18 mm (E), 12 mm (F) beneath the apical bud. Blots A thru E were reacted with anti-ptGRPl sera; blot F was reacted with pre-immune sera. Bar at upper right in A = 250 µm. Blots were photographed using a Zeiss Photomicroscope 2 on Kodak T-Max film, ASA 400. All exposures were for 1 s. Printing exposure times were as follows: A, 15 s; B, 20 s; C, 24 s; D, 26 s; E, 30 s; F, 50 s; P, pith; C, cortex.
developing petunia stem cut approximately 4 mm below the bud (Fig. 6), the xylem does not form a continuous ring but is present only at the leaf traces. Since, as shown in Figure 5, A and B (sections 2 and 6 mm beneath the bud), ptGRP1 is present within the vascular system as a continuous ring between cortex and pith with no discontinuities, this protein must be (at least) localized to cells of the phloem and/or cambium.

**DISCUSSION**

In this paper we have shown that an antibody raised against a synthetic peptide comprising amino acids 22 through 37 of the mature ptGRP1 protein reacts with a single protein of 23 kD. In addition, cell fractionation studies have shown that it is likely that ptGRP1 is a cell wall protein. We have also found that this protein (or the amino terminal portion of the protein) is very labile. We found that ptGRP1 is partially stabilized when tissue is ground in the presence of high concentrations of non-ionic detergents and protease inhibitors and completely stabilized only when the tissue was either ground in 2X Laemmli buffer (4.6% SDS) and immediately heated before the grindate had completely thawed, or ground in high alkali (0.5 M NaOH).

The lability we found for ptGRP1 is so far unique for a cell wall structural protein. Members of all three classes of these type of proteins, including fbGRP1.8 (the cell wall protein most related to ptGRP1) have either been isolated or subjected to cell fractionation studies under conditions in which pt-GRP1 is completely degraded (1, 3, 8). We theorize that this difference in lability between ptGRP1 and all other cell wall proteins may be due to this protein’s tertiary structure.

We had previously proposed that amino acids 42–357 of the mature ptGRP1 protein were capable of forming an 8 stranded β-pleated sheet (4). This proposal was based on the fact that this region of the protein was 75% Gly with an addition 10% of the residues either Ala or Ser, allowing the sequence of this region to be written as (Gly-X)₈n, where X is generally Gly, Ala or Ser. This formula is similar to poly-L-glycine (Gly-Gly)ₐ and to fibron (Gly-Ala)ₙ, two peptides capable of forming a β-pleated sheet. A space-filling model of portions of the ptGRP1 protein showed that there was no steric hindrance to the formation of such a structure (4). Further, in this proposed model, the first 41 hydrophilic amino-terminal amino acids of this protein would largely be projected away from the slightly hydrophobic body of the remainder of the molecule. In addition, being hydrophilic (it contains 13 of the 20 charged particles of the protein), this portion of the molecule would be incapable of interaction with the hydrophobic β-pleated sheet region and thus would lie in an extremely exposed and unprotected position. This model would therefore predict that the hydrophilic portion of the protein would be extremely labile and susceptible to degradation by either exo- or endo-peptidases. Since the antibody we used in these experiments was raised against this portion of the molecule, it is possible that the tertiary structure of the protein could account for the lability we observed. Further, since only ptGRP1, of all described cell wall proteins (including fbGRP1.8), contains a short hydrophilic amino terminal region, this uniqueness in its primary and tertiary structures might account for its (as yet) unique lability.

In addition to its unique lability, ptGRP1 also differs from all other sequenced cell wall proteins, in that it contains no tyrosine. This is particularly significant as the formation of intramolecular isodityrosine linkages are likely involved in the insolubilization of extensin (6, 13) and it has been proposed that these same type of linkages may be involved in the insolubilization of fbGRP1.8 (7.4% tyrosine) within the cell wall. It is not yet determined whether ptGRP1 becomes insolubilized in the cell wall. Data presented in this paper have shown that there are decreasing amounts of extractable ptGRP1 present as both leaves and stems mature. These results, however, are consistent either with the antigen’s insolubilization or its degradation as the plant develops. Although ptGRP1 contains no tyrosine, insolubilization of this protein could occur via the O-glycosylation of serine residues (ptGRP1 contains no other known glycosylation sites), or it could simply become physically trapped within the cell wall matrix.

We have also shown that the expression of the ptGRP1 protein like other plant cell wall proteins appears to be under developmental control. Highest amounts of extractable pt-GRP1 are found in the bud with lesser amounts of extractable antigen present as distance from the bud increases in both leaves (Fig. 3) and stem (Fig. 5). This pattern of protein accumulation parallels that previously found for the steady state levels of ptGRP1 message (5). In addition, we found that like soybean seed coat extensin and fbGRP1.8, ptGRP1 is expressed only in a discrete subset of cell types, this latter protein being specific to the vascular tissue, with significant but low levels of the protein present in the epithelium (or in a layer of cells directly beneath the epidermis).

Although both ptGRP1 and fbGRP1.8 protein are both present in the vascular tissue it is likely that they are not their species respective homologs. fbGRP1.8 has been shown to be specific only to protoxylem cells (9) while we have shown in this report that the ptGRP1 gene must be expressed within...
the vascular tissue in at least the cambium and/or the phloem. In addition, significant but low levels of ptGRP1 were found to also be expressed in the epidermis or in a layer of cells directly beneath the epidermis. These results indicate that ptGRP1 is produced in at least two different cell types, neither of which is protoxylem. In addition to differing cell specificities, ptGRP1 contains much lower levels of the polar amino acids serine, asparagine glutamine and threonine than does fbGRP1.8. As mentioned above, ptGRP1 unlike fbGRP1.8 contains no tyrosine and has an hydrophilic amino terminal sequence. In addition, fbGRP1.8 appears to be expressed in cell cultures while ptGRP1 is not. These differences make it likely that these two genes are not homologs but are two different members of the same class of cell wall proteins.

From the data presented above, that different members of a single class of cell wall proteins have differing narrow cell specificities (9), and from the fact that soybean seed coat extensin has been also show to exhibit a similar narrow cell specificity (3), it is interesting to speculate that a similar type of specificity may be found for each gene family member of each class of cell wall protein. Although much further work is necessary to prove or disprove this theory, the data presented here and elsewhere indicate that the role that cell wall structural proteins play in the development and maturation of the cell wall is likely to be a highly specific and complex process.

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LITERATURE CITED