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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

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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
reb1-1	*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).

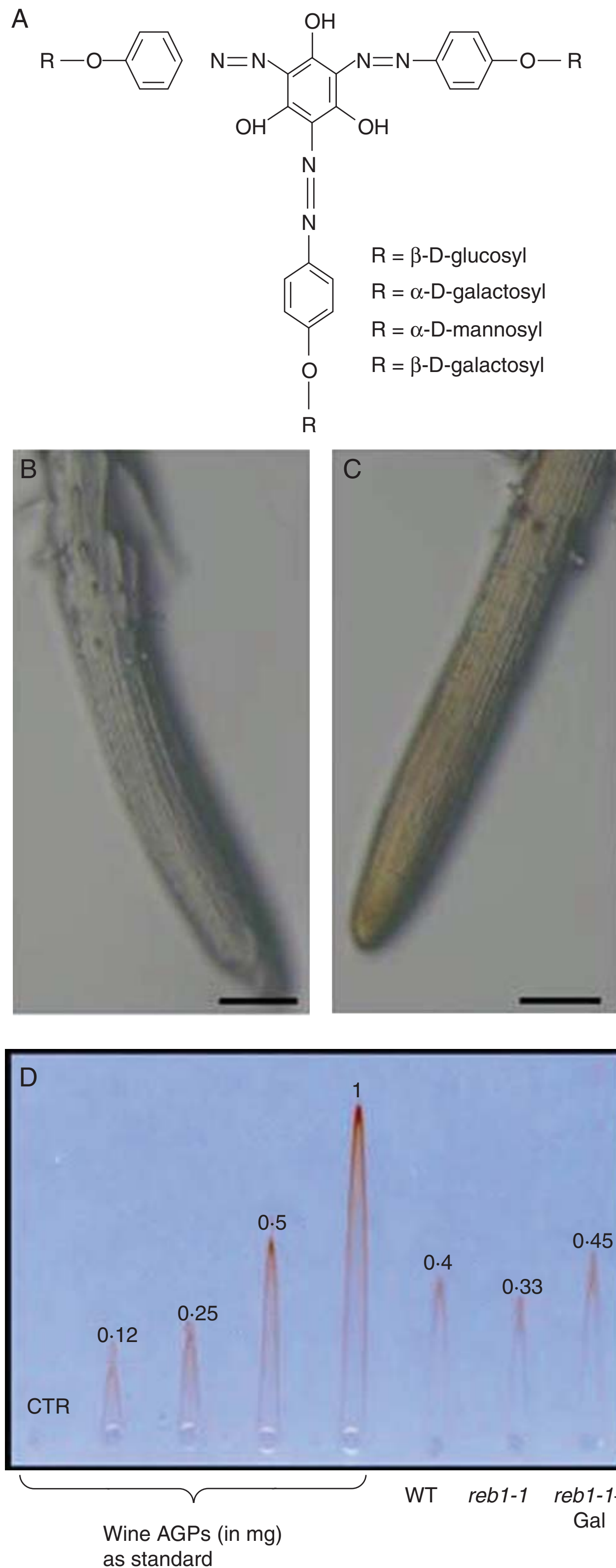
At the subcellular level, AGPs can be found in the cell wall, in the apoplast or anchored to the plasma membrane via a GPI anchor attached to the C-ter domain of the AGP backbone (Youl *et al.*, 1998; Svetek *et al.*, 1999). Cleavage of the GPI-anchor by phospholipases results on the release of the glycoprotein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



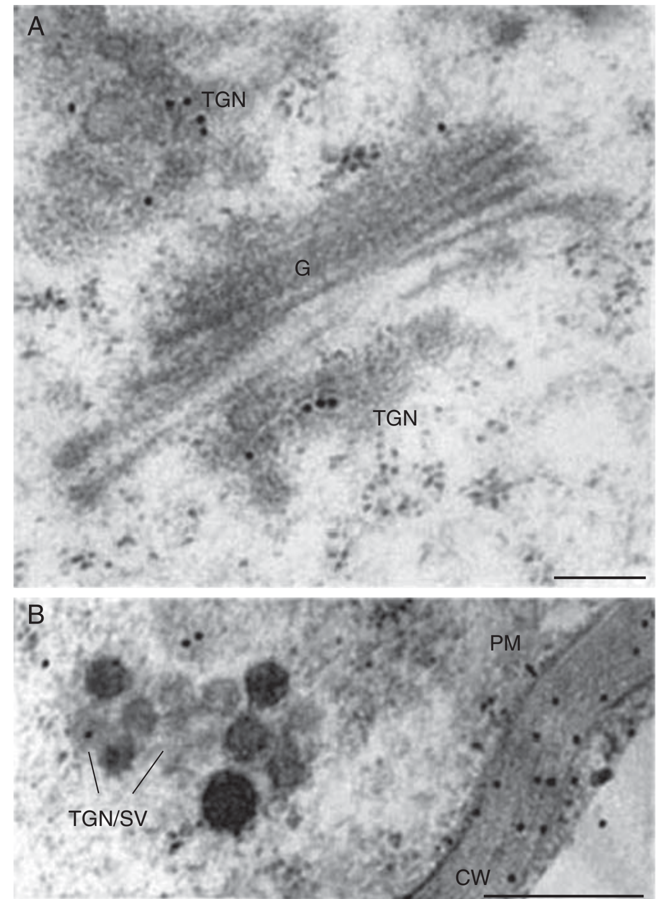


Fig. 2. Electron micrographs of arabidopsis root cells labelled with the anti-AGP antibody JIM14. Note the immuno-gold labelling of Golgi cisternae (G) and the *trans*-Golgi network (TGN) in (A), and secretory vesicles (SV), the plasma membrane (PM) and the cell wall (CW) in (B). AGPs are known to be 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-dehydroproline (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-Dehydroproline is known to interfere with the hydroxylation of proline residues and hence the production of Hyp residues leading to a reduction

Fig. 1. The Yariv reagent. (A) chemical structures of different phenylglycosides including active Yariv (β-D-glucosyl and β-D-galactosyl) and inactive Yariv (α-D-galactosyl and α-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 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 *et al.* (1996)
JIM8	AG from sugar beet	Unknown	Pennell *et al.* (1991)
JIM13	AGP from carrot	β-D-GlcA-(1,3)-α-D-GalA-(1,2)-α-L-Rha	Yates and Knox (1994); Yates *et al.* (1996)
JIM14	AGP from carrot	Unknown	Yates and Knox (1994); Yates *et al.* (1996)
JIM15	AGP from carrot	Unknown	Yates and Knox (1994); Yates *et al.* (1996)
JIM16	AGP from carrot	Unknown	Yates and Knox (1994); Yates *et al.* (1996)
LM2	AGP from rice	β-Linked GlcA	Smallwood *et al.* (1994); Yates *et al.* (1996)
MAC204	AGP from pea	Unknown	Bradley *et al.* (1988); Pennell *et al.* (1989)
MAC207	AGP from pea	β-GlcA-(1,3)-α-GalA-(1,2)-Rha	Bradley *et al.* (1988); van den Bosch *et al.* (1989)
MAC265	AGP fom pea	Unknown	van den Bosch *et al.* (1989)
PCBC3	AGP from *Nicotiana alata* style	Unknown	Fergusson *et al.* (1999)
CCRC-M7	Rhamnogalacturonan I	β-(1,6)-Gal chain carrying one or more Ara residues	Steffan *et al.* (1995)
PN 16·4B4	AG from *Nicotiana glutinosa*	Unknown	Norman *et al.* (1986)
MH4·3E5	AG from *Nicotiana tabacum*	Unknown	Hahn *et al.* (1987)
LM6	Sugar beet arabinan	α-(1,5)-L-linked arabinosyl heptasaccharide	Lee *et al.* (2005)
LM14	AGP from carrot	Arabinose- and galactose-enriched carbohydrate chains	Moller *et al.* (2008)
Antiserum			
Le-AGP1	Proteic backbone from tomato	Lysine-rich subdomain of Le-AGP1 proteic backbone	Gao *et al.* (1999)
Reagent			
Yariv reagent	AGP	n.a.	Yariv *et al.* (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.ccrc.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 *Lycopercison 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 lycopercisum*) (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 Gal_4; 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 (Motose *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

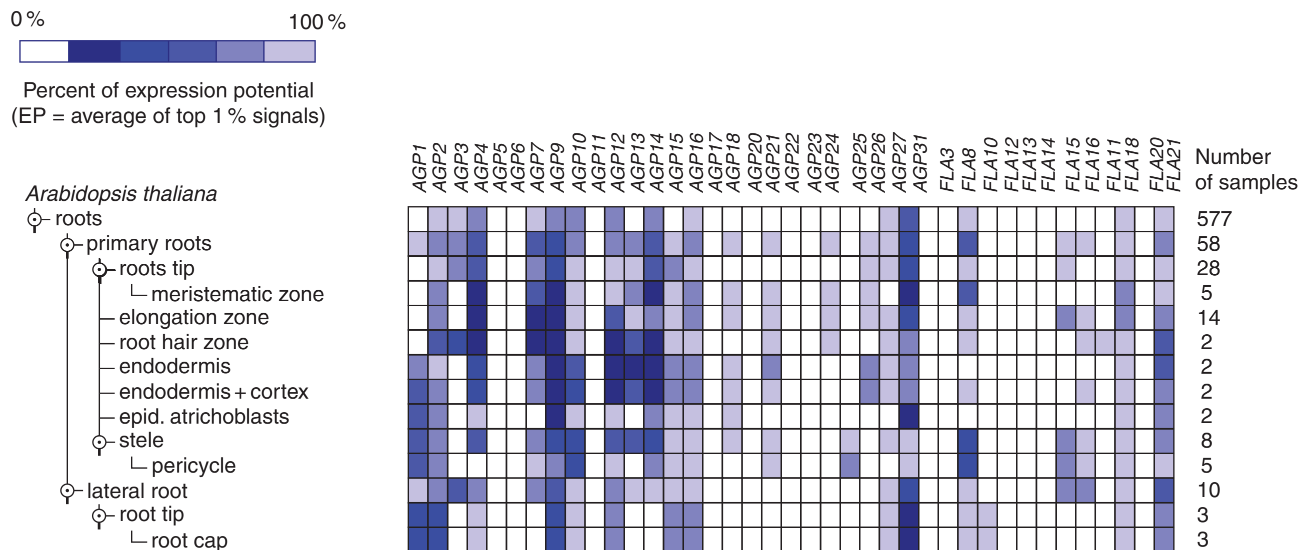


Fig. 3. Relative level of expression of AGP and FLA genes in different root tissues of *Arabidopsis thaliana*. The figure was generated using Genevestigator (Zimmermann *et al.*, 2004).

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* (*murus 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 (*Anguila anguila*) 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, Driouich 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 *At*AGP30, *Daucus carota* AGP1 (*Dc*AGP1), a close homologue of *At*AGP30, 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 *At*AGP30 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 *At*AGP30 caused a general decrease in the perception of the hormone abscissic acid. This led to the proposal by Gens *et al.* (2000) and Wagner and Kohorn (2001) that *At*AGP30 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. *At*AGP30 is expressed in non-hairy epidermal cells (atrichoblasts) 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 abscissic acid-dependent manner. *At*AGP30 is also expressed in cortical, endodermal and vascular tissues of the mature part of the roots (van Hengel *et al.*, 2004). *At*AGP31 is a close homologue of *At*AGP30 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 (Kawamato *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. *At*FLA1 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* (*r*esistant to *a*grobacterium *t*ransformation *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 Yariv 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 *At*AGP17 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 *Le*AGP-1 and *Na*AGP4 (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 treads, 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 progess 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; Driouich *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; Driouich *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).

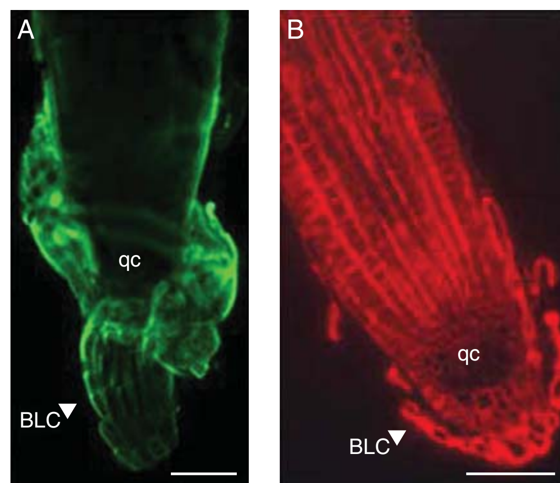


FIG. 4. Arabidopsis root tips and associated border-like cells labelled with anti-AGP antibodies: JIM13 (A) and JIM14 (B). Note the strong labelling associated with border-like cells in (A) (secondary antibody conjugated to FITC) and the lack of labelling in the quiescent centre in (B) (secondary antibody 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 μm.

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 *qua1-1* 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 *qua1-1* 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 alata* 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 (Ra*e 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 (*Camelia 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).

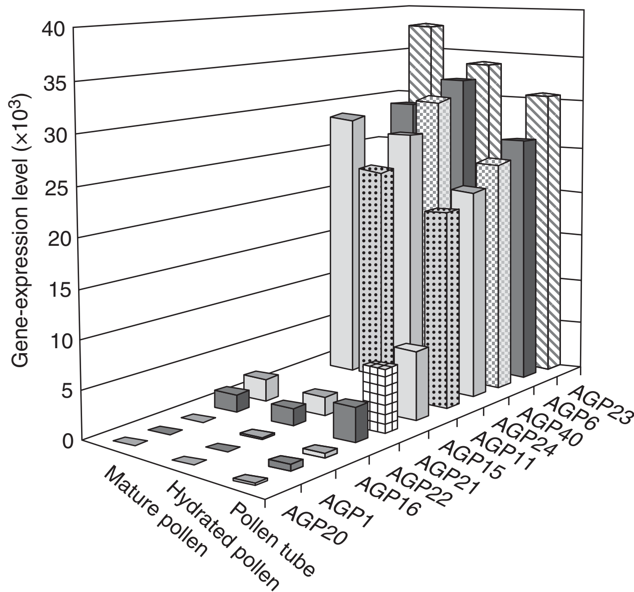


FIG. 5. ATH1 genome array showing the gene-expression level for the different AGPs, at three stages of development: mature pollen, hydrated pollen and pollen tube (according to Wang *et al.*, 2008).

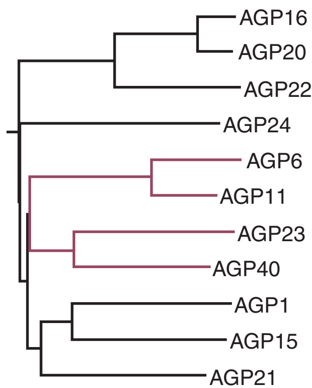


FIG. 6. Clustal W sequence homology alignment for the 11 pollen AGPs. AGP6, AGP11, AGP23 and AGP40 are the only four pollen-specific AGPs in arabidopsis. AGP6 and AGP11 present the highest similarity scores and are closely related to AGP23 and AGP40 (highlighted in red)

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 methylesterases (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 wilsonni* and *Pinus densiflora*), the angiosperm monocots (*Lilium longiflorum* and *Lolium perenne*) and eudicots (*Brassica napus*, *Beta vulgaris*, *Nicotiana tabacum*, *Nicotiana alata* 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

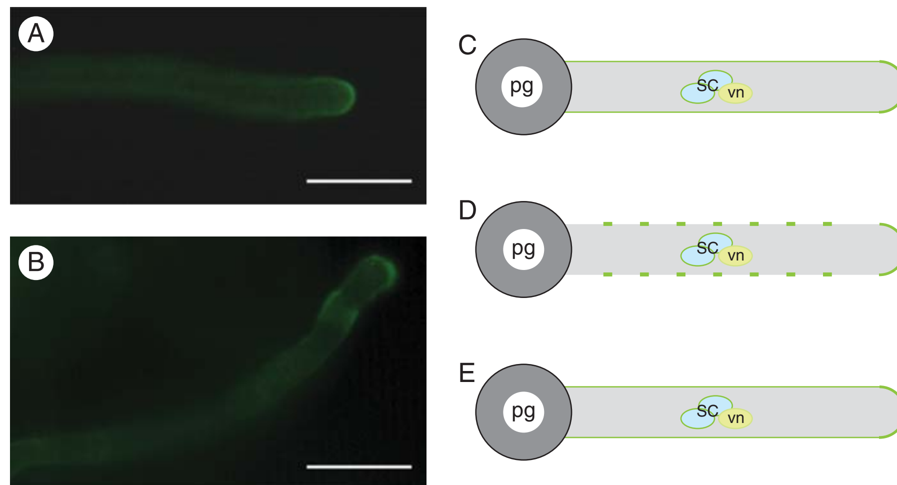


Fig. 7. Immunolocalization of AGPs in *Arabidopsis thaliana* pollen tube and the most-common AGP-labelling patterns observed in pollen tubes. (A) Cell surface immuno-labelling of *A. thaliana* pollen tube with LM2 and (B) with MAC207. Immuno-labelling patterns of AGP epitopes (in green) found in (C) *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 µm 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 µm min^{-1} based on the length of the pollen tubes after 6 h) (Jauh and Lord, 1996), *N. tabacum* (average of 2·7 µm min^{-1} based on the length of the pollen tubes after 5 h) (Mollet *et al.*, 2002) or arabidopsis (average of 1·3 µm 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 (*Os*AGP) 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						
Cycas revoluta	JIM13, LM2	EM/CF	*In vitro*	PG and PT	JIM13: strong labelling of the intine and PT; LM2: strong labelling PT, no labelling of the intine wall	Yatomi *et al.* (2002)
Ginkgoales						
Ginkgo biloba	JIM13, LM2	EM/CF	*In vitro*	PG and PT	JIM13 and LM2: strong labelling of the intine and PT wall	Yatomi *et al.* (2002)
Pinales						
Podocarpus macrophyllus	JIM13, LM2	EM/CF	*In vitro*	PG and PT	JIM13: labelling of the intine and PT wall	Yatomi *et al.* (2002)
Podocarpus nagi					LM2 : weak labelling	
Abies veitchii	JIM13, LM2	EM/CF	*In vitro*	PG and PT	JIM13: labelling of the intine and PT wall LM2 : no labelling of the PT, labelling of the intine wall	Yatomi *et al.* (2002)
Cedrus deodara	JIM13, LM2	EM/CF	*In vitro*	PG and PT	JIM13: strong labelling of the intine and PT wall	Yatomi *et al.* (2002)
Chamaecyparis obtusa					LM2 : no labelling	
Picea wilsonii	LM2	FM	*In vitro*	PT	Whole PT wall	Chen *et al.* (2008)
	LM2	FM	*In vitro*	De-exined PG	Intine surface	Fang *et al.* (2008)
Picea meyeri	LM2	FM	*In vitro*	PT	Periodic ring-like pattern along the whole PT wall	Chen *et al.* (2007)
Pinus densiflora	JIM13, LM2	EM/CF	*In vitro*	PG and PT	JIM13 and LM2: intine wall of PG. Whole PT wall (tip and back) and secretory vesicles LM2: generative cell wall	Mogami *et al.* (1999); Yatomi *et al.* (2002)
Pinus bungeana	LM2	FM	*In vitro*	De-exined PG	Intine surface	Fang *et al.* (2008)
Pinus banksiana	JIM13, LM2	EM/CF	*In vitro*	PG and PT	JIM13and LM2: labelling of the intine and PT wall	Yatomi *et al.* (2002)
Pinus rigida						
Cryptomeria japonica						
Pinus elliottii	JIM13, LM2	EM/CF	*In vitro*	PG and PT	JIM13: labelling of the intine and PT wall LM2: weak labelling of the PT, no labelling of the intine	Yatomi *et al.* (2002)
Angiosperms						
Monocot						
Liliales						
Lilium longiflorum	JIM13	FM	*In vitro*	PG protoplast	Cell surface	Zhao *et al.* (2004)
	JIM13	EM/HPF	*In vivo*	PT	PM, inner cell wall and secretory vesicles	Roy *et al.* (1997)
	JIM13-16, LM2, MAC207	FM	*In vitro* and *in vivo*	PT	PT tip wall	Jauh and Lord (1996) Mollet *et al.* (2002)
	JIM13, LM2, MAC207	FM	*In vitro* and *in vivo*	Pectinase treated PT	Ring-like pattern along the whole PT wall	Jauh and Lord (1996)
	βGlcY (30 μM)	LM	*In vitro*	PT	PT tip wall, arrest of growth	Mollet *et al.* (2002)
	JIM13, LM2, MAC207	EM/CF	*In vivo*	PT	PM, secretory vesicles and generative cell membrane	Jauh and Lord (1996)
Poales						
Lolium perenne	JIM13	EM/CF	*In vivo*	PG formation	Microspore wall	Wisniewska and Majewska-Sawka (2006)
Eudicot						
Magnoliales						
Annona cherimola	JIM13	FM	*In vitro*	PT	Whole PT wall	Mollet *et al.* (2002)
	βGlcY (30 μM)	LM	*In vitro*	PT	Expanded region from the tip, arrest of growth	

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

* 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 *Os*AGP. The *Os*AGP 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 *Os*AGP 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 (Lamport *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

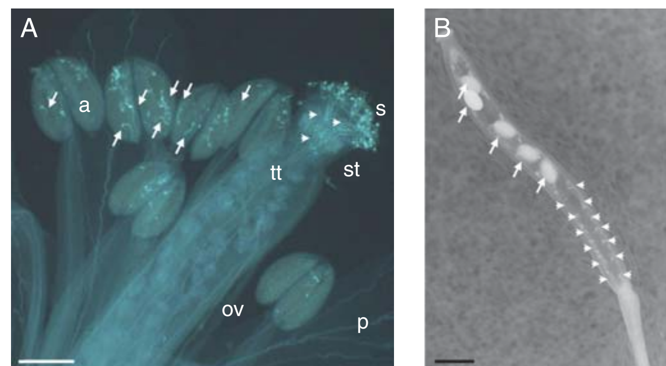


FIG. 8. Triple-null arabidopsis mutant plant for *AGP6*, *AGP11* and *AGP40*. (A) Decolourized aniline blue-stained flower. The anthers show a significant number of pollen tubes growing inside (arrows). Nevertheless some pollen grains maintain their normal germination behaviour and pollen tubes are visibly 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 (*Nt*TTS) and the Pistil-specific Extensin-Like Protein III (PELPIII)] from *N. tabacum* (AGP*Na*1, 120-kDa and *Na*TTS) 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, PELPIII and *Nt*TTS are not classical AGPs. The 120-kDa and PELPIII have chimeric structures with both AGP and extensin properties (Lind *et al.*, 1994; Schultz *et al.*, 1997; Bosch *et al.*, 2001) whereas *Nt*TTS has an unusual protein to sugar ratio, 65 : 35 by weight, and a basic pI (Cheung and Wu, 1999). The AGP*Na*1, 120-kDa and PELPIII 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 AGP*Na*1 and t-Ara; t-Gal; 1,3-linked Gal; 1,6-linked Gal and 1,3,6-linked Gal in 120-kDa and PELPIII) but additionally the 1,2-linked arabinosyl residues generally found in extensin are found in the 120-kDa and PELPIII (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 *Nt*TTS mRNA display a reduced fertility (Cheung *et al.*, 1995). In contrast, deglycosylated *Nt*TTS looses 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 *Nt*TTS 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 *Nt*TTS from the top of the style to the ovary. Moreover, the pollen tubes are able to deglycosylate the *Nt*TTS 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). PELPIII is also localized in the intercellular matrix of the transmitting tissue in unpollinated styles. In pollinated styles, PELPIII 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 *Nt*TTS, PELPIII 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 *Na*TTS, 120-kDa and PELPIII 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-hydrid and pull-down assays, that three pollen-tube proteins can interact with the C-terminal domain of the stylar 120-kDa and *Na*TTS: (1) an S-RNase binding protein, a putative E3 ubiquitin ligase, (2) a C2 domain-containing protein (*Na*PCCP) that can bind lipids and regulates cellular processes and (3) a putative cysteine protease that may degrade vacuolar proteins (Lee *et al.*, 2008). The *Na*PCCP 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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LITERATURE CITED

van Aelst AC, van Went JL. 1992. Ultrastructural immuno-localization of pectins and glycoproteins in *Arabidopsis thaliana* pollen grains. *Protoplasma* **168**: 14–19.

Albersheim P, Darvill A, Augur C, *et al*. 1992. Oligosaccharins: oligosaccharides regulatory molecules. *Accounts of Chemical Research* **25**: 77–83.

Anand S, Tyagi AK. 2010. Characterization of a pollen-preferential gene OSIAGP from rice (*Oryza sativa* L. subspecies *indica*) coding for an arabinogalactan protein homologue, and analysis of its promoter activity during pollen development and pollen tube growth. *Transgenic Research* **19**: 385–397.

Andème-Onzighi C, Sivaguru M, Judy-March J, Baskin TI, Driouich A. 2002. The *reb1-1* mutation of *Arabidopsis* alters the morphology of trichoblasts, the expression of arabinogalactan proteins and the organisation of cortical microtubules. *Planta* **215**: 949–958.

Bacic A, Moody SF, Clarke AE. 1988. Structural analysis of secreted root slime from maize. *Plant Physiology* **80**: 771–777.

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. 2006. The role of root exudates in rhizosphere interactions with plants and others organisms. *Annual Review in Plant Biology* **57**: 233–266.

Baldwin TC, Domingo C, Schindler T, Seetharaman G, Steacey N, Roberts K. 2001. DcAGP1, a secreted arabinogalactan protein, is related to a family of basic proline-rich proteins. *Plant Molecular Biology* **5**: 421–435.

Balestrini R, Lanfranco L. 2006. Fungal and plant gene expression in arbuscular mycorrhizal symbiosis. *Mycorrhiza* **16**: 509–524.

Baskin TI, Bertzner AS, Hoggart R, Cork A, Williamson RE. 1992. Root morphology mutants in *Arabidopsis thaliana*. *Australian Journal of Plant Physiology* **19**: 427–437.

Berry AM, Rasmussen U, Bateman K, Huss-Danell K, Lindwall S, Bergman B. 2002. Arabinogalactan proteins are expressed at the symbiotic interface in root nodule of *Alnus* spp. *New Phytologist* **155**: 469–479.

Boisson-Dernier A, Kessler SA, Grossniklaus U. 2011. The walls have ears: the role of plant CrRLK1Ls in sensing and transducing extracellular signals. *Journal of Experimental Botany* **62**: 1581–1591.

Borges F, Gomes G, Gardner R, *et al*. 2008. Comparative transcriptomics of *Arabidopsis* sperm cells. *Plant Physiology* **148**: 1168–1181.

Borner GHH, Sherrier DJ, Stevens TJ, Arkin IT, Dupree P. 2002. Prediction of glycosylphosphatidylinositol-anchored proteins in *Arabidopsis*.: a genomic analysis. *Plant Physiology* **129**: 486–499.

Borner GHH, Lilley KS, Stevens TJ, Dupree P. 2003. Identification of glycosylphosphatidylinositol-anchored proteins in *Arabidopsis*: a proteomic and genomic analysis. *Plant Physiology* **132**: 568–577.

Borner GHH, Sherrier DJ, Weimar T, *et al*. 2005. Analysis of detergent-resistant membranes in *Arabidopsis*: evidence for plasma membrane lipid rafts. *Plant Physiology* **137**: 104–116.

van den Bosch KA, Bradley DJ, Knox JP, Perotto S, Butcher GW, Brewin NJ. 1989. Common components of the infection thread matrix and the intercellular space identified by immunocytochemical analysis of pea nodules and uninfected roots. *EMBO Journal* **8**: 335–341.

Bosch M, Knudsen JS, Derksen J, Mariani C. 2001. Class III pistil-specific extensin-like proteins from tobacco have characteristics of arabinogalactan proteins. *Plant Physiology* **125**: 2180–2188.

Bosch M, Derksen J, Mariani C. 2003. A functional study of stylar hydroxyproline-rich glycoproteins during pollen tube growth. *Sexual Plant Reproduction* **16**: 87–98.

Bradley DJ, Wood EA, Larkins AP, Galfre G, Butcher GW, Brewin NJ. 1988. Isolation of monoclonal antibodies reacting with peribacteroid membranes and other components of pea root nodules containing *Rhizobium leguminosarum*. *Planta* **173**: 149–160.

van Buuren ML, Malonado-Mendoza IE, Trieu AT, Blaylock LA, Harrison MJ. 1999. Novel genes induced during an arbuscular mycorrhizal (AM) symbiosis formed between *Medicago truncatula* and *Glomus versiforme*. *Molecular Plant–Microbe Interactions* **12**: 171–181.

Cannesan MA, Durand C, Burel C, *et al*. 2012. Effect of arabinogalactan proteins from the root caps of *Pisum sativum* and *Brassica napus* on *Aphanomyces euteiches* zoospores chemotaxis and germination. *Plant Physiology*, in press. http://dx.doi.org/10.1104/pp.112.198507.

Cervone F, Hahn GH, De Lorenzo G, Darvill A, Albersheim P. 1989. Host–pathogen interactions. XXXIII. A plant protein converts a fungal pathogenesis factor into an elicitor of plant defense responses. *Plant Physiology* **90**: 542–548.

Chabaud M, Venard C, Defaux-Petras A, Bécard G, Barker DG. 2002. Targeted inoculation of *Medicago truncatula in vitro* root cultures reveals *MtENOD11* expression during early stages of infection by arbuscular mycorrhizal fungi. *New Phytologist* **156**: 265–273.

Chen C-G, Pu Z-Y, Moritz RL, *et al*. 1994. Molecular cloning of a gene encoding an arabinogalactan-protein from pear (*Pyrus communis*) cell suspension culture. *Proceedings of the National Academy of Sciences of the USA* **91**: 10305–10309.

Chen KM, Wu GL, Wang YH, *et al*. 2008. The block of intracellular calcium release affects the pollen tube development of *Picea wilsonii* by changing the deposition of cell wall components. *Protoplasma* **233**: 39–49.

Chen T, Teng N, Wu X, *et al*. 2007. Disruption of actin filaments by latrunculin B affects cell wall construction in *Picea meyeri* pollen tube by disturbing vesicle trafficking. *Plant Cell Physiology* **48**: 19–30.

Cheung AY, Wu HM. 1999. Arabinogalactan proteins in plant sexual reproduction. *Protoplasma* **208**: 87–98.

Cheung AY, Wang H, Wu HM. 1995. A floral transmitting tissue-specific glycoprotein attracts pollen tubes and stimulates their growth. *Cell* **82**: 383–393.

Cheung AY, Wu HM, Di Stilio V, *et al*. 2000. Pollen–pistil interactions in *Nicotiana tabacum*. *Annals of Botany* **85** (Suppl. A): 29–37.

Coimbra S, Duarte C. 2003. Arabinogalactan proteins may facilitate the movement of pollen tubes from the stigma to the ovules in *Actinidia deliciosa* and *Amaranthus hypochondriacus*. *Euphytica* **133**: 171–178.

Coimbra S, Almeida J, Junqueira V, Costa M, Pereira LG. 2007. Arabinogalactan proteins as molecular markers in *Arabidopsis thaliana* sexual reproduction. *Journal of Experimental Botany* **58**: 4027–4035.

Coimbra S, Costa ML, Jones BJ, Mendes MA, Pereira LG. 2009. Pollen grain development is compromised in *Arabidopsis agp6 agp11* null mutants. *Journal of Experimental Botany* **60**: 3133–3142.

Coimbra S, Costa ML, Mendes MA, Pereira A, Pinto J, Pereira LG. 2010. Early germination of *Arabidopsis* pollen in a double null mutant for the arabinogalactan protein genes AGP6 and AGP11. *Sexual Plant Reproduction* **23**: 199–205.

Costa ML, Pereira LG, Coimbra S. 2011. Arabidopsis pollen specific AGPs are essential for pollen development and fitness. In: *Workshop on Molecular Mechanisms Controlling Flower Development*, 14–17 June 2011, Maratea, Italy.

Cooper JB, Varner JE. 1983. Selective inhibition of proline hydroxylation by 3,4-dehydroproline. *Plant Physiology* **73**: 324–328.

Creelman RA, Mullet JE. 1997. Oligosaccharins, brassinolides, and jasmonates: non traditional regulators of plant growth, development and gene expression. *The Plant Cell* **9**: 1211–1223.

Cruz-Garcia F, Hancock CN, Kim D, McClure BA. 2005. Stylar glycoproteins bind to S-RNase *in vitro*. *The Plant Journal* **42**: 295–304.

Dardelle F, Lehner A, Ramdani Y, *et al*. 2010. Biochemical and immunocytological characterizations of *Arabidopsis* pollen tube cell wall. *Plant Physiology* **153**: 1563–1576.

Ding L, Zhu JK. 1997. A role for arabinogalactan-proteins in root epidermal cell expansion. *Planta* **203**: 289–294.

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

Dolan L, Linstead P, Roberts K. 1995. An AGP epitope distinguishes a central metaxylem initial from other vascular initials in the *Arabidopsis* roots. *Protoplasma* **189**: 145–155.

Downie JA. 2010. The roles of extracellular proteins, polysaccharides and signals in the interactions of rhizobia with legumes roots. *FEMS Microbiology Reviews* **34**: 150–170.

Dresselhaus T, Márton ML. 2009. Micropylar pollen tube guidance and burst: adapted from defense mechanisms? *Current Opinion in Plant Biology* **12**: 773–780.

Driouich A, Baskin TI. 2008. Intercourse between cell wall and cytoplasm exemplified by arabinogalactan proteins and cortical microtubules. *American Journal of Botany* **95**: 1491–1497.

Driouich A, Zhang GF, Staehelin LA. 1993. Effect of Brefeldin A on the structure of the Golgi apparatus and on the synthesis and secretion of proteins and polysaccharides in sycamore suspension culture cells. *Plant Physiology* **101**: 1363–1373.

Driouich A, Durand C, Vicré-Gibouin M. 2007. Formation and separation of root border cells. *Trends in Plant Science* **12**: 14–19.

Driouich A, Durand C, Cannesan MA, Percoco G, Vicré-Gibouin M. 2010. Border cells versus border-like cells: are they alike? *Journal of Experimental Botany* **61**: 3827–3831.

Driouich A, Cannesan MA, Dardelle F, *et al*. 2012. Unity is strength: the power of border cells and border like cells in relation with plant

defense. In:Vivanco JM, Baluska F, eds. *Secretions and exudates in biological systems*. Heidelberg: Springer, 91–108.

Du H, Simpson RJ, Moritz RL, Clarke AE, Bacic A. 1994. lsolation of the protein backbone of an arabinogalactan-protein from the styles of *Nicotiana alata* and characterization of a corresponding cDNA. *The Plant Cell* **6**: 1643–1653.

Du H, Clarke AE, Bacic A. 1996. Arabinogalactan-proteins: a class of extracellular matrix proteoglycans involved in plant growth and development. *Cell Biology* **6**: 411–414.

Durand C, Vicré-Gibouin M, Follet-Gueye ML, *et al*. 2009. The organization pattern of root border like cells of *Arabidopsis* is dependent on cell wall homogalacturonan. *Plant Physiology* **150**: 1411–1421.

Ellis M, Egelund J, Schultz CJ, Bacic A. 2010. Arabinogalactan proteins: key regulators at the cell surface. *Plant Physiology* **153**: 403–419.

Esquerré-Tugaye MT, Mazau D. 1974. Effect of a fungal disease on extension, the plant cell wall glycoprotein. *Journal of Experimental Botany* **25**: 509–513.

Estévez J, Kieliszewski MJ, Khitrov N, Somerville C. 2006. Characterization of synthetic hydroxyproline-rich proteoglycans with arabinogalactan-protein and extensin motifs in *Arabidopsis*. *Plant Physiology* **142**: 458–470.

Fang K, Wang Y, Yu T, *et al*. 2008. Isolation of de-exined pollen and cytological studies of the pollen intines from *Pinus bungeana* Zucc. Ex Endl. and *Picea wilsonii* Mast. *Flora* **203**: 332–340.

Feijó J, Malhó R, Obermeyer G. 1995. Ion dynamics and its possible role during *in vitro* pollen germination and tube growth. *Protoplasma* **187**: 155–167.

Ferguson C, Bacic A, Anderson MA, Read SM. 1999. Subcellular distribution of arabinogalactan proteins in pollen grains and tubes as revealed with a monoclonal antibody raised against stylar arabinogalactan proteins. *Protoplasma* **206**: 105–117.

Fernando DD, Quinn CR, Brenner E, Owens JN. 2010. Male gametophyte development and evolution in Gymnosperms. *International Journal of Plant Developmental Biology* **4**: 47–63.

Fincher GB, Stone BA, Clarke AE. 1983. Arabinogalactan-proteins: structure, biosynthesis and function. *Annual Review of Plant Physiology* **34**: 47–70.

Francis KE, Lam SY, Copenhaver GP. 2006. Separation of *Arabidopsis* pollen tetrads is regulated by QUARTET1, a pectin methylesterase gene. *Plant Physiology* **142**: 1004–1013.

Fry SC, Aldington S, Hetherington PR, Aitken J. 1993. Oligosaccharides as signals and substrates in the plant cell wall. *Plant Physiology* **103**: 1–5.

Gage DJ, Margolin W. 2000. Hanging by a thread: invasion of legume plants by rhizobia. *Current Opinion in Microbiology* **3**: 613–617.

Gane AM, Weinhandl JA, Bacic A, Harris PJ. 1994. Histochemistry and composition of the cell walls of styles of *Nicotiana alata* Link et Otto. *Planta* **195**: 217–225.

Gane AM, Craik D, Munro SLA, Howlett GJ, Clarke AE, Bacic A. 1995. Structural analysis of the carbohydrate moiety of arabinogalactan-proteins from stigmas and styles of *Nicotiana alata*. *Carbohydrate Research* **277**: 67–85.

Gao M, Showalter AM. 1999. Yariv reagent treatment induces programmed cell death in *Arabidopsis* cell cultures and implicated arabinogalactan proteins involvement. *The Plant Journal* **19**: 321–331.

Gao M, Kieliszewski MJ, Lamport DTA, Showalter AM. 1999. Isolation, characterization and immunolocalization of a novel, modular tomato arabinogalactan-protein corresponding to the *LeAGP1* gene. *The Plant Journal* **18**: 43–55.

Gaspar YM, Nam J, Schultz CJ, *et al*. 2004. Characterization of the Arabidopsis lysine-rich arabinogalactan-protein *AtAGP17* mutant (rat1) that results in a decreased efficiency of *Agrobacterium* transformation. *Plant Physiology* **135**: 2162–2171.

Geitmann A, Steer M. 2006. The architecture and properties of the pollen tube cell wall. In:Malhó R, ed. *The pollen tube: a cellular and molecular perspective (Plant Cell Monographs)*. Heidelberg: Springer: 177–200.

Genre A, Chabaud M, Timmers T, Bonfante P, Barker DG. 2005. Arbuscular mycorrhizal fungi elicits a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. *The Plant Cell* **17**: 3489–3499.

Gens JS, Fujiki M, Pickard BG. 2000. Arabinogalactan proteins and wall associated kinases in a plasmalemmal reticulum with specialized vesicles. *Protoplasma* **212**: 115–134.

Gerster J, Allard S, Robert LS. 1996. Molecular characterization of two *Brassica napus* pollen-expressed genes encoding putative arabinogalactan proteins. *Plant Physiology* **110**: 1231–1237.

Gianinazzi-Pearson V, Denarié J. 1997. Red carpet genetic programmes for root endosymbioses. *Trends in Plant Science* **10**: 371–372.

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

Goldraij A, Kondo K, Lee CB, *et al*. 2006. Compartmentalization of S-RNase and HT-B degradation in self-incompatible *Nicotiana*. *Nature* **439**: 805–810.

de Graaf BHJ, Knuiman BA, Derksen J, Mariani C. 2003. Characterization and localization of the transmitting tissue-specific PELPIII proteins of *Nicotiana tabacum*. *Journal of Experimental Botany* **54**: 55–63.

Grennan AK. 2007. Lipid raft in plants. *Plant Physiology* **143**: 1083–1085.

Hahn MG, Lerner DR, Fitter MS, Norman PM, Lamb CJ. 1987. Characterization of monoclonal antibodies to protoplast membranes of *Nicotiana tabacum* identified by an enzyme-linked immunosorbent assay. *Planta* **171**: 453–465.

Hancock CN, Kent L, McClure BA. 2005. The stylar 120-kDa glycoprotein is required for S-specific pollen rejection in *Nicotiana*. *The Plant Journal* **43**: 716–723.

Harris PJ, Ferguson LR, Roberton AM, Mckenzie RJ, White JB. 1992. Cell wall histochemistry and anatomy of taro (*Colocasia esculenta*). *Australian Journal of Botany* **40**: 207–222.

Hasegawa Y, Nakamura S, Kakizoe S, Sato M, Nakamura N. 1998. Immunocytochemical and chemical analyses of Golgi vesicles isolated from the germinated pollen of *Camellia japonica*. *Journal of Plant Research* **111**: 421–429.

Hawes MC, Gunawardena U, Miyasaka S, Zhao X. 2000. The role of border cells in plant defense. *Trends in Plant Science* **5**: 128–133.

van Hengel AJ, Roberts K. 2002. Fucosylated arabinogalactan-proteins are required for full root cell elongation in Arabidopsis. *The Plant Journal* **32**: 105–113.

van Hengel AJ, Roberts K. 2003. *At*AGP30, an arabinogalactan-protein in the cell walls of the primary root, plays a role in root regeneration and seed germination. *The Plant Journal* **36**: 256–270.

van Hengel AJ, van Kammen A, de Vries SC. 2002. A relationship between seeds development, arabinogalactan proteins (AGPs) and the AGP mediated promotion of somatic embryogenesis. *Physiologia Plantarum* **114**: 637–644.

van Hengel AJ, Barber C, Roberts K. 2004. The expression patterns of arabinogalactan-protein AtAGP30 and GLABRA2 reveal a role for abscisic acid in the early stages of root epidermal patterning. *The Plant Journal* **39**: 70–83.

Hepler PK, Vidali L, Cheung AY. 2001. Polarized cell growth in higher plants. *Annual Review of Cell and Developmental Biology* **17**: 159–187.

Hervé C, Marcus SE, Knox JP. 2011. Monoclonal antibodies, carbohydrate-binding modules, and the detection of polysaccharides in plant cell walls. In: Popper Z, ed. *The plant cell wall: methods and protocols*. New York, NY: Humana Press, 103–113.

Hird DL, Worrall D, Hodge R, Smartt S, Paul W, Scott R. 1993. The anther-specific protein encoded by the *Brassica napus* and *Arabidopsis thaliana* A6 gene displays similarity to β-1,3-glucanases. *The Plant Journal* **4**: 1023–1033.

Honys D, Twell D. 2004. Transcriptome analysis of haploid male gametophyte development in Arabidopsis. *Genome Biology* **5**: R85.

Hu Y, Qin Y, Zhao J. 2006. Localization of an arabinogalactan protein epitope and the effects of Yariv phenylglycoside during zygotic embryo development of *Arabidopsis thaliana*. *Protoplasma* **229**: 21–31.

Huang L, Cao J-S, Zhang A-H, Ye YQ. 2008. Characterization of a putative pollen-specific arabinogalactan protein gene, BcMF8, from *Brassica campestris* ssp. *chinensis*. *Molecular Biology Reports* **35**: 631–639.

Jauh GY, Lord EM. 1996. Localization of pectins and arabinogalactan-proteins in lily (*Lilium longiflorum* L.) pollen tube and style, and their possible roles in pollination. *Planta* **199**: 251–261.

Johnson KL, Jones BJ, Bacic A, Schultz CJ. 2003. The fasciclin arabinogalactan protein-like arabinogalactan proteins of Arabidopsis: a multigene family of putative cell adhesion molecules. *Plant Physiology* **133**: 1911–1925.

Johnson KL, Kibble NAJ, Bacic A, Schultz CJ. 2011. A fasciclin like arabinogalactan protein (FLA) mutant of *Arabidopsis thaliana*, *fla1*, shows defects in shoot regeneration. *PLoS One* **6** (9) e25154.

Johnson MA, Lord EM. 2006. Extracellular guidance cues and intracellular signaling pathways that direct pollen tube growth. In:Malho R, ed. *The pollen tube: a cellular and molecular perspective (Plant Cell Monographs)*. Heidelberg: Springer, 223–242.

Journet E-P, El-Gachtouli N, Vernoud V, *et al*. 2001. *Medicago truncatula ENOD11*: a novel RPRP-encoding early nodulin gene expressed during mycorrhization in arbuscule-containing cells. *Molecular Plant–Microbe Interactions* **14**: 737–748.

Kawamoto T, Noshiro M, Shen M, *et al*. 1998. Structural and phylogenetic analyses of RGD-CAP/beta ig-h3, a fasciclin-like adhesion protein expressed in chick chondrocytes. *Biochimica et Biophysica Acta* **1395**: 288–292.

Kessler S, Shimosato-Asano H, Friderike Keinath N, *et al*. 2010. Conserved molecular components for pollen tube reception and fungal invasion. *Science* **330**: 968–971.

Kieliszewski MJ, Shpak E. 2001. Synthetic genes for the elucidation of glycosylation codes for arabinogalactan-proteins and other hydroxyproline-rich glycoproteins. *Cell and Molecular Life Sciences* **58**: 1386–1398.

Kikuchi S, Ohinata A, Tsumuraya Y, Hashimoto Y, Kaneko Y, Matsushima H. 1993. Production and characterization of antibodies to the beta-1,6-galactotetraosyl group and their interaction with arabinogalactan proteins. *Planta* **190**: 525–535.

Kim JE, Jeong HW, Nam JO, *et al*. 2002. Identification of motifs in the fasciclin domains of the transforming growth factor-b-induced matrix protein big-h3 that interact with the avb5 integrin. *Journal of Biological Chemistry* **277**: 46159–46165.

Kjellbom P, Snogerup L, Stohr C, Reuzeau C, McCabe PF, Pennell RI. 1997. Oxidative crosslinking of plasma membrane arabinogalactan proteins. *The Plant Journal* **12**: 1189–1196.

Knee EM, Gong F-C, Gao M, *et al*. 2001. Root mucilage from pea and its utilization by rhizosphere bacteria as a sole carbon source. *Molecular Plant–Microbe Interactions* **14**: 775–784.

Knox JP. 1995. Developmental regulated proteoglycans and glycoproteins of the plant cell surface. *Federation of American Societies for Experimental Biology Letters* **9**: 1004–1012.

Knox JP. 1997. The use of antibodies to study the architecture and developmental regulation of plant cell walls. *International Review of Cytology* **171**: 79–120.

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

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

Kobayashi Y, Motose H, Iwamoto K, Fukuda H. 2011. Expression and genome-wide analysis of the xylogen-type gene family. *Plant and Cell Physiology* **52**: 1095–1106.

Kumar A, McClure B. 2010. Pollen–pistil interactions and the endomembrane system. *Journal of Experimental Botany* **61**: 2001–2013.

Lalanne E, Honys D, Johnson A, *et al*. 2004. SETH1 and SETH2, two components of the glycosylphosphatidylinositol anchor biosynthetic pathway, are required for pollen germination and tube growth in *Arabidopsis*. *The Plant Cell* **16**: 229–240.

Lamport DT, Kieliszewski MJ, Showalter AM. 2006. Salt-stress upregulates arabinogalactan-proteins: using salt-stress to analyse AGP function. *New Phytologist* **169**: 479–492.

Laus MC, Logman TJ, Lamers GE, Van Brussel AAN, Carlson RW, Kijne JW. 2006. A novel polar surface polysaccharide from *Rhizobium leguminosarum* binds host plant lectin. *Molecular Microbiology* **59**: 1704–1713.

Lee CB, Swatek KN, McClure B. 2008. Pollen proteins bind to the C-terminal domain of *Nicotiana alata* pistil arabinogalactan proteins. *Journal of Biological Chemistry* **283**: 26965–26973.

Lee CB, Kim S, McClure B. 2009. A pollen protein, NaPCCP, that binds pistil arabinogalactan proteins also binds phosphatidylinositol 3-phophate and associates with the pollen tube endomembrane system. *Plant Physiology* **149**: 791–802.

Lee KJD, Sakata Y, Mau S-L, *et al*. 2005. Arabinogalactan proteins are required for apical cell extension in the moss *Physcomitrella patens*. *The Plant Cell* **17**: 3051–3065.

Lennon KA, Lord EM. 2000. *In vivo* pollen tube cell of *Arabidopsis thaliana*. I. Tube cell cytoplasm and wall. *Protoplasma* **214**: 45–56.

Lennon KA, Roy S, Hepler PK, Lord E. 1998. The structure of the transmitting tissue of *Arabidopsis thaliana* (L.) and the path of pollen tube growth. *Sexual Plant Reproduction* **11**: 49–59.

Lerouxel O, Choo TS, Séveno M, *et al*. 2002. Rapid structural phenotyping of plant cell wall mutants by enzymatic oligosaccharide fingerprinting. *Plant Physiology* **130**: 1754–1763.

Levitin B, Richter D, Markovich I, Zik M. 2008. Arabinogalactan proteins 6 and 11 are required for stamen and pollen function in *Arabidopsis*. *The Plant Journal* **56**: 351–363.

Li J, Yu M, Geng L-L, Zhao J. 2010. The fasciclin-like arabinogalactan protein gene, FLA3, is involved in microspore development of *Arabidopsis*. *The Plant Journal* **64**: 482–497.

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

Li YQ, Bruun L, Pierson ES, Cresti M. 1992. Periodic deposition of arabinogalactan epitopes in the cell wall of pollen tubes of *Nicotiana tabacum* L. *Planta* **158**: 422–427.

Li YQ, Faleri C, Geitmann A, Zhang H-Q, Cresti M. 1995. Immunogold localization of arabinogalactan proteins, unesterified and esterified pectins in pollen grains and pollen tubes of *Nicotiana tabacum* L. *Protoplasma* **189**: 26–36.

Lind JL, Bacic A, Clarke A, Anderson MA. 1994. A style specific hydroxyproline-rich glycoprotein with properties of both extensins and arabinogalactan proteins. *The Plant Journal* **6**: 491–502.

Lind JL, Bönig I, Clarke AE, Anderson MA. 1996. A style-specific 120-kDa glycoprotein enters pollen tubes of *Nicotiana alata in vivo*. *Sexual Plant Reproduction* **9**: 75–86.

Liu C, Mehdy MC. 2007. A nonclassical arabinogalactan protein gene highly expressed in vascular tissues, AGP31, is transcriptionally repressed by methyl jasmonic acid in *Arabidopsis*. *Plant Physiology* **145**: 863–874.

McCabe PF, Valentine TA, Forsberg LS, Pennell RI. 1997. Soluble signals from cells identified at the cell wall establish a developmental pathway in carrot. *The Plant Cell* **9**: 2225–2241.

McCubbin AG, Kao TH. 2000. Molecular recognition and response in pollen and pistil interactions. *Annual Review of Cell and Developmental Biology* **16**: 333–364.

Ma H, Zhao J. 2010. Genome-wide identification, classification, and expression analysis of the arabinogalactan protein gene family in rice (*Oryza sativa* L.). *Journal of Experimental Botany* **61**: 2647–2668.

Ma W, Muthreich N, Liao C, *et al*. 2010. The mucilage proteome of maize (*Zea mays* L.) primary roots. *Journal of Proteome Research* **9**: 2968–2976.

Majeswska-Sawka A, Rodriguez-Garcia I. 2006. Immunodetection of pectin and arabinogalactan protein epitopes during pollen exine formation of *Beta vulgaris* L. *Protoplasma* **228**: 41–47.

Malhó R, Read N, Trewavas A, Pais MS. 1995. Calcium channel activity during pollen tube growth and reorientation. *The Plant Cell* **7**: 1173–1184.

Mashiguchi K, Asami T, Suzuki Y. 2009. Genome-wide identification, structure and expression studies, and mutant collection of 22 early nodulin-like protein genes in Arabidopsis. *Bioscience Biotechnology Biochemistry* **73**: 2452–2459.

Matthysse AG, Kijne JW. 1998. Attachment of *Rhizobiaceae* to plant cells. In:Spaink HP, Kondorosi A, Hooykaas PJJ, eds. *The rhizobiaceae*. Dordrecht: Kluwer Academic, 135–249.

Mogami N, Nakamura S, Nakamura N. 1999. Immunolocalization of the cell wall components in *Pinus densiflora* pollen. *Protoplasma* **206**: 1–10.

Moller I, Marcus SE, Haeger A, *et al*. 2008. High-throughput screening of monoclonal antibodies against plant cell wall glycans by hierarchical clustering of their carbohydrate microarray binding profiles. *Glycoconjugate Journal* **25**: 37–48.

Mollet JC, Kim S, Jauh GY, Lord EM. 2002. Arabinogalactan proteins, pollen tube growth, and the reversible effects of Yariv phenylglycoside. *Protoplasma* **219**: 89–98.

Mollet JC, Faugeron C, Morvan H. 2007. Cell adhesion, separation and guidance in compatible plant reproduction. *Annual Plant Reviews* **25**: 69–90.

Mongrand S, Morel J, Laroche J, *et al*. 2004. Lipid raft in higher plant cells: purification and characterization of of triton X-100-insoluble microdomains from tobacco plasma membrane. *Journal of Biological Chemistry* **279**: 36277–36286.

Moody SF, Clarke AE, Bacic A. 1988. Structural analysis of secreted lime from wheat and cowpea roots. *Phytochemistry* **27**: 2857–2861.

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

Nam J, Mysore KS, Zheng C, Knue MK, Mathysse AG, Gelvin SB. 1999. Identification of T-DNA tagged Arabidopsis mutants that are resistant to transformation by *Agrobacterium*. *Molecular and General Genetics* **261**: 429–438.

Nguema-Ona E, Andeme-Onzighi C, Aboughe-Angone S, *et al*. 2006. The reb1-1 mutation of Arabidopsis: effect on the structure and localization of galactose-containing cell wall polysaccharides. *Plant Physiology* **140**: 1406–1417.

Nguema-Ona E, Bannigan A, Chevalier L, Baskin TI, Driouich A. 2007. Disruption of arabinogalactan-proteins disorganizes cortical microtubules in the root of *Arabidopsis thaliana*. *The Plant Journal* **52**: 240–251.

Nguema-Ona E, Moore JP, Fagerström A, *et al*. 2012. Profiling the main cell wall polysaccharides of tobacco leaves using high throughput techniques. *Carbohydrate Polymers* **88**: 939–949.

Norman PM, Wingate VPM, Fitter MS, Lamb CJ. 1986. Monoclonal antibodies to plant plasma-membrane antigens. *Planta* **167**: 452–459.

Nothnagel E. 1997. Proteoglycans and related components in plant cells. *International Review of Cytology* **174**: 195–191.

Palanivelu R, Johnson MA. 2010. Functional genomics of pollen tube–pistil interactions in *Arabidopsis*. *Biochemical Society Transactions* **38**: 593–597.

Park BS, Kim JS, Kim SH, Park YD. 2005. Characterization of a pollen preferential gene, BAN102, from chinese cabbage. *The Plant Cell* **14**: 1–8.

Pattathil S, Avci U, Baldwin D, *et al*. 2010. A comprehensive toolkit of plant cell wall glycan-directed monoclonal antibodies. *Plant Physiology* **153**: 514–525.

Pennell RI, Knox JP, Scofield GN, Selvendran RR, Roberts K. 1989. A family of abundant plasma membrane-associated glycoproteins related to the arabinogalactan proteins is unique to flowering plants. *Journal of Cell Biology* **108**: 1967–1977.

Pennell RI, Janniche L, Kjellbom P, Scofield GN, Peart JM, Roberts K. 1991. Developmental regulation of a plasma membrane arabinogalactan protein epitope in oilseed rape flowers. *The Plant Cell* **3**: 1317–1326.

Pereira LG, Coimbra S, Oliveira H, Monteiro L, Sottomayor M. 2005. Expression of arabinogalactan protein genes in pollen tubes of *Arabidopsis thaliana*. *Planta* **223**: 374–380.

Pina C, Pinto F, Feijó JA, Becker JD. 2005. Gene family analysis of the *Arabidopsis* pollen transcriptome reveals biological implications for cell growth, division control, and gene expression regulation. *Plant Physiology* **138**: 744–756.

Pitzschke A, Hirt H. 2010. New insights into an old story: *Agrobacterium* induced tumour formation in plants by plant transformation. *EMBO Journal* **29**: 1021–1032.

Pogson BJ, Davies C. 1995. Characterization of a cDNA encoding the protein moiety of a putative arabinogalactan protein from *Lycopersicon esculentum*. *Plant Molecular Biology* **28**: 347–352.

Preuss D. 2002. Sexual signaling on a cellular level: lesson from plant reproduction. *Molecular Biology of the Cell* **13**: 1803–1805.

Puhlmann J, Bucheli E, Swain MJ, *et al*. 1994. Generation of monoclonal antibodies against plant cell wall polysaccharides. I. Characterization of a monoclonal antibody to a terminal α-(1→2)-linked fucosyl-containing epitope. *Plant Physiology* **104**: 699–710.

Qi W, Fong C, Lamport DTA. 1991. Gum arabic glycoprotein is a twisted hairy rope. *Plant Physiology* **96**: 848–855.

Qin Y, Zhao J. 2006. Localization of arabinogalactan proteins in egg cells, zygotes, and two-celled proembryos and effects of beta-D-glucosyl Yariv reagent on egg cell fertilization and zygote division in *Nicotiana tabacum*. *Journal of Experimental Botany* **57**: 2061–2074.

Qin Y, Chen D, Zhao J. 2007. Localization of arabinogalactan proteins in anther, pollen, and pollen tube of *Nicotiana tabacum* L. *Protoplasma* **231**: 43–53.

Qin Y, Leydon AR, Manziello A, *et al*. 2009. Penetration of the stigma and style elicits a novel transcriptome in pollen tubes, pointing to genes critical for growth in a pistil. *PLoS Genetics* **5**(8): e1000621. doi 10·1371/journal.pgen.1000621.

Qiu X, Wu Y Z, Du S, Erickson L. 1997. A new arabinogalactan protein-like gene expressed in the pollen of alfalfa. *Plant Science* **124**: 41–47.

Qu Y, Egelund J, Gilson PR, *et al*. 2008. Identification of a novel group of putative *Arabidopsis thaliana* β-(1,3)-galactosyltransferases. *Plant Molecular Biology* **68**: 43–59.

Rae AL, Harris PJ, Bacic A, Clarke AE. 1985. Composition of the cell walls of *Nicotiana alata* Link et Otto pollen tubes. *Planta* **166**: 128–133.

Raghavan V. 1997. Gene expression during pollen development. *In:* Molecular embryology of flowering plants. Cambridge: Cambridge University Press, 90–119.

Rathbun EA, Naldrett MJ, Brewin NJ. 2002. Identification of a family of extensin-like glycoproteins in the lumen of *Rhizobium*-induced infection threads in pea root nodules. *Molecular Plant–Microbe Interactions* **15**: 350–359.

Rhee SY, Osborne E, Poindexter PD, Somerville CR. 2003. Microspore separation in the *quartet 3* mutants of *Arabidopsis* is impaired by a defect in a developmentally regulated polygalacturonase required for pollen mother cell wall degradation. *Plant Physiology* **133**: 1170–1180.

Ringli C. 2010. Monitoring the outside: cell wall-sensing mechanisms. *Plant Physiology* **153**: 1445–1452.

Roudier F, Fernandez AG, Fujita M, *et al*. 2005. COBRA, an *Arabidopsis* extracellular glycosylphosphatidylinositol anchored protein, specifically controls highly anisotropic expansion through its involvement in cellulose microfibril orientation. *The Plant Cell* **17**: 1749–1763.

Roy S, Eckard KJ, Lancelle S, Kepler PK, Lord EM. 1997. High-pressure freezing improves the ultrastructural preservation of *in vivo* grown lily pollen tubes. *Protoplasma* **200**: 87–98.

Roy S, Jauh GY, Hepler PK, Lord EM. 1998. Effects of Yariv phenylglycoside on cell wall assembly in the lily pollen tube. *Planta* **204**: 450–458.

Samaj J, Braun M, Baluska F, Ensikat H-J, Tsumuraya Y, Volkmann D. 1999. Specific localization of arabinogalactan protein epitopes at the surface of maize root hairs. *Plant and Cell Physiology* **40**: 874–883.

Sardar HS, Yang J, Showalter AM. 2006. Molecular interactions of arabinogalactan-proteins with cortical microtubules and F-actin in Bright-Yellow-2 tobacco cultured cells. *Plant Physiology* **142**: 1469–1479.

Schultz CJ, Harrison MJ. 2008. Novel plant and fungal AGP like proteins in the *Medicago truncatula–Glomus intraradices* arbuscular mycorrhizal symbiosis. *Mycorrhiza* **18**: 403–412.

Schultz CJ, Hauser K, Lind JL, *et al*. 1997. Molecular characterisation of a cDNA sequence encoding the backbone of a style-specific 120-kDa glycoprotein which has features of both extensins and arabinogalactan-proteins. *Plant Molecular Biology* **35**: 833–845.

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

Schultz CJ, Johnson KL, Currie G, Bacic A. 2000. The classical arabinogalactan protein gene family of *Arabidopsis*. *The Plant Cell* **12**: 1751–1768.

Schultz CJ, Rumsewicz MP, Johnson KL, Jones BJ, Gaspar YM, Bacic A. 2002. Using genomic resources to guide research directions: the arabinogalactan protein gene family as a test case. *Plant Physiology* **129**: 1448–1463.

Seifert GJ, Roberts K. 2007. The biology of arabinogalactan proteins. *Annual Review in Plant Biology* **58**: 137–161.

Seifert GJ, Barber C, Wells B, Dolan L, Roberts K. 2002. Galactose biosynthesis in *Arabidopsis*: genetic evidence for substrate channeling from UDP-D-galactose into cell wall polymers. *Current Biology* **12**: 1840–1845.

Serpe MD, Nothnagel EA. 1994. Effects of Yariv phenyl glycosides on rose cell-suspensions: evidence for the involvement of arabinogalactan proteins in cell proliferation. *Planta* **193**: 542–550.

Shi H, Kim Y, Guo Y, Stevenson B, Zhu JK. 2003. The *Arabidopsis* SOS5 locus encodes a putative cell surface adhesion protein and is required for normal cell expansion. *The Plant Cell* **15**: 19–32.

Showalter AM. 2001. Arabinogalactan proteins: structure, expression and function. *Cellular and Molecular Life Sciences* **58**: 1399–1417.

Showalter AM, Keppler B, Lichtenberg J, Gu D, Welch LR. 2010. A bioinformatic approach to the identification, classification and analysis of hydroxyproline rich glycoproteins. *Plant Physiology* **153**: 485–513.

Shpak E, Barbar E, Leykam JF, Kieliszewski MJ. 2001. Contiguous hydroxyproline residues direct hydroxyproline arabinosylation in *Nicotiana tabacum*. *Journal of Biological Chemistry* **276**: 11272–11278.

Simon-Plas F, Perraki A, Bayer E, Gerbeau-Pissot P, Mongrand S. 2011. An update on plant membrane raft. *Current Opinion in Plant Biology* **14**: 642–649.

Smallwood M, Beven A, Donovan N, *et al*. 1994. Localization of cell wall protein in relation to the developmental anatomy of the carrot root apex. *The Plant Journal* **5**: 237–246.

Smallwood M, Yates EA, Willats WGT, Martin H, Knox JP. 1996. Immunochemical comparison of membrane-associated and secreted arabinogalactan proteins in rice and carrots. *Planta* **198**: 452–459.

Sommer-Knudsen J, Clarke AE, Bacic A. 1996. A galactose-rich, cell-wall glycoprotein from styles of *Nicotiana alata*. *The Plant Journal* **9**: 71–83.

Sørensen I, Domozych D, Willats WGT. 2010. How have plant cell walls evolved? *Plant Physiology* **153**: 366–372.

Speranza A, Taddei AR, Gambellini G, Ovidi E, Scoccianti V. 2009. The cell wall of kiwifruit pollen tubes is a target for chromium toxicity: alterations to morphology, callose pattern and arabinogalactan protein distribution. *Plant Biology* **11**: 179–193.

Steffan W, Kova P, Albersheim P, Darvill AG, Hahn MG. 1995. Characterization of a monoclonal antibody that recognizes an arabinosylated beta-1,6-D-galactan epitope in plant complex carbohydrates. *Carbohydrate Research* **275**: 295–307.

Sun W, Zhao ZD, Hare MC, Kieliszewski MJ, Showalter AM. 2004. Tomato LeAGP-1 is a plasma membrane-bound, glycosylphosphatidylinositol-anchored arabinogalactan-protein. *Physiologia Plantarum* **120**: 319–327.

Sun W, Xu J, Yang J, Kieliszewski MJ, Showalter AM. 2005. The lysine-rich arabinogalactan-protein subfamily in arabidopsis: gene expression, glycoprotein purification and biochemical characterization. *Plant and Cell Physiology* **46**: 975–984.

Svetek J, Yadav MP, Nothnagel EA. 1999. Presence of a glycosylphosphatidylinositol lipid anchor on rose arabinogalactan proteins. *Journal of Biological Chemistry* **274**: 14724–14733.

Takata R, Tokita K, Mori S, *et al*. 2010. Degradation of carbohydrate moieties of arabinogalactan-proteins by glycoside hydrolases from *Neurospora crassa*. *Carbohydrate Research* **345**: 2516–2522.

Tan L, Leykam JF, Kieliszewski MJ. 2003. Glycosylation motifs that direct arabinogalactan addition to arabinogalactan-proteins. *Plant Physiology* **132**: 1362–1369.

Tan L, Qiu F, Lamport DTA, Kieliszewski MJ. 2004. Structure of a hydroxyproline (Hyp)-arabinogalactan polysaccharide from repetitive Ala-Hyp expressed in transgenic *Nicotiana tabacum*. *Journal of Biological Chemistry* **279**: 13156–13165.

Tan L, Varnai P, Lamport DTA, *et al*. 2010. Plant *O*-glycosylation arabinogalactans are composed of repeating trigalactosyl subunits with short bifurcated side chains. *Journal of Biological Chemistry* **285**: 24575–24583.

Tang W, Ezcurra I, Muschietti J, McCormick S. 2002. A cysteine-rich extracellular protein, LAT52, interacts with the extracellular domain of the pollen receptor kinase LePRK2. *The Plant Cell* **14**: 2277–2287.

Tang X-C, He Y-Q, Wang Y, Sun M-X. 2006. The role of arabinogalactan proteins binding to Yariv reagents in the initiation, cell developmental fate, and maintenance of microspore embryogenesis in *Brassica napus* L. cv. Topas. *Journal of Experimental Botany* **57**: 2639–2650.

The Arabidopsis Genome Initiative. 2000. Analysis of the genome sequence of the Flowering plant *Arabidopsis thaliana*. *Nature* **408**: 796–814.

Tryfona T, Liang H-C, Kotake T, *et al*. 2010. Carbohydrate structural analysis of wheat flour arabinogalactan protein. *Carbohydrate Research* **345**: 2648–2656.

Tsumuraya Y, Ogura K, Hashimoto Y, Mukoyama H, Yamamoto S. 1988. Arabinogalactan-proteins from primary and mature roots of radish (*Raphanus sativus* L.). *Plant Physiology* **86**: 155–160.

Tsyganova AV, Tsyganov VE, Findlay KC, Borisov AY, Tikhonovich IA, Brewin NJ. 2009. Distribution of legume arabinogalactan proteins-extensin (AGPE) glycoproteins in symbiotically defective pea mutants with abnormal infection threads. *Cell and Tissue Biology* **3**: 93–102.

Velasquez SM, Ricardi MM, Dorosz JG, *et al*. 2011. *O*-Glycosylated cell wall proteins are essential in root hair growth. *Science* **332**: 1401–1403.

Vicré M, Santaella C, Blanchet S, Gateau A, Driouich A. 2005. Root border-like cells of *Arabidopsis*: microscopical characterization and role in the interaction with rhizobacteria. *Plant Physiology* **138**: 998–1008.

Vlad F, Spano T, Vlad D, Boudaher F, Ouelhadj A, Kalaitzis P. 2007. *Arabidopsis* prolyl-4 hydroxylases are differentially expressed in response to hypoxia, anoxia and mechanical wounding. *Physiologia Plantarum* **130**: 471–483.

Wagner TA, Kohorn BD. 2001. Wall-associated kinases are expressed throughout plant development and are required for cell expansion. *The Plant Cell* **13**: 303–318.

Walker TS, Bais HP, Grotewold E, Vivanco JM. 2003. Root exudation and rhizosphere biology. *Plant Physiology* **132**: 44–51.

Wang H, Wu HM, Cheung AY. 1993. Developmental and pollination regulation of the accumulation and glycosylation of a transmitting tissue-specific proline-rich glycoprotein. *The Plant Cell* **5**: 1639–1650.

Wang HJ, Huang JC, Jauh GY. 2010. Pollen germination and tube growth. *Advances in Botanical Research* **54**: 1–52.

Wang Y, Zhang WZ, Song LF, Zou JJ, Su Z, Wu WH. 2008. Transcriptome analyses show changes in gene expression to accompany pollen germination and tube growth in *Arabidopsis*. *Plant Physiology* **148**: 1201–1211.

Wen F, van Etten HD, Tsaprailis G, Hawes MC. 2007. Extracellular proteins in pea roots tip and border cell exudates. *Plant Physiology* **143**: 773–783.

Willats GTW, Knox JP. 1996. A role for arabinogalactan-proteins in plant cell expansion: evidence from studies on the interaction of β-glucosyl Yariv reagent with seedlings of *Arabidopsis thaliana*. *The Plant Journal* **9**: 919–925.

Williams A, Wilkinson A, Krehenbrink M, Russo DM, Zorreguieta A, Downie JA. 2008. Glucomannan-mediated attachment of *Rhizobium leguminosarum* to pea root hairs is required for competitive nodule infection. *Journal of Bacteriology* **190**: 4706–4715.

Wiśniewska E, Majewska-Sawka A. 2006. Cell wall polysaccharides in differentiating anthers and pistils of *Lolium perenne*. *Protoplasma* **228**: 65–71.

Wu HM, Wang H, Cheung AY. 1995. A pollen tube growth stimulatory glycoprotein is deglycosylated by pollent tubes and displays a glycosylation gradient in the flower. *Cell* **82**: 395–403.

Wu HM, Wong E, Ogdahl J, Cheung AY. 2000. A pollen tube growth-promoting arabinogalactan protein from *Nicotiana alata* is similar to the tobacco TTS protein. *The Plant Journal* **22**: 167–176.

Wu Y, Williams M, Bernard S, Driouich A, Showalter AM, Faik A. 2010. Functional identification of two nonredundant Arabidopsis (1,2)fucosyltransferases specific to arabinogalactan proteins. *Journal of Biological Chemistry* **285**: 13638–13645.

Xie D, Ma L, Samaj J, Xu C. 2011. Immunohistochemical analysis of cell wall hydroxyproline-rich glycoproteins in the roots of resistant and susceptible wax gourd cultivars in response to *Fusarium oxysporum* f. sp. *Benincasae* infection and fusaric acid treatment. *Plant Cell Report* **30**: 1555–1569.

Xie F, Williams A, Edwards A, Downie JA. 2012. A plant arabinogalactan-like glycoprotein promotes a novel type of polar surface attachment by *Rhizobium leguminosarum*. *Molecular Plant–Microbe Interactions* **25**: 250–258.

Xu J, Tan L, Lamport DTA, Showalter AM, Kieliszewski MJ. 2008. The *O*-Hyp glycosylation code in tobacco and Arabidopsis and a proposed role of Hyp-glycans in secretion. *Phytochemistry* **69**: 1631–1640.

Yang J, Kieliszewski MJ, Showalter AM. 2005. The lysine-rich arabinogalactan-protein subfamily in *Arabidopsis*: gene expression, glycoprotein purification and biochemical characterization. *Plant and Cell Physiology* **46**: 975–984.

Yariv J, Rapport MM, Graf L. 1962. The interaction of glycosides and saccharides with antibody to the corresponding phenylazo glycosides. *Biochemical Journal* **85**: 383–388.

Yariv J, Lis H, Katchalski E. 1967. Precipitation of arabic acid and some seed polysaccharides by glycosylphenylazo dyes. *Biochemical Journal* **105**: p1C–2C.

Yates EA, Knox JP. 1994. Investigations into the occurrence of plant cell surface epitopes in exudate gums. *Carbohydrate Polymers* **24**: 281–286.

Yates EA, Valdor J-F, Haslam SM, *et al*. 1996. Characterization of carbohydrate structural features recognized by anti arabinogalactan-protein monoclonal antibodies. *Glycobiology* **6**: 131–139.

Yatomi R, Nakamura S, Nakamura N. 2002. Immunochemical and cytochemical detection of wall components of germinated pollen of Gymnosperms. *Grana* **41**: 21–28.

Youl JJ, Bacic A, Oxley D. 1998. Arabinogalactan-proteins from *Nicotiana alata* and *Pyrus communis* contain glycosyl-phosphatidylinositol membrane anchors. *Proceedings of the National Academy of Sciences of the USA* **95**: 7921–7926.

Yu J, Wang J, Lin W, Li S, Li H, *et al*. 2005. The genomes of *Oryza sativa*: a history of duplications. *PLoS Biology* **3**: e38. doi: 10.1371/journal.pbio.0030038.

Zang Y, Yang J, Showalter A. 2011. AtAGP18, a lysine-rich arabinogalactan protein in *Arabidopsis thaliana*, functions in plant growth and development as a putative co-receptor for signal transduction. *Plant Signaling and Behaviour* **6**: 855–857.

Zhao J, Mollet JC, Lord EM. 2004. Lily (*Lilium longiflorum* L.) pollen protoplast adhesion is increased in the presence of the peptide SCA. *Sexual Plant Reproduction* **16**: 227–233.

Zhao ZD, Tan L, Showalter AM, Lamport DTA, Kieliszewski MJ. 2002. Tomato LeAGP-1 arabinogalactan-protein purified from transgenic tobacco corroborates the Hyp contiguity hypothesis. *The Plant Journal* **31**: 431–444.

Zimmermann P, Hirsch-Hoffmann M, Hennig L, Gruissem W. 2004. Genevestigator. Arabidopsis microarray database and analysis toolbox. *Plant Physiology* **136**: 2621–2632.