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In-situ analysis of pectic polysaccharides in seed mucilage and at the root surface of *Arabidopsis thaliana*

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Abstract. Pectic polysaccharides are a complex set of macromolecules of the primary cell wall matrix with distinct structural domains. The biosynthesis, organisation and function of these domains within cell wall matrices are poorly understood. An immersion immunofluorescence labelling technique was developed for the in-situ analysis of pectic polysaccharides at the surface of seeds and seedlings of *Arabidopsis thaliana* (L.) Heynh., and used to investigate the occurrence of pectic homogalacturonan (HG) and rhamnogalacturonan-I (RG-I) epitopes. Seed mucilage appeared to consist of two regions: a highly methyl-esterified HG was a major component throughout the mucilage, while an inner region with relatively low porosity was stabilized by calcium-based HG cross-linking. The small size and transparency of *Arabidopsis* roots allowed the occurrence of pectic HG and RG-I epitopes at root surfaces to be directly determined on whole-mount preparations. Pectic epitopes were not distributed evenly over root surfaces and were notably absent from lateral root apices and from the surface of root hairs. The use of defined antibody probes in the immersion immunolabelling protocol will be useful for the analysis of the influence of growth conditions and genetic factors on pectic polysaccharides in *Arabidopsis*.

Key words: *Arabidopsis* (pectic polysaccharides) – Homogalacturonan – Mucilage – Pectin – Polysaccharide – Root surface

Introduction

Pectic polysaccharides are structurally complex, multifunctional molecules of the matrix of primary cell walls of all land plants. The defining feature of pectic polysaccharides is the presence of a galacturonan backbone that can be substituted and modified in various ways. The homopolymer of (1 → 4)-α-linked D-galacturonic acid (homogalacturonan, HG) can have various states of methyl-esterification and may be acetylated or substituted with xylose or apiose (O'Neill et al. 1990; Mohnen 1999). A highly conserved branched domain, consisting of an HG backbone substituted with complex side chains, is known as rhamnogalacturonan-II (RG-II) (Mohnen 1999; Vidal et al. 2000). A domain consisting of repeats of the [→ 2)-α-L-rhamnose-(1 → 4)-α-D-galacturonic acid (1 →] dimer can be substituted with galactose and/or arabinose-rich side chains and is known as rhamnogalacturonan-I (RG-I) (O'Neill et al. 1990; Mohnen 1999).

The precise details of the nature of the attachments and interactions of the pectic domains are unknown although they are thought to form a network in which cellulose microfibrils and hemicellulose components are embedded. Some pectic polysaccharides have the capacity to form gels, the properties of which will influence matrix properties such as tensile and compression strengths and porosity. Pectic polysaccharides are also important in defence responses and contribute to defensive and developmental signalling pathways. Discrete populations of pectic domains occur in relation to pit fields on the inner face of primary cell walls (Orfila and Knox 2000), and on the opposite side of the wall at the middle lamellae, pectin is known to contribute to cell adhesion (Jarvis 1984). However, the occurrence and role of pectic polysaccharides at the outer face of unadhered cell walls at plant surfaces is less well documented. In many cases, a layer of pectin is thought to occur between the cell wall and the cuticle in aerial parts of land plants (Juniper and Jeffree 1983). Antibody probes for defined oligosaccharide structures in pectic domains allow the occurrence and nature of pectin at the

Abbreviations: CDTA = cyclohexanediamine-N,N,N′,N′-tetraacetate; FITC = fluorescein isothicyanate; HG = homogalacturonan; HRGP = hydroxyproline-rich glycoprotein; PBS = phosphate-buffered saline; RG = rhamnogalacturonan

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plant surface to be rapidly and reliably determined. In particular, the small size of *Arabidopsis* seeds and seedlings make it feasible to map the occurrence of pectic domains by immersion labelling of whole-mount preparations. Such an approach can provide insight into the occurrence and function of pectin at plant surfaces and, in addition, may provide a useful tool for the analysis of the structure-function relationships of pectic polysaccharides in general. *Arabidopsis,* a member of the Brassicaceae, is myxospermous, i.e. its seeds secrete an enveloping mucilage when they come in to contact with water. Three reports have recently described the differentiation of the *Arabidopsis* seed coat and its mucilage-producing outer cell layer (Beeckman et al. 2000; Western et al. 2000; Windsor et al. 2000). Here we report the use of antibodies for the in situ analysis of pectic HG epitopes within *Arabidopsis* seed mucilage and of the regulated occurrence of HG and RG-I side chain epitopes at the surface of *Arabidopsis* seedling roots.

Material and methods

Plant materials and growth conditions

The *Arabidopsis thaliana* (L.) Heynh. genotypes used in this study were the C24 wild type (Lehle Seeds, Round Rock, Tex., USA), the Landsberg *erecta* wild type and the mucilage-deficient mutant *transparent testa glabrous-1* (*ttg1*) (provided by A. Marchant, University of Nottingham, UK). All seedlings were germinated and grown for up to 15 days in 10-cm tissue culture Petri dishes (Sterilin, Staffordshire, UK) on a solid medium consisting of 2.2 g l^{-1} Murashige and Skoog basal medium (Sigma, Poole, Dorset, UK) supplemented with 10 g l^{-1} sucrose and 2 g l^{-1} Phytagel (Sigma). Seedlings were grown with a 16-h photoperiod (50 μmol m^{-2} s^{-1}) at 22 °C. Seeds were imbibed in de-ionised water for 20 h at room temperature before labelling with antibodies or dyes.

Cytochemical probes

Seed mucilage was examined with the cationic dye ruthenium red and with Calcofluor (Sigma). Both were dissolved in de-ionised water and used at final concentrations of 20 μg ml^{-1} and 25 μg ml^{-1} respectively. Imbibed seeds were incubated in dye solutions for 10 min and de-stained by extensive washing in de-ionised water before examination. Ruthenium red staining was examined with bright-field illumination, while Calcofluor staining was examined using epifluorescent UV irradiation.

Monoclonal antibodies

The monoclonal antibodies used in this report have all been described previously and unless stated otherwise were rat hybridoma supernatants. JIM5 and JIM7 bind to relatively low-ester and high-ester HG, respectively (Knox et al. 1990). For a recent examination of the structure of the HG epitopes bound by these antibodies see Willats et al. (2000a). PAM1 is a phage display monoclonal antibody that binds specifically to large de-esterified blocks of HG (Willats et al. 1999a). LM5 recognises an epitope of (1 → 4)-β-D-galactan (Jones et al. 1997) and LM6 an epitope of (1 → 5)-α-L-arabinan (Willats et al. 1998). Both of these epitopes occur in the side chains of RG-I. LM1 binds to hydroxyproline-rich glycoproteins (HRGPs) (Smallwood et al. 1995).

Immunolabelling procedures

Imbibed seeds or roots excised from 10- to 15-day-old seedlings were fixed for 30 min in 4% paraformaldehyde in 50 mM Pipes, 5 mM $MgSO_4$, and 5 mM EGTA. Following fixation, seeds or roots were washed in phosphate-buffered saline (PBS: prepared from a 10 × stock solution −80 g NaCl, 2 g KCl, 28.6 g $Na_2HPO_4 \cdot 12H_2O$ and 2 g KH_2PO_4 in 1 l de-ionised H_2O, pH 7.2) and then incubated for 1 h in primary antibody diluted in PBS containing 5% (w/v) fat-free milk powder (5%M/PBS). Rat hybridoma supernatants were used at 10-fold dilutions and PAM1 was used at a concentration of approximately 10^{11} phage particles ml^{-1} (corresponding to an approximately 1/10 dilution of phage prepared by polyethylene glycol precipitation, Willats et al. 1999a). Roots or seeds were washed by gently rocking in PBS (five changes, 2 min per wash) prior to incubation for 1 h in secondary antibody. For visualisation of hybridoma antibody binding, the secondary antibody was anti-rat-IgG coupled to fluorescein isothiocyanate (FITC; Sigma). For visualisation of PAM1 binding, a secondary antibody was prepared by conjugating an anti-M13 antibody (Pharmacia, Uppsala, Sweden) to FITC using a protein conjugation kit (Sigma). All secondary antibodies were used at dilutions of 1/100 in 5%M/PBS. After washing in PBS, roots or seeds were mounted in anti-fade agent (Citifluor; Agar Scientific, Dover, UK) and examined on an Olympus BH-2 microscope equipped with epifluorescent irradiation. In some cases imbibed seeds were incubated in 0.1 M sodium carbonate for 1 h or 50 mM trans-1,2-cyclohexanediamine-N,N,N′,N′-tetraacetate (CDTA) pH 7.0 for 4 h. Seeds were then washed three times with de-ionised water prior to immunolabelling.

In some cases, intact and unfixed seedlings or seeds were immunolabelled using a modified non-destructive protocol that enabled seeds and seedlings to be grown following labelling. In the case of seedlings, material was immunolabelled as described except that Hepes buffer (20 mM, pH 7.0) was used for all incubations instead of PBS. Antibody stocks free of toxic preserving agents were prepared. For hybridoma supernatants this was achieved by dialysis against Hepes buffer. For PAM1 and all secondary antibodies, stocks were prepared in Hepes buffer by centrifugation filtration (Amicon, Stonehouse, Gloucs., UK). Following labelling, material was examined immediately (without anti-fade reagents). Following examination material was washed in de-ionised water and planted directly into compost. Seeds were immunolabelled as described for fixed material, except that they were examined without anti-fade reagents. After examination seeds were sterilised in 10% household bleach for 20 min and washed 6 times in de-ionised water. Seeds were germinated and grown for up to 15 days, as described above, prior to transfer to compost.

Immuno-dot assays

Aqueous extracts of pectic components in seed mucilage were analysed by immuno-dot assays. Approximately 5,000 wild-type (C24) seeds were imbibed in 1 ml of de-ionised water or 50 mM CDTA (pH 7.0) for 20 h at room temperature after which the extract was filtered through glass wool. Five-fold serial dilutions (in de-ionised water) were prepared from the extract and applied to nitrocellulose as 1-μl aliquots. Nitrocellulose membranes were then air-dried at room temperature for at least 30 min. All subsequent treatments were at room temperature. Membranes were blocked with 5%M/PBS for 1 h prior to incubation for 1.5 h in primary antibody solution. Hybridoma supernatants of JIM5 and JIM7 were diluted 1/10 in 5%M/PBS while PAM1 was used at a concentration of approximately 10^{11} phage particles per ml (see above). Membranes were washed extensively in PBS containing 0.1% (v/v) Tween 20 prior to incubation for 1.5 h in

secondary antibody solution. For detection of JIM5 and JIM7 binding, the secondary antibody was anti-rat horseradish peroxidase conjugate (Sigma) while for detection of PAM1 binding the secondary antibody was anti-M13 horseradish peroxidase (Pharmacia). Both secondary antibodies were diluted 1/1,000 in 5%M/PBS. Membranes were again washed as described above prior to development in substrate solution (25 ml de-ionised water, 5 ml methanol containing 10 mg/ml 4-chloro-1-naphthol, 30 µl 6% (v/v) H_2O_2).

Results

Structure and properties of Arabidopsis *seed mucilage*

Seed mucilages are thought to largely consist of pectic polysaccharides (Fahn 1990; Western et al. 2000). It is known that the mucilage of *Arabidopsis* seeds can be visualised with the cationic dye ruthenium red indicating acidic pectic components (Western et al. 2000) and as shown in Fig. 1a. The application of Calcofluor, a fluorescent probe for β1,4-glycans, resulted in abundant fluorescence labelling of the mucilage, as shown in Fig. 1b, indicating the presence of cellulose in addition to pectic polysaccharides. Cellulose has previously been reported in to be present in *Brassica* seed mucilage (Frey-Wyssling 1976). The thickness of the mucilage gel visualised by ruthenium red and Calcofluor was approximately 30% of the seed width at the middle of its length. The porosity of the mucilage was examined using fluorescently (FITC)-tagged dextrans. Free FITC and FITC coupled to a 40,000-M_r dextran moved freely through the mucilage (data not shown) whereas FITC coupled to a 150,000-M_r dextran was to some extent excluded from the mucilage, particularly from the inner region as shown in Fig. 1c.

A highly methyl-esterified HG is an abundant pectic component of Arabidopsis *seed mucilage*

The pectic components of the mucilage surrounding *Arabidopsis* seeds were directly investigated further using anti-pectin monoclonal antibodies. An immersion immunolabelling procedure of whole seeds was found to be directly applicable for the in situ analysis of the pectic components of intact seed mucilage. The anti-HG monoclonal antibodies JIM5 and JIM7 indicated not only the presence of HG within the seed mucilage but also structures and domains within the mucilage as shown in Fig. 2. Both JIM5 and JIM7 bind to a range of methyl-esterified epitopes of HG. Neither antibody binds optimally to HG with very low degrees of esterification, and the binding of JIM5, but not JIM7, is also reduced against HG with high levels of esterification (Willats et al. 2000a). JIM7 labelling was strong throughout the mucilage and its binding indicated rays of mucilage that appeared to reflect points of secretion from seed coat epidermal cells. However, the JIM7 epitope appeared to be absent from the surface of the testa itself (Fig. 2a). In contrast, JIM5 bound most strongly to an inner region of the mucilage that corresponded to the region that most effectively excluded the 150,000-M_r dextran (Fig. 1c) as shown in Fig. 2b. JIM5 also bound to the borders of the epidermal cells at the seed surface and columella structures at the centre of the cells (Fig. 2b). These observations indicated that the pectic component of *Arabidopsis* seed mucilage consisted of relatively highly methyl-esterified HG throughout the mucilage with a more de-esterified HG component in the inner region. These observations were extended with the use of PAM1, a phage display monoclonal antibody that recognises long stretches of de-esterified blocks of HG that are capable of being cross-linked through calcium (Willats et al. 1999a). PAM1 showed no binding to seed mucilage as shown in Fig. 2c. However, when imbibed seeds were treated with sodium carbonate for 1 h prior to antibody labelling (a treatment that removes methyl ester groups), PAM1 binding was abundant in the mucilage and especially in the outer regions of the mucilage (Fig. 2g). This observation confirmed that the HG component of the mucilage is extensively methyl-esterified and contains no de-esterified HG block structures. It is possible however, that the inner region of untreated mucilage may contain some de-esterified regions of HG that are not accessible to the PAM1 phage particles due to the low porosity of this region (as discussed above).

The lack of PAM1 binding to untreated seed mucilage suggested that the structure and integrity of mucilage was not maintained to any great extent by a calcium-HG gel system. Possible calcium-mediated gel structures within the mucilage were explored further using the calcium-chelator CDTA. Treatment with CDTA prior to probing with JIM5 and JIM7 did not result in extensive loss or dissolution of the mucilage (Fig. 2e,f). However, the mucilage increased in volume, and the binding of JIM5 was observed throughout the mucilage, which may indicate the expansion of the inner region (Fig. 2f). Clearly, although overall mucilage structure is not maintained by calcium-mediated HG links, the apparent increase in volume of mucilage suggested that the treatment resulted in loosening of some components and that calcium-mediated HG linkages contribute to maintaining a more highly cross-linked region of the mucilage closest to the seed.

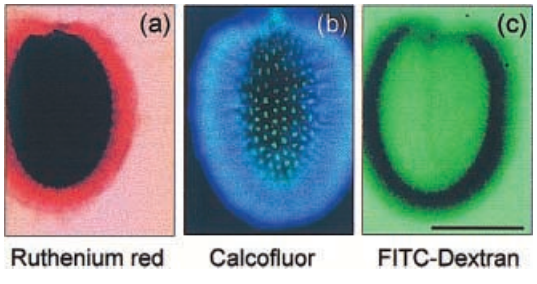

Fig. 1a–c. Cytochemistry of intact *Arabidopsis* seed mucilage. The mucilage surrounding imbibed *Arabidopsis* seeds (C24 wild type) was stained with ruthenium red (**a**) and calcofluor (**b**). The porosity of the mucilage layers was examined with FITC-dextran (MW 150 kDa), which was excluded from the inner (dark) layer of mucilage (**c**). Bar = 500 µm

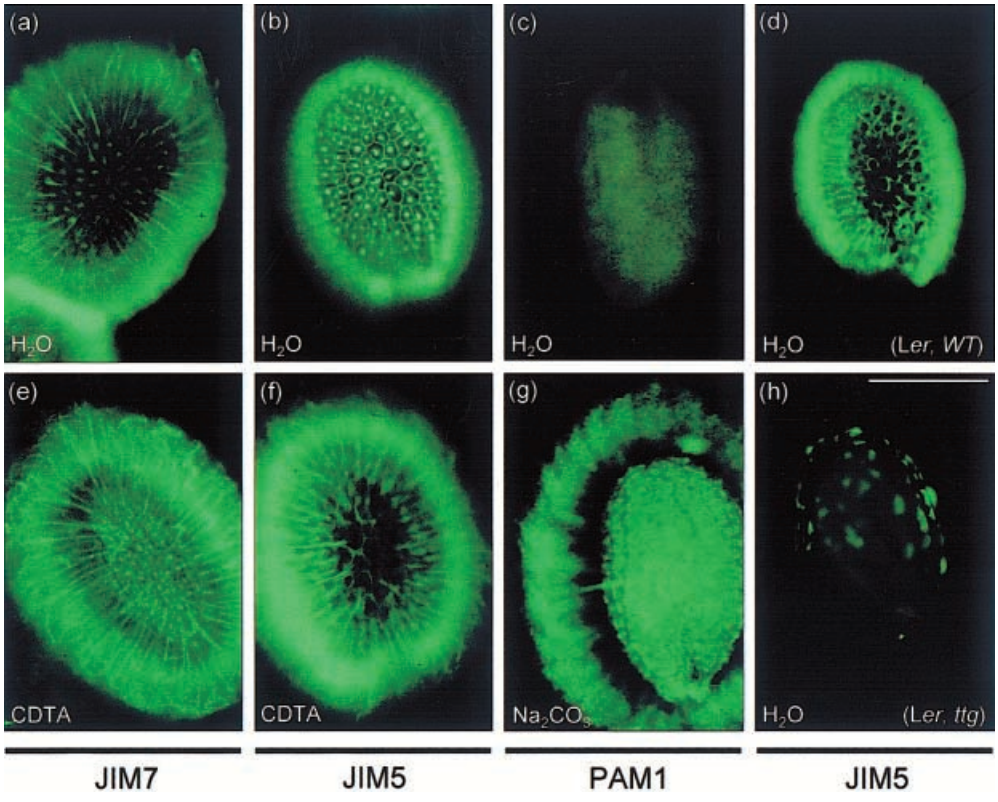

Fig. 2a–h. Immersion immunofluorescence labelling of intact *Arabidopsis* seed mucilage and testa. Seeds of the C24 (**a–c, e–g**) or Landsberg *erecta* (L*er*) ecotypes (**d**), and seeds of the *ttg* mutant (**h**) were immersion-labelled with JIM7 (**a, e**) JIM5 (**b, d, f h**), and PAM1 (**c, g**). In some cases prior to immunolabelling seeds were treated with a calcium chelator (CDTA) (**e**) and (**f**), or Na_2CO_3 (**g**). Bar = 500 μm

Immuno-dot assays of material released from seeds during incubation with CDTA confirmed the above observations (Fig. 3). The most abundant epitope recovered was that recognised by JIM7. However, equivalent amounts of the JIM7 epitope were retrieved from extractions in water and CDTA, indicating that calcium did not have a role in maintaining this component of the mucilage (Fig. 3). This suggests that the mucilage contains a subset of highly methyl-esterified HG that is water-soluble. This component will be lost during immunolabelling washes and not observed by immersion immunofluorescence procedures. This may be the outer layer of mucilage that is stained weakly by ruthenium red that is lost upon shaking in water as observed by Western et al. (2000). The epitopes recognised by JIM5 and, at a very low level, PAM1 occurred more abundantly in CDTA-solubilised material compared with material extracted with water. This confirmed the presence of a low abundance of HG components stabilised within the mucilage by calcium cross-links. The appearance of rings in the JIM5 immuno-dot assay of the CDTA-soluble material suggests the presence of a range of structurally distinct pectic components that are specifically held in the mucilage by calcium cross-links (Willats and Knox 1999). These components may be involved in maintaining the inner region of the mucilage that appears to have a relatively low porosity. Monoclonal antibodies LM5 and LM6 did not bind to the mucilage indicating the absence of (1 → 4)-β-D-galactan and (1 → 5)-α-L-arabinan from the mucilage pectin (data not shown).

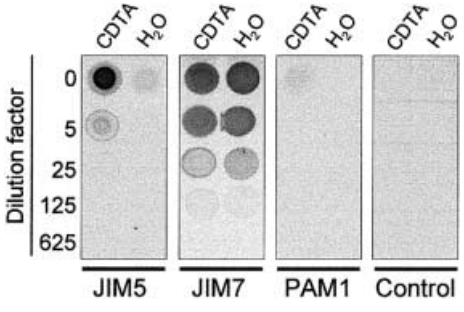

Fig. 3. Immuno-dot assays of HG components released from *Arabidopsis* seed mucilage. Homogalacturonan was extracted by incubation of seeds in calcium chelator (*CDTA*) or water (*H_2O*) and detected using JIM7, JIM5 and PAM1. The primary antibody was omitted during the labelling of control blots. A highly methyl-esterified water-soluble HG component is present in addition to a chelator-soluble HG component

The applicability of the immersion immunofluorescence approach for the examination and possible selection of mutants with altered mucilage occurrence or morphology was determined by the analysis of seeds of the *transparent testa glabrous* (*ttg*) mutant that is known not to produce mucilage. As shown in Fig. 2h, JIM5 binding was confined to small patches at the surface of *ttg* seeds. The *ttg* mutation is in the Landsberg *erecta* (L*er*) ecotype background. JIM5 labelling of wild-type L*er* seed is shown in Fig. 2d, and indicates subtle differences between C24 and L*er* ecotypes, most notably a different pattern of labelling of the testa.

Homogalacturonan and RG-I pectic epitopes at the root surface

The immersion immunofluorescence whole-mount labelling technique was also found to be applicable to the analysis of pectic epitopes at the surface of seedling roots. Immersion immunolabelling of intact 10- to 15-day-old *Arabidopsis* seedlings indicated that epitopes occurring in HG and RG-I were abundant on the root surface and had regulated patterns of occurrence as shown in Fig. 4. JIM5 showed intense labelling of the root surface apart from the apex of lateral roots as shown in Fig. 4a–c. In contrast, the JIM5 epitope occurred abundantly at the radicle apex and also bound to cells in the process of sloughing off (Fig. 4d). This was a consistent observation indicating a biochemical distinction between the surfaces of the radicle apex and lateral root apices. JIM7 labelling patterns were similar to those of JIM5 though often weaker (data not shown). The intensity of immunofluorescence often declined in a proximal direction as shown in short lateral roots in Fig. 4c – although the extent of such gradients in binding were variable. These observations indicated that the degree of methyl-esterification of HG was not even over the root surface, or that levels of HG varied. The de-esterified block-specific antibody PAM1 bound to the seedling root surface but only at junctions between cells as shown in Fig. 4e, indicating the presence of de-esterified block structures of HG that are likely to participate in cell-to-cell adhesion. In contrast to JIM5 and JIM7, the occurrence of the PAM1 epitope did not diminish in a proximal direction. In this way PAM1 is a useful probe to visualize cell files at the root surface and the apparent twisting of cells files in the root body that occurs during root growth.

(1 → 4)-β-D-Galactan and (1 → 5)-α-L-arabinan are known to occur as features of the side chains of RG-I. Antibodies to epitopes in these components have indicated an extensive regulation of these structures in the context of cell wall architecture and in relation to cell proliferation and cell differentiation (Jones et al. 1997; Willats et al. 1998, 1999b; McCartney et al. 2000; Orfila and Knox 2000). These epitopes were also found on the surface of roots of *Arabidopsis* seedlings. In some cases the (1 → 4)-β-D-galactan epitope was associated with loosely bound cells at the radicle apex as seen in Fig. 4f, but was generally absent from lateral root apices (Fig. 4g). The (1 → 5)-α-L-arabinan epitope was found to occur abundantly over the radicle apex and lateral root apices as shown in Fig. 4h and sometimes just at the distal region of the lateral root apex (Fig. 4i).

It is noteworthy that in no case were HG or RG-I pectic epitopes found at the surface of root hairs (Fig. 4a). Root hairs were present in all preparations examined and could be readily visualised with anti-HRGP monoclonal antibody (Fig. 4j). This indicates that pectic epitopes are differently regulated at the unadhered surface of trichoblasts – being abundant on

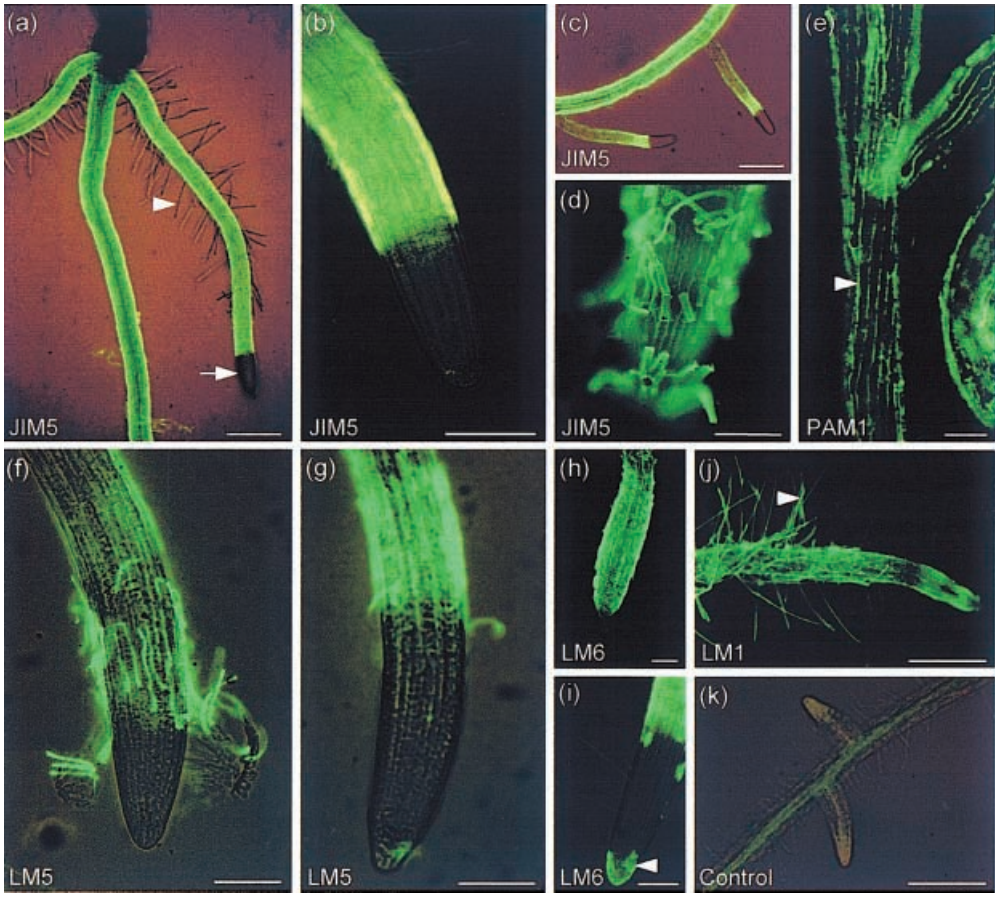

Fig. 4a–k. Immersion immunofluorescence labelling of the surface of *Arabidopsis* roots. Roots were immunolabelled with JIM5 (**a–d**), PAM1 (**e**), LM5 (**f, g**) and LM6 (**h, i**). The JIM5 HG epitope was present at the surface of epidermal cells but absent from the surface of root hairs (*arrowhead* in **a**). The JIM5 epitope was abundant at the radicle root apex (**d**) but absent from lateral root apices (**a–c**). The PAM1 HG epitope was restricted to cell junctions at root surfaces (*arrowhead* in **e**). The LM5 (**f, g**) and the LM6 (**h, i**) epitopes were generally absent from lateral root apices, although in some cases LM6 labelled the distal regions of root apices (*arrowhead* in **i**). An anti-HRGP probe, LM1 (**j**) was used as a positive control and labelled root hair surfaces (*arrowhead*). Omission of a primary antibody (**k**) provided a negative control. Bars = 300 μm (**a, c, e, j, k**), 100 μm (**b, d ,f–i**)

exposed regions of cell wall other than root hair itself. It is possible that the pectin is masked or modified in some way at the root hair surface.

Non-destructive immersion-labelling of seeds and seedlings

The observations described and shown above, were all obtained using fixed excised roots and fixed seeds. However, with minor modifications to the standard protocol (as described in *Materials and methods*) it was found that intact non-fixed seedlings and seeds could be labelled non-destructively and subsequently grown to maturity. Essentially, this involved the use of Hepes-based buffers in the place of PBS. In all cases, the labelling patterns obtained using the non-destructive procedures were the same as those using PBS-based protocols.

Discussion

Pectic components of seed mucilage

The use of antibodies in the direct labelling procedure described here has indicated the presence of an extensively methyl-esterified HG component and a more tightly cross-linked inner region of the mucilage that may be stabilized by calcium-HG cross-linking. Determinations of the monosaccharide composition of *Arabidopsis* seed mucilage have indicated an abundance of rhamnose and galacturonic acid, suggesting the occurrence of rhamnogalacturonan components in addition to HG (Goto 1985; Western et al. 2000). Immunolabelling of seed mucilage with probes for epitopes of (1 → 4)-*β*-D-galactan and (1 → 5)-α-L-arabinan, which are two of the most common structural features of RG-I side chains (Mohnen 1999), resulted in no binding (data not shown). This indicates that these structures are not present on RG-I in mucilage, an observation that is supported by the low level of arabinose that has been observed in *Arabidopsis* seed mucilage (Goto 1985). Galactose has been reported to occur at a relatively low level in seed mucilage of the Sendai ecotype (Goto 1985) and appears to be more abundant in the Columbia-2 ecotype (Western et al. 2000). It is possible that RG-I without side chains or with novel structural features is present. Alternatively, the high level of rhamnose may reflect the presence of RG-II or other rhamnose-containing polymers. It may also be of significance that a growth-regulating rhamnose-containing disaccharide has been isolated from germinating *Arabidopsis* seeds (Yokatani-Tomita et al. 1998).

Calcium cross-linking of de-esterified HG is known to be a major component of gel formation within the primary cell wall matrix. In the case of seed mucilage, such links contribute only to the inner regions of mucilage. There may be biosynthetic and functional limitations in generating a large extracellular calcium-de-esterified HG gel that would require both pectin methyl esterases and calcium. High methoxyl HG domains can form gels in vitro and these can be stabilised by the presence of solutes such as sucrose (Jarvis 1984). The extent of the formation of high-methoxyl HG gels in plant cell walls is not known. However, HG domains are generally highly methyl-esterified when first deposited in cell walls and it seems feasible that supra-molecular pectic structures could be generated from high-methoxyl HG. The HG component of seed mucilage could also be stabilised by interaction with other components such as cellulose. The presence of cellulose in *Arabidopsis* seed mucilage is indicated by the binding of Calcofluor to the seed mucilage (Fig. 1b) and has previously been reported to occur in *Brassica* seed mucilage (Frey-Wyssling 1976). Recent studies on the development of mucilage-secreting cells of the *Arabidopsis* testa have described its packing between cell walls and plasma membranes in surface cells (Beeckman et al. 2000; Western et al. 2000; Windsor et al. 2000). The nature of mucilage prior to release upon imbibition is unknown.

The functions of seed mucilage, and hence its required properties are not clearly understood. Mucilage may have roles in imbibition, adhesion to soil, seed dispersal, accumulation of heavy metals, defence, or germination (McCully 1999; Western et al. 2000; Windsor et al. 2000). Several mutants of *Arabidopsis* are known that do not produce mucilage and these include *ttg* (as shown in Fig. 2), *glabrous2* and *apetala2* (Jofuku et al. 1994; Leon-Kloosterziel et al. 1994). The mutated genes encode regulatory proteins that are involved in testa development and are not directly involved in polysaccharide biosynthesis. However, *Arabidopsis* seed mucilage may provide an interesting system for the study of the influence of genetic factors on pectin biosynthesis and pectin interaction with other polysaccharide or protein components.

Pectic polysaccharides at the root surface

Iterative plant growth of apical meristems extends the surface of plants. In the case of the root, this surface extension is within the soil environment and provides anchorage and access to water and nutrients. The function(s) of pectic polysaccharides at the root surface in relation to growth and physiology and possibly rhizosphere interactions are uncertain (McCully 1999). In addition to contributing to the mechanical properties of epidermal cells they may influence the pH of the root surface, which is known to vary with cell elongation (Peters and Felle 1999).

The most significant observation reported here is the regulation of HG and RG-I components during root growth. Neither HG nor RG-I components were detected on the surface of root hairs, although they were present on other surface regions of trichoblasts. This may indicate that other components, such as HRGPs, may cover the surface of tip-growing regions of cell walls. It is of interest that the JIM5 epitope has been detected at the surface of tip-growing pollen tubes

in certain species (Li et al. 1996). It is also noteworthy that HG epitopes were absent from the apex surface of lateral roots but occurred on the surface of the radicle apex and of sloughing root-cap cells. This may relate to the distinct developmental origins of these apices. Although pectic polysaccharides of surface and internal cell walls have been examined in sections of roots in a range of species, including *Arabidopsis*, (Knox et al. 1990; Lynch and Staehelin 1992, 1995; Freshour et al. 1996; Dolan et al. 1997) differences between radicle and lateral root apices have not been reported.

Conclusion

The small size of the *Arabidopsis* seedlings can make analysis by many biochemical and cell biological procedures difficult. However, the small size of seedlings, and particularly the transparency of the roots, makes direct analysis of intact seedlings by the procedures described feasible. Such an approach is particularly useful when used in combination with defined antibody probes. In this report we have focused on pectic polysaccharides but other cell wall components such as HRGPs and arabinogalactan-proteins can also be visualized on plant surfaces (Šamaj et al. 1999; Willats et al. 2000b). The fact that this can also be done on living plants and the plants then grown on for further studies, seed collection or experimentation makes this a useful new methodology for the study of cell wall components. The variability of pectic epitope occurrence in seed mucilage and at the surface of *Arabidopsis* roots provides a useful resource for the analysis of the effects of environmental and genetic factors on pectic structure and function.

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