

INVITED REVIEW: PART OF A SPECIAL ISSUE ON ROOT BIOLOGY
**Arabinogalactan proteins in root and pollen-tube cells: distribution
and functional aspects**

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- **Background** Arabinogalactan proteins (AGPs) are complex proteoglycans of the cell wall found in the entire plant kingdom and in almost all plant organs. AGPs encompass a large group of heavily glycosylated cell-wall proteins which share common features, including the presence of glycan chains especially enriched in arabinose and galactose and a protein backbone particularly rich in hydroxyproline residues. However, AGPs also exhibit strong heterogeneities among their members in various plant species. AGP ubiquity in plants suggests these proteoglycans are fundamental players for plant survival and development.
- **Scope** In this review, we first present an overview of current knowledge and specific features of AGPs. A section devoted to major tools used to study AGPs is also presented. We then discuss the distribution of AGPs as well as various aspects of their functional properties in root tissues and pollen tubes. This review also suggests novel directions of research on the role of AGPs in the biology of roots and pollen tubes.

Key words: Arabinogalactan proteins, cell wall, root, pollen tube, growth, development, plant–microbe interactions, sexual reproduction, plant proteoglycans, *Arabidopsis*, *Nicotiana*.

GENERAL INTRODUCTION

Arabinogalactan proteins (AGPs) are a family of non-enzymatic cell surface hydroxyproline-rich glycoproteins (HRGPs) (see Table 1 for a list of abbreviations used in the text). These proteoglycans, analogous to animal proteoglycans, are found throughout the entire plant kingdom from bryophytes (e.g. *Physcomitrella patens*; Lee *et al.*, 2005) to angiosperms (e.g. *Arabidopsis thaliana*; Schultz *et al.*, 2000). AGPs have been implicated in a large number of biological functions throughout the plant life cycle and many are specifically associated with development and reproduction. AGPs encompass a large group of cell-wall proteins which share common features, including their ability to bind to β -D-glucosyl Yariv reagent (Yariv *et al.*, 1967; see Fig. 1A), their typical arabinogalactosylated glycomodules, and many other features associated with their protein and nucleic sequences such as the presence of numerous hydroxyproline (HyP)-based sites of O-glycosylation, the possibility of being anchored to the plasma membrane, or the existence of many functional domains (often putative) that confer several possible biological functions (for reviews, see Seifert and Roberts, 2007; Ellis *et al.*, 2010).

The protein backbone of AGPs is synthesized by members of a large multigene family consisting of 52–82 members in *Arabidopsis thaliana* (Schultz *et al.*, 2002; Johnson *et al.*, 2003; Showalter *et al.*, 2010) and 69 members in rice (*Oryza sativa*) (Ma and Zhao, 2010). AGPs are characterized by the extensive O-glycosylation of the protein backbone which takes place post-translationally in the Golgi apparatus (see

also Fig. 2). Typically the carbohydrate moiety accounts for >90 % w/w of the mass of the glycoprotein and consists predominantly of arabinose and galactose residues, although other ‘minor’ sugars including rhamnose, fucose, glucuronic acid and xylose are also present (Nothnagel, 1997; Showalter, 2001). The glycan composition of AGPs can vary greatly between species, between organs within the same species and may even be developmentally regulated within the same organ in different cell-types (Tsumuraya *et al.*, 1988; Pennell *et al.*, 1991). The two glycans most commonly found in AGPs are (1) a short oligo-arabinoside chain of three to four residues, and (2) a larger β -1,3-linked galactan backbone with 1,6-linked side chains containing galactose, arabinose and, often, rhamnose and glucuronic acid (Kieliszewski and Shpak, 2001; Tan *et al.*, 2010; Tryfona *et al.*, 2010). AGP glycomodules have been proposed to adopt different spatial configurations that modulate AGP functionality (Fincher *et al.*, 1983; Qi *et al.*, 1991; Showalter, 2001). Although AGPs are known as O-glycosylated proteins (Showalter, 2001; Seifert and Roberts, 2007) discrete N-glycosylation sites have also been reported to be present in the protein backbone (Du *et al.*, 1996). The O-glycosylation of AGPs occurs predominantly on HyP residues, and less often on serine and threonine residues (Showalter, 2001). Proline hydroxylation is performed by a multigene family of enzymes, proline-4-hydroxylases (P4-Hs) (Vlad *et al.*, 2007) that are believed to act within the endoplasmic reticulum. Using a synthetic gene strategy and engineering, a variety of consensus motifs to study glycosylation patterns in living plant cells (Kieliszewski and

TABLE 1. List of abbreviations

AG	Arabinogalactan
AGPs	Arabinogalactan proteins
AGPE	Arabinogalactan protein-extensins
AM	Arbuscular mycorrhizas
ENOD	Early nodulin
FLA	Fasciclin-like AGP
GPI	Glycosylphosphatidylinositol
GTs	Glycosyltransferases
HRGPs	Hydroxyproline-rich glycoproteins
Hyp	Hydroxyproline
mAb	Monoclonal antibodies
nsLTP	Non-specific lipid transfer protein
P4-Hs	Proline-4-hydroxylases
<i>reb1-1</i>	root epidermal bulger
PELPIII	Pistil-specific extensin-like protein III
TTS	Transmitting-tract-specific
XTH	Xyloglucan endotransglucosylase hydrolase

Shpak, 2001; Shpak *et al.*, 2001; Tan *et al.*, 2003, 2004; Estevez *et al.*, 2006; Xu *et al.*, 2008) it was shown that *O*-glycosylation leading to arabinosylation occurs preferentially on clustered Ser-(Hyp)4 contiguous sequences (contigs), whereas *O*-glycosylation leading to arabinogalactosylation occurs on Hyp-alanine, Hyp-serine, Hyp-threonine and Hyp-valine contigs. Based on the amino acid sequence and composition, AGPs were initially categorized into classical AGPs (consisting of a P/Hyp-rich domain heavily *O*-glycosylated, a hydrophobic C-terminal (C-ter) domain required for anchorage to the plasma membrane, and a signal peptide sequence) and non-classical AGPs (sometimes *N*-glycosylated and lacking the C-ter domain). However, this classification has subsequently been altered based on a better knowledge of plant genomes [Arabidopsis, The Arabidopsis Genome Initiative (2000); rice, Yu *et al.* (2005)], along with the development of several algorithms using a biased amino acid composition and the presence of signal peptides (Schultz *et al.*, 2002; Showalter *et al.*, 2010) or BLAST-based alignment (Borner *et al.*, 2002; Schultz *et al.*, 2002; Johnson *et al.*, 2003). Thus AGPs are now classified into classical AGPs (characterized by a signal peptide, a P/Hyp rich domain and C-ter domain), AG peptides (short classical AGPs), fasciclin-like AGPs (FLA), Lys-rich AGPs, non-specific lipid transfer protein (nsLTP)-like AGPs and early nodulin (ENOD)-like AGPs (Schultz *et al.*, 2000, 2002; Johnson *et al.*, 2003; Sun *et al.*, 2005; Yang *et al.*, 2005; Mashiguchi *et al.*, 2009; Ma and Zhao, 2010). The term chimeric AGPs was used to describe classical AGPs that harbour an additional protein domain such as FLAs, Lys-rich, nsLTP-like AGPs and plastocyanin-like AGPs. Some chimeric AGPs were found to lack the C-ter domain responsible for the glycosylphosphatidylinositol (GPI) anchorage as shown for FLAs (Johnson *et al.*, 2003). All the chimeric AGPs and several other GPI-anchored proteins were predicted (though mostly not experimentally proven) to contain arabinogalactan type II (AG-II) glycomodules (Borner *et al.*, 2002, 2003).

At the subcellular level, AGPs can be found in the cell wall, in the apoplast or anchored to the plasma membrane via a GPI anchor attached to the C-ter domain of the AGP backbone (Youl *et al.*, 1998; Svetek *et al.*, 1999). Cleavage of the GPI-anchor by phospholipases results on the release of the

glycoprotein into the cell wall (Schultz *et al.*, 1998; Borner *et al.*, 2002, 2003). Interestingly, some GPI-anchored AGPs were shown to be associated with specific microdomains on the plasma membrane, the so-called lipid rafts (Borner *et al.*, 2005; Grennan, 2007; Simon-Plas *et al.*, 2011). Finally, AGPs can also be found in secretions, including root exudates and the extracellular media of cultured cells.

AGPs occur ubiquitously in land plants and have been isolated from a wide range of plant organs and cell types, from the flowers to root caps and border cells, and from pollen tubes to root hairs. They have been implicated to be involved in a variety of functions (Seifert and Roberts, 2007) including cell proliferation (Serpe and Nothnagel, 1994), cell expansion (Willats and Knox, 1996), programmed cell death (Gao and Showalter, 1999), pollen-tube growth (Wu *et al.*, 1995; Coimbra *et al.*, 2010), xylem differentiation (Motosé *et al.*, 2004), somatic embryogenesis (van Hengel *et al.*, 2002), zygotic division and embryo development (Hu *et al.*, 2006; Qin and Zhao, 2006). Recent reviews have described the structure and biology of AGPs (Seifert and Roberts, 2007; Ellis *et al.*, 2010) highlighting the importance of these glycoproteins in plant survival and development. Here, we focus on the distribution and functions of AGPs in root tissues and pollen tubes.

PROBES AND TOOLS TO STUDY AGPS IN PLANTA

Several tools and probes have been developed and used to study AGP distribution and function in various plant species and organs. The most well-known and commonly used reagent is β -D-glucosyl Yariv that was generated 50 years ago (Yariv *et al.*, 1962, 1967). Yariv and co-workers discovered that a phenylglycoside containing β -D-glucosyl residues (active Yariv) was able to bind and precipitate AGPs whereas related phenylglycosides containing α -D-mannosyl or α -D-galactosyl units (non-active Yariv) were not (Fig. 1A). Since then, Yariv reagents (both active and non-active) are commonly used in most, if not all, plant laboratories working on AGPs worldwide. Active Yariv has been largely used to isolate and quantify AGPs from a number of species [pear, Chen *et al.* (1994); arabidopsis, Schultz *et al.* (2000); tobacco, Du *et al.* (1994)]. It has also been widely used to investigate the function of AGPs *in vivo* based on its ability to bind AGPs and to interfere with their dynamics within the cell wall and plasma membrane. For instance, it has been well documented that active Yariv inhibits expansion and morphogenesis of root and pollen-tube cells (Willats and Knox, 1996; Mollet *et al.*, 2002; Nguema-Ona *et al.*, 2007).

AGP-directed monoclonal antibodies (mAbs) (Table 2) are another class of valuable probes that have been pivotal for deciphering AGP localization and function (Puhlmann *et al.*, 1994; Knox, 1997). Most of the available mAbs reactive to AGPs are specific for epitopes associated with the carbohydrate moieties; although a few have specificity toward the protein backbone (Table 2). Similarly to active Yariv, some of the anti-AGP mAbs (e.g. JIM13) have been used to unravel the involvement of specific epitopes in controlling cell growth and morphogenesis in arabidopsis (van Hengel and Roberts, 2002; Nguema-Ona *et al.*, 2007). Furthermore a

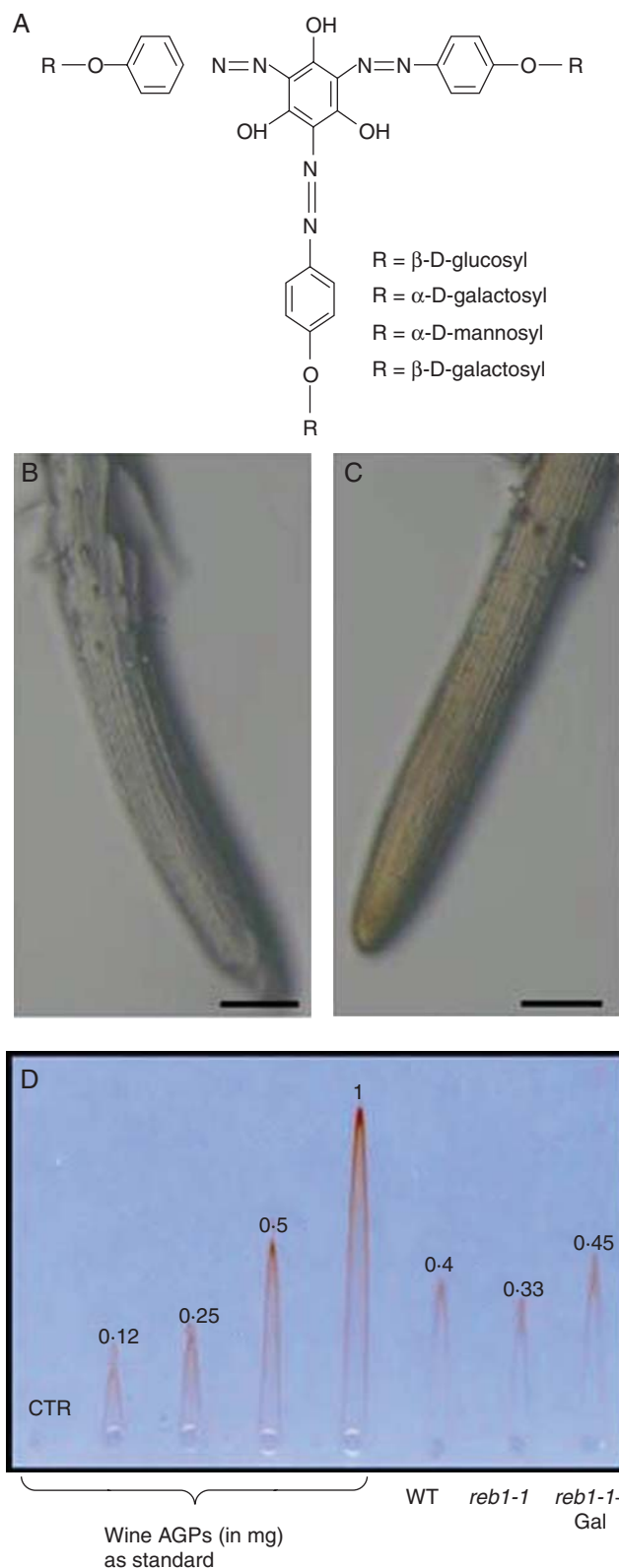


FIG. 1. The Yariv reagent. (A) chemical structures of different phenylglycosides including active Yariv ($\beta\text{-D-glucosyl}$ and $\beta\text{-D-galactosyl}$) and inactive Yariv ($\alpha\text{-D-galactosyl}$ and $\alpha\text{-D-mannosyl}$), commercially available at www.biosupplies.com.au. (B) Histochemical staining of arabidopsis root with inactive Yariv. (C) Histochemical staining of arabidopsis root with

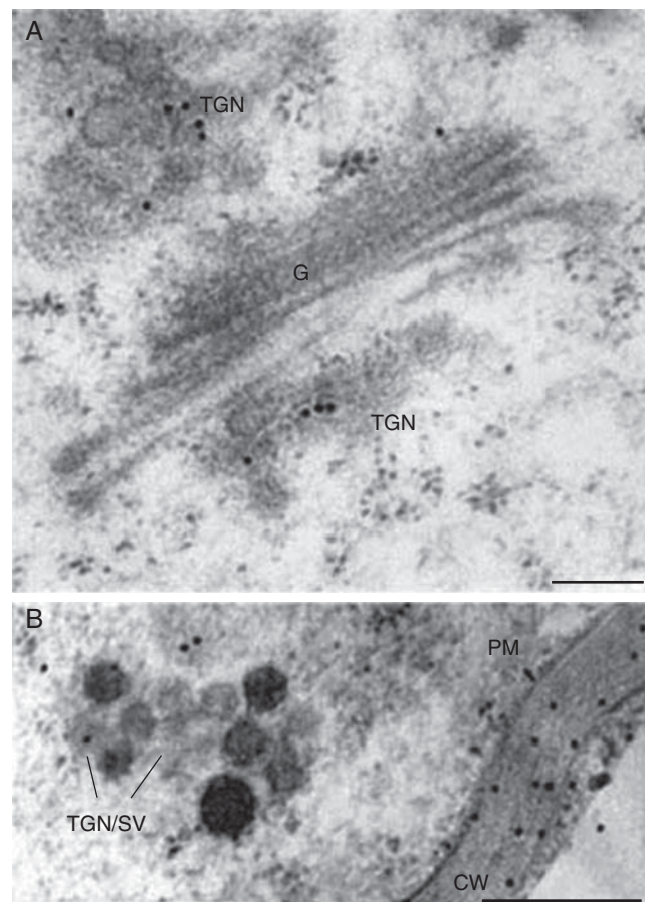


FIG. 2. Electron micrographs of arabidopsis root cells labelled with the anti-AGP antibody JIM14. Note the immuno-gold labelling of Golgi cisternae (G) and the trans-Golgi network (TGN) in (A), and secretory vesicles (SV), the plasma membrane (PM) and the cell wall (CW) in (B). AGPs are known to be assembled within the endomembrane system. Roots were prepared by the high-pressure freezing technique and the immunogold labelling was performed as described in Driouich *et al.* (1993). Scale bars = 0.2 μm .

large body of information regarding the spatial distribution of AGPs in plant tissues has been obtained employing mAbs in combination with a variety of imaging technologies (see below).

Other chemical agents including inhibitors of AGP biosynthesis are 3,4-dehydropoline (Cooper and Varner, 1983; Vicré *et al.*, 2005), ethyl 3,4 dihydroxy benzoate and α,α -dipyridyl (Velasquez *et al.*, 2011). These have been used to investigate AGP functions. It is noteworthy that these agents are not specific for AGPs but also interfere with the biosynthesis of other HRGPs including extensin. 3,4-Dehydropoline is known to interfere with the hydroxylation of proline residues and hence the production of Hyp residues leading to a reduction

active Yariv (note the reddish colour of the root). (D) Rocket electrophoresis of AGPs extracted from the roots of wild-type (WT) *Arabidopsis thaliana*, from the *reb1-1* mutant or from the *reb1-1* mutant grown in the presence of 10 mM galactose. The numbers at the top of rockets correspond to AGP quantities (in mg). CTR = 0 mg. Note that AGP content is recovered after addition of galactose (Gal) to *reb1-1* mutant (0.45 mg). Standard AGPs were extracted and purified from red wine. Scale bars in (B) and (C) = 200 μm .

TABLE 2. List of reagent and antibodies directed against AGP carbohydrate epitopes and proteic backbone

Probe	Antigen	Epitope recognized	References
mAb			
JIM4	AGP from carrot	β-D-GlcA-(1,3)-α-D-GalA-(1,2)-α-L-Rha	Yates <i>et al.</i> (1996)
JIM8	AG from sugar beet	Unknown	Pennell <i>et al.</i> (1991)
JIM13	AGP from carrot	β-D-GlcA-(1,3)-α-D-GalA-(1,2)-α-L-Rha	Yates and Knox (1994); Yates <i>et al.</i> (1996)
JIM14	AGP from carrot	Unknown	Yates and Knox (1994); Yates <i>et al.</i> (1996)
JIM15	AGP from carrot	Unknown	Yates and Knox (1994); Yates <i>et al.</i> (1996)
JIM16	AGP from carrot	Unknown	Yates and Knox (1994); Yates <i>et al.</i> (1996)
LM2	AGP from rice	β-Linked GlcA	Smallwood <i>et al.</i> (1994); Yates <i>et al.</i> (1996)
MAC204	AGP from pea	Unknown	Bradley <i>et al.</i> (1988); Pennell <i>et al.</i> (1989)
MAC207	AGP from pea	β-GlcA-(1,3)-α-GalA-(1,2)-Rha	Bradley <i>et al.</i> (1988); van den Bosch <i>et al.</i> (1989)
MAC265	AGP from pea	Unknown	van den Bosch <i>et al.</i> (1989)
PCBC3	AGP from <i>Nicotiana alata</i> style	Unknown	Fergusson <i>et al.</i> (1999)
CCRC-M7	Rhamnogalacturonan I	β-(1,6)-Gal chain carrying one or more Ara residues	Steffan <i>et al.</i> (1995)
PN 16-4B4	AG from <i>Nicotiana glutinosa</i>	Unknown	Norman <i>et al.</i> (1986)
MH4-3E5	AG from <i>Nicotiana tabacum</i>	Unknown	Hahn <i>et al.</i> (1987)
LM6	Sugar beet arabinan	α-(1,5)-L-linked arabinosyl heptasaccharide	Lee <i>et al.</i> (2005)
LM14	AGP from carrot	Arabinose- and galactose-enriched carbohydrate chains	Moller <i>et al.</i> (2008)
Antiserum			
Le-AGP1	Proteic backbone from tomato	Lysine-rich subdomain of Le-AGP1 proteic backbone	Gao <i>et al.</i> (1999)
Reagent			
Yariv reagent	AGP	n.a.	Yariv <i>et al.</i> (1967)

AG, Arabinogalactan; AGP, arabinogalactan protein; GalA, galacturonic acid; GlcA, glucuronic acid; Rha, rhamnose; mAb, monoclonal antibody; n.a., not applicable.

For more information see the Plant Cell Wall Monoclonal Antibody Database at <http://glycomics.ccruc.uga.edu/wall2/jsp/abIndex.jsp>

in O-glycosylation of AGPs among other HRGP (Cooper and Varner, 1983). Ethyl 3,4 dihydroxy benzoate binds to the active site of P4-Hs while α,α-dipyridyl chelates a co-factor required for P4-Hs activity (Velasquez *et al.*, 2011).

While chemical reagents and mAbs often recognize the carbohydrate moiety of AGPs, the cloning of a single AGP gene followed by its functional characterization, as well as the generation of mutants, has helped to assign functions to single AGP genes (Shi *et al.*, 2003; Seifert and Roberts, 2007; Coimbra *et al.*, 2009, 2010). In this context, genomic resources have proven highly valuable in the identification and cloning of AGP genes (Schultz *et al.*, 2002; Ma and Zhao, 2010; Showalter *et al.*, 2010). In addition, the development of publicly available microarray analyses, as well as bioinformatic tools to ‘mine’ such datasets, has enabled prediction of the expression and possible physiological roles of a given AGP in specific organs and tissues under specific experimental conditions. Using this approach (Schultz *et al.*, 2002; Johnson *et al.*, 2003; Showalter *et al.*, 2010) it has also been shown that AGPs are most frequently expressed in floral tissues and pollen tubes, as well as in root tissues. More interestingly, Showalter *et al.* (2010) have shown that different AGP genes are co-expressed (AGPs with other AGPs including FLAs, AG peptides and chimeric AGPs) and are also often concurrently expressed with other HRGPs such as extensin. AGPs may also be co-expressed with genes involved in their post-translational modification including P4-Hs, and wall peroxidases, as well as with certain glycosyltransferases (GTs) genes involved in the synthesis of other cell-wall components (e.g. homogalacturonans that are suggested to be co-secreted with some AGPs; Showalter *et al.*, 2010). Finally, fusion proteins such as *Lycopersicon esculentum* AGP1:GFP have also been generated and used to investigate localization and

function of AGPs (Zhao *et al.*, 2002; Sun *et al.*, 2004; Sardar *et al.*, 2006; Nguema-Ona *et al.*, 2007).

AGPS IN ROOTS: DISTRIBUTION AND FUNCTIONAL ASPECTS

Distribution of AGPs in roots: localization of AGPs in root tissues of arabidopsis and others species

Staining and localization of AGPs in plant tissues was initially made possible by the use of active Yariv as a histochemical probe (Fig. 1B, C). This led to the discovery of AGPs in taro mucilage (*Colocasia esculenta*) (Harris *et al.*, 1992), tomato roots (*Solanum lycopersicum*) (Pogson and Davies, 1995), styles of tobacco (*Nicotiana alata*) (Gane *et al.*, 1994) and *Brassica napus* microspores (Tang *et al.*, 2006). However, major progress in mapping AGP distribution in root tissues was made using the various AGP-directed mAbs and polyclonal antibodies generated over the years by several laboratories (Knox, 1997; Pattathil *et al.*, 2010; Hervé *et al.*, 2011). The vast majority of these antibodies are directed against carbohydrate epitopes (Table 2). An antiserum which recognizes the protein core of LeAGP1 has also been generated (Gao *et al.*, 1999).

Several anti-AGP mAbs raised against AGP fractions isolated from carrot cell cultures at different stages of development have been generated [MAC207, Pennell *et al.* (1989); JIM14-16, Knox *et al.* (1991); LM2, Smallwood *et al.* (1996)]. Some of these AGP-associated epitopes were shown to accompany differentiation of cambium cells during the secondary thickening in *Arabidopsis thaliana* root (Dolan and Roberts, 1995). Epitopes recognized by the JIM14 mAb were associated with sieve tubes of the phloem in the

secondary thickened roots, while the JIM13 mAb was associated with young differentiated xylem cells (Dolan and Roberts, 1995; Dolan *et al.*, 1995). JIM13 has also been shown to specifically localize to the root cap and border-like cells in seedlings of *Arabidopsis thaliana* (Fig. 2) (Vicré *et al.*, 2005). JIM4 is another AGP-directed mAb recognizing a plasma membrane-associated epitope which has also been used for root tissue labelling (Knox *et al.*, 1989, 1991). The epitope appears at a very early stage in the formation of the vascular pattern in carrot and has been proposed as a marker of cell identity in the developing pericycle cells of carrot (Knox *et al.*, 1991). In maize roots, the AGP epitopes recognized by the mAb LM2 have been localized to the growing tips of root hairs, while a β -(1,6)-galactan epitope associated with AGPs (recognized by a polyclonal antiserum Gal4; Kikuchi *et al.*, 1993) has been found over the entire root surface including root hairs (Samaj *et al.*, 1999). Together, these observations indicate that AGP-associated epitopes are developmentally regulated in plant roots and that modulation of AGP expression occurs during cell development and positioning of cells within the apex. Along with AGPs, other HRGPs including extensins and chimeric AGPs are distributed over plant cell surfaces of growing and differentiating tissues and are believed to play a role in plant cell morphogenesis (Knox, 1995).

Sequencing of plant genomes including *Arabidopsis thaliana* (The Arabidopsis Genome Initiative, 2000) and *Oryza sativa* (Yu *et al.*, 2005), along with the development of bioinformatics and molecular techniques, has allowed the mapping of AGP genes in the roots of arabidopsis based on their expression patterns. Using Genevestigator (Zimmermann *et al.*, 2004) (Fig. 3), 15–20 AGP genes were found to be expressed in roots under different environmental conditions. However, few of them have been subjected to in-depth analyses. Screening of arabidopsis mutants has also allowed identification of patterns of expression for specific genes. A number of genes reflecting the diversity of AGP types has been found to be

specifically expressed in root tissues including: the non-classical AGP *AtAGP30* (At2g33790; van Hengel and Roberts, 2003) expressed in the arabidopsis root elongating zone and *AtAGP31* (At1g28290; Liu and Mehdy, 2007); FLA genes, *SOS5* (AtFLA4; At3g46550; Shi *et al.*, 2003) and *AtFLA1* (At5g55730; Johnson *et al.*, 2011), also expressed in growing arabidopsis roots; nsLTP-like AGP family member *AtXYP2* (At2g13820; Kobayashi *et al.*, 2011) the closest homologue of *Zinnia elegans* *ZeXYP* previously shown to play a role in xylem differentiation (Motosé *et al.*, 2004) and a Lys-rich AGP gene, *AtAGP17*, expressed in arabidopsis roots (Gaspar *et al.*, 2004). The functions of these AGPs are discussed below.

Functional properties of AGPs in roots

Role in root growth and morphogenesis. A number of studies have shown that AGPs are important for root development. Willats and Knox (1996) showed that treatment of *Arabidopsis thaliana* seedlings with active Yariv causes a disruption in root growth and abnormal morphology. Both cell elongation and expansion were affected, leading to short and swollen roots. Swelling (e.g. radial expansion) was mostly associated with epidermal cells. Indeed, the epidermal cells more exposed to the reagent exhibited radial expansion. Interestingly, root cap and meristem did not appear to be affected by the treatment (Vicré *et al.*, 2005) suggesting that sensitivity to active Yariv varies between different tissues probably because of differences in reactivity of different AGPs to the reagent. Interestingly, the arabidopsis mutant *reb1-1* (root epidermal bulger) displayed a root morphology similar to that obtained by active Yariv treatment. The root of the *reb1-1* mutant was clearly shown to lack a subpopulation of AGPs normally present in wild-type root (Baskin *et al.*, 1992; Ding and Zhu, 1997) confirming that AGPs are required for the control of oriented cell expansion in elongating roots (Willats and Knox, 1996). Extending on this, Andème-Onzighi *et al.* (2002) have shown that the swelling

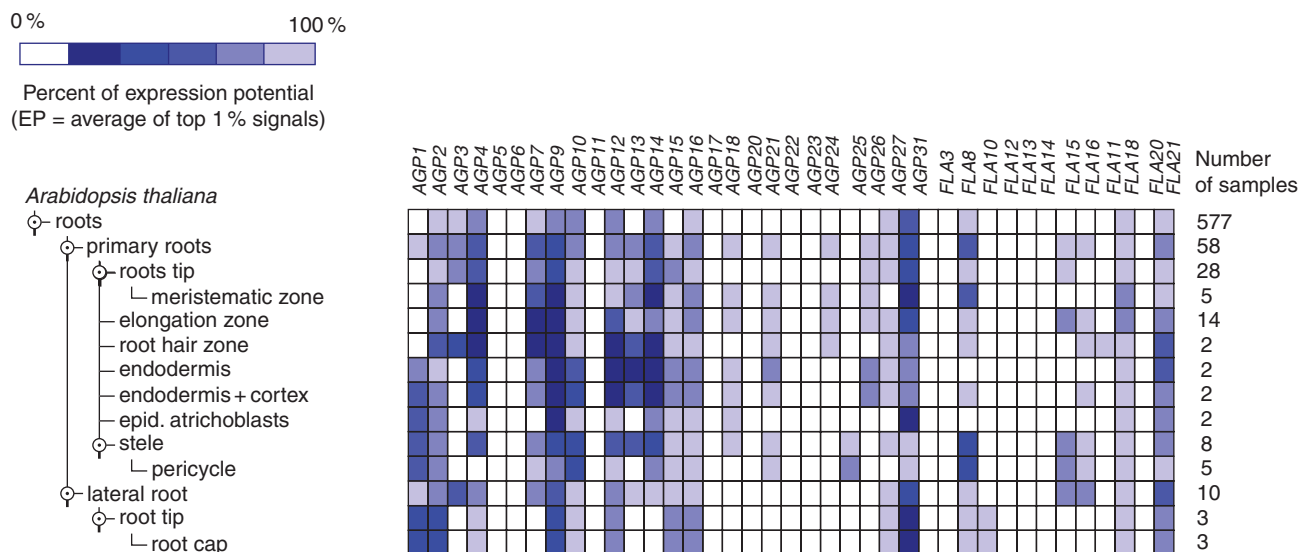


FIG. 3. Relative level of expression of AGP and FLA genes in different root tissues of *Arabidopsis thaliana*. The figure was generated using Genevestigator (Zimmermann *et al.*, 2004).

phenotype of the mutant is restricted to trichoblasts and that certain AGP epitopes (recognized by the mAbs LM2 and JIM14) are absent from the swollen cells.

As postulated by Ding and Zhu (1997), the *REB1-1* gene was later shown to encode for a UDP D-glucose 4-epimerase that converts D-glucose into D-galactose (Seifert *et al.*, 2002), providing galactose to AGPs and xyloglucans (Nguema-Ona *et al.*, 2006), and supporting a link between the normal carbohydrate structure of AGPs and the control of cell expansion. Supplementation of the growth media of the *reb1-1* mutant with 10 mM galactose restored the wild-type phenotype and AGP content in the root of the mutant (Fig. 1D). The importance of an unaltered carbohydrate moiety of AGPs in controlling root cell elongation was also confirmed for another arabidopsis mutant, *mur1* (*muris 1*), deficient in a mannose 4,6 dehydratase, involved in the biosynthesis of fucose (van Hengel and Roberts, 2002). The *mur1* mutant contains fewer terminal fucose residues on AGP glycans than the wild type and leads to changes in the cross-electrophoresis pattern of root AGPs and alterations of root morphology and elongation (van Hengel and Roberts, 2002). The addition of an eel (*Anguilla anguilla*) lectin that specifically binds to terminal fucose residues associated with AGPs to the media used to grow wild-type seedlings caused their roots to phenocopy those of *mur1* mutants. This led to the conclusion that fucosylated AGPs are important for controlling root growth and development.

Further investigations of the *reb1-1* mutant showed that swollen trichoblasts also displayed disorganized cortical microtubules (Andème-Onzighi *et al.*, 2002; Nguema-Ona *et al.*, 2006) suggesting a link between the cytoskeleton and plasma membrane/cell wall AGPs. To validate and extend on this finding, arabidopsis seedlings were treated with active Yariv or anti-AGP mAbs and cortical microtubules organization examined. Interestingly, within 30 min of treatment, cortical microtubules were shown to lose their transverse organization and detach from the inner side of the plasma membrane (Nguema-Ona *et al.*, 2007). Concomitantly, disruption of AGP distribution at the outer side of the plasma membrane was observed (Nguema-Ona *et al.*, 2007). Tobacco BY-2 suspension-cultured cells also exhibited microtubule and actin networks disorganization in response to Yariv treatment (Sardar *et al.*, 2006).

Whether and how AGP and cytoskeleton elements are interconnected is yet to be elucidated. Nevertheless, it has been proposed that the flow of information could be directed from the cell wall to the cytoplasm or reversely, from the cytoplasm to the cell wall (Nguema-Ona *et al.*, 2007). Here, AGP could interact with molecular factors such as receptor-like kinases for signal transduction into the cytoplasm (Ringli, 2010; Boisson-Dernier *et al.*, 2011). The rapid effect of active Yariv treatment on cortical microtubules organization (within a few minutes) pleads for the closeness of AGPs and elements of the cytoskeleton. The ability of AGPs to be inserted in the external leaflet of the plasma membrane via a GPI-anchor (Schultz *et al.*, 1998) places them in proximity to microtubules and suggests that this property may play a role in the interconnection. Sardar *et al.* (2006) showed that both microtubule and actin-disorganizing drugs had an effect on AGP localization, and active Yariv could disorganize, in a reversible manner,

both actin and microtubules in BY-2 cells, supporting the possibility of bidirectional flow of information. They also suggested the implication of phospholipase D, wall-associated kinases and lectin receptor kinases as potential candidates contributing to the cell wall–plasma membrane–cytoskeleton continuum. These potential interactors could act as linkers between AGPs and microtubules (Sardar *et al.*, 2006) possibly at specific plasma membrane domains such as lipid rafts (Mongrand *et al.*, 2004; Grennan, 2007). However, Driouch and Baskin (2008) suggested the potential interactors could be part of a scaffold where cortical microtubules are essentially required for controlling the orientation of cellulose microfibrils via GPI-anchored partners such as COBRA protein (Roudier *et al.*, 2005) or AGPs.

In addition to pharmacological studies that target several AGP populations (above), investigations focused on a particular AGP gene and its product have also linked biological functions to a given AGP. To date, about a dozen AGP-encoding genes have been shown to be expressed in root tissues of *Arabidopsis thaliana* and other species (e.g. *Oryza sativa*). The expression of some of these genes is regulated by factors including phytohormones. AGP30 is the first non-classical AGP shown to be specifically expressed in roots. It is a histidine-rich AGP that plays a role in root regeneration and seed germination (van Hengel and Roberts, 2003). Similarly to AtAGP30, *Daucus carota* AGP1 (*DcAGP1*), a close homologue of AtAGP30, is specifically expressed in roots and found abundantly in carrot cell cultures (Baldwin *et al.*, 2001). Disruption of AGPs with active Yariv at the root surface of an arabidopsis *agp30* mutant deficient in AtAGP30 displays a less severe phenotype than the wild type, supporting the lack of some AGPs in the roots of this mutant. More interestingly, the lack of AtAGP30 caused a general decrease in the perception of the hormone abscisic acid. This led to the proposal by Gens *et al.* (2000) and Wagner and Kohorn (2001) that AtAGP30 may be part of a signalling pathway involving cell wall-associated kinases (Gens *et al.*, 2000; Wagner and Kohorn, 2001) or leucine-rich repeat receptor kinases (Tang *et al.*, 2002) at the cell surface. AtAGP30 is expressed in non-hairy epidermal cells (atrachoblasts) of the meristematic and elongating zones where they might be involved in maintaining the shape of these cell lines (van Hengel *et al.*, 2004). Its co-localization with the GLABRA2 supports a role in the early stage of root epidermal patterning in an abscisic acid-dependent manner. AtAGP30 is also expressed in cortical, endodermal and vascular tissues of the mature part of the roots (van Hengel *et al.*, 2004). AtAGP31 is a close homologue of AtAGP30 and is a non-classical AGP with a cysteine-rich C-terminal PAC (proline-rich protein and AGP, containing cysteine) domain, a proline-rich domain and a histidine-rich domain. AtAGP31 gene is strongly expressed in vascular tissues including the phloem and primary xylem (Liu and Mehdy, 2007) and has been suggested to play a role in root development. Interestingly, AtAGP31 expression is repressed in the presence of a wounding stress or wounding-associated factors such as methyl-jasmonate, implicating a role in abiotic stress responses. Another subgroup of chimeric AGPs, the FLA, has also been shown to play a role in the control of morphogenesis. Under salinity stress, the lack of the *SOS5/FLA4*

gene in the *sos5/fla4* mutant leads to a reduction in root growth and abnormal expansion of epidermal, cortical and endodermal cells (Shi *et al.*, 2003). SOS5/FLA4 protein is strongly expressed in the root cortex and vascular tissues but weakly in epidermal cells and root hairs. It is a plasma membrane-associated AGP that contains two fasciclin-like domains and a C-terminal GPI anchor (Shi *et al.*, 2003). Fasciclin domains are known to play a role in cell adhesion in animals, insects and algae (Kawamoto *et al.*, 1998). Interestingly, root cells of *sos5* mutant have thinner and loosened cell walls compared with wild type. Shi *et al.* (2003) speculated that SOS5 proteins may aggregate or interact through fasciclin domains with other cell-wall components to form a network capable of maintaining cell-wall structure and proper cell expansion under salt stress. AtFLA1 is another FLA which has recently been shown to play a role in lateral root development and shoot regeneration from root tissues in *Arabidopsis thaliana* (Johnson *et al.*, 2011). It is expressed in the mature vasculature of lateral roots and the elongation zone of the primary root. The function of AtFLA1 gene is unclear but one possibility is that it is involved in regulating cell differentiation and expansion in the newly formed lateral roots (Johnson *et al.*, 2011) but may also have a role in defining cell fate and identity, a role that has been already proposed based on differential expression of specific AGP-associated epitopes as markers of cell fate (McCabe *et al.*, 1997). Such a possibility remains to be demonstrated with the generation and use of anti-AtFLA1 mAbs.

Role in microbe interaction with roots. Apart from their important roles in root development, AGPs present in roots, specifically in root-released border cells/border-like cells and in exudates are important for plant–microorganism interactions within the rhizosphere. The role of root exudates in interactions between plant roots and microbes has been reviewed by Bais *et al.* (2006). The interactions are described as positive, when they lead to symbiotic associations with mycorrhizal fungi and soil bacteria, or biocontrol agents. In contrast, they are classified as negative when they lead to plant parasitism or pathogenesis (Bais *et al.*, 2006). Exudates (containing mucilage and metabolites) are root secretions that contribute significantly to defining the microenvironment surrounding root surfaces wherein specific populations of microbes are attracted and can develop (Walker *et al.*, 2003). Mucilage contains high molecular-weight cell-wall components including AGPs (Bacic *et al.*, 1988; Moody *et al.*, 1988) as observed in cowpea (*Vigna unguiculata*) (Knee *et al.*, 2001), and maize root mucilage (Ma *et al.*, 2010).

The unexpected finding that AGPs influence root interactions with microbes was found by studying an arabidopsis mutant resistant to transformation by *Agrobacterium tumefaciens*, the *rat1* (resistant to *agrobacterium* transformation 1) (Nam *et al.*, 1999). Gaspar *et al.* (2004) showed that absence of AtAGP17/RAT1 expression in the mutant suppresses *Agrobacterium tumefaciens* ability to colonize arabidopsis roots. Pre-treatment of wild-type roots with active Yarrowia prior to transformation with *agrobacterium* also prevents root colonization (Gaspar *et al.*, 2004) and led Gaspar *et al.* (2004) to suggest that AtAGP17 deficiency may either affect a physical association between the root cell surface and

bacteria at the initial stage of infection, or interfere with a signalling cascade in which AtAGP17 acts as an elicitor-like signalling molecule (Gaspar *et al.*, 2004). The later assumption is supported by the finding that under-expression/lack of AtAGP17 prevents modulation of the content of salicylic and of certain pathogenesis-related proteins (e.g. PR1) leading to the resistant phenotype. It is well known that during infection by *Agrobacterium tumefaciens* and subsequent nodule formation, some plant defence mechanisms are repressed (Pitzschke and Hirt, 2010). Also, it is interesting to note that other Lys-rich AGPs such as LeAGP-1 and NaAGP4 (Gilson *et al.*, 2001) have been found to respond to wounding and to pathogen attack. For instance, NaAGP4, a close homologue of AtAGP17, is repressed following leaf infection with the necrotrophic pathogen *Botrytis cinerea*, contrasting with extensin overexpression under the same conditions (Gilson *et al.*, 2001). It is possible that AGP genes required for growth and development such as LeAGP1 or NaAGP4 are repressed under stress conditions to allow extensin-dependent cell wall cross-linking/strengthening to take over as a defence response.

Additionally, studies of interactions between symbiotic bacteria such as *Rhizobium leguminosarum* and pea roots during nodule formation have provided further evidence for the role of AGPs during root colonization by micro-organisms. Nodule formation starts with entrapping of rhizobia between legume root hairs and development of an infection thread which grows inward from the root hair toward epidermal and cortical cells of the root (reviewed by Gage and Margolin, 2000). A chimeric population of AGPs [called arabinogalactan protein-extensins (AGPE)] has been identified as the major component of the infection thread lumen (Rathbun *et al.*, 2002). Immuno-localization with the MAC265 mAb (van den Boesch *et al.*, 1989) revealed that AGPE are located both at the root surface and in the infection threads, and can be cross-linked by peroxidases (see also Kjellbom *et al.*, 1997 for oxidative cross linking of plasma membrane AGPs). Rathbun *et al.* (2002) hypothesized that physical and biochemical properties of AGPE may have an important influence on the progress of tissue and cell colonization by *Rhizobium*, probably by surrounding the bacteria in the infection thread or by regulating the growth of the infection thread itself. Symbiotically defective mutants of pea (*Pisum sativum*) can fail to induce formation of infection threads. Tsyganova *et al.* (2009) showed that, in addition to recognizing new infection threads in the infection zone and mature infection threads in the nitrogen-fixing zone of root nodule in wild type and symbiotically deficient strains MAC265 labelled the intercellular spaces of infected nodule tissue and small cytoplasmic vesicles in symbiotically deficient strains. This suggests that the targeted secretion of the matrix glycoprotein recognized by the MAC265 mAb is closely correlated with growth of the infection thread (Tsyganova *et al.*, 2009).

In addition to their role during development of infection threads, AGPs also play a role during early stages of nodule formation between *Rhizobia* and legumes. Downie (2010) reviewed the steps leading to a successful nodule formation prior to infection. While nod (nodulation) factors remain the major determinant for host specificity and nodule initiation, other factors such as bacterial exopolysaccharides and plant

lectins can influence legume nodulation. Indeed, it was shown that attraction could also be mediated by bacterial cell wall glucomannan and plant lectins from pea (*Pisum sativum*) and favour a polar attachment *in vitro* or an attraction *in vivo* (Laus *et al.*, 2006; Williams *et al.*, 2008), in the absence of nod factors. It is known that rhizobia and agrobacteria attach in a polar manner to plant root surface (Matthysse and Kijne, 1998). Recently, Xie *et al.* (2012) identified an AGP in root exudates of pea that is able to induce a polar attachment of *Rhizobium*. Interestingly, the purified glycoprotein is recognized by the anti-AGP mAbs JIM13 and LM2, but not by MAC265, specific for AGPE. Treatments of the glycoprotein with proteases or glycosidases suppress the polar attachment, suggesting that both the carbohydrate moiety and the protein backbone are required for function (Xie *et al.*, 2012). Xie *et al.* (2012) proposed that AGP could provide a source of nutrients for soil bacteria (Knee *et al.*, 2001) and indirectly act in a complementary manner to the previously described legume-specific polar attachment mediated by specific plant lectins and bacterial glucomannans (Laus *et al.*, 2006).

Much more ancient symbioses have been established between plants and fungi, forming arbuscular mycorrhizas (AM) and actinorhizes. After spore germination, establishment of the symbiosis includes hyphal branching, apressorium development after contacting the root, colonization of the root cortex, formation of intracellular arbuscules and, concomitantly, production of an extraradical mycelium from which spores are eventually formed (Balestrini and Lanfranco, 2006). The molecular similarity between the legumes/rhizobia and legumes/AM has suggested a common genetic programme for both symbioses (Gianinazzi-Pearson and Denarié, 1997). Van Buuren *et al.* (1999) also showed that, among several cell-wall genes required for wall metabolism activities necessary to favour AM colonization, AGPs and HRGP transcripts were identified in cells containing arbuscules (see also Balestrini and Lanfranco, 2006). The presence of AGPs at the symbiotic interface was clearly demonstrated by Berry *et al.* (2002). More recently, Schultz and Harrison (2008) proposed that plant AGPs could physically interact with the mycorrhizal fungus *Glomus intraradices* AGP-like proteins in the apoplastic compartment hosting the arbuscules. In this context, early nodulin ENOD11, a putative cell-wall proline-rich protein, having a low overall tyrosine content (Journet *et al.*, 2001; Chabaud *et al.*, 2002), is inducible upon simple adhesion of the AM fungus to the root surface. This protein was shown to play a role during the early stages of root nodulation and root colonization by AM in epidermal and cortical cells, including reorganization of host cytoplasm and construction of a novel apoplastic compartment, the pre-penetration apparatus where the fungus is destined to penetrate (Genre *et al.*, 2005). Interestingly, ENOD11 is also expressed during root hair nodule formation, as well as in non-symbiotic root tissues such as root cap and root border cells (Journet *et al.*, 2001), supporting the suggestion that ENOD11 may be responsible for the elastic properties of cell walls under these conditions.

In a pathogenesis context, few data on the role of AGPs at the root surface are available, while other HRGPs (e.g. extensins) (Esquerré-Tugaye and Mazau, 1974) are known to be involved in defence mechanisms. Xie *et al.* (2011) compared the distribution of HRGPs, including AGPs, in the roots of

resistant and susceptible wax gourds prior to and after infection by *Fusarium oxysporum* or treatment with fusaric acid. In the absence of infection, the resistant cultivar constitutively expresses more extensin-associated epitopes than the susceptible cultivar. Upon infection, expression of extensin-associated epitopes decreased significantly in epidermal cells of the susceptible cultivar, while it remained unchanged in the resistant one. Interestingly, the anti-AGP-associated epitope recognized by the mAb CCRC-M7 (Steffan *et al.*, 1995) was enhanced in the resistant cultivar, indicating that this epitope is likely to contribute to resistance. In contrast, other AGP-associated epitopes (recognized by LM2 and JIM16) were equivalently expressed in both cultivars before and after infection.

Further evidence for a role of AGPs in interactions with the rhizosphere was provided by arabidopsis root border-like cells. Border cells and border-like cells are released from the root tip either individually or as a group of attached cells (Hawes *et al.*, 2000; Driouch *et al.*, 2007). They play a major role in plant–microbe interactions within the rhizosphere and provide protection to the root (Hawes *et al.*, 2000; Vicré *et al.*, 2005; Driouch *et al.*, 2012). In addition to root cap-secreted exudates which contain high molecular components including AGPs (Knee *et al.*, 2001; Xie *et al.*, 2012), border-like cells also synthesize and secrete significant amount of AGPs at their surface (Fig. 4) (Vicré *et al.*, 2005; Wen *et al.*, 2007; Cannesan *et al.*, 2012). The proteome of border cell exudates plays a role in protecting the cap against infection (Wen *et al.*, 2007). Hawes *et al.* (2000) listed functions associated with border cells and it is likely that many are AGP-dependent. Recently, it was shown that AGPs secreted by pea roots are capable of attracting zoospores of the pathogenic oomycete *Aphanomyces euteiches* and then inhibiting their germination (Cannesan *et al.*, 2012).

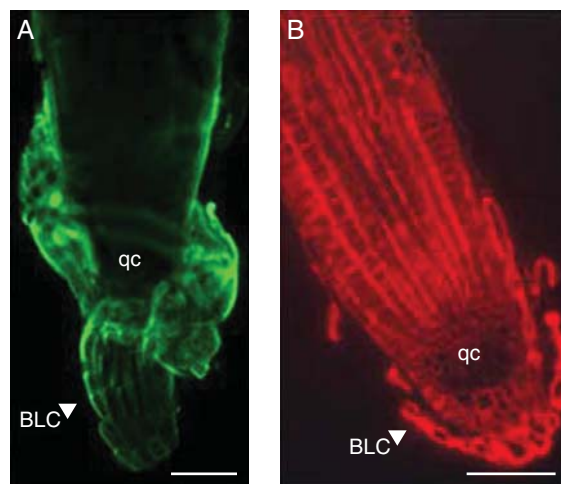


FIG. 4. Arabidopsis root tips and associated border-like cells labelled with anti-AGP antibodies: JIM13 (A) and JIM14 (B). Note the strong labelling associated with border-like cells in (A) (secondary antibody conjugated to FITC) and the lack of labelling in the quiescent centre in (B) (secondary antibody conjugated to TRITC). The image in (A) is of an intact root while the image in (B) is of a sectioned root (as described in Nguema-Ona *et al.*, 2006). Abbreviations: BLC, border-like cells; qc, quiescent centre. Scale bars = 100 μ m.

In addition to AGPs, root cap-secreted exudates, root- and border cell-released enzymes involved in degradation and remodelling of cell wall glycoproteins and polysaccharides such as xyloglucan endotransglucosylase hydrolase (XTH) and beta-galactosidase (Wen *et al.*, 2007) have been described. Their presence opens the possibility for novel modes of action. Thus, it is of interest to establish whether specific oligosaccharides released from AGPs in the rhizosphere could act as signals of infection. Such a function has been already proposed in the oligosaccharins theory (Albersheim *et al.*, 1992; Creelman and Mullet, 1997) and was demonstrated for pectin-derived oligosaccharides (Cervone *et al.*, 1989) and xyloglucan-derived oligosaccharides from plant cell walls (Fry *et al.*, 1993). Root border-like cells of arabidopsis were shown to be highly enriched in AGPs that are likely to be released in the rhizosphere. In addition, the association of a rhizobacterium with root cells was shown to be AGP-dependent since the use of drugs acting on AGP abolished the interaction (Vicré *et al.*, 2005). More interestingly, the homogalacturonan-deficient *qual-1* arabidopsis mutant has an altered release pattern of border-like cells that is accompanied by the secretion of abundant mucilage particularly enriched in AGPs and xylogalacturonan (Durand *et al.*, 2009; Driouich *et al.*, 2010). These molecules are suggested to enhance the protective capacity of border cells of the *qual-1* mutant (Driouich *et al.*, 2010, 2012).

AGPS IN POLLEN TUBE AND OTHER FLOWER ORGANS: DISTRIBUTION AND FUNCTIONAL ASPECTS

Double fertilization in flowering plants requires the targeted delivery of sperm by the pollen tube. After landing on compatible stigmatic cells, pollen germinates and forms a tube that grows deep inside the specialized transmitting tissue of the pistil to arrive precisely at a specific target, the embryo sac. Typically, one pollen tube terminates its journey by entering a synergid cell and bursting to release the sperm nuclei, in a process called pollen tube reception. Unlike most plant cells, pollen tubes grow in a polarized way, restricted to the tip area. During pollen-tube growth, callose plugs are laid down at regular intervals behind the growing tip, and the area adjacent to the plug becomes vacuolated, which serves to maintain a region of concentrated cytoplasm containing organelles, the vegetative nucleus and the two sperm cells, near the tip (Wang *et al.*, 2010). Tip growth is made possible through co-ordinated cellular activities, including a dynamic actin cytoskeleton system, targeted exocytosis, and regulated endocytosis (Hepler *et al.*, 2001). One of the main features of growing pollen tubes is a tip-focused calcium gradient which is thought to be maintained by influx of extracellular calcium through calcium channels active at the extreme end of the growing tip (Feijó *et al.*, 1995; Malhó *et al.*, 1995). The grow trajectory is adjusted and apparently dependent on an intricate network of signalling events, largely unidentified, and probably involving molecules of different kinds (Preuss, 2002; Johnson and Lord, 2006; Mollet *et al.*, 2007; Palanivelu and Johnson, 2010; Wang *et al.*, 2010). Among them, AGPs have attracted much attention. Indeed, AGPs prevail in stigma exudates, style transmitting tissues, pollen

grains and pollen tubes in many species (Cheung *et al.*, 1995; Wu *et al.*, 1995) and their implications in pollen formation, pollen-grain hydration, pollen-tube growth, cell-wall deposition, pollen-tube guidance and pollen-tube rejection during the gametophytic S-incompatibility are described.

Biochemical evidence for AGP occurrence in pollen tubes

Due to difficulties of extracting sufficient cell-wall material, only a few studies have focused on the biochemical characterization of the pollen-tube cell wall and more specifically on AGPs. In *Nicotiana glauca* pollen-tube cell walls, the content of HyP is low (2.4 mol%) and linkage analyses of a total cell-wall extract did not reveal any type-II arabinogalactosyl residues generally found in AGPs (Rae *et al.*, 1985), suggesting that AGPs are a minor component of pollen-tube cell wall. The total cell-wall extract was mainly composed of α -1,5-linked arabinan, a large amount of callose and low amount of cellulose (Rae *et al.*, 1985). A more recent study on *Arabidopsis thaliana* pollen-tube cell walls revealed that typical type-II arabinogalactan residues of AGPs (i.e. 1,6-linked Gal; 1,3,6-linked Gal; 1,3-linked Gal; t-Ara and t-Gal) accounted for 47.5 % of the total galactosyl residues (Dardelle *et al.*, 2010). The remaining galactosyl residues (1,4-linked Gal and 1,4,6-linked Gal) are typical of type-I arabinogalactan side chains of the pectic polysaccharide rhamnogalacturonan-I (Dardelle *et al.*, 2010). Extraction and fractionation of Golgi vesicles from Japanese camellia (*Camellia japonica*) pollen tubes also revealed that the vesicles are enriched in protein with significant amount of galactosyl and arabinosyl residues. Moreover, the content of the vesicles reacted strongly with JIM13, indicating that they were enriched in AGPs (Hasegawa *et al.*, 1998). Similarly, in *Lilium longiflorum*, SDS-PAGE and immuno-blot analyses of the total protein from pollen tubes probed with JIM13 or stained with the active Yariv displayed several bands greater than 97 kDa (Jauh and Lord, 1996). Together these studies indicate that AGPs occur in pollen-tube cell walls and that variation in AGP content, structure and size exists.

Microarray data of arabidopsis pollen development (Honys and Twell, 2004; Pina *et al.*, 2005) clearly showed that pollen expresses a unique subclass of genes. The sperm cell transcriptome also indicated that male gametes have different gene expression from pollen grains (Borges *et al.*, 2008). Recently, the pollen-tube gene-expression profile was evaluated (Wang *et al.*, 2008; Qin *et al.*, 2009) and a significantly different set of genes found to be expressed. Intriguingly, some genes were only expressed if the pollen tubes were growing through the pistil tissues, meaning that this interaction is central and that pistil-dependent gene expression exists. Pollen-tube gene-expression data showed high expression levels for some AGPs in pollen grains and pollen tubes (Fig. 5). Four of these genes, two classical AGPs (AGP6 and AGP11) and two AG-peptides (AGP23 and AGP40), are present only in pollen grains and tubes. These two AGP groups exhibit a high degree of similarity, although AG-peptides are very different molecules, typically consisting of fewer than 30 amino acid residues (Fig. 6).

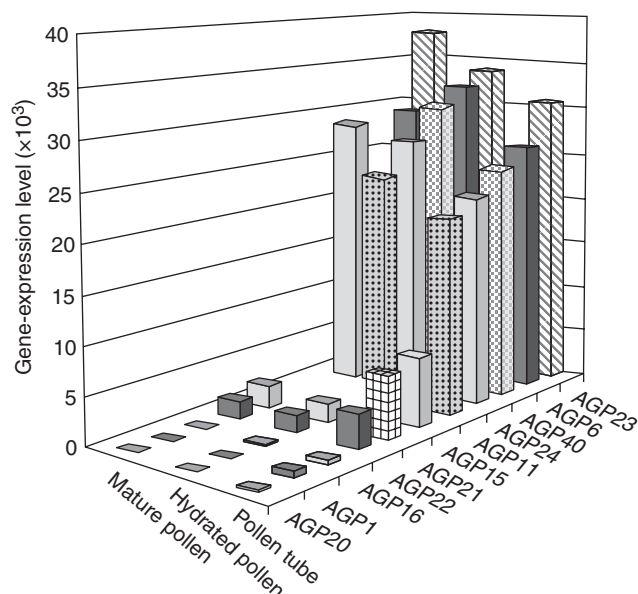


FIG. 5. ATH1 genome array showing the gene-expression level for the different AGPs, at three stages of development: mature pollen, hydrated pollen and pollen tube (according to Wang *et al.*, 2008).

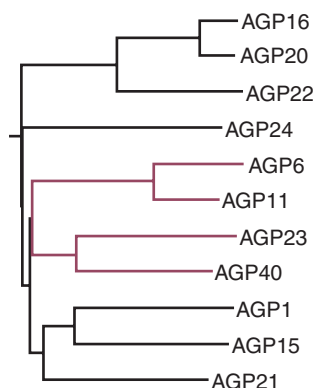


FIG. 6. Clustal W sequence homology alignment for the 11 pollen AGPs. AGP6, AGP11, AGP23 and AGP40 are the only four pollen-specific AGPs in arabidopsis. AGP6 and AGP11 present the highest similarity scores and are closely related to AGP23 and AGP40 (highlighted in red)

Distribution of AGPs at different stages of pollen formation and pollen-tube growth

Use of AGP-specific mAbs to study AGP distribution in pollen and pollen tubes. The cell wall of pollen grains is composed of two layers, the intine, the inner wall, and the exine, the outer wall, (Wang *et al.*, 2010). The pollen-tube cell wall in plants such as arabidopsis, tobacco and lily, with the exception of the tip is composed of two layers; the inner cell wall is enriched in callose and the outer cell wall is composed mostly of pectins, hemicellulose, cellulose and AGPs (Dardelle *et al.*, 2010). At the tip, only the outer layer is present (Geitmann and Steer, 2006; Dardelle *et al.*, 2010). In *Arabidopsis thaliana*, during pollen-grain formation in the anther at the beginning of meiosis, the labelling obtained with the mAbs JIM8 and JIM13 is selectively found in the cell walls of

microsporocytes (Coimbra *et al.*, 2007). After meiosis, the four haploid microspores called tetrads are surrounded by a thick wall composed of callose, pectin and hemicelluloses. Following degradation of the thick wall with β -(1,3)-glucan hydrolases (Hird *et al.*, 1993), pectin methylsterases (Francis *et al.*, 2006), polygalacturonases (Rhee *et al.*, 2003) and other enzymes secreted by the tapetal cells, the microspores are released and AGP epitopes recognized by JIM8 and JIM13 are strongly detected in the cytoplasm and the outer surface of the microspores (Coimbra *et al.*, 2007). After the first pollen mitosis, the bicellular pollen contains the generative and the vegetative cells. At this stage, the membrane of the generative cell is strongly labelled. After the second mitosis, the generative cell produces the two sperm cells with their membranes still strongly labelled (Coimbra *et al.*, 2007). The specific labelling of the two male gametes is maintained in the mature pollen grains (van Aelst and van Went, 1992), and in pollen tubes grown *in vitro* (Coimbra *et al.*, 2007) and *in vivo* (Lennon and Lord, 2000). In addition, a subset of AGP epitopes recognized by MAC207 is also present in the intine wall of the mature pollen grain close to the plasma membrane (van Aelst and van Went, 1992). Other mAbs such as LM2 do not show any cell-specific labelling during pollen formation (Coimbra *et al.*, 2007). The cell wall of *in vitro*-grown pollen tubes displays different specificities towards the mAbs. The pollen-tube tip is more strongly labelled than the shank with LM2 and MAC207 antibodies (Fig. 7A, B, E) (Pereira *et al.*, 2005; Dardelle *et al.*, 2010). In contrast, no labelling is detected over the pollen-tube cell wall with JIM13 and JIM8 mAb (Dardelle *et al.*, 2010).

In other species investigated including the gymnosperms (*Cycas revoluta*, *Ginkgo biloba*, *Picea wilsonii* and *Pinus densiflora*), the angiosperm monocots (*Lilium longiflorum* and *Lolium perenne*) and eudicots (*Brassica napus*, *Beta vulgaris*, *Nicotiana tabacum*, *Nicotiana glauca* and *Solanum peruvianum*), AGP epitopes are detected in the intine wall of the pollen grains close to the plasma membrane (Table 3). In the pollen-tube cell wall, several AGP labelling patterns have been described (Table 3 and Fig. 7). In spruce (*Picea meyeri*) and kiwifruit (*Actinidia deliciosa*), a periodic ring-like deposition of AGP epitopes along the pollen tube length is observed using LM2 and JIM13, respectively (Fig. 6D) (Chen *et al.*, 2007; Speranza *et al.*, 2009). With MAC207, no visible labelling is detected in *A. deliciosa* pollen tubes (Speranza *et al.*, 2009). In *L. longiflorum*, JIM13-16, MAC207 and LM2 labelling is restricted to the pollen-tube tip (Fig. 7C) (Jauh and Lord, 1996; Mollet *et al.*, 2002). Whereas in *N. tabacum*, no visible labelling is observed in the pollen-tube cell wall using the MAC207 (Li *et al.*, 1992) or PCBC3 (Fergusson *et al.*, 1999) and uniform labelling of the entire pollen-tube cell wall is observed with JIM13 (Qin *et al.*, 2007). Interestingly, treatments of the pollen tubes of *N. tabacum* and *L. longiflorum* with pectinase and/or cellulase, uncover AGP epitopes organized as a periodic deposition of ring-like structure along the entire pollen tube (Fig. 7D) (Li *et al.*, 1992; Jauh and Lord, 1996). These results have been confirmed at sub-cellular level where AGP epitopes are found unevenly distributed over the plasma membrane along the pollen tube (Roy *et al.*, 1997; Fergusson *et al.*, 1999). Finally, within the pollen-tube cell, AGP epitopes are also detected, in many species, associated

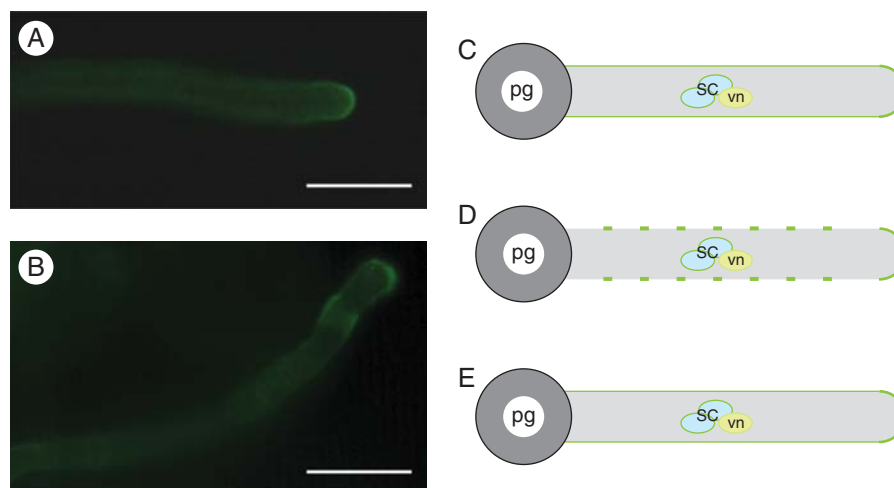


FIG. 7. Immunolocalization of AGPs in *Arabidopsis thaliana* pollen tube and the most-common AGP-labelling patterns observed in pollen tubes. (A) Cell surface immuno-labelling of *A. thaliana* pollen tube with LM2 and (B) with MAC207. Immuno-labelling patterns of AGP epitopes (in green) found in (C) *Lilium longiflorum*, (D) *Picea meyeri*, *Actinidia deliciosa* and *Lilium longiflorum* after enzyme treatments, and (E) *Podocarpus nagi*, *Picea wilsonii*, *Pinus densiflora*, *Annona cherimola*, *Arabidopsis thaliana* and *Nicotiana tabacum* with JIM13. Abbreviations: pg, pollen grain; sc, sperm cells; vn, vegetative nucleus. Scale bars in (A) and (B) = 20 μm . The drawings in (C–E) are not to scale. The detection of AGP epitopes in the sperm cell membranes is generally deduced from electron microscopy observations (see also Dardelle *et al.*, 2010).

with secretory vesicles, the generative and the sperm cell membranes (Table 3). Differences in the labelling patterns observed with JIM13 and MAC207 antibodies are not easy to explain as the recognized epitopes are supposed to be identical (Table 3). By comparing the labelling patterns, the differences might be due to (a) the species, (b) the organization of the epitopes in the pollen-tube cell wall and/or the structure of the AGPs, (c) the accessibilities of the epitopes, (d) the type of pistil (wet versus dry stigma, hollow versus solid style), (e) the *in vitro* culture conditions and (f) probably the growth rate of the pollen tubes observed *in vitro* and *in vivo*. The pollen-tube growth in gymnosperms is generally slower (0.01–0.05 $\mu\text{m min}^{-1}$ based on the length of the pollen tubes after 72 h) (Yatomi *et al.*, 2002; Fernando *et al.*, 2010) than that observed for angiosperms such as *L. longiflorum* (approx. 10.1 $\mu\text{m min}^{-1}$ based on the length of the pollen tubes after 6 h) (Jauh and Lord, 1996), *N. tabacum* (average of 2.7 $\mu\text{m min}^{-1}$ based on the length of the pollen tubes after 5 h) (Mollet *et al.*, 2002) or *Arabidopsis* (average of 1.3 $\mu\text{m min}^{-1}$ based on the length of the pollen tubes after 16 h) (Dardelle *et al.*, 2010).

It is interesting to notice the recurrent occurrence of the LM2 and JIM13 epitopes associated with AGPs in pollen tubes of all the plant families analysed, and the occurrence of additional AGP-associated epitopes in higher plants, as shown in Table 2 in the different taxa. This observation prompts two interesting thoughts on AGPs diversity, occurrence and biology. First, AGP-associated epitopes in pollen tubes are more complex and diverse in higher plants when compared with other taxa. This reflects the complexity of the AGP-glycans' biosynthetic machinery or their evolutionary relationships (see recent review on evolution of plant cell walls; Sørensen *et al.*, 2010). Second, in the case of AGPs, and contrasting with other polysaccharide-rich cell-wall components, the assembly of AGPs within the endomembrane system and their transport to the cell wall are still poorly understood.

AGP genes and proteins expressed in angiosperm pollen. Pollen development requires expression of early genes after meiosis, and late genes encoding products involved in cell-wall formation, pollen maturation, pollen recognition, pollen germination and pollen-tube growth. The late genes are expressed after pollen mitosis and represent the pollen-specific genes (Raghavan, 1997). In *Arabidopsis*, several AGP genes including *AtAGP6* (At5g14380) and *AtAGP11* (At3g01700) were shown to be associated with pollen-tube development (Levitin *et al.*, 2008; Coimbra *et al.*, 2010). Their functions are described below. AGP genes expressed during pollen development were also studied in other plant species. *Brassica campestris BcMF8* is a pollen-specific gene. Its deduced protein backbone presents features of a classical AGP (Huang *et al.*, 2008). By differential screening, Gerster *et al.* (1996) characterized two highly homologous AGP genes from *B. napus*, *Sta 39-4* and *Sta 39-3* which were shown to be pollen-specific. *BcMF8* shares high sequence identity with those two putative pollen-expressed AGP genes, and a lower similarity with the classical AGP genes *AtAGP11* and *AtAGP6*. Anther pollen-specific AGP gene *PO2* from alfalfa (*Medicago sativa*) was also characterized by Qiu *et al.* (1997) using differential screening technology. *BAN102* from Chinese cabbage, another pollen-preferential gene which shared sequence similarity to *AtAGP23*, was isolated by Park *et al.* (2005). In rice (*Oryza sativa*), several AGP-encoding genes were dominantly expressed in anthers (Ma and Zhao, 2010) and the two classical AGP-encoding genes, *OsAGP7* and *OsAGP10*, were highly expressed in pollen (Ma and Zhao, 2010). Although expression of *OsAGP7* and *OsAGP10* parallels that of *AtAGP6* and *AtAGP11* they do not have the closest phylogenetic relationship. In fact the phylogenetically closest rice genes exhibiting higher homology with *AGP6* and *AGP11* have a different expression pattern. Anand and Tyagi (2010) isolated an AGP (*OsAGP*) cDNA (586 bp) from rice preferentially expressed in the inflorescence and

TABLE 3. Overview from several plant species of AGP localization during pollen-grain formation, and in pollen tubes grown in vitro and in vivo

Species	Probe	Microscopy*	Culture	Cell type [†]	Labelling pattern [†]	References
Gymnosperms						
Cycadales						
<i>Cycas revoluta</i>	JIM13, LM2	EM/CF	<i>In vitro</i>	PG and PT	JIM13: strong labelling of the intine and PT; LM2: strong labelling PT, no labelling of the intine wall	Yatomi <i>et al.</i> (2002)
Ginkgoales						
<i>Ginkgo biloba</i>	JIM13, LM2	EM/CF	<i>In vitro</i>	PG and PT	JIM13 and LM2: strong labelling of the intine and PT wall	Yatomi <i>et al.</i> (2002)
Pinales						
<i>Podocarpus macrophyllus</i>	JIM13, LM2	EM/CF	<i>In vitro</i>	PG and PT	JIM13: labelling of the intine and PT wall	Yatomi <i>et al.</i> (2002)
<i>Podocarpus nagi</i>					LM2 : weak labelling	
<i>Abies veitchii</i>	JIM13, LM2	EM/CF	<i>In vitro</i>	PG and PT	JIM13: labelling of the intine and PT wall	Yatomi <i>et al.</i> (2002)
					LM2 : no labelling of the PT, labelling of the intine wall	
<i>Cedrus deodara</i>	JIM13, LM2	EM/CF	<i>In vitro</i>	PG and PT	JIM13: strong labelling of the intine and PT wall	Yatomi <i>et al.</i> (2002)
<i>Chamaecyparis obtusa</i>					LM2 : no labelling	
<i>Picea wilsonii</i>	LM2	FM	<i>In vitro</i>	PT	Whole PT wall	Chen <i>et al.</i> (2008)
	LM2	FM	<i>In vitro</i>	De-exined PG	Intine surface	Fang <i>et al.</i> (2008)
<i>Picea meyeri</i>	LM2	FM	<i>In vitro</i>	PT	Periodic ring-like pattern along the whole PT wall	Chen <i>et al.</i> (2007)
<i>Pinus densiflora</i>	JIM13, LM2	EM/CF	<i>In vitro</i>	PG and PT	JIM13 and LM2: intine wall of PG. Whole PT wall (tip and back) and secretory vesicles	Mogami <i>et al.</i> (1999); Yatomi <i>et al.</i> (2002)
					LM2: generative cell wall	
<i>Pinus bungeana</i>	LM2	FM	<i>In vitro</i>	De-exined PG	Intine surface	Fang <i>et al.</i> (2008)
<i>Pinus banksiana</i>	JIM13, LM2	EM/CF	<i>In vitro</i>	PG and PT	JIM13and LM2: labelling of the intine and PT wall	Yatomi <i>et al.</i> (2002)
<i>Pinus rigida</i>						
<i>Cryptomeria japonica</i>						
<i>Pinus elliotii</i>	JIM13, LM2	EM/CF	<i>In vitro</i>	PG and PT	JIM13: labelling of the intine and PT wall	Yatomi <i>et al.</i> (2002)
					LM2: weak labelling of the PT, no labelling of the intine	
Angiosperms						
Monocot						
Liliales						
<i>Lilium</i>	JIM13	FM	<i>In vitro</i>	PG protoplast	Cell surface	Zhao <i>et al.</i> (2004)
<i>longiflorum</i>	JIM13	EM/HPF	<i>In vivo</i>	PT	PM, inner cell wall and secretory vesicles	Roy <i>et al.</i> (1997)
	JIM13-16, LM2, MAC207	FM	<i>In vitro</i> and <i>in vivo</i>	PT	PT tip wall	Jauh and Lord (1996)
	JIM13, LM2, MAC207	FM	<i>In vitro</i> and <i>in vivo</i>	Pectinase treated PT	Ring-like pattern along the whole PT wall	Mollet <i>et al.</i> (2002)
	βGlcY (30 μM)	LM	<i>In vitro</i>	PT	PT tip wall, arrest of growth	Jauh and Lord (1996)
	JIM13, LM2, MAC207	EM/CF	<i>In vivo</i>	PT	PM, secretory vesicles and generative cell membrane	Mollet <i>et al.</i> (2002)
Poales						
<i>Lolium perenne</i>	JIM13	EM/CF	<i>In vivo</i>	PG formation	Microspore wall	Wisniewska and Majewska-Sawka (2006)
Eudicot						
Magnoliales						
<i>Annona cherimola</i>	JIM13	FM	<i>In vitro</i>	PT	Whole PT wall	Mollet <i>et al.</i> (2002)
	βGlcY (30 μM)	LM	<i>In vitro</i>	PT	Expanded region from the tip, arrest of growth	

Brassicales <i>Arabidopsis thaliana</i>	LM2, JIM13,	FM	<i>In vitro</i>	PT	LM2 and MAC207: stronger labelling at the tip than the back	Dardelle <i>et al.</i> (2010), Pereira <i>et al.</i> (2005)
	MAC207 JIM8, JIM13,	FM	<i>In vivo</i>	PG formation	JIM13: no labelling JIM8 and JIM13: strong labelling of microsporocyte wall, microspore wall, generative membrane and then sperm cells from microspore stage to mature pollen and in the pollen tube	Coimbra <i>et al.</i> (2007)
	MAC207, LM2 MAC207, JIM8	EM/PF	<i>In vitro</i>	PG	MAC207 and LM2: no specific labelling pattern MAC207: Intine wall close to the PM	van Aelst and van Went (1992)
	LM2, JIM13, MAC207	EM/PF	<i>In vivo</i>	PG and PT	JIM8: sperm cell membrane PG: intine	Lennon and Lord (2000)
<i>Brassica napus</i> Caryophyllales <i>Beta vulgaris</i>	JIM8	FM	<i>In vivo</i>	PG formation	PT: sperm cell membrane Tetrad wall, PM of mature PG, sperm cell membrane during anthesis	Pennel <i>et al.</i> (1991)
	MAC207 JIM4, JIM8	FM FM	<i>In vitro</i>	PG PG formation	PM In the callose wall of the tetrad	Pennel <i>et al.</i> (1989) Majewska-Sawka and Rodriguez-Garcia (2006)
Solanales <i>Nicotiana tabacum</i>	JIM13, LM2	EM/CF			JIM4: no labelling JIM13: strong gold labelling in the callose wall of the tetrad JIM8 and LM2: weak gold labelling in the callose wall of the tetrad	
	PCBC3, MAC207, JIM8, JIM13	EM/CF	<i>In vitro</i>	PG and PT	PCBC3: PG: PM	Fergusson <i>et al.</i> (1999)
	MAC207	FM	<i>In vitro</i>	PT Cellulase treated PT Pectinase treated PT	PT: discontinuous labelling at the PM along the PT, between the inner and outer cell wall layers, in secretory vesicles and callose plugs MAC207, JIM8, JIM13 : no labelling No labelling No tip labelling, strong periodic ring-like pattern back from the tip	Li <i>et al.</i> (1992)
	MAC207, JIM8	EM/CF	<i>In vitro</i>	PG and PT	No tip labelling, weak periodic ring-like pattern back from the tip	
	βGlcY (30 μM) JIM4, JIM13, LM2,	LM FM	<i>In vitro</i> <i>In vitro</i>	PT PG formation and PT	PG: uniform in the intine PT: discontinuous labelling along the outer wall of the PT. Labelling in the secretory vesicles and generative cell membrane. No tip labelling No labelling, no arrest of PT growth JIM13: microspore wall and whole PT	Li <i>et al.</i> (1995)
	βGlcY (50–100 μM) JIM13	LM EM/CF	<i>In vitro</i> <i>In vivo</i>	PT growth PG and PT	JIM4 and LM2: weak labelling Slow down of PT growth	Mollet <i>et al.</i> (2002) Qin <i>et al.</i> (2007)
	PCBC3	EM/CF	<i>In vitro</i>	PG and PT	PG: intine wall and vesicles PT: cell wall, generative and sperm cell membranes PG: PM and generative cell membrane PT: between inner and outer cell wall layers. No labelling at the tip	Fergusson <i>et al.</i> (1999)
	JIM13 JIM13, JIM8 JIM14, MAC207	FM EM/CF	<i>In vitro</i> <i>In vitro</i>	PT PT	Whole PT with periodic brighter ring-like pattern along the PT and at the tip JIM8, 13 and 14: cytoplasm, PM and the outer cell wall JIM13: tube tip wall MAC207: no labelling	Speranza <i>et al.</i> (2009)

* EM, Electron microscopy; FM, fluorescence microscopy; LM, light microscopy; CF, chemical fixation of the sample; PF, plunge freezing fixation; HPF, high-pressure freezing fixation.

† PT, Pollen tube, PG, pollen grain; PM, plasma membrane.

the deduced gene and protein sequence showed homology with the AGP-encoding gene *AGP23* from arabidopsis. An effort was made to correlate the expression and promoter activity data with the possible function of *OsAGP*. The *OsAGP* promoter shows activity after the microspores have been separated from the callose wall and therefore is categorized as a late pollen-expressing gene with maximum level of expression in pollen grains from mature flowers. The *OsAGP* promoter is also active during germination and pollen-tube growth (Anand and Tyagi, 2010).

Functional aspects of AGPs in pollen tubes and styles

AGPs in pollen-grain and pollen-tube development. Pollen-tube growth is coupled with deposition of new wall material at the tip resulting in an increase in pollen tube length. It has been recently proposed that pollen-tube AGPs are sent to the apex, where they function in signalling processes related to pollen-tube guidance (Lampert *et al.*, 2006; Zang *et al.*, 2011). With pollen-tube continuous growth, AGPs are recycled by endocytosis, to be reused or to be sent for degradation in the multivesicular bodies (M. L. Costa *et al.*, F.C. Universidade do Porto, unpubl. res.). Cell-wall components such as pectins or glycosylated proteins (e.g. AGPs) are known to carry complex and structurally diverse carbohydrate decorations. In addition, it is believed that different signalling molecules could bind to specific cell-wall carbohydrates (including those associated with AGPs) to modulate cell wall integrity and function. AGPs could act as co-receptors to sense extracellular signals and interact with transmembrane proteins, possibly receptor kinases or ion channels, to initiate signalling by triggering various intracellular events (Boisson-Dernier *et al.*, 2011; Zang *et al.*, 2011).

Treatments of *N. tabacum* pollen tubes with 30 μM active Yariv and the control, inactive Yariv, do not reveal red staining at the pollen-tube tip and do not affect growth (Mollet *et al.*, 2002). In contrast, active Yariv reagent stains the pollen-tube tip of *L. longiflorum* and causes the growth of the pollen tube to be arrested (Table 3) (Mollet *et al.*, 2002) without stopping vesicular secretion at the tip but disorganizing the cell wall architecture and composition due to abnormal callose deposition (Roy *et al.*, 1998). Upon removal of the active Yariv from the culture medium, pollen-tube growth resumes by emergence of a new pollen-tube tip behind the arrested tip (Mollet *et al.*, 2002). These data suggest that AGPs are important in the deposition of new cell-wall material during pollen-tube growth. Using higher concentrations of active Yariv (50 and 100 μM) and different culture conditions Qin *et al.* (2007) have also shown a significant reduction in pollen-tube growth in *N. tabacum*.

AGP6 and *AGP11* are two arabidopsis genes which are strongly and specifically expressed in pollen grains and pollen tubes (Figs 5 and 6) (Levitin *et al.*, 2008; Coimbra *et al.*, 2009). To determine whether the *agp6 agp11* mutant affected pollen-tube growth and seed set, female gametophytes at the pollination stage were analysed in emasculated flowers pollinated with wild-type and double-mutant pollen, using confocal laser-scanning microscopy with the conclusion that *AGP6* and *AGP11* are necessary for proper pollen-tube growth as well as for preventing untimely pollen-grain

germination (Coimbra *et al.*, 2010). The level of expression of all AGPs expressed in pollen tubes was monitored in *agp6 agp11* and in single knockout mutants for *agp6* and for *agp11* by real-time PCR. Based on the expression levels of the pollen-specific AGPs, and depending on the pollen-tube growth medium conditions (M. L. Costa *et al.*, F.C. Universidade do Porto, unpubl. res.), a consistent up-regulation of *AGP40*, a pollen-specific AG-peptide, was obtained in pollen grains and pollen tubes of the double null mutant *agp6 agp11*, and a more moderate over-expression of *AGP23* (also a pollen-specific AG-peptide) (Figs 5 and 6). Early results from *agp40* null-mutant characterization show no alteration in pollen grain development but illustrate a reduction in pollen-grain fitness, making it the ideal candidate for multiple knockouts. The triple mutant *agp6 agp11 agp40* was obtained and characterized (Fig. 8A, B) (Costa *et al.*, 2011). It showed an even more penetrant phenotype than the double mutant, with a significant reduction in seed production (Fig. 8B) and a higher number of early germinating pollen tubes inside the anthers (Fig. 8A). The strong distortion obtained in the germination percentage of *agp6 agp11* pollen and pollen-tube length, together with an early germination inside the anther, for some of these double-mutant pollen tubes, encouraged another experiment to further analyse this phenotype. So, in order to understand the mode of action of these AGPs, an Affymetrix ATH1 genome array in the *agp6 agp11* double null-mutant pollen tube was performed (Coimbra *et al.*, unpubl. res.). An unexpectedly high number of genes showed altered expression levels in pollen tubes that lack the two cell-wall proteoglycans, *AGP6* and *AGP11*, strengthening the idea that these molecules are involved in complex phenomena. Both calcium and signalling-related genes were altered, in agreement with the known roles of such phenomena in pollen-tube growth. The presumed involvement of AGPs in signalling cascades was also reinforced. Cysteine-rich proteins have been proposed to play a role in recognition and fertilization, and it was thus quite relevant that such genes were found to be

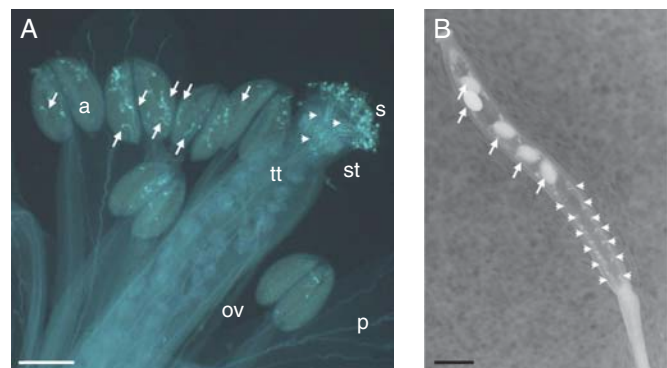


FIG. 8. Triple-null arabidopsis mutant plant for *AGP6*, *AGP11* and *AGP40*. (A) Decolourized aniline blue-stained flower. The anthers show a significant number of pollen tubes growing inside (arrows). Nevertheless some pollen grains maintain their normal germination behaviour and pollen tubes are visibly growing through the transmitting tissue (arrowheads). (B) The mature silique of the triple null mutant presents a reduce number of seeds (arrows) and many ovules remain attached to the placenta unfertilized and degenerated (arrowheads). Abbreviations: a, anther; ov, ovary; p, petal; s, stigma; st, style; tt, transmitting tract. Scale bars = 150 μm .

differentially expressed. This microarray study was complemented with experiments using two hybrids of yeast which revealed that some AGP interactors are present during pollen-tube growth. These results are being presented and discussed elsewhere by S. Coimbra and colleagues. It is known that molecules involved in pollination and stress/defence responses are evolutionarily related (Dresselhaus and Márton, 2009; Kessler et al., 2010) and one of the most altered gene ontology groups on the *agp6 agp11* array is stress, with several PR genes and heat-shock gene expression significantly distorted. Members of these families are involved in reproductive processes in other systems. It is thus of interest to investigate the role of these two AGPs and/or their partners, in the last stages of pollen–pistil interaction, sperm recognition and double fertilization.

It is well known that certain AGPs are GPI-anchored to the plasma membrane (Youl et al., 1998; Svetek et al., 1999). The collective importance of the GPI-anchor of arabidopsis proteins has been addressed through the study of insertional mutations disrupting *SETH1* and *SETH2* genes, which encode homologues of known components of the GPI anchor synthesis complex (Lalanne et al., 2004). Interestingly, the mutant plants showed drastic impairment of pollen germination and pollen-tube growth probably because the mutations affect a large number of GPI-anchored proteins simultaneously. Classical AGPs, AG-peptides, FLAs, phytocyanins and lipid transfer proteins, as well as β -1,3-glucanases, are among the GPI-anchored proteins identified in arabidopsis pollen tubes by transcriptomic and proteomic analyses (Lalanne et al., 2004). The question remains, however, to what extent the GPI-anchor of a particular protein affects its function. In arabidopsis, previous studies demonstrated that proteins with FAS domains may function as adhesion molecules (Kim et al., 2002). FLA3 is involved in microspore development and may affect pollen intine formation, possibly by impacting on cellulose deposition. In FLA3-overexpressing transgenic plants, defective elongation of the stamen filament and reduced female fertility led to short siliques with low seed set, suggesting that ectopic expression of FLA3 may reduce or disrupt cell growth (Li et al., 2010).

Role of stylar AGPs during pollen-tube growth. During pollen-tube growth through the style, navigation is controlled at the surface of the specialized transmitting-tract epidermis in plants with a hollow style such as *L. longiflorum* or in the intercellular matrix of the transmitting tract tissue in plants with a solid style like tobacco, tomato, *Arabidopsis*, *Actinidia* and *Amaranthus* where AGPs are abundant (Jauh and Lord, 1996; Li and Showalter, 1996; Lennon et al., 1998; Cheung et al., 2000; Coimbra and Duarte, 2003; de Graff et al., 2003; Coimbra et al., 2007). In lily (*L. longiflorum*), analysis of the stylar exudates by SDS–PAGE and Western blot indicates that the electrophoretic mobility of AGPs ranged from 97 to 200 kDa as revealed with JIM13 and active Yariv staining (Jauh and Lord, 1996). Similarly, AGPs from solid styles of tobacco ranged from 45 to 140 kDa (Cheung et al., 2000; de Graff et al., 2003). Several tobacco AGPs [Transmitting-Tract Specific (*NtTTS*) and the Pistil-specific Extensin-Like Protein III (PELP III)] from *N. tabacum* (AGPNa1, 120-kDa and *NtTTS*) and

N. alata have been purified from the stylar transmitting-tract tissue and characterized (Wang et al., 1993; Du et al., 1994, 1996; Lind et al., 1994; Sommer-Knudsen et al., 1996; Bosch et al., 2001). The 120-kDa, PELP III and *NtTTS* are not classical AGPs. The 120-kDa and PELP III have chimeric structures with both AGP and extensin properties (Lind et al., 1994; Schultz et al., 1997; Bosch et al., 2001) whereas *NtTTS* has an unusual protein to sugar ratio, 65 : 35 by weight, and a basic pI (Cheung and Wu, 1999). The AGPNa1, 120-kDa and PELP III are composed mainly of arabinose (45 %, 55 % and 48 %) and galactose (52 %, 45 % and 50 %), respectively, with the main linkages found in AGPs (t-Ara; 1,3,6-linked Gal in AGPNa1 and t-Ara; t-Gal; 1,3-linked Gal; 1,6-linked Gal and 1,3,6-linked Gal in 120-kDa and PELP III) but additionally the 1,2-linked arabinosyl residues generally found in extensin are found in the 120-kDa and PELP III (Lind et al., 1994; Gane et al., 1995; Du et al., 1996; Bosch et al., 2001).

Studies on *N. tabacum* and *N. alata* have shown that the glycosylated stylar TTS are able to attract and promote the growth of the pollen tubes *in vitro* (Wu et al., 2000) and transgenic tobacco plants with reduced level of *NtTTS* mRNA display a reduced fertility (Cheung et al., 1995). In contrast, deglycosylated *NtTTS* loses these properties, suggesting an important biological role of glycosylation (Cheung et al., 1995). During the growth of the pollen tubes in the extracellular matrix of the transmitting tissue, the loosely associated *NtTTS* is detected at the pollen-tube cell wall surface, in the inner callose wall and at the pollen-tube tip. The guidance of the pollen tubes is presumably promoted by a gradient of increasing glycosylation of the *NtTTS* from the top of the style to the ovary. Moreover, the pollen tubes are able to deglycosylate the *NtTTS* proteins and may incorporate the sugars (Wu et al., 1995). Together, these studies suggest that the carbohydrate moieties of AGPs may have several functions including signalling and/or as nutritional molecules (Cheung et al., 2000).

In *N. alata* unpollinated styles, the 120-kDa protein epitopes are evenly distributed in the extracellular matrix of the transmitting tissue (Lind et al., 1996). In pollinated styles with compatible pollen, the 120-kDa is concentrated in the inner wall adjacent to the pollen-tube cell wall and can be incorporated by the growing pollen tubes as shown by immunolabelling in the cytoplasm and vesicle/vacuole-like structures (Lind et al., 1996; Goldraij et al., 2006). With incompatible pollen, similar labelling was observed (Lind et al., 1996). PELP III is also localized in the intercellular matrix of the transmitting tissue in unpollinated styles. In pollinated styles, PELP III epitopes are no longer detected in the transmitting tract but found associated with the pollen-tube callose wall and callose plugs (de Graff et al., 2003). Unlike *NtTTS*, PELP III is apparently unmodified by the expanding pollen tubes (de Graff et al., 2003) and is not involved in promoting pollen-tube growth and attraction (Bosch et al., 2003). Interestingly, the stylar *NtTTS*, 120-kDa and PELP III are able to bind *in vitro* to S-RNases (Cruz-Garcia et al., 2005), the stylar ribonucleases responsible for the degradation of the pollen-tube rRNA and growth arrest of the S-incompatible pollen in the Solanaceae gametophytic system (McCubbin and Kao, 2000). These data suggest that

AGPs may be involved indirectly in the S-pollen rejection by forming a complex with the S-RNase that may promote the initial interaction with the growing pollen tubes (Cruz-Garcia *et al.*, 2005). Among these three glycoproteins, the 120-kDa is the more polymorphic in size, ranging from 80 kDa to 120 kDa between different compatible and incompatible plants, and suppression of the 120-kDa by RNAi resulted in a deficient S-specific pollen rejection. Thus, the 120-kDa is probably one of the partners required for the gametophytic S-rejection (Hancock *et al.*, 2005). More recently, Lee *et al.* (2008) showed using yeast two-hybrid and pull-down assays, that three pollen-tube proteins can interact with the C-terminal domain of the stylar 120-kDa and NaTTS: (1) an S-RNase binding protein, a putative E3 ubiquitin ligase, (2) a C2 domain-containing protein (NaPCCP) that can bind lipids and regulates cellular processes and (3) a putative cysteine protease that may degrade vacuolar proteins (Lee *et al.*, 2008). The NaPCCP is associated with the pollen tube plasma membrane and may function in the internal transport of the 120-kDa in the endomembrane system toward the vacuoles where the 120-kDa and S-RNase have been localized (Lee *et al.*, 2009; reviewed by Kumar and McClure, 2010).

Mostly studied in tobacco, the results indicate that stylar AGPs play important roles in pollen-tube growth, guidance, nutrition and pollen-tube rejection. Similar functions may be found in other species but experimental data are required in order to draw a general conclusion.

CONCLUSIONS AND PERSPECTIVES

AGPs are fascinating cell wall molecules because of their diversity in terms of glycan structures and functional properties. In the last decade, major advances have been made in understanding some of the functions of these proteoglycans in roots, pollen tubes and other organs. However, future challenges are numerous and diverse with structural and functional characterization of individual AGPs as outstanding questions. The role of AGPs in plant interaction with microorganisms, particularly in roots, is relatively poorly understood, although AGPs were known for a while to be present in roots and secreted in the rhizosphere. Interesting studies have been recently reported on the implication of AGPs in the interaction between the root and microorganisms. They offer enthusiastic and exciting new research directions to study the biology of AGPs. More particularly, the recent finding that AGPs in root exudates of pea are able to induce a polar attachment of *Rhizobium* (Xie *et al.*, 2012) support an active role of AGPs in plant–microbe interaction (see also Gaspar *et al.*, 2004). Apart from this role in symbiotic interaction, it has been shown recently that AGPs isolated from pea root tip are also capable of attracting zoospores of the pathogenic oomycete *Aphanomyces euteiches* responsible for root rot disease then inhibiting their germination (Cannesan *et al.*, 2012). Although investigation of AGP distribution at the cellular and tissue levels has been made possible through the use of several AGP-directed mAbs, most, if not all, of the mAbs are directed toward AG-glycan epitopes which are heterogeneous and structurally complex. Such complexity makes structural determination difficult, but efforts should be concentrated towards detailed characterization of the O-glycans of AGPs,

including root and pollen-tube AGPs. This is a key issue as few complete glycan structures for native AGPs have been reported thus far (Kieliszewski and Shpak, 2001; Tan *et al.*, 2010; Tryfona *et al.*, 2010). Furthermore, if we were to relate structure to function, it would be of importance to elucidate AG-structures using appropriate analytical tools as has been performed for other cell-wall components, e.g. xyloglucan (Lerouxel *et al.*, 2002; Nguema-Ona *et al.*, 2006, 2012). AG-moieties are composed of up to 100 sugar residues, and oligosaccharides consisting of various sugar residues could be released by specific glycosidases (Takata *et al.*, 2006; Tryfona *et al.*, 2010). The released oligosaccharides could be then tested to assess their potential role in controlling development, morphogenesis, or protection of root cells against pathogens. Also, mutant analysis has proven fundamental to functional analysis of many cell-wall components and is also likely to be a useful avenue for investigating the functions of AGPs.

Another highly challenging issue is to investigate the biosynthesis of AG-glycans. First, the GTs involved will have to be identified and characterized. AGP-associated glycan epitopes have already been found in association with Golgi cisternae, the *trans*-Golgi network and secretory vesicles (Fig. 2). Efforts to identify GTs (Qu *et al.*, 2008; Ellis *et al.*, 2010; Wu *et al.*, 2010) involved in the biosynthesis of these carbohydrate chains have been undertaken recently. Secondly, studies will have to be conducted on how these enzymes are organized within the endomembrane system and how such an organization leads to structural diversity. The third line of research would be to study whether and how the AG-moieties are remodelled by glycohydrolases in the apoplastic compartment.

Clearly advanced structural understanding, elucidation of mechanisms of AG biosynthesis, assessment of their potential role in cell interaction with microorganisms, their functions in pollen-tube growth and pollen–pistil interaction are all exciting questions for future research on AGPs.

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