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RESEARCH PAPER

# Expression analyses of AtAGP17 and AtAGP19, two lysine-rich arabinogalactan proteins, in *Arabidopsis*

J. Yang[1], Y. Zhang[2], Y. Liang[2] & A. M. Showalter[2]

1 College of Biological Science and Technology, Fuzhou University, Fuzhou, Fujian, China

2 Department of Environmental and Plant Biology, Molecular and Cellular Biology Program, Ohio University, Athens, OH, USA

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**Correspondence**

A. M. Showalter, Department of Environmental and Plant Biology, 315 Porter Hall, Ohio University, Athens, OH 45701-2979, USA.

E-mail: showalte@ohio.edu

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## ABSTRACT

**AtAGP17 and AtAGP19 are members of the lysine-rich arabinogalactan protein (AGP) subfamily in *Arabidopsis*. Detailed anatomical analysis of promoter activity of the *AtAGP19* gene was carried out using transgenic *Arabidopsis* plants expressing a $P_{AtAGP19}$:*GUS* fusion. *AtAGP19* promoter activity was tissue-specific and associated with vascular bundles, particularly differentiating xylem elements. Peptide-specific antibodies were raised against the Lys-rich regions of AtAGP17 and AtAGP19 and used to study the organ-specific expression patterns of these two AGPs. AtAGP17 and AtAGP19 were most abundant in roots and flowers, moderately abundant in stems, seedlings and siliques and virtually absent in leaves. Antibodies specific for AtAGP17 and AtAGP19, as reported here, represent valuable tools for understanding the biology of these two AGPs.**

## INTRODUCTION

Arabinogalactan proteins (AGPs) are a family of hyperglycosylated proteins within the hydroxyproline-rich glycoprotein (HRGP) superfamily. Protein backbones of AGPs are rich in Pro/Hyp (hydroxyproline), Ser, Ala and Thr and are modified by the addition of type II arabinogalactan polysaccharides and arabinose oligosaccharides to Hyp residues. AGPs are present in all lower and higher plants studied to date (Nothnagel 1997; Gaspar *et al.* 2001; Showalter 2001; Seifert & Roberts 2007; Ellis *et al.* 2010).

AGP antibodies are valuable tools to probe the expression and function of AGPs. Given that AGPs are typically 10% protein and 90% carbohydrate by weight, most of the protein backbone of a typical AGP is coated with sugar. Consequently, most of the antibodies for AGPs developed against native AGPs recognise the carbohydrate epitopes on AGPs. The JIM and MAC antibodies, JIM4, JIM8, JIM13, JIM15 and MAC207, are examples of such AGP antibodies (Knox 1997). These antibodies are useful for examining organ-, tissue- and cell-specific expression of AGPs sharing common carbohydrate epitopes; however, they cannot discriminate among individual AGPs and may cross-react with other macromolecules bearing the same carbohydrate epitopes, such as pectin (Kreuger & van Holst 1996; Showalter 2001).

AGPs are divided into several classes: classical AGPs, lysine-rich classical AGPs, AGP peptides, fasciclin-like AGPs (FLAs) and other chimeric AGPs (Schultz *et al.* 2002; Showalter *et al.* 2010). Classical AGPs typically consist of an N-terminal signal peptide, a Pro/Hyp-rich AGP central domain and a C-terminal glycosylphosphatidylinositol (GPI) lipid anchor addition sequence (Schultz *et al.* 1998). Lysine-rich AGPs are a subclass of the classical AGPs and contain a short Lys-rich region within the Pro/Hyp-rich AGP central domain. This Lys-rich region is not glycosylated and allows for production and recognition of peptide-specific antibodies (Gao *et al.* 1999; Zhang *et al.* 2003; Yang & Showalter 2007).

Seven Lys-rich AGPs were identified from different plant species: LeAGP1 in tomato (Li & Showalter 1996; Gao *et al.* 1999), NaAGP4 in *Nicotiana alata* (Gilson *et al.* 2001), AtAGP17, 18 and 19 in *Arabidopsis* (Schultz *et al.* 2002; Sun *et al.* 2005), CsAGP1 in cucumber (Park *et al.* 2003) and PtaAGP6 in pine (Zhang *et al.* 2003). Expression of LeAGP1, PtaAGP6 and AtAGP18 was characterised on the protein level (Gao & Showalter 2000; Zhang *et al.* 2003; Yang & Showalter 2007). Antibodies were raised against peptides encompassing the unique Lys-rich subdomains of LeAGP1 (PAPAPSKGKVKGKKGKKHNA), PtaAGP6 (HHKRKKKKKKHHH) and AtAGP18 (PAPAPSKHKKTTKKSKKHQA). Western analyses revealed similar expression patterns of LeAGP1 and AtAGP18; protein levels of both AGPs were high in roots, flowers, stems and young fruits and low in leaves. LeAGP1 and PtaAGP6 were both localised to differentiating xylem elements; LeAGP1 was also abundant in stylar transmitting tissues (Gao *et al.* 1999; Gao & Showalter 2000; Zhang *et al.* 2003; Yang & Showalter 2007).

AtAGP17 and AtAGP19 share 37.7% amino acid sequence similarity and 27.9% amino acid sequence identity. Genetic

expression of *AtAGP17* and *19* was previously characterised at the organ level *via* Northern blotting and RT-PCR (Gaspar *et al.* 2004; Sun *et al.* 2005; Yang *et al.* 2007). Interestingly, although the *AtAGP17* transcript was barely detectable in roots, a T-DNA knockdown mutant of *AtAGP17* displayed abnormal phenotypes only in the roots (*i.e.,* reduced *Agrobacterium* binding). The tissue-specific expression patterns of these two genes and accumulation of the corresponding proteins at the organ level was never characterised, until now.

Here, we report on the tissue-specific promoter activity of *AtAGP19* and the production and use of peptide-specific antibodies for AtAGP17 and AtAGP19 to examine accumulation of these AGPs at the protein level. The AtAGP17- and AtAGP19-specific antibodies reported here represent new additions to the small family of antibodies specific for single AGP family members in their glycosylated forms and will facilitate further analyses to probe the biology and function of these two Lys-rich AGPs.

## MATERIALS AND METHODS

### Bioinformatics

Peptide sequences used for antibody production were aligned with ClustalW (http://www.ebi.ac.uk/clustalw). AGP gene identification numbers (either *Arabidopsis* AGI locus numbers or Genbank accession numbers) are as follows: *AtAGP17*, At2g23130; *AtAGP18*, At4g37450; *AtAGP19*, At1g68725; *LeAGP1*, X99147.

*Plant material and growth conditions*

*Arabidopsis thaliana* (ecotype Columbia-0) plants were grown in soil in either a growth room or a growth chamber at 22 °C under 16 h light/8 h dark conditions. Alternatively, *Arabidopsis* seedlings were grown on Murashige and Skoog (MS) plates consisting of 4.3 $g \cdot l^{-1}$ MS basal salts (Caisson Laboratories, Logan, UT, USA), 1% sucrose and 0.8% agar (pH 5.8), unless indicated otherwise. The plates were kept at 24 °C under long-day conditions.

### Histology

Leaves, hypocotyls and stems of T2 transgenic *Arabidopsis* plants harbouring the $P_{AtAGP19}$:*GUS* reporter gene construct were stained for GUS activity as described previously (Yang *et al.* 2007). At least 10 independent transgenic lines were tested. WT samples were included as negative controls, and no staining of WT samples was observed. Immediately following GUS staining, samples were fixed overnight in Safefix II (Fisher Scientific, Hampton, NH, USA), dehydrated in an ethanol series (30%, 50%, 60%, 70%, 80%, 90%, 95%, and 100%, each for 2 h) and embedded in paraffin. Sections 8–25-μm thick were cut with a rotary microtome, depending on the intensity of staining. Sections were dried for 24 h, dewaxed in Citrisolv and rehydrated. Rehydrated sections were either mounted directly in Permount or counter-stained with 1% aqueous Safranin O for 15 min or phloroglucinol/HCl for a few seconds. Safranin O-stained sections were dehydrated and mounted. Phloroglucinol/HCl-stained slides were viewed immediately under a microscope.

### Production of AtAGP17 and 19 antibodies

Previous reports demonstrated the feasibility of raising specific antibodies against the unique Lys-rich subdomains of Lys-rich AGPs (Gao *et al.* 1999; Zhang *et al.* 2003; Yang & Showalter 2007). The same approach was used to produce antibodies against AtAGP17 and 19. Before peptide injection, rabbit pre-immune sera were screened, and rabbits with the lowest immune responses to *Arabidopsis* total leaf proteins were chosen for production of antibodies. Peptides (20 amino acids in length) were synthesised (Genemed Synthesis, San Francisco, CA, USA) encompassing the Lys-rich regions of the AGPs (Fig. 1).

The synthetic peptides were conjugated to keyhole limpet haemocyanin (KLH) to promote immune responses in rabbits. Peptide-specific antibodies were purified by passing antisera through affinity columns conjugated to the synthetic peptides, and they are referred to as (anti-peptide) antibodies. The flow-through portions from the affinity columns were also collected and used in analyses; these were designated as antisera. Purified antibodies in PBS buffer, antisera and pre-immune sera were stored in aliquots at −80 °C; they were also stable at 4 °C for at least 1 month.

### Western blotting

Total proteins were extracted from *Arabidopsis* seedlings, leaves, roots, stems, flowers and siliques according to an established method (Weigel & Glazebrook 2002). Electrophoresis and transfer of proteins were carried out using the Mini-PROTEAN 3 Electrophoresis Cell and Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad, Hercules, CA, USA). Protein samples were run on 5–10% SDS-PAGE gels with Precision Plus Protein All Blue Standards (Bio-Rad) and electro-blotted to a PVDF membrane (Bio-Rad).

Duplicate PVDF membranes were either stained in 0.1% (w/v) Coomassie blue R-250 or immunoblotted. For immunoblotting, the membrane was treated with blocking buffer (5% non-fat dry milk, 0.05% Tween 20, PBS) for 1 h at room temperature (RT) and then incubated with primary antibodies for at least 3 h at RT or overnight at 4 °C. Purified AtAGP17 and 19 antibodies were diluted to between 1:500 and 1:1000; pre-immune sera and antisera were diluted to between 1:100 and 1:500. Membranes were washed (0.05% Tween 20, PBS) before incubation with goat anti-rabbit alkaline phosphatase-conjugated secondary antibodies (Sigma-Aldrich, St. Louis, MO, USA) or goat anti-rabbit horseradish

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LeAGP1  --PAPAPSKGKVKGKKGKKHNA 20
AtAGP18 --PAPAPSKHKKTTKKSKKHQA 20
AtAGP17 --PAPALTKHKKKTKKHKTAPA 20
AtAGP19 PAPAPAPTKHKRK-HKHKRHH- 20
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**Fig. 1.** Alignment of AtAGP17 and AtAGP19 peptides used for antibody production and their homologous sequences from AtAGP18 and LeAGP1. Peptide sequences used for production of PAP antibodies (for LeAGP1) and AtAGP18 antibodies are from previous reports (Gao *et al.* 1999; Yang & Showalter 2007). '*' indicates identical amino acids. ':' indicates strongly conserved residues. '.' indicates weakly conserved residues.

peroxidase-conjugated secondary antibodies (Pierce, Rockford, IL, USA).

### Dot blotting

Synthetic peptides (1 μl) of 0.4, 1, 4, 20 and 100 μM were spotted directly onto nitrocellulose membranes. The membranes were allowed to dry completely after sample loading. Blocking and immunoblotting were the same as described for Western blotting.

## RESULTS

### *AtAGP19* expression is specific and developmentally controlled

*AtAGP19* promoter activity was studied at the tissue level. Consistent with whole mount GUS staining in leaves (Yang *et al.* 2007), all leaf cells were stained in newly developed leaves (Fig. 2a). As the leaf matured, *AtAGP19* expression decreased in the adaxial half of the leaf (Fig. 2b) and was later restricted to the epidermis on both sides of the leaf as well as to the vascular bundles (Fig. 2c). Both xylem and phloem displayed GUS staining (Fig. 2d). Eventually, *AtAGP19* expression was not detectable in old/senescent leaves.

In hypocotyls undergoing secondary cell wall thickening, *AtAGP19* GUS activity was found in the xylem, vascular cambium, phloem and periderm, substantiating a previous report on the presence of AGPs in the periderm (Dolan & Roberts 1995). The collapsing outer layers, including the epidermis, cortex and endodermis, did not show GUS activity (Fig. 3a and b). In secondary xylem, xylem parenchyma cells and developing xylem vessel elements were stained, but differentiated vessels containing lignified secondary cell walls were not stained (Fig. 3c–e). Staining in the periderm decreased in hypocotyls at later developmental stages (Fig. 3e).

In young inflorescence stems where high *AtAGP19* expression was observed, virtually all cells were stained (Fig. 3f and g). Vascular bundles, phloem, procambium, xylem parenchyma cells and developing xylem vessels displayed GUS activity (Fig. 3h–k). As development progressed, staining faded from the pith region first (Fig. 3j). GUS staining was also associated with fibre cells in the stem interfascicular region (Fig. 3j and k) and hypocotyl secondary xylem region (Fig. 3e) even after they were lignified, suggesting additional roles in living fibre cells.

The above observations show that *AtAGP19* promoter activity was consistently associated with vascular bundles in *Arabidopsis* plants. Detailed analyses demonstrated that *AtAGP19* transcription was high in xylem elements but absent in developed xylem elements.

### Specificity and reactivity of antibodies

Antibodies were generated against the Lys-rich regions of AtAGP17 and 19 (Fig. 1) and purified by affinity chromatography against their respective synthetic peptides. The flow-through from the affinity column still contained unremoved antibodies and was referred to as antiserum. The purified antibodies were first tested for their specificity against synthetic peptides encompassing the Lys-rich regions of four homologous AGPs, namely AtAGP17, 18 and 19 and LeAGP1. The AtAGP17 antibodies only recognised the AtAGP17 peptide and did not recognise the peptides for AtAGP18, AtAGP19 or LeAGP1. Similarly, the AtAGP19 antibodies did not bind to peptides for AtAGP17, AtAGP18 or LeAGP1, but only recognised the peptide it was raised against (Fig. 4). Therefore, both anti-peptide antibodies were specific for their respective AGP Lys-rich regions. In addition to being specific, the antibodies exhibited strong reactivity against the peptides. Similar to the antibodies, the AtAGP17 and 19 antisera were specific against their respective peptides. Although the AtAGP19 antiserum recognised the LeAGP1 peptide at 100 μM, this did not cause a cross-reactivity problem since AtAGP19 and LeAGP1 are found in different plant species. Furthermore, the corresponding pre-immune sera did not produce any signals against any of the peptides.

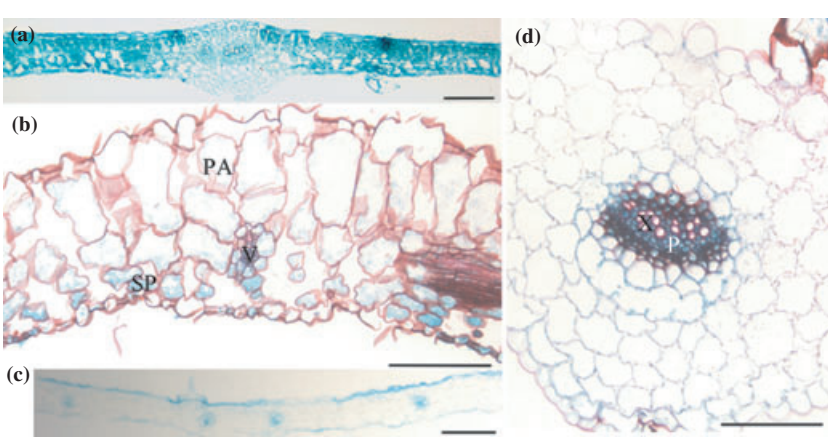


**Fig. 2.** Expression of *AtAGP19* in *Arabidopsis* leaves. Leaves of transgenic *Arabidopsis* plants harbouring the $P_{AtAGP19}$:*GUS* reporter gene construct were stained and sectioned. a: A transverse section of a young leaf (7 days old) showing strong GUS staining in leaf epidermal and mesophyll cells. b: In developing leaves, GUS staining gradually diminished from the adaxial portion but was retained longer in the abaxial portion (spongy mesophyll cells and abaxial epidermis). PA = palisade mesophyll; SP = spongy mesophyll; V = vascular bundle. c: GUS activity of the *AtAGP19* promoter was confined to vascular tissues in mature leaves. d: A transverse section of a 4-week-old petiole showing that GUS activity was associated with both xylem and phloem. X = xylem; P = phloem; (b) and (d) were counter-stained with Safranin O after GUS staining and sectioning. Bars = 100 μm.

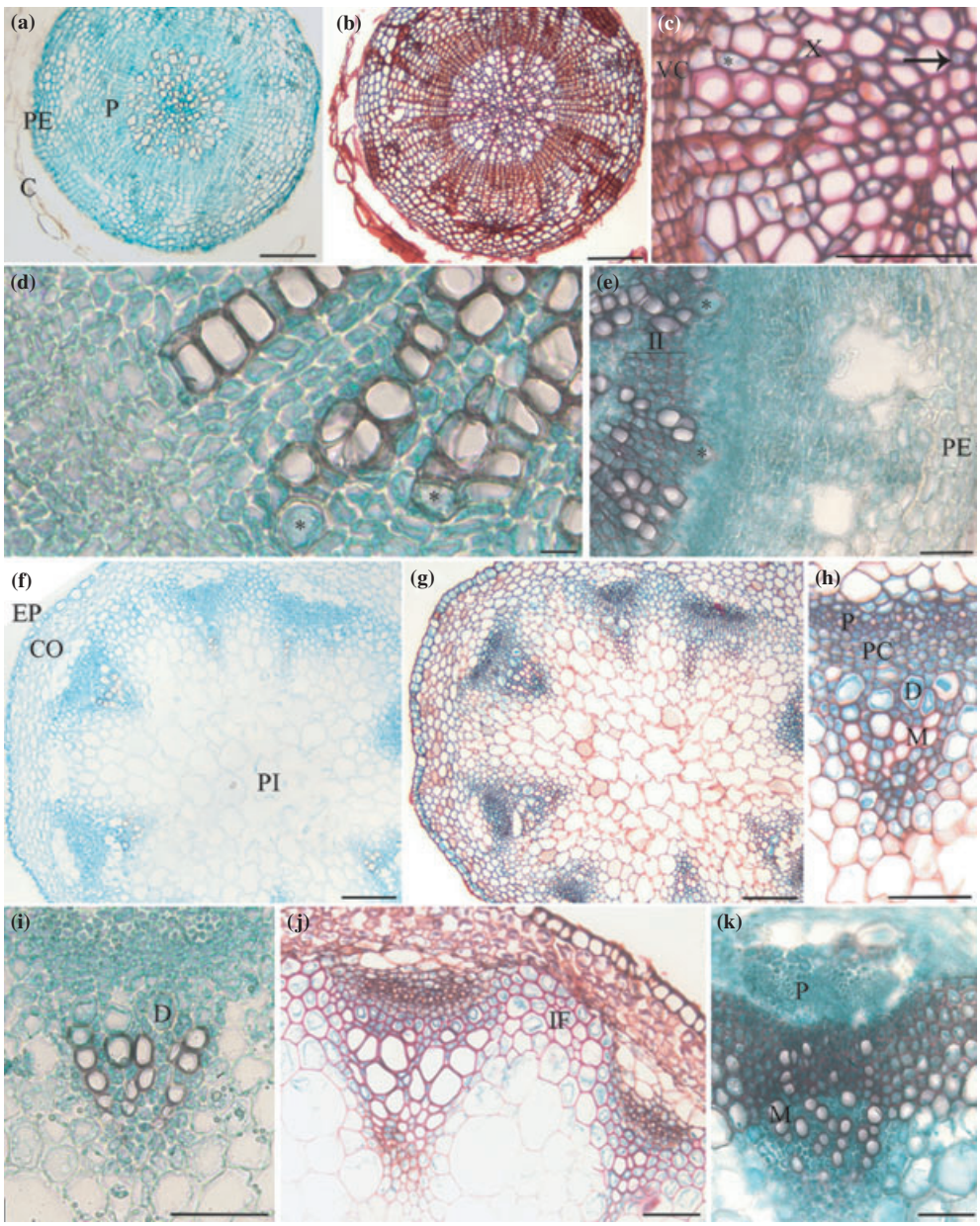


**Fig. 3.** Promoter activity of *AtAGP19* in *Arabidopsis* hypocotyls and stems. Hypocotyls and stems of transgenic *Arabidopsis* plants harbouring the $P_{AtAGP19}$:*GUS* reporter gene construct were stained and sectioned. a and b: Transverse sections of a 4-week-old hypocotyl undergoing secondary cell wall thickening, showing GUS staining in all cells. c: A magnified view of secondary xylem in (b). Differentiating vessels and xylem parenchyma cells displayed GUS staining. d and e: Transverse sections of hypocotyls at later stages of secondary cell wall thickening. f and g: Transverse sections of a young stem showing GUS staining in all cells. h and i: Enlarged views of a vascular bundle. Mature xylem elements were not stained. j and k: Transverse sections of stems at later developmental stages. (a) and (f) were GUS stained only; (b), (c), (g), (h) and (j) were counter-stained with Safranin O; (d), (e), (i) and (k) were stained with phloroglucinol/HCl. II, phase II region in hypocotyl secondary thickening. C, disintegrating layers of epidermis, cortex and endodermis. CO = cortex; D = developing metaxylem; EP = epidermis; IF = interfascicular region; M = developed metaxylem; P = phloem; PC = procambium; PE = periderm; PI = pith; VC = vascular cambium; X = xylem; Asterisks indicate developing vessel elements. The arrow indicates a xylem parenchyma cell. Bars = 100 μm in (a), (b), (f) and (g) and 50 μm in (c) to (e) and (h) to (k).

The AtAGP17 antibodies and antiserum were also tested against purified EGFP-AtAGP17 fusion protein obtained previously (Fig. 5a). Pre-immune serum of AtAGP17 did not recognise the fusion protein, while its antiserum and antibodies did. The AtAGP17 antiserum could detect as little as 250 ng of EGFP-AtAGP17 (Fig. 5b). The smears above 250 kD in most lanes were expected, given that the mobility of AGPs in gels is retarded and diffuse due to carbohydrate microheterogeneity. In addition, a series of dilutions of the AtAGP17 antibodies and antiserum was tested for reactivity against 1 μg of purified EGFP-AtAGP17. Minimal dilutions of 1:1000 for AtAGP17 antibodies and 1:500 for the antiserum

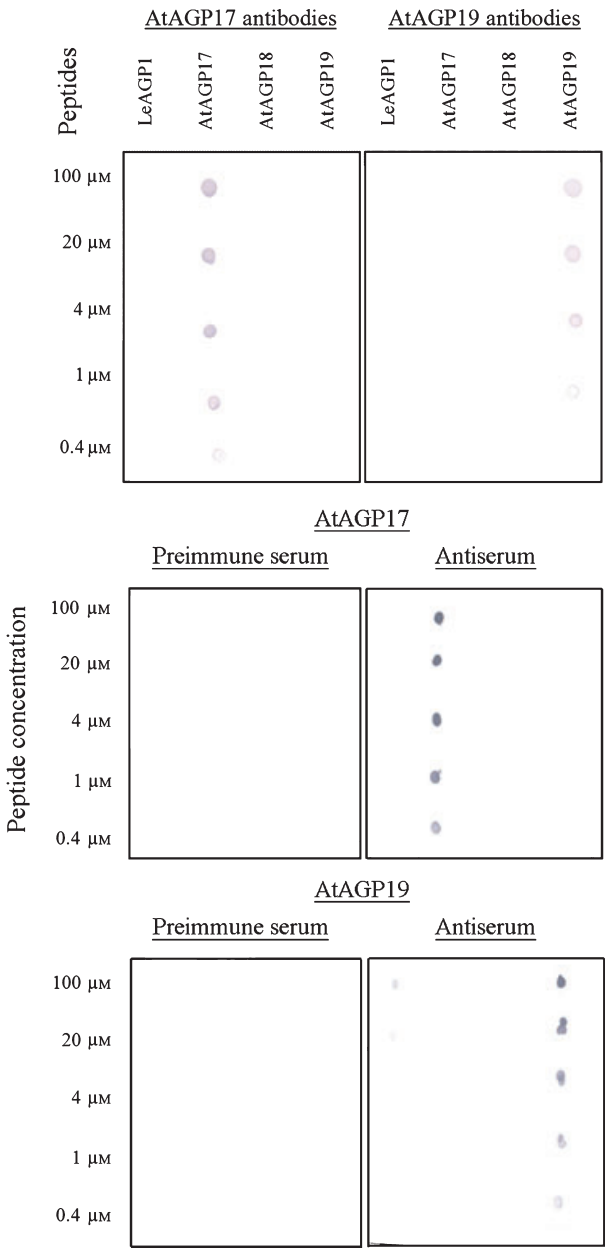


**Fig. 4.** Antibody and antiserum specificity tests using Western dot blots of synthetic peptides examined with the AtAGP17 and AtAGP19 antibodies and antisera. Blots were made by loading 1 μl of LeAGP1, AtAGP17, AtAGP18 and AtAGP19 synthetic peptides at the indicated concentrations onto a nitrocellulose membrane. The blots were air dried, blocked and then used to test specificity and reactivity of the AtAGP17 and AtAGP19 antibodies and antisera.

generated detectable colorimetric signals (data not shown). The purified anti-AtAGP19 antibodies did not react with EGFP-AtAGP17, consistent with its known specificity (data not shown).

### Western blotting with AtAGP17 and 19 antibodies and antisera

Total proteins were extracted from *Arabidopsis* seedlings and various organs, and the organ-specific expression of AtAGP17 and AtAGP19 was examined using their specific antibodies.

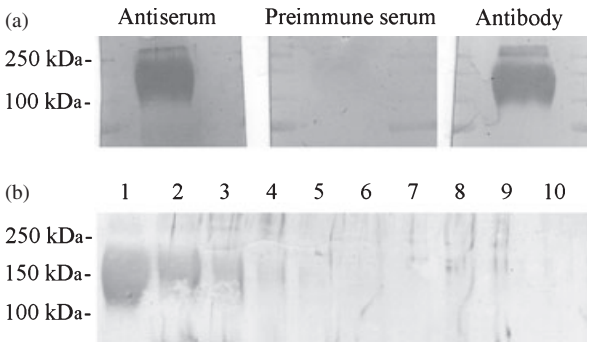


**Fig. 5.** Western blot analysis of purified EGFP-AtAGP17 using the AtAGP17 antibodies and antiserum. a: Ten μg of purified EGFP-AtAGP17 (Sun *et al.* 2005) were loaded in each lane and incubated with antiserum (1:200 dilution), pre-immune serum (1:200 dilution) and affinity-purified antibodies (1:500 dilution) of AtAGP17. b: Different quantities of purified EGFP-AtAGP17 fusion protein incubated with the AtAGP17 antiserum (1:300 dilution). Lane 1, 5 μg; Lane 2, 2.5 μg; Lane 3, 1 μg; Lane 4, 500 ng; Lane 5, 250 ng; Lane 6, 75 ng; Lane 7, 50 ng; Lane 8, 10 ng; Lane 9, 1 ng; Lane 10, 100 pg.

The mature protein backbones of AtAGP17 and 19 contain 138 and 196 amino acids, respectively (excluding signal peptides and the C-terminal regions replaced by GPI anchors). Expression of AtAGP17 and 19 varied in each organ in terms of the intensity and molecular weight (MW) ranges of antibody reactivity. The smears were of different size ranges (37–250 kD) on the Western blots, indicating that AtAGP17 and AtAGP19 were differentially glycosylated in different organs, but similarly glycosylated within any given organ (Figs 6 and 7). AtAGP19 levels were highest in roots and flowers, followed by seedlings, stems and siliques. Little AtAGP19, however, was detected in rosette leaves (Fig. 6).

Surprisingly, unlike the AtAGP19 antibodies, AtAGP17 antibodies could not produce a detectable signal on the Western blot. Its antiserum was then used to detect the organ-specific expression pattern of AtAGP17. Similar expression patterns of AtAGP17 and AtAGP19 were obtained with their individual antiserum (Fig. 7). Namely, both AtAGP17 and 19 were most abundant in roots, flowers and stems and less abundant or undetectable in the other organs examined. Incubating an identical membrane with pre-immune serum produced virtually no signal (data not shown).

## DISCUSSION

AtAGP17, 18 and 19 are Lys-rich classical AGPs in *Arabidopsis* (Schultz *et al.* 2002; Sun *et al.* 2005). These three genes are homologous to four other AGP genes characterised to varying extents in other plant species (Gao *et al.* 1999; Gilson *et al.* 2001; Park *et al.* 2003; Zhang *et al.* 2003). Here, we report on the tissue-specific promoter activity of *AtAGP19* as well as the protein expression patterns of AtAGP17 and 19.

Expression of *AtAGP19* on the tissue level, as revealed by anatomical analysis of transgenic plants harbouring the $P_{AtAGP19}$*:GUS* fusion, corroborates our earlier finding at the organ level that *AtAGP19* expression was spatially and developmentally controlled. Notably, vasculature and young organs displayed the highest amounts of GUS staining. In

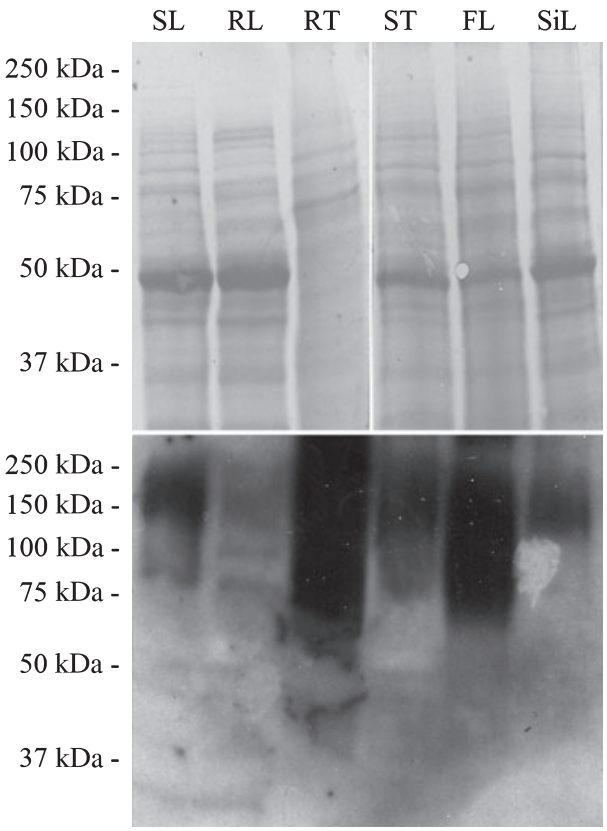


**Fig. 6.** Western blot analysis of *Arabidopsis* seedlings and organs using the AtAGP19 antibodies. Total proteins (30 μg per lane) from *Arabidopsis* 12-day-old seedlings (SL), 35-day-old rosette leaves (RL), roots (RT), stems (ST), flowers (FL) and siliques (SiL) were loaded. Identical PVDF membranes were either stained with Coomassie blue (top panel) or incubated with the AtAGP19 antibodies overnight (bottom panel).

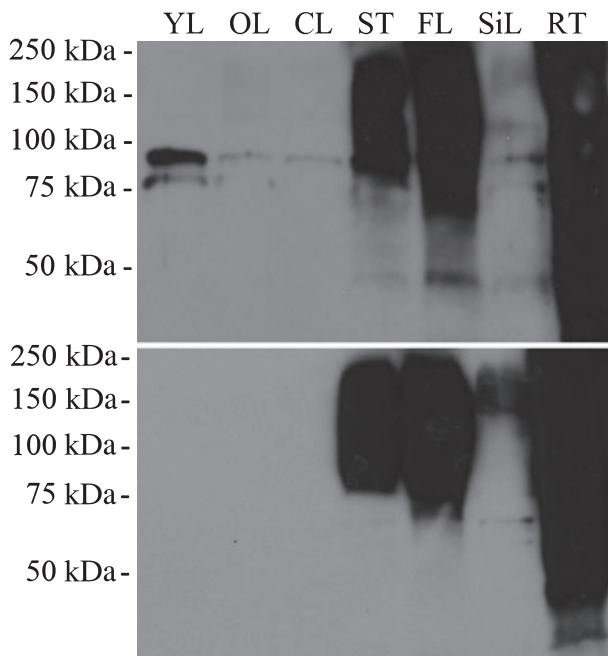


**Fig. 7.** Western blot analysis of *Arabidopsis* seedlings and organs using the AtAGP17 and AtAGP19 antisera. Total protein (60 μg per lane) from *Arabidopsis* young leaves (YL), old leaves (OL), cauline leaves (CL), stems (ST), flowers (FL), green siliques (SiL) and roots (RT) was loaded. An identical protein gel was stained with Coomassie blue to ensure the quality of protein samples (data not shown). Duplicate membranes were incubated with AtAGP17 (top) or AtAGP19 (bottom) antisera (1:200 dilution). Another identical membrane did not give any signal when incubated with pre-immune sera (1:200 dilution, data not shown). Consistent results were obtained in two independent experiments.

this study, we extend our analysis to show that *AtAGP19* promoter-directed GUS activity was high in young cells and diminished as the cells matured; this phenomenon is consistent with AGP19's proposed role in cell expansion (Yang *et al.* 2007).

*AtAGP19* promoter activity is consistently associated with the vascular tissues throughout the plant. In particular, *AtAGP19* promoter activity is found in vascular cambium, procambium, xylem parenchyma cells and differentiating xylem vessels in hypocotyls and stems. Similar to *AtAGP19*, many other AGP genes show xylem-specific or -preferential expression patterns. Two such examples are provided by *in situ* hybridisation of two homologous FLA genes, *AtFLA11*and *ZeFLA11*. The *AtFLA11* transcript is restricted to *Arabidopsis* stem and silique sclerenchyma cells and co-regulated with secondary cell wall cellulose synthase genes (Brown *et al.* 2005; Ito *et al.* 2005; Persson *et al.* 2005), whereas *ZeFLA11* is specifically expressed in differentiating xylem elements with reticulate type wall thickenings in *Zinnia* (Dahiya *et al.* 2006). In addition to the above correlations between AGP expression and xylem differentiation, xylogen, a chimeric AGP, is unequivocally established as a mediator in xylem differentiation (Motose *et al.* 2001). In *Zinnia* stems, xylogen is expressed in procambium and immature xylem cells. A double xylogen mutant in *Arabidopsis* (*atxyp1 atxyp2*) has discontinuous leaf venation patterns (Motose *et al.* 2004).

Important progress in characterising AGPs has been made with AGP antibodies. AGPs are implicated in pattern formation and vascular development, with their spatially and temporally controlled expression in different stages of xylem differentiation (Knox *et al.* 1989, 1991; Stacey *et al.* 1990, 1995; Dolan & Roberts 1995; Dolan *et al.* 1995; Schindler *et al.* 1995; Casero *et al.* 1998). While most antibodies recognise the carbohydrate epitopes on AGPs and are not able to discriminate among individual AGPs, a limited number of antibodies against AGP core proteins are also available, allowing the examination of individual AGPs. PAP and anti-AGPB are antibodies against the Lys-rich regions of LeAGP1 and PtaAGP6, respectively. These antibodies allow the recognition and detection of specific, glycosylated AGPs, since the Lys-rich region imparts specificity and also allows accessibility of the antibody to this non-glycosylated region of the native molecule (Gao *et al.* 1999; Zhang *et al.* 2003). With peptide-specific antibodies, LeAGP1 and PtaAGP6 were immunolocalised to differentiating xylem elements and functionally associated with secondary cell wall thickening and xylem differentiation (Gao & Showalter 2000; Zhang *et al.* 2003).

The AtAGP17 and 19 antibodies and antisera generated using synthetic peptides, demonstrate sufficient specificity

with respect to the synthetic peptides and the EGFP-AtAGP17 fusion protein. However, AtAGP17 antibodies are not able to detect AtAGP17 in total protein preparations. In contrast, AtAGP17 antisera and pre-immune serum allow specific antigen detection in total protein preparations; pre-immune sera either do not react at all or react only to a minimal degree. Furthermore, the AtAGP19 antibodies and antiserum produced Western blotting results that were similar, but not identical, to those obtained with the AtAGP17 antiserum, adding to the validity of the data and specificity of the two antisera.

Protein expression patterns obtained in this study are very similar for AtAGP17 and AtAGP19, despite the relatively low sequence similarity/identity between these two homologous proteins. Both are very abundant in roots and flowers, moderate in stems and siliques and low in leaves. Furthermore, the organ-specific expression patterns of AtAGP17 and AtAGP19 are reminiscent of LeAGP1 and AtAGP18 (Gao & Showalter 2000; Yang & Showalter 2007). Moreover, it appears that AGP17 and 19 as well as AGP18 undergo similar glycosylation reactions in any given organ, leading to the hypothesis that the extent of AGP glycosylation is organ-specific and independent of the AGP core protein. Such organ-specific AGP glycosylation may be critical for biological functions unique to a particular organ. Indeed, it would be informative to tag and express different AGP genes under the control of various organ-specific promoters and examine the extent of glycosylation, carbohydrate composition and glycosidic linkages associated with such AGPs.

Although the reported pattern of expression for AtAGP19 is largely in line with its genetic expression data (Yang *et al.* 2007), this is not the case for AtAGP17. Surprisingly, little AtAGP17 was detected in leaves, while a large amount of AtAGP17 was found in roots; this is opposite to what would be expected based on Northern blotting data reported in different studies, all of which found that the *AtAGP17* transcript level was barely detectable in roots (Gaspar *et al.* 2004; Sun *et al.* 2005; Yang *et al.* 2007). Clearly, the amount of mRNA does not always agree with the amount of protein, and the inconsistency between the AtAGP17 protein and mRNA levels here is particularly noteworthy considering that *rat1*, an *AtAGP17* mutant, displayed altered phenotypes only in the roots, but not in other parts of the plant (Nam *et al.* 1999; Gaspar *et al.* 2004). Consequently, future work on AtAGP17 protein distribution in *Arabidopsis* roots at the tissue level would be worthwhile.

With the research convenience and specificity offered by anti-peptide antibodies and antisera, work can be performed in the future to detect the distribution of these individual Lys-rich AGPs at the cell and tissue levels in order to elucidate their detailed expression patterns. Additionally, these antibodies may be useful in isolating and identifying interacting molecular partners for these AGP molecules. Both of these approaches can contribute to a better functional understanding of these particular AGP molecules.

## ACKNOWLEDGEMENTS

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## REFERENCES

Brown D.M., Zeef L.A.H., Ellis J., Goodacre R., Turner S.R. (2005) Identification of novel genes in *Arabidopsis* involved in secondary cell wall formation using expression profiling and reverse genetics. *Plant Cell*, **17**, 2281–2295.

Casero P.J., Casimiro I., Knox J.P. (1998) Occurence of cell surface arabinogalactan-protein and extensin epitopes in relation to pericycle and vascular tissue development in the root apex of four species. *Planta*, **204**, 252–259.

Dahiya P., Findlay K., Roberts K., McCann M.C. (2006) A fasciclin-domain containing gene, *ZeFLA11*, is expressed exclusively in xylem elements that have reticulate wall thickening in the stem vascular systems of *Zinnia elegas* cv Envy. *Planta*, **223**, 1281–1291.

Dolan L., Roberts K. (1995) Secondary thickening in roots of *Arabidopsis thaliana*: anatomy and cell surface changes. *New Phytologist*, **131**, 121–128.

Dolan L., Linstead P.J., Roberts K. (1995) An AGP epitope distinguishes a central metaxylem initial from other vascular initials in the *Arabidopsis* root. *Protoplasma*, **189**, 149–155.

Ellis M., Egelund J., Schultz C.J., Antony B. (2010) Arabinogalactan-proteins (AGPs): key regulators at the cell surface? *Plant Physiology*, **153**, 403–419.

Gao M., Showalter A.M. (2000) Immunolocalization of LeAGP-1, a modular arabinogalactan-protein, reveals its developmentally regulated expression in tomato. *Planta*, **210**, 865–874.

Gao M., Kieliszewski M.J., Lamport D.T.A., Showalter A.M. (1999) Isolation, characterization and immunolocalization of a novel, modular tomato arabinogalactan-protein corresponding to the *LeAGP-1* gene. *Plant Journal*, **18**, 43–55.

Gaspar Y., Johnson K.L., McKenna J.A., Bacic A., Schultz C.J. (2001) The complex structures of arabinogalactan-proteins and the journey towards understanding function. *Plant Molecular Biology*, **47**, 161–176.

Gaspar Y.M., Nam J., Schultz C.J., Lee L.-Y., Gilson P.R., Gelvin S.B., Bacic A. (2004) Characterization of the Arabidopsis lysine-rich arabinogalactan-protein *AtAGP17* mutant (*rat1*) that results in a decreased efficiency of Agrobacterium transformation. *Plant Physiology*, **135**, 2162–2171.

Gilson P., Gaspar Y.M., Oxley D., Youl J.J., Bacic A. (2001) NaAGP4 is an arabinogalactan protein whose expression is suppressed by wounding and fungal infection in *Nicotiana alata*. *Protoplasma*, **215**, 128–139.

Ito S., Suzuki Y., Miyamoto K., Ueda J., Yamaguchi I. (2005) AtFLA11, a fasciclin-like arabinogalactan-protein, specifically localized in screlenchyma cells. *Bioscience, Biotechnology, and Biochemistry*, **69**, 1963–1969.

Knox J.P. (1997) The use of antibodies to study the architecture and developmental regulation of plant cell walls. *International Review of Cytology*, **171**, 79–120.

Knox J.P., Day S., Roberts K. (1989) A set of cell surface glycoproteins forms an early marker of cell position, but not cell type, in the root apical meristem of *Daucus carota* L. *Development*, **106**, 47–56.

Knox J.P., Linstead P.J., Peart J., Cooper C., Roberts K. (1991) Developmentally regulated epitopes of cell surface arabinogalactan proteins and their relation to root tissue pattern formation. *Plant Journal*, **1**, 317–326.

Kreuger M., van Holst G.-J. (1996) Arabinogalactan proteins and plant differentiation. *Plant Molecular Biology*, **30**, 1077–1086.

Li S.-X., Showalter A.M. (1996) Cloning and developmental/stress-regulated expression of a gene encoding a tomato arabinogalactan protein. *Plant Molecular Biology*, **32**, 641–652.

Motose H., Sugiyama M., Fukuda H. (2001) An arabinogalactan protein(s) is a key component of a fraction that mediates local intercellular communication involved in tracheary element differentiation of *Zinnia* mesophyll cells. *Plant and Cell Physiology*, **42**, 129–137.

Motose H., Sugiyama M., Fukuda H. (2004) A proteoglycan mediates inductive interaction during plant vascular development. *Nature*, **429**, 873–878.

Nam J., Mysore K.S., Zheng C., Knue M.K., Gelvin S.B. (1999) Identification of T-DNA tagged *Arabidopsis* mutants that are resistant to transformation by *Agrobacterium*. *Molecular and General Genetics*, **261**, 429–438.

Nothnagel E.A. (1997) Proteoglycans and related components in plant cells. *International Review of Cytology*, **174**, 195–291.

Park M.H., Suzuki Y., Chono M., Knox J.P., Yamaguchi I. (2003) *CsAGP1*, a gibberellin-responsive gene from cucumber hypocotyls, encodes a classical arabinogalactan protein and is involved in stem elongation. *Plant Physiology*, **131**, 1450–1459.

Persson S., Wei H., Milne J., Page G.P., Somerville C.R. (2005) Identification of genes required for cellulose synthesis by regression analysis of public microarray data sets. *Proceedings of the National Academy of Sciences, USA*, **102**, 8633–8638.

Schindler T., Bergfeld R., Schopfer P. (1995) Arabinogalactan proteins in maize coleoptiles: developmental relationship to cell death during xylem differentiation but not to extension growth. *Plant Journal*, **7**, 25–36.

Schultz C., Gilson P., Oxley D., Youl J., Bacic A. (1998) GPI-anchors on arabinogalactan-proteins: implications for signalling in plants. *Trends in Plant Science*, **3**, 426–431.

Schultz C.J., Rumsewicz M.P., Johnson K.L., Jones B.J., Gaspar Y.M., Bacic A. (2002) Using genomic resources to guide research directions. The arabinogalactan protein gene family as a test case. *Plant Physiology*, **129**, 1448–1463.

Seifert G.J., Roberts K. (2007) The biology of arabinogalactan proteins. *Annual Review of Plant Biology*, **58**, 137–161.

Showalter A.M. (2001) Arabinogalactan-proteins: structure, expression and function. *Cellular and Molecular Life Sciences*, **58**, 1399–1417.

Showalter A.M., Keppler B.D., Lichtenberg J., Gu D., Welch L.R. (2010) A bioinformatics approach to the identification, classification, and analysis of hydroxyproline-rich glycoproteins. *Plant Physiology*, **153**, 485–513.

Stacey N.J., Roberts K., Knox J.P. (1990) Patterns of expression of the JIM4 arabinogalactan-protein epitope in cell cultures and during somatic embryogenesis in *Daucus carota* L. *Planta*, **180**, 285–292.

Stacey N.J., Roberts K., Carpita N.C., Wells B., McCann M.C. (1995) Dynamic changes in cell surface molecules are very early events in the differentiation of mesophyll cells from *Zinnia elegans* into tracheary elements. *Plant Journal*, **8**, 891–906.

Sun W., Xu J., Yang J., Kieliszewski M.J., Showalter A.M. (2005) The lysine-rich arabinogalactan-protein subfamily in *Arabidopsis*: gene expression, glycoprotein purification and biochemcal characterization. *Plant and Cell Physiology*, **46**, 975–984.

Weigel D., Glazebrook J. (2002) *Arabidopsis: a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.

Yang J., Showalter A.M. (2007) Expression and localization of AtAGP18, a lysine-rich arabinogalactan-protein in Arabidopsis. *Planta*, **226**, 169–179.

Yang J., Sardar H.S., McGovern K.R., Zhang Y., Showalter A.M. (2007) A lysine-rich arabinogalactan-protein in *Arabidopsis* is essential for plant growth and development, including cell division and expansion. *Plant Journal*, **49**, 629–640.

Zhang Y., Brown G., Whetten R., Loopstra C.A., Neale D., Kieliszewski M.J., Sederoff R.R. (2003) An arabinogalactan protein associated with secondary cell wall formation in differentiating xylem of loblolly pine. *Plant Molecular Biology*, **52**, 91–102.