Planta (2011) 233:675–683
DOI 10.1007/s00425-010-1331-6

ORIGINAL ARTICLE

AtAGP18 is localized at the plasma membrane and functions in plant growth and development

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Received: 20 September 2010 / Accepted: 30 November 2010 / Published online: 17 December 2010

Abstract Arabinogalactan-proteins (AGPs) are a family of highly glycosylated hydroxyproline-rich glycoproteins (HRGPs). *AtAGP17*, *18* and *19* comprise the lysine-rich classical AGP subfamily in *Arabidopsis*. Overexpression of GFP–AtAGP17/18/19 fusion proteins in *Arabidopsis* revealed localization of the fusion proteins on the plant cell surface of different organs. Subcellular localization of the fusion proteins at the plasma membrane was further determined by plasmolysis of leaf trichome cells. To elucidate AtAGP17/18/19 function(s), these AGPs were expressed without the green fluorescent protein (GFP) tag under the control of 35S cauliflower mosaic virus promoter. In contrast to AtAGP17/AtAGP19 overexpressors which showed phenotypes identical to wild-type plants, AtAGP18 overexpressors displayed several phenotypes distinct from wild-type plants. Specifically, these overexpressors had smaller rosettes and shorter stems and roots, produced more branches and had less viable seeds. Moreover, these AtAGP18 overexpressors exhibited similar phenotypes to tomato LeAGP-1 overexpressors, suggesting these two AGP genes may have similar function(s) in *Arabidopsis* and tomato.

Keywords *Arabidopsis* · Arabinogalactan-proteins · Green fluorescent protein · Overexpression · Transgenic plant

Abbreviations

ABA	Abscisic acid
AGP	Arabinogalactan-protein
CaMV	Cauliflower mosaic virus
GFP	Green fluorescent protein
GPI	Glycosylphosphatidylinositol
GUS	β-Glucuronidase
MS	Murashige and Skoog

Introduction

Arabinogalactan-proteins (AGPs) are a diverse family of hyperglycosylated hydroxyproline-rich glycoproteins (HRGPs) which decorate cell surfaces in different plant species (Fincher et al. 1983; Seifert and Roberts 2007). Most, but not all, AGPs consist of more than 90% carbohydrate and less than 10% protein (Nothnagel 1997). The protein backbone is rich in proline/hydroxyproline, alanine, serine and threonine. There are many structurally diverse members in the AGP family. Using a bioinformatics approach involving searching for Pro-, Ala-, Ser- and Thr-rich proteins encoded by the *Arabidopsis* genome, Schultz et al. (2002) and subsequently Showalter et al. (2010) identified 52 and 85 putative AGP genes, respectively. Based on their protein sequences, these AGPs can be

Electronic supplementary material The online version of this article (doi:10.1007/s00425-010-1331-6) contains supplementary material, which is available to authorized users.

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divided into several classes, including the classical AGPs, lysine-rich classical AGPs, AG peptides, fasciclin-like AGPs (FLAs), and other chimeric AGPs.

There are three members in the lysine-rich classical AGP subfamily in *Arabidopsis*: *AtAGP17* (Locus Identifier At2g23130), *AtAGP18* (Locus Identifier At4g37450) and *AtAGP19* (Locus Identifier At1g68725). Each of these three family members encodes an N-terminal signal peptide, a central Pro/Hyp-rich AGP domain that is interrupted by a short basic lysine-rich region near its C-terminus, and a C-terminal glycosylphosphatidylinositol (GPI) anchor addition sequence. Several *Arabidopsis* mutants were examined in order to elucidate functions of these AGPs. The *Arabidopsis rat1* (resistant to *Agrobacterium* transformation) mutant with a T-DNA insertion in the promoter region of *AtAGP17* exhibits reduced binding of *Agrobacterium*, resulting in resistance to *Agrobacterium* root transformation (Gaspar et al. 2004). An *AtAGP18* RNAi mutant demonstrates defective ovule development as functional megaspores fail to enlarge and divide (Acosta-Garcia and Vielle-Calzada 2004). The null T-DNA insertion mutant of *AtAGP19* displayed a pleiotropic phenotype including smaller and lighter green leaves, shorter stems, slower growth, fewer lateral roots, less seeds, and abnormal cell numbers and cell sizes in several organs (Yang et al. 2007).

Previous work in our laboratory focused on LeAGP-1, a lysine-rich AGP in tomato. To examine the subcellular localization of LeAGP-1, a GFP–LeAGP-1 fusion protein was expressed in tobacco (*Nicotiana tabacum* BY-2) cells and the fusion protein was found to be localized to the plasma membrane and in Hechtian threads (Sun et al. 2004b). Overexpression of LeAGP-1 in tomato plants caused an overbranching phenotype. These plants were short, had multiple branches and produced less seeds than the control plants. As cytokinin-overexpressing plants also have a similar bushy phenotype (Li et al. 1992), and cytokinins and other plant growth hormones (auxins and ABA) can regulate LeAGP-1 mRNA expression, LeAGP-1 probably functions in plant growth and development via cytokinin/auxin signaling (Sun et al. 2004a).

Here, we report on the use of an overexpression approach to explore the functions of three homologous AGP genes, namely *AtAGP17*, *AtAGP18* and *AtAGP19*. A series of GFP fusion protein constructs *35S-ss-GFP-AtAGP17/18/19* (*35S*, 35S cauliflower mosaic virus promoter (CaMV 35S promoter); *ss*, LeAGP-1 signal peptide sequence; *GFP*, green fluorescent protein) were delivered into *Arabidopsis* plants to study the subcellular localization of these AGPs. Another series of constructs without GFP (*35S-AtAGP17/18/19*) were constructed and overexpressed in *Arabidopsis* plants to observe phenotypes.

Materials and methods

Plant materials and growth conditions

Arabidopsis ecotype Columbia was used in this study. Plants were grown in soil in a growth room under long-day conditions (16 h day/8 h night cycle) for 2 weeks to harvest seedlings, or for 5 weeks to harvest flowers, stems and rosette leaves. Plants grown on Murashige and Skoog (MS) vertical plates with 1% sucrose for 2 weeks were used to harvest roots. These materials were used for organ-specific expression of AtAGP17.

RNA extraction and RT-PCR

Total RNA was extracted with the RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) and used for RT-PCR with the OneStep RT-PCR Kit (Qiagen, Valencia, CA, USA). The primers used for organ-specific AtAGP17 expression were 5′-ATA AGC CTA AAC CCA CAT CTC CCG-3′ and 5′-CAT GAG ACA AAT GGG AGA GGA TCA-3′. The primers for actin as an internal control were 5′-GTG CTC GAC TCT GGA GAT GGT GTG-3′ and 5′-CGG CGA TTC CAG GGA ACA TTG TGG-3′.

Overexpression constructs

A schematic representation of all the overexpression constructs and their related information are shown in Fig. S1.

For construction of *35S-ss-GFP*, the *pUC-ss-GFP* construct was digested with *Bam*HI and *Sac*I, and the *ss-GFP* fragment was subcloned into binary vector pBI121 between the *Bam*HI site and the *Sac*I site to replace the GUS gene under the control of CaMV 35S promoter (Fig. S2a). The *35S-ss-GFP-AtAGP17/18/19* chimeric gene constructs were produced as described previously (Sun et al. 2005; Yang 2006).

For construction of the vector control which contains only a hygromycin resistant gene, the pMDC45 binary vector was digested with *Pst*I and then self-ligated after removal of the small fragment containing the non-essential DNA sequences between the two *Pst*I sites (Fig. S2b). To create the *35S-AtAGP17/18/19* constructs (i.e., 17NG, 18NG, 19NG), the *AtAGP17/18/19* coding sequence was amplified from genomic DNA and inserted between the *Kpn*I site and the *Pac*I site on binary vector pMDC45 (Fig. S2c). Primer pairs used for amplifying the *AtAGP17/18/19* coding sequence were 5′-GGG GTA CCC CTC TCA ACT AAT TAC AAA TTA TGA CTC G-3′ and 5′-CCT TAA TTA AGG TTA TTA GAA GGC TAG AAC AAG TAG AG-3′ for the *AtAGP17* coding sequence; 5′-GGG GTA CCC CTC CAA ATT TTA ACA AAA TTA TGG ATC-3′ and 5′-CCT TAA TTA AGG TTA GAA TGC CAT AAC

GAG AAC G-3′ for the *AtAGP18* coding sequence; and 5′-GGG GTA CCT AGC TTC CTC CAC AAC ACA ATG-3′ and 5′-CCT TAA TTA AGG TTA GGC TGT CAT AGC AAG TAG-3′ for the *AtAGP19* coding sequence. The AtAGP17/18/19 DNA sequence in the *35S-AtAGP17/18/19* constructs were sequenced in both directions at the Ohio University Genomics Facility (Fig. S3).

Plant transformation and screening of transgenic plants

The constructs were transformed into *Agrobacterium* strain LBA4404 by electroporation using the GENE PULSER II system (Bio-Rad, Hercules, CA, USA) and then transformed into *Arabidopsis* plants by the floral dip method (Clough and Bent 1998). Transformants harboring the *35S-ss-GFP* or *35S-ss-GFP-AtAGP17/18/19* construct were selected using kanamycin. Transformants harboring the vector control or *35S-AtAGP17/18/19* construct were selected using hygromycin.

Genetic analyses of transgenic plants

Leaves from transgenic plants were used to extract DNA with the Extract-N-Amp Plant PCR Kit (Sigma-Aldrich, St. Louis, MO, USA). Alternatively, the CTAB method was used to extract genomic DNA (Weigel and Glazebrook 2002). PCR was performed to verify the presence of the construct. The primers specific to the hygromycin resistance gene were 5′-GGT TTC CAC TAT CGG CGA GTA CTT CTA C-3′ and 5′-AGA TCG TTA TGT TTA TCG GCA CTT TGC ATC-3′, or 5′-GGC GTC GGT TTC CAC TAT CG-3′ and 5′-TCA TTG ACT GGA GCG AGG CG-3′.

Total RNA was extracted from transgenic plant leaves and used for RT-PCR. For 17NG and 19NG plants, 35-day-old young leaves were used; for 18NG plants, 50-day-old cauline leaves were used. Primers for mRNA expression levels of *AtAGP17/18/19* were *AtAGP17*, 5′-ATA AGC CTA AAC CCA CAT CTC CCG-3′ and 5′-CAG CTC CCA CCA TTT GTA TCA-3′; *AtAGP18*, 5′-GCT CCG GCG AAA ACT CCA ACT G-3′ and 5′-AAC CGC TCC CAC CGC TAC ATT C-3′; *AtAGP19*, 5′-CTG CTC TCA TCT CTT CCT TTA GTG-3′ and 5′-ATT GAG CCA CAT TAC TGC TCT TCC-3′.

Western blotting

Total protein was extracted with protein extraction buffer (50 mM Tris–HCl pH 7, 10 mM potassium chloride, 1 mM EDTA, 0.1 mM magnesium chloride, 8% sucrose, 1 mM phenylmethanesulphonyl fluoride) and centrifuged at 10,000×*g* for 10 min at 4°C. The supernatant was collected and quantified by the Lowry assay using the DC Protein Assay (Bio-Rad, Hercules, CA, USA). Protein samples were separated on a 10% SDS-PAGE gel using a Mini-PROTEAN 3 Electrophoresis Cell (Bio-Rad, Hercules, CA, USA) and transferred to a PVDF membrane (Bio-Rad, Hercules, CA, USA) at 100 V for 1 h in a Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad, Hercules, CA, USA).

For Western blotting, the PVDF membrane was blocked with 5% milk in TBST (0.05% Tween 20, 1XTBS) for 1 h and incubated with the GFP antibody (Clontech, Mountain View, CA, USA) diluted 1:1,000 for 1 h. It was then incubated with alkaline phosphatase-conjugated secondary antibody (Sigma-Aldrich, St. Louis, MO, USA) diluted 1:500 for 1 h and color was developed in color substrate solution (0.5 mM NBT, 0.5 mM BCIP, 0.1 M Tris–HCl, 0.1 M NaCl, pH 9.5).

Fluorescence microscopy and confocal laser scanning microscopy

Different plant organs were observed under a Nikon fluorescence microscope or a Zeiss confocal laser scanning microscope LSM510 (Zeiss, Germany) with an excitation laser at 488 nm and a 510–550 nm emission filter.

Plasmolysis

Arabidopsis leaves were incubated in 4% NaCl for 10–15 min and were degassed in a vacuum desiccator for 2 min to facilitate infiltration and induce plasmolysis.

AtAGP18 expression of ABA treated *Arabidopsis* seedlings

Arabidopsis wild-type seeds were sterilized and sown on half strength MS plates containing 0.5% sucrose. The plates were kept in the dark at 4°C to stratify the seeds for 2–3 days and then transferred to the tissue culture room at 22°C under long-day conditions. On days 6 and 12, the seedlings were transferred to MS liquid media containing 20 μM ABA or no ABA (mock) and allowed to culture on a shaker at 120 rpm for 2 days. The expression levels of *AtAGP18* in the seedlings were examined by total RNA extraction and RT-PCR. The primers were the same as those used for mRNA expression of *AtAGP18*.

Results

Organ-specific expression of *AtAGP17*

RT-PCR results showed *AtAGP17* was expressed in seedlings, flowers, stems and rosette leaves, and weakly expressed in roots (Fig. 1). These results are in agreement with previous Northern blot analyses from our lab and are also consistent with the microarray data from

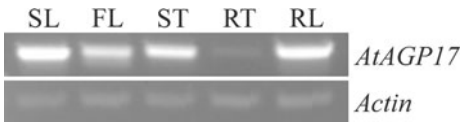

Fig. 1 RT-PCR results of *AtAGP17* expression. *SL* seedlings, *FL* flowers, *ST* stems, *RT* roots, *RL* rosette leaves

Genevestigator (Yang et al. 2007; http://www.genevestigator.ethz.ch). Surprisingly, Western blotting with AtAGP17 antiserum shows that AtAGP17 is highly expressed in roots and flowers, moderately expressed in stems and weakly expressed in siliques and leaves (Yang et al. 2010). This finding demonstrates that the mRNA expression level is not always consistent with the level of protein expression.

In order to examine *AtAGP17* expression at the tissue level, a $P_{AtAGP17}$*:GUS* (*GUS*, *β*-glucuronidase) construct was made and introduced into *Arabidopsis* wild-type plants. The promoter sequence used in this construct included 1.2 kb of DNA upstream of the start codon since a previous study showed 0.9 kb of this promoter region was insufficient to drive the expression of *AtAGP17* (data not shown). T1 transgenic $P_{AtAGP17}$*:GUS* lines were identified by PCR (Fig. S4); however, GUS staining was not detected in the T1 transgenic lines in various organs, while GUS staining of positive controls ($P_{AtAGP18}$*:GUS* and $P_{AtAGP19}$*:GUS* T2 plants) showed distinct staining patterns consistent with previous published data (Yang et al. 2007; Yang and Showalter 2007), indicating this 1.2 kb promoter region was not able to drive *AtAGP17* expression.

GFP-AtAGP17/18/19 were expressed in transgenic plants

The *35S-ss-GFP-AtAGP17/18/19* overexpression constructs were transferred into *Arabidopsis* wild-type plants by floral dip transformation. For convenience, transgenic plants were named 17 over, 18 over and 19 over, respectively. RT-PCR was performed to examine *AtAGP17* mRNA expression levels in the transgenic plants (Fig. S5a). *AtAGP17* mRNAs in the transgenic plants were from both the endogenous gene and the *GFP-AtAGP17* transgene. As a result, the RT-PCR product of *AtAGP17* mRNA from the transformants was considerably more than that from wild type. To verify the presence of the GFP-AtAGP17 fusion protein, Western blotting was carried out using GFP antibody. Protein samples were boiled for 3 min prior to loading to release the GFP from the GFP–AtAGP17 fusion protein since a previous study showed heating releases the GFP from the fusion protein (Sun et al. 2004b). As Fig. S5b shows, GFP (about 28 kDa) was present in 17 over, but was absent in WT.

Green fluorescence was also observed in different transgenic lines, indicating expression of the GFP-AtAGP17/18/19 fusion proteins. The GFP-AtAGP17/18/19 fusion proteins were localized to the cell surface in different plant organs, including root, leaf, stem and flower (Figs. 2, S6).

To examine the subcellular localization of some of these GFP fusion proteins, leaf trichome cells of 17 over and 18 over were plasmolyzed and enhanced GFP fluorescence was observed at the plasma membrane; however, no Hechtian strands were observed (Fig. 3).

AtAGP17/19 overexpressors showed no phenotype

To overexpress AtAGP17/18/19 without the GFP tag, the *35S-AtAGP17/18/19* constructs as well as a vector control were transformed into *Arabidopsis* wild-type plants. For convenience, the transformants were named 17NG (AtAGP17 overexpressor, no GFP tag), 18NG, 19NG and VC (vector control), respectively. Ten 17NG lines and 17 19NG lines were identified by PCR and their RNA expression levels were determined (Figs. S7, S8 and data not shown). Phenotypes of these lines, however, were not different from wild-type plants with respect to plant height, branch number, and reproduction. There was only one 19NG line which was smaller and shorter compared with the wild-type plants. This might be due to the disruption of some important genes by random insertion of the transgene.

AtAGP18 overexpressors had several phenotypes

Thirty-two 18NG lines were identified to contain the *35S-AtAGP18* construct (Fig. 4a and data not shown). Among these lines, 18 lines (slightly more than half) displayed several phenotypes distinct from wild-type and vector control (VC) plants (Fig. 4c). Furthermore, RNA was extracted from 50-day-old cauline leaves of individual plants and RT-PCR was performed to monitor *AtAGP18* expression. The 18NG lines had high *AtAGP18* expression, while the VC lines had low *AtAGP18* expression as did wild-type plants (Fig. 4b). The various phenotypes of 18NG lines are discussed below.

The differences between 18NG lines and the wild-type plants were not dramatic during the first 3 weeks after germination. During the 4th week and later, when the wild-type plants developed long inflorescence stems, 18NG plants developed multiple short branches from the rosette. The length of these stems was usually less than one-fifth of the wild-type stem, and the branching number was usually more than twice that of the wild type, resulting in multiple flower buds crowded near the rosette. Moreover, the rosette of 18NG plants was smaller than that of the wild type, and

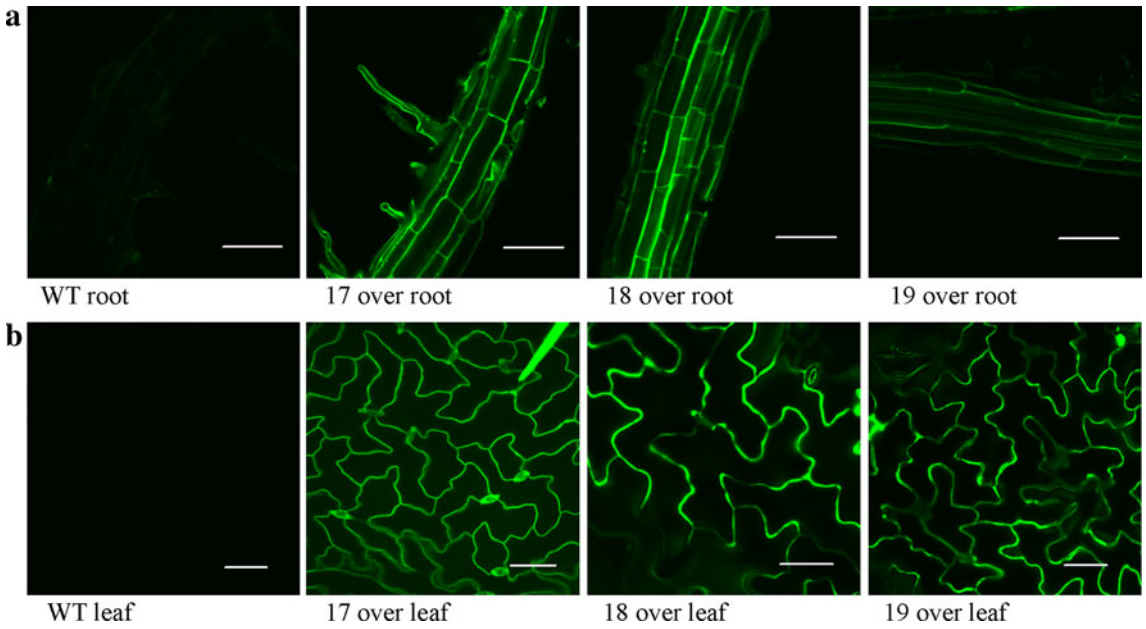

Fig. 2 GFP-AtAGP17/18/19 was expressed in transgenic plants and localized on the plant cell surface. **a** Roots of WT, 17 over, 18 over and 19 over plants. **b** Leaves of WT, 17 over, 18 over and 19 over plants. *Bars* 50 μm

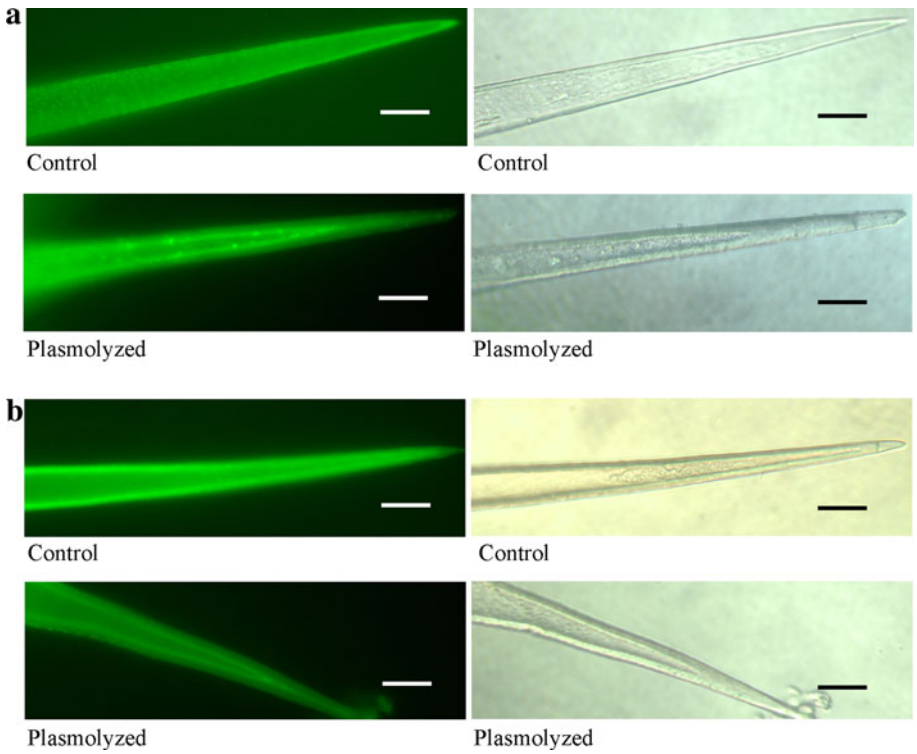

Fig. 3 AtAGP17/18 was localized at the plasma membrane of leaf trichomes. **a** Fluorescent image (*left*) and bright field image (*right*) of 17 over leaf trichome before (control) and after plasmolysis. **b** Fluorescent image (*left*) and bright field image (*right*) of 18 over leaf trichome before (control) and after plasmolysis. *Bars* 20 μm

some rosette leaves showed an epinastic phenotype (Fig. 4c, d).

The reproductive system in 18NG plants was also affected. Although 18NG plants had the same flowering time as wild-type plants, they usually produced smaller and sterile siliques (Fig. 4d). As a result, most of the 18NG lines were sterile, and even the partially fertile lines still had less seeds than the wild type. When flowers were dissected, some flowers from 18NG plants were found to have short stamens that presumably could not function normally (Fig. 4d).

The 18NG lines also had shorter primary roots and less lateral roots than the wild type and VC lines (Fig. 4d). These plants without the normal root system might have difficulty

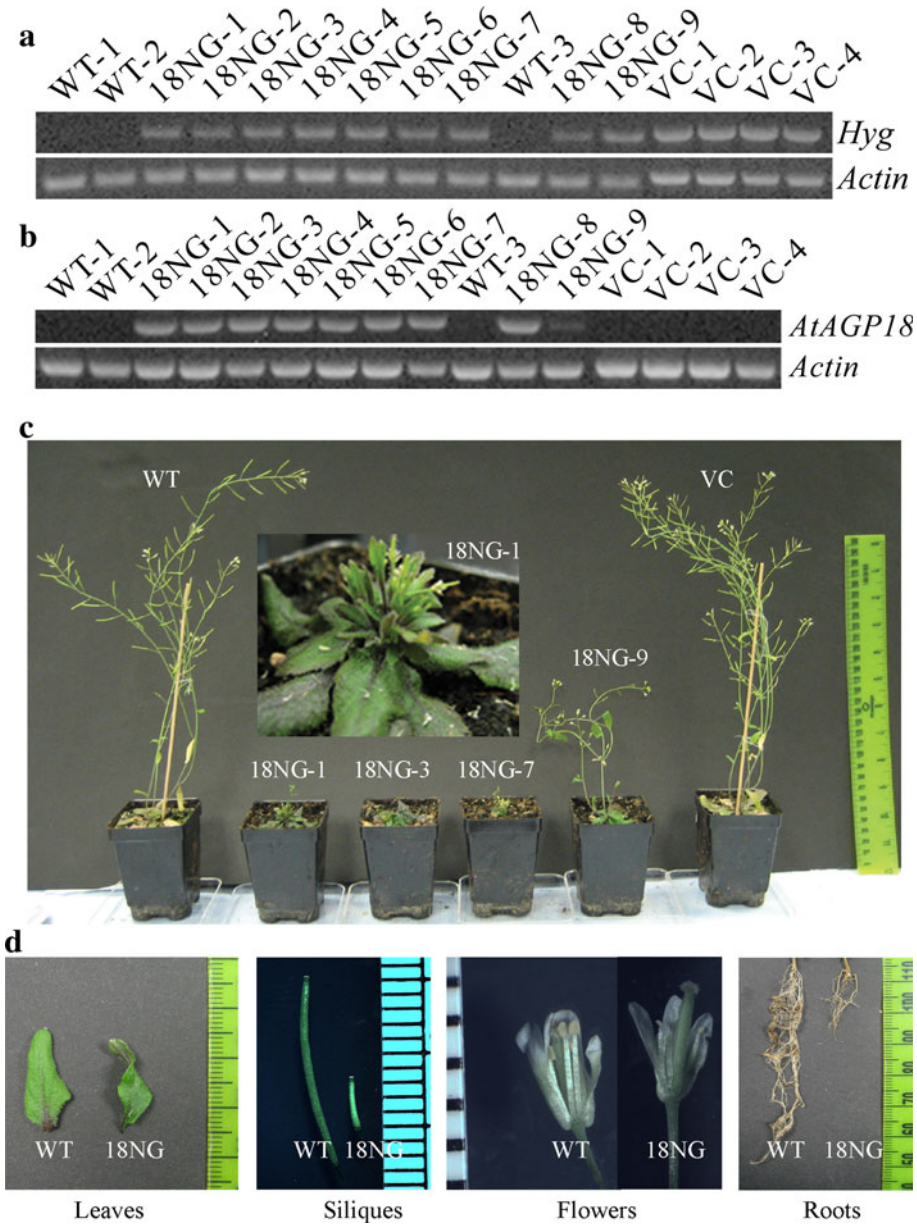

Fig. 4 Genetic analyses and phenotypes of AtAGP18 overexpression plants (18NG). **a** PCR results with hygromycin resistance gene primers of WT, 18NG and VC plants. **b** RT-PCR results showing the expression levels of AtAGP18 mRNA in 50-day-old cauline leaves in WT, 18NG and VC plants. **c** Forty-six-day-old WT, 18NG and VC plants. The *inset* shows a magnified picture of one of the 18NG plants. **d** Phenotypes of 18NG plants

obtaining water and nutrients from the soil, and as a result, some lines died 4–6 weeks after germination.

Abscisic Acid (ABA) down-regulated *AtAGP18* expression

Microarray data of hormone-treated plants from the TAIR website (http://www.arabidopsis.org) showed that ABA down-regulated *AtAGP18* expression (Fig. 5a). In order to test the validity of this microarray data, an RT-PCR experiment was designed. Specifically, 6- and 12-day-old seedlings were treated with 20 μM ABA before isolating RNA for RT-PCR to monitor *AtAGP18* expression. It was determined that this ABA treatment resulted in yellowing of the seedlings and a marked reduction in *AtAGP18* mRNA levels, consistent with the microarray data (Fig. 5b, c).

Discussion

Subcellular localization of AtAGP17/18/19

The lysine-rich AGPs including AtAGP17/18/19 in *Arabidopsis* and LeAGP-1 in tomato are a subfamily of classical AGPs, with AGP 17 and 18 as well as LeAGP-1 being predicted to have a GPI anchor (Schultz et al. 2002). LeAGP-1 was shown to be localized on the plasma membrane via a GPI anchor (Sun et al. 2004b). GFP–AtAGP17 and GFP–AtAGP18 were also expressed in tobacco cell

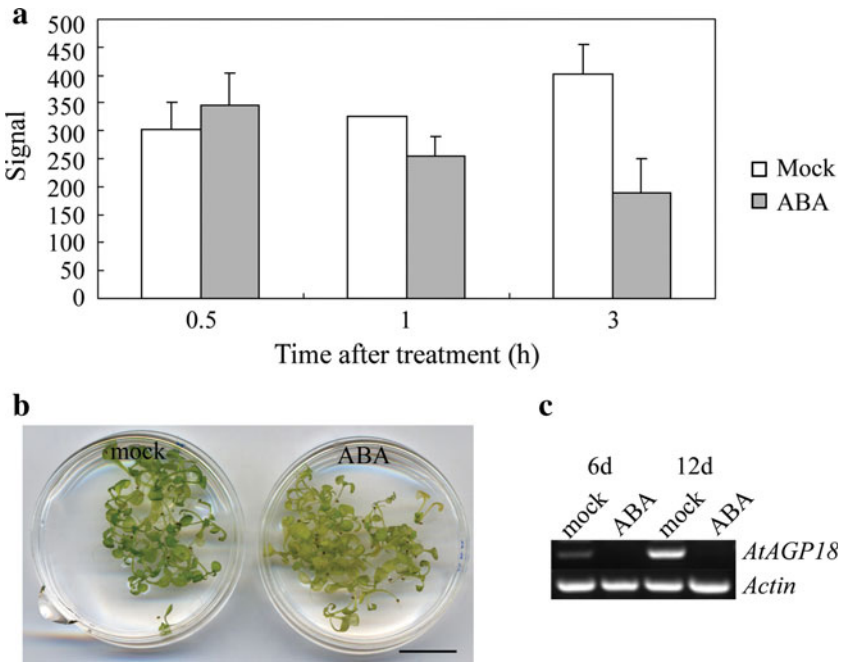

Fig. 5 *AtAGP18* expression was down-regulated by ABA treatment. **a** Response of *AtAGP18* expression to ABA treatment from the TAIR database. Seven-day-old seedlings were treated with 10 μM ABA for 30 min, 1 h and 3 h. *Y* axis is the signal strength from the microarray data. **b** Twelve-day-old seedlings treated without (mock) or with 20 μM ABA for 2 days. *Bar* 1 cm. Seedlings treated with 20 μM ABA became yellow. **c** RT-PCR results of *AtAGP18* expression following treatment of 6- and 12-day-old seedlings without (mock) or with 20 μM ABA for 2 days. The *left two lanes* were 6-day-old seedlings; the *right two lanes* were 12-day-old seedlings

cultures and GFP fluorescence was observed on the plasma membrane and in Hechtian strands (connections between the plasma membrane and the cell wall) after plasmolysis (Sun et al. 2005; Yang and Showalter 2007). In this study, leaf trichome cells from transgenic *Arabidopsis* plants expressing GFP–AtAGP17/18 were plasmolyzed with 4% NaCl. Although uniformly distributed fluorescence was still present in the space between the plasma membrane and the cell wall, enhanced fluorescence was observed at the plasma membrane, indicating the presence of the fusion protein at the plasma membrane. This is consistent with previous GFP work in tobacco cell cultures as well as biochemical studies of other GPI-anchored AGPs (Youl et al. 1998; Sherrier et al. 1999; Svetek et al. 1999). The localization of these lysine-rich AGPs at the plasma membrane, particularly here *in planta*, in contrast to previous plant cell culture work, provides some insight into exploring their possible functions. For example, these GPI-anchored AGPs may be associated with lipid rafts and involved with cell signaling (Borner et al. 2005; Johnson and Ingram 2005).

AtAGP18 and overexpression phenotypes

Transgenic lines overexpressing GFP–LeAGP-1 were short and bushy, had less fruit and smaller seeds compared with wild-type and GFP control plants (Sun et al. 2004a). Here, a similar method was used to overexpress GFP-AtAGP17/18/19 in *Arabidopsis*. At the same time, GFP was also overexpressed in *Arabidopsis* as a control. Transgenic plants overexpressing GFP–AtAGP17 (15 independent lines) or GFP–AtAGP19 (five independent lines) were not significantly different from wild-type plants, while three independent lines overexpressing GFP–AtAGP18 displayed phenotypes similar to the GFP–LeAGP-1 overexpressors in tomato.

Although transgenic plants overexpressing GFP–AtAGP18 suggested potential functions in vegetative growth and reproduction, there were still some concerns. First, the number of independent transgenic lines of GFP–AtAGP18 was limited. Second and most important, although the GFP control plants showed no phenotypes, the possibility that GFP in the fusion protein affected the plant phenotypes could not be excluded. To exclude this potential effect by GFP, AtAGP17/18/19 lacking a GFP tag were overexpressed in *Arabidopsis*. Multiple lines of 18NG plants had several phenotypes different from the wild type, with respect to branch numbers, stem length, rosette size, root length and seed numbers. The phenotypes of the 18NG plants were identical to that of the GFP–AtAGP18 overexpressors, indicating that overexpression of AtAGP18 alone is responsible for the observed phenotypes.

RT-PCR of *AtAGP18* with 15 18NG plants which displayed abnormal phenotypes showed high levels of *AtAGP18* mRNA. RT-PCR was also performed on some of these transgenic plants that had wild-type phenotypes. Some of them had expression levels similar to the wild type, while others had high expression levels. Thus, the

phenotypes were not completely correlated with the expression levels. One explanation for this is that the AtAGP18 expression was detected at the RNA level, but not at the protein level, and high mRNA expression does not necessarily guarantee high protein expression.

Among the three overexpressor lines (AtAGP17/18/19), only AtAGP18 had phenotypes distinguishing it from the wild type. This is probably because the endogenous expression level of AtAGP18 is generally the highest among these three genes in wild-type plants based on Northern blot results from our lab (Sun et al. 2005) as well as publicly available microarray data (Yang 2006). For example, in seedlings, the expression intensity of *AtAGP18* is about three times that of *AtAGP17* and is more than five times that of *AtAGP19*. In roots, flowers, stems and leaves, the difference in expression intensity for these three genes are similar to, or even greater than that of seedlings (Yang 2006). In addition, Northern blot analysis shows the expression pattern of these three AGPs is organ-specific. *AtAGP17* is expressed moderately in leaves, stems and flowers, but not in roots. In contrast, the expression pattern of *AtAGP18* shows a high level in flowers and moderate level in roots, stems and young leaves, which is nearly identical to the expression pattern of *LeAGP-1* (Li and Showalter 1996; Sun et al. 2005). The *AtAGP19* expression pattern is similar to *AtAGP18* but much weaker (Sun et al. 2005). Moreover, comparing the protein sequence of LeAGP-1 to AtAGP17/18/19 reveals AtAGP18 shares the highest sequence identity/similarity (45.2%/62.3%) with LeAGP-1 compared with AtAGP17 (40.3%/57.2%) and AtAGP19 (36.1%/45.7%) (Yang and Showalter 2007). Considering all the above information on these three AGPs, it makes sense that transgenic plants overexpressing AtAGP18 had phenotypes similar to transgenic plants overexpressing LeAGP-1.

It is also interesting to contrast this overexpression of AtAGP18 with the production of RNAi plants with reduced AtAGP18 mRNA levels. The RNAi plants showed only reduced seed set but no other developmental defects (Acosta-Garcia and Vielle-Calzada 2004), while the AtAGP18 overexpressing plants showed a much more severe phenotype which not only affected seed set, but also many other aspects of development. This more dramatic phenotype may be attributed to the ectopic distribution and overproduction of AtAGP18 under the control of the CaMV 35S promoter.

A number of genes co-expressed with AtAGP18 were obtained via the *Arabidopsis thaliana* Co-Response Database (Yang and Showalter 2007). Interestingly, these genes include some receptors on the plasma membrane such as receptor-like kinases (RLKs) which may be related to hormone signaling. AGPs may be associated with RLKs since immunofluorescence labeling showed colocalization of AGP epitopes and some RLKs (Gens et al. 2000). Thus, AtAGP18 may be involved in a signal transduction cascade by binding some plant growth factors and bringing these growth factors to the transmembrane receptor to facilitate their interaction (Showalter 2001; Sun et al. 2004a). While further evidence is required to support this hypothesis, it is clear that AtAGP18 has functional roles in vegetative growth and reproduction.

Acknowledgments We are particularly grateful to Dr. Li Tan in the Department of Chemistry and Biochemistry at Ohio University for providing the *pUC-ss-GFP* construct. This work was supported by the National Science Foundation [IBN-0110413].

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