The Organization Pattern of Root Border-Like Cells of Arabidopsis Is Dependent on Cell Wall Homogalacturonan\textsuperscript{1,2}[C][W]

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Border-like cells are released by Arabidopsis (Arabidopsis thaliana) root tips as organized layers of several cells that remain attached to each other rather than completely detached from each other, as is usually observed in border cells of many species. Unlike border cells, cell attachment between border-like cells is maintained after their release into the external environment. To investigate the role of cell wall polysaccharides in the attachment and organization of border-like cells, we have examined their release in several well-characterized mutants defective in the biosynthesis of xyloglucan, cellulose, or pectin. Our data show that among all mutants examined, only qua1-1 and qua2-1, which have been characterized as producing less homogalacturonan, had an altered border-like cell phenotype as compared with the wild type. Border-like cells in both lines were released as isolated cells separated from each other, with the phenotype being much more pronounced in qua1-1 than in qua2-1. Further analysis of border-like cells in the qua1-1 mutant using immunocytochemistry and a set of anti-cell wall polysaccharide antibodies showed that the loss of the wild-type phenotype was accompanied by (1) a reduction in homogalacturonan-JIM5 epitope in the cell wall of border-like cells, confirmed by Fourier transform infrared microspectrometry, and (2) the secretion of an abundant mucilage that is enriched in xylogalacturonan and arabinogalactan-protein epitopes, in which the cells are trapped in the vicinity of the root tip.

Higher plants rely on their roots to acquire water and other nutrients in the soil to grow and develop (Esau, 1977). At the tip of every growing root is a conical covering consisting of several layers of cells called the root cap that plays a major role in root protection and its interaction with the rhizosphere (Rougier, 1981; Baluška et al., 1996; Barlow, 2003). Root tips of most plant species produce a large number of cells programmed to separate from the root cap and to be released into the external environment (Hawes et al., 2003). This process occurs through the action of cell wall-degrading enzymes that solubilize the interconnections between root cap peripheral cells, causing the cells to separate from each other and from the root as populations of single cells (Hawes et al., 2003). Because of their specific position at the interface between root and soil, these living cells are defined as root border cells. It has been shown that the number of these cells per root varies between plant families: from about 100 (e.g. the Solanaceae family) to several thousands (e.g. 10,000 or more for the Pinaceae; Hawes et al., 2003). It has also been suggested that species of the Brassicaceae family including Arabidopsis (Arabidopsis thaliana) do not produce border cells (Hawes et al., 2003). Indeed, the Arabidopsis root tip does not produce isolated border cells per se, but it does produce and release cells that remain attached to each other, forming a block of several cell layers called border-like cells (Vicré et al., 2005; Fig. 1). This also occurs in other Brassicaceae species, including rape-seed (Brassica napus), mustard (Brassica juncea), and Brussels sprout (Brassica oleracea gemmifera), indicating that such an organization might be specific to this family (Driouich et al., 2007).

The unique organization pattern of Arabidopsis border-like cells (e.g. they do not disperse individually) suggests that they might have a specific cell wall composition and/or structure that makes them resistant to cell wall-hydrolyzing enzymes or that the enzymes are not present or not functional (Driouich et al., 2007).
et al., 2007). The only information on cell wall composition of Arabidopsis border-like cells was obtained from immunocytochemical studies, in which it has been shown that the cell wall of border-like cells is rich in pectic homogalacturonan and arabinogalactan-proteins, two wall polymers believed to be involved in cell adhesion in plants (Vicré et al., 2005). Based on this observation, we postulated that pectic polysaccharides of the cell wall may serve as a glue to cement border-like cells together, leading to that particular organization (Vicré et al., 2005).

The cell wall of higher plants comprises mainly polysaccharides and proteoglycans. Cell wall polysaccharides are assembled into complex macromolecules, including cellulose, hemicellulose, and pectin. Cellulose forms microfibrils, which constitute an ordered, fibrous phase, whereas pectin and hemicellulose form an amorphous matrix phase surrounding the microfibrils (Cosgrove, 1997). Pectins constitute a highly complex family of cell wall polysaccharides, including homogalacturonan, rhamnogalacturonan I, and rhamnogalacturonan II. Homogalacturonan domains consist of \( \alpha\)-D-(1\(\rightarrow\)4)-GalUA residues, which can be methyl esterified, acetylated, and/or substituted with \( \beta\)-(1\(\rightarrow\)3)-Xyl residues to form xylogalacturonan (Schols et al., 1995; Willats et al., 2001; Vincken et al., 2003). Deesterified blocks of homogalacturonan can be cross-linked by calcium, leading to the formation of a gel that is believed to be involved in cell adhesion (Jarvis et al., 2003). Rhamnogalacturonan I consists of a backbone of up to 100 repeats of the disaccharide \( \alpha\)-(1\(\rightarrow\)4)-GalUA-(1\(\rightarrow\)2)-rhamnose, which carries complex and variable side chains. The rhamnose residues are commonly substituted with polymeric \( \beta\)-(1\(\rightarrow\)4)-linked D-galactosyl residues and/or \( \alpha\)-(1\(\rightarrow\)5)-linked L-arabinosyl residues (Ridley et al., 2001). Rhamnogalacturonan II is a highly complex but conserved molecule consisting of a homogalacturonan-like backbone substituted with four different side chains containing specific sugars (O’Neill et al., 2004).

Xyloglucan is the major hemicellulosic polysaccharide of the primary wall of dicotyledonous plants, and it consists of a \( \beta\)-D-(1\(\rightarrow\)4)-glucan backbone to which are attached side chains containing xylosyl, galactosyl-xylosyl, or fucosyl-galactosyl-xylosyl residues. Xyloglucan is the principal polysaccharide that cross-links the cellulose microfibrils. The xyloglucan-cellulose network forms a major load-bearing structure that contributes to the control of cell expansion (Hayashi, 1989; Cosgrove, 1999).

Glycoproteins, such as arabinogalactan-proteins, are also present in the cell wall matrix (Showalter, 1993; Seifert and Roberts, 2007). Arabinogalactan-proteins are highly glycosylated members of the Hyp-rich glycoprotein superfamily. Many of these glycoproteins, the so-called classical arabinogalactan-proteins, are anchored to the plasma membrane by a glycosylphosphatidylinositol anchor and have the potential to bind both cell wall components (Immerzeel et al., 2006) and cytosolic cortical microtubules (Schultz et al., 2002; Sardar et al., 2006; Nguema-Ona et al., 2007). These proteoglycans have been implicated in many aspects of plant life, including cell expansion, cell signaling and communication, embryogenesis, and wound response (Johnson et al., 2003; Seifert and Roberts, 2007; Driouich and Baskin, 2008).

Although cell-to-cell interaction is a fundamental feature of plant growth and development, the molec-
ular bases of intercellular adhesion and its loss are not fully understood (Roberts et al., 2002; Jarvis et al., 2003; Willats et al., 2004). This study aims at investigating the role of cell wall polysaccharides in cell attachment and the organization of border-like cells in Arabidopsis. To this end, we took advantage of the recent characterization of several Arabidopsis mutants affected in the biosynthesis of different classes of cell wall polysaccharides, including pectin, xyloglucan, and cellulose. We thus examined the pattern of border-like cells released by the root tip of selected Arabidopsis mutants using microscopy and immunocytochemistry. These mutants are (1) *qua1-1* (Arioli et al., 1998; Madson et al., 2003), which all have been reported to be possibly affected in pectin biosynthesis; (2) *mur2-1* (Vanzin et al., 2002) and *mur3* (Murus et al., 2002, 2007), which make altered xyloglucan and cellulose, and (3) *radially swollen1* (*rsw1*) and *korrigan1* (*kor1*), which are affected in cellulose biosynthesis (Arioli et al., 1998; Nicol et al., 1998).

Our data show that the organization of border-like cells had a wild-type phenotype in all of the mutants examined except in *qua1-1* and *qua2-1*. In both of these mutants, border-like cells had lost the wild-type phenotype, as they were released as single cells separated from each other. This phenotype was far more pronounced in *qua1-1* than in *qua2-1*. Further analysis of *qua1-1* using immunocytochemistry and Fourier transform infrared microscopy showed a substantial loss of homogalacturonan content in border-like cells. In addition, border-like cells in the *qua1-1* mutant secreted an abundant mucilage enriched in xylogalacturonan and arabinogalactan-protein epitopes.

### RESULTS

#### Morphological Characterization of Border-Like Cells in Cell Wall-Altered Mutants of Arabidopsis

A major function of the cell wall is to maintain cell-to-cell contact and tissue cohesion. To investigate the cell wall carbohydrates involved in border-like cell organization and attachment in Arabidopsis, we examined root cap morphology in mutants known to be defective in the biosynthesis of different classes of cell wall polysaccharides, including cellulose, hemicellulose, and pectin (Table I).

We first examined border-like cell organization in two hemicellulose mutants, *mur2-1* and *mur3*, known to be deficient in genes encoding for a fucosyltransferase and a galactosyltransferase, two enzymes involved in xyloglucan biosynthesis (Vanzin et al., 2002; Madson et al., 2003). As illustrated in Figure 1, no difference was observed in either border-like cell production or organization between wild-type and *mur3* or *mur2-1* root tips (Fig. 1, compare A, C, and D). Similarly, two cellulose-defective mutants, *kor1* and *rsw1*, did not show any alteration in the overall organization of the same cells (Fig. 1, E and F). Nevertheless, root tips of the mutant *rsw1*, whose phenotype is temperature sensitive (Arioli et al., 1998), produced fewer border-like cells at 30°C than at 20°C.

We next examined border-like cell phenotypes in mutant lines displaying abnormal pectin content. Reduced cell adhesion was reported for the *epc1-1* mutant, deficient in a gene encoding a putative pectin-glycosyltransferase (Singh et al., 2005). Observation of the root tip in this mutant showed that the organization pattern of border-like cells is similar to that of the wild type (Fig. 1G). Similarly, the organization of the cells in the *arad1-1* mutant, deficient in a gene encoding an α-1,5-arabinofuranosyltransferase involved in rhamnogalacturonan I biosynthesis (Harholt et al., 2006), was not altered (Fig. 1H).

In contrast, examination of border-like cells in the mutant *qua1-1*, which is deficient in a putative glycosyltransferase involved in homogalacturonan biosynthesis and known to exhibit reduced cell adhesion (Bouton et al., 2002), revealed a different organization of border-like cells as compared with the wild type. As shown in Figure 1I, *qua1-1* border-like cells were not present as organized files of attached cells but were separated from each other and dispersed individually in the vicinity of the root tip. Furthermore, these cells were frequently seen embedded in an abundant layer

### Table I. List of Arabidopsis mutants used in this study and description of the root border-like cell organization in each of them

<table>
<thead>
<tr>
<th>Mutant Name</th>
<th>Gene Code</th>
<th>Border-Like Cell Organization</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>mur2-1</em></td>
<td>At2g03220</td>
<td>Border-like cells organized in files</td>
<td>Vanzin et al. (2002)</td>
</tr>
<tr>
<td><em>mur3</em></td>
<td>At2g03270</td>
<td>Border-like cells organized in files</td>
<td>Madson et al. (2003)</td>
</tr>
<tr>
<td><em>Cellulose</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>kor1</em></td>
<td>At5g49720</td>
<td>Border-like cells organized in files</td>
<td>Nicol et al. (1998)</td>
</tr>
<tr>
<td><em>rsw1</em></td>
<td>At4g32410</td>
<td>Reduced border-like cell formation at 30°C</td>
<td>Arioli et al. (1998)</td>
</tr>
<tr>
<td><em>Pectin</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>qua1-1</em></td>
<td>At3g25140</td>
<td>Isolated border-like cells and thick mucilage</td>
<td>Bouton et al. (2002)</td>
</tr>
<tr>
<td><em>qua2-1</em></td>
<td>At1g78240</td>
<td>Isolated border-like cells and sometimes thick mucilage</td>
<td>Moulle et al. (2007)</td>
</tr>
<tr>
<td><em>arad1-1</em></td>
<td>At2g35100</td>
<td>Border-like cells organized in files</td>
<td>Harholt et al. (2006)</td>
</tr>
<tr>
<td><em>Undefined function</em></td>
<td>At3g55830</td>
<td>Border-like cells organized in files</td>
<td>Singh et al. (2005)</td>
</tr>
</tbody>
</table>
of mucilage (Fig. 1I). Interestingly, careful and daily examination of border-like cell production during root growth revealed that the mucilage is secreted concomitantly during border-like cell formation and release from the root tip. A quite similar but less pronounced phenotype of border-like cells was also observed in the qua2-1 mutant (Fig. 1J), which is deficient in a putative methyltransferase involved in pectin biosynthesis (Mouille et al., 2007). Separation of the cells in qua2-1 was also accompanied by mucilage secretion, although less frequently than in the qua1-1 mutant (Fig. 1I).

Together, these observations demonstrate that, unlike in the wild type and cellulose- and xyloglucan-defective mutants, root tips of mutants altered in homogalacturonan biosynthesis produced border-like cells that are separated from each other. This suggests that biosynthesis of homogalacturonan is required for the normal organization of border-like cells in Arabidopsis.

**Histochemical Staining of Border-Like Cells**

As the phenotype was more pronounced in qua1-1 than in qua2-1, we focused only on the former for all of the following histochemical and immunocytochemical analyses. Histochemical staining of root tips with calcofluor white or ruthenium red showed that border-like cells are stained with both dyes in wild-type and qua1-1 plants. In contrast, the mucilage surrounding the cells of the mutant was only stained with ruthenium red but not with calcofluor white, suggesting that the mucilage contains acidic polymers such as pectin but not β-glucans (Fig. 2).

**Immunolocalization of Polysaccharide Epitopes in the Cell Wall of Border-Like Cells and the Secreted Mucilage of the Homogalacturonan-Defective Mutant**

We used immersion immunofluorescence labeling (Willats et al., 2001; Vicré et al., 2005) to gain insights into the distribution of polysaccharide epitopes at the surface of border-like cells and in associated mucilage of the qua1-1 mutant (Table I; Supplemental Data).

**Xyloglucan Immunostaining**

To investigate the occurrence of xyloglucan, we probed root tips with the CCRC-M1 monoclonal antibody (mAb). As shown in Figure 3, the antibody bound strongly to border-like cells produced by wild-type and mutant root tips. In contrast, the secreted mucilage was not labeled (Fig. 3, C and D). Close examination of the labeling pattern revealed the presence of labeled filament-like structures at the surface of border-like cells. These structures can sometimes be seen peeling off from the surface of the cells.

**Pectin Immunostaining**

To localize pectin epitopes, we immunolabeled root tips with anti-pectin mAbs, including JIM5, LM5, LM6, and LM8. Staining with JIM5 was detectable at the surface of border-like cells, with nearly no difference in localization between the wild type and the mutant (Fig. 4, A and B). Labeling of the mucilage was also observed in the qua1-1 line, although very weakly (Fig. 4B). Immersion immunofluorescence staining, which allows detection of epitopes at the surface of the tissue only, did not detect any differences in terms of intensity of fluorescence between the qua1-1 mutant and the wild type. However, as reduction in homogalacturonan in the cell walls of qua1-1 was reported to be particularly associated with the tricellular junction in suspension-cultured cells (Leboeuf et al., 2005), we performed the same labeling experiment with JIM5 on cryofixed and resin-embedded sections of root tips of the mutant and the wild type (Fig. 4). Under these conditions, we found a significant loss of JIM5 staining.
in cell walls of border-like cells in the *qua1-1* mutant compared with the wild type (Fig. 4, C and D). Such a decrease in the amount of homogalacturonan in border-like cells of *qua1-1* was further confirmed using Fourier transform infrared microspectrometry analysis (see below).

Arabinan epitopes recognized by the mAb LM6 were strongly detected in the cell wall of border-like cells released by the *qua1-1* mutant and the wild-type root tips (Fig. 5, A and B). The mucilage was rarely and weakly labeled in the *qua1-1* mutant (Fig. 5B). The LM5 antibody recognizing galactan epitopes also stained border-like cells in the wild type and the mutant but not the mucilage (data not shown).

Labeling with the mAb LM8 specific for xylogalacturonan occurred in the outer surface of border-like cells as well as in the mucilage from wild-type and mutant plants (Fig. 5). Interestingly, staining of the mucilage appeared stronger around isolated cells of the *qua1-1* mutant (Fig. 5, D and E).

**Immunostaining of Arabinogalactan-Proteins**

We also examined the distribution of epitopes associated with arabinogalactan-proteins in border-like cells and mucilage using the mAb JIM13 known to stain these cells in Arabidopsis wild-type plants (Vicré et al., 2005). As shown in Figure 6A, a uniform staining of cell wall of border-like cells in the wild type with the mAb JIM13 was observed. In contrast, in *qua1-1*, staining of the cell wall of border-like cells was either diffuse or not present at all (Fig. 6, B and C). In contrast, the secreted mucilage showed a very strong staining in the mutant (Fig. 6, B and C). These observations were confirmed using the mAb LM2 that also recognizes arabinogalactan-protein epitopes (data not shown).

Together, the immunolabeling findings demonstrate that isolated border-like cells produced by the *qua1-1* mutant express fewer homogalacturonan epitopes than the wild type and that the abundant mucilage they produce is enriched in arabinogalactan-proteins and xylogalacturonan polysaccharides.

**Fourier Transform Infrared Microspectrometry Analysis of Border-Like Cells**

The analysis of Fourier transform infrared data using principal component analysis revealed two distinct clusters of values for border-like cells, indicating significant spectral differences between the *qua1-1* mutant and the wild type (Fig. 7A). We compared the two mean second derivative spectra of the *qua1-1* mutant and the wild type in the 920 to 1,780 cm⁻¹ region (Fig. 7B). Differences were noticed at the following wave numbers characteristic of cell wall pectic polysaccharides: 1,740, 1,600, 1,414, 1,243, and 1,018 cm⁻¹. The peak at 1,740 cm⁻¹ is specific to carboxylic esters, probably linked to pectins (Kačuráková et al., 2002);
the peaks at 1,600 and 1,414 cm\(^{-1}\) are characteristic of carboxylate ion stretches for pectin (Marry et al., 2000); the peak at 1,243 cm\(^{-1}\) is assigned to C-O vibrations in pectic polysaccharides (Kacˇura´kova´ et al., 2002); and the peak at 1,018 cm\(^{-1}\) corresponds to uronic acid of pectins (Coimbra et al., 1998).

Interestingly, all of these peaks attributed to pectins are reduced in the qua1-1 mutant. These results are consistent with an alteration in GalUA content in the qua1-1 mutant.

DISCUSSION
The goal of this study was to investigate the role of cell wall polysaccharides in the control of intercellular attachment of border-like cells that are released by Arabidopsis root tips as sheets of cells rather than as single cells. To this end, border-like cells from several well-defined mutants deficient in the biosynthesis of cellulose, xyloglucan, or pectin were examined using microscopy and immunocytochemistry. Among all of the mutants studied, only the qua1-1 mutant, and to a lesser extent qua2-1, presented an altered phenotype of border-like cells in which adhesion between groups of cells was lost. Such a loss of adhesion led to a release of single border-like cells that secreted abundant mucilage and whose walls contained reduced amounts of homogalacturonan. These findings indicate that homogalacturonan is involved in the maintenance of adhesion between border-like cells in Arabidopsis and show that the loss of cell-to-cell contact is accompanied by secretion of substantial amounts of arabino-galactan-proteins and xylogalacturonan-containing mucilage, in which the cells are trapped and kept in the vicinity of the root tip.

Homogalacturonan Is Involved in Border-Like Cell Attachment in Arabidopsis

In almost all plant families, border cells are released from the root tip as populations of individually separated single cells (Hawes et al., 2003). In pea (Pisum sativum), for instance, one of the most studied species, border cells are programmed to separate from each other upon their release from the root tip. In contrast, Brassicaceae species, including Arabidopsis, do not release border cells per se; instead, they release border-like cells that remain attached together as sheets of...
cells rather than being separated from each other (Vicré et al., 2005). Thus, unlike in pea, cell adhesion in border-like cells is maintained after their release. Based on the finding that homogalacturonan epitopes (recognized by the mAb JIM5) are abundant in the cell wall of these cells, we postulated that pectin might be necessary in maintaining their unique organization (Vicré et al., 2005). This hypothesis was tested in this study using different available cell wall-defective mutants.

We found that border-like cells could be released individually in the pectin-defective mutant qua1-1, to a lesser extent in qua2-1, and not at all in cellulose-defective (kor1 and rsu1) or xyloglucan-defective (mur2-1 and mur3) mutants or other pectin mutants studied. Therefore, maintenance of adhesion between groups of border-like cells in Arabidopsis seems to depend on QUA genes, mostly on QUA1. It has been shown that mutation in QUA1 (GAUT8) and QUA2 resulted in dwarfed plant phenotypes and reduced cell adhesion in the hypocotyl, and the corresponding gene products were suggested to be involved in homogalacturonan synthesis (Bouton et al., 2002; Mouille et al., 2007). The cell walls of qua1-1 and qua2-1 were shown to have a 25% and 50% reduction, respectively, in homogalacturonan content (quantification made from 4-week-old whole plants or from the leaves only) as compared with the wild type (Bouton et al., 2002; Mouille et al., 2007). Although these studies have not measured the extent of homogalacturonan reduction in each organ specifically, including the root cap and border-like cells, they have established a correlation between homogalacturonan content and cell adhesion. They also indicate that even a partial decrease in this polysaccharide content (25%–50%) is sufficient to induce a significant loss of cell adhesion. Therefore, the important loss of cell adhesion observed in border-like cells released by the qua1-1 mutant is possibly related to a loss of homogalacturonan content in these cells. This is supported by (1) the Fourier transform infrared microspectrometry data, which revealed a significant alteration in the content of homogalacturonan in border-like cells of the qua1-1 mutant (Fig. 7B), and (2) the immunolabeling result on fixed/resin-embedded root tip sections, where staining of border-like cells with JIM5 was significantly reduced. Thus, the QUA1 gene functions in the production of homogalacturonan by root border-like cells.

Figure 7. Fourier transform infrared microspectrometric analysis of border-like cells. Projection of the 60 preprocessed spectra for the wild type and the qua1-1 mutant in the principal component analysis space (A) and mean second derivative absorbance spectra for the wild type and the qua1-1 mutant (B) are shown. PC, Principal component. [See online article for color version of this figure.]
Separation of Border-Like Cells in the *qua1-1* Mutant

It is interesting that our findings on border-like cell separation support the reduced cell adhesion reported in *qua1-1* and *qua2-1* (Bouton et al., 2002; Mouille et al., 2007) but do not support the cell adhesion defect in hypocotyl tissues reported for the *epc1-1* mutant (Singh et al., 2005). A more recent study on a new mutant allele of this gene, *epc1-2*, as well as on *epc1-1* itself showed that neither of these mutations affected cell adhesion (Bown et al., 2007), which was attributed to differences in growth conditions.

Border-Like Cells of Arabidopsis as a Novel and Suitable System for Cell Wall Mutant Screening

Identification of Arabidopsis mutants with altered structure and synthesis of cell wall polymers has been...
fundamental in increasing our understanding of the function of certain wall polysaccharides in relation to plant development. Nevertheless, selection of cell wall mutants is a difficult task, and many approaches have been recently developed for rapidly screening Arabidopsis cell wall mutants. Among other methods, cell wall mutants have been successfully identified on the basis of developmental phenotypes (Baskin et al., 1992; Arioli et al., 1998). Fourier transform infrared microspectrometry analysis (Chen et al., 1998; Mouille et al., 2003), quantification of monosaccharide composition by gas chromatography (Reiter et al., 1997), and matrix-assisted laser desorption ionization-time of flight spectrometry (Lerouxel et al., 2002). In this study, we show that based on a direct observation of root border-like cells of mutants using light microscopy, it was possible to obtain valuable information on the function of cell wall molecules. This revealed that homogalacturonan reduction in the qua1-1 mutant leads to peculiar border-like cell phenotypes easily observed with a light microscope without any staining or further treatment of the roots. Moreover subtle alteration in border-like cell morphology can also be easily monitored. The method needs neither staining of the sample nor any specific treatment apart from immersing the root tip in water. It is simple, reproducible, and fast. We estimated the overall time from seedling collection to the final observation of border-like cells to be approximately 4 min. As a consequence, we believe that direct observation of border-like cells at the light microscope level is a well-adapted method for fast, easy, and inexpensive screening for mutants altered in key developmental processes such as cell attachment and morphology. Therefore, we propose to use it as an alternative and simple approach for accurate detection of novel mutants defective in cell wall structure and function.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Plant material used was wild-type Arabidopsis (Arabidopsis thaliana ecotypes Columbia and Wassilewskija) and the following mutants: mur2-1 (Vainzini et al., 2002), mur3 (Madson et al., 2003), rsw1 (Arioli et al., 1998), rsw1-1 (Singh et al., 2003), qua2-1 (Mouille et al., 2007), rsw1-1 (Harholt et al., 2006; these mutants were in the Columbia background), qua1-1 (Bouton et al., 2002), and kor1 (Nicol et al., 1998; these mutants were in the Wassilewskija background). Seeds were surface sterilized with 35% (v/v) ethanol for 5 min followed by immersion in 35% (v/v) bleach for 5 min. After several washes in sterile distilled water, the seeds were sown on agar-solidified nutrient medium (Baskin et al., 1992). Growth conditions were identical to those described by Víceré et al. (2005). Seeds were grown in vertically orientated square petri dishes in 16-h-day/8-h-night cycles at 24°C for 15 d. The rsw1 mutant was grown at 24°C for 11 d before being transferred to 30°C for 4 d before observation as described by Arioli et al. (1998). Mutant seeds were obtained either directly from the authors who previously described and characterized the mutant or from the Salk Institute.

Formation of Border-Like Cells and Their Associated Mucilage in the qua1-1 Mutant

Ten 11-d-old qua1-1 seedlings were aseptically placed into a droplet of sterile water on autoclaved microscope slides. Border-like cells and mucilage were carefully removed from the root tip using a razor blade. The seedlings were replaced in petri dishes containing the growing medium. The production of both mucilage and border-like cells was observed daily for 6 d by placing the petri dishes on an inverted bright-field microscope stage.

Light Microscopy and Histochemical Staining

Roots were mounted on glass microscope slides in a drop of water and directly examined for morphological analyses using bright-field illumination. Staining of cellulose with calciofluor white M2R (Sigma) was performed as described previously by Andemé-Onzighi et al. (2002). Fresh roots were incubated with the fluorescent probe (1 mg L−1) for 30 min in the dark. After careful washes with distilled water, the roots were observed using a microscope equipped with UV fluorescence (excitation filter, 359 nm; barrier filter, 461 nm). For cytochemical staining of pectins, roots were treated with a solution of 0.05% (w/v) ruthenium red dye (Sigma) in deionized water for 10 to 15 min, then washed extensively in deionized water. Roots were mounted as described above and observed using a bright-field microscope. Images were acquired with a Leica DFC 300 FX camera. For each line, 50 to 60 roots were observed.

Immunofluorescence Labeling

The anti-pectin mAbs used in this study were JIM5, which recognizes homogalacturonan (Wills et al., 2000), LM8, which recognizes a xylogalacturonan-associated epitope (Wills et al., 2004), LM3 specific to (1→4)-β-D-galactan (Jones et al., 1997), and LM6 specific to (1→3)-α-L-arabinan (Wills et al., 1998). The mAb CCRC-M1, which is specific for fucosylated side chains of xyloglucan (Pühlmann et al., 1994), was used to label xyloglucan. Arabino-galactan-proteins were stained by two specific mAbs, JIM13 and LM2 (Knox et al., 1991; Smallwood et al., 1996; Yates et al., 1996). The secondary antibodies used were either fluorescein isothiocyanate (FITC)-conjugated goat anti-rat (JIM5, LM5, LM6, JIM13, and LM2) or FITC-conjugated sheep anti-mouse (CCRC-M1) IgGs (Sigma).

Roots of 15- to 17-d-old seedlings were fixed for 30 min in 4% (w/v) paraformaldehyde and 1% (v/v) glutaraldehyde in 50 mM PIPES, pH 7, and 1 mM CaCl2 (adapted from Wills et al., 2001). Roots were washed in 50 mM PIPES, 1 mM CaCl2, pH 7, and incubated for 30 min in a blocking solution of 3% (w/v) low-fat dried milk in phosphate-buffered saline (PBS), pH 7.2. After being carefully rinsed in PBS containing 0.05% (v/v) Tween 20 (PBST), roots were incubated overnight at 4°C with the primary antibody (dilution 1:5 in 0.1% [v/v] PBST). After five washes with 0.05% PBST, roots were incubated with the appropriate secondary antibody (dilution 1:50 in 0.1% PBST) for 2 h at 30°C. Roots were rinsed in 0.05% PBST, mounted in anti-fade solution (Citifluor AF2; Agar Scientific), and examined using a confocal laser-scanning microscope (Leica TCS SP2 AOBS; excitation filter, 488 nm; barrier filter, 500–600 nm). Control experiments were performed by omission of the primary antibody. An average of 10 to 15 root apices were examined for each antibody.

Fixed Resin-Embedding Root Tip Sections and Immunofluorescence Labeling

Wild-type and qua1-1 root apices of 15 d (2–3 mm long) were fixed in a freezing medium composed of MES buffer (20 mM MES, pH 5.5, 2 mM CaCl2·2H2O, and 2 mM KCl) containing 200 mM Suc and 10% glycerol. Three to four roots were placed in gold platelet carriers prefilled with the same freezing medium and frozen with the EMPACT freezer (Leica Microsystems) as described previously by Studer et al. (2001).

The root apices were then freeze substituted in anhydrous acetone and 0.5% (w/v) uranyl acetate at −90°C for 72 h. The temperature was gradually raised (2°C h−1) to −60°C and stabilized during 12 h to −30°C and stabilized again during 12 h to −15°C. The root apices were washed twice with anhydrous ethanol for 15 min. Embedding was performed at −15°C in a solution of ethanol and London Red (30%, 50%, and 75% [v/v] 8 h each step. Finally, two infiltrations (24 h each) of pure resin were achieved. Polymerization was realized at −15°C, under UV light, for 48 h.

The polymerized root apices were sectioned with a diamond knife (Diatome) on an ultramicrotome (UCT; Leica). Semithin sections (300 nm) were collected on glass well microscope slides coated with poly-l-lys.

The sections were then incubated, for 5 min, in Tris-buffered saline (50 mM Tris-HCl, pH 7.4, and 0.9% [w/v] NaCl) containing 0.2% (w/v) bovine serum albumin.
albunin and 0.01% (v/v) Tween 20, also named 0.01% TBST. After this, they were incubated for 30 min in a blocking solution of 3% (w/v) low-fat dried milk in 0.01% TBST. The semithin sections were rinsed with 0.01% TBST and incubated overnight at 4°C with the primary antibody JIM5 (dilution 1:5 in 0.01% TBST). After five washes with 0.01% TBST, the sections were incubated with the secondary FITC-conjugated goat anti-rabbit antibody (dilution 1:50 in 0.01% TBST) for 2 h at 30°C. The sections were rinsed in 0.01% TBST followed by distilled water washes, mounted in citifluor AF2 and observed in the same conditions as chemically fixed roots. An average of 10 to 15 root apices were examined for the wild type and the qua1-1 mutant.

Fourier Transform Infrared Microspectrometry

Sample Preparation and Spectral Acquisition

Fourier transform infrared microspectrometric analysis was carried out using a BrukerIFS 88 FTIR spectrometer fitted with a Bruker IFSCOMP E II microscope and a MCT detector. For the wild type and the qua1-1 mutant, 15 seedlings were selected. Root tips were placed on a BaF2 window, which exhibits no interference over the wavelength range chosen for the study (i.e. 600–4000 cm⁻¹). A drop of water was used to release border-like cells. The sample area to be analyzed was determined prior to the measurement using a video camera. The focused area was then adjusted to a diameter of 30 μm using a 36× cassegrain objective in order to analyze a representative number of border-like cells. The working mode for spectral analysis was transmittance. Absorbance spectra were recorded after 32 scans with a resolution of 2 cm⁻¹. Four replicates were made in different parts of border-like cells, producing a total of 60 spectra for the wild type and the qua1-1 mutant. Only the 921 to 1776 cm⁻¹ spectral region was kept for the chemometrics analysis because of its high molecular information content.

Spectral Data Pretreatments and Chemometrics Analysis

All spectral data analyses were computed with Matlab (version 7.1; Math Works) and the PLS Chemometrics Toolbox (Eigenvector Research). The spectra were preprocessed in order to obtain representative absorances for our samples. The first step was to normalize each spectrum (maximum absorbance equal to 1) in order to suppress the variance due to the path length variability between samples. Second derivative absorbance spectra were obtained in a second step with the well-known algorithm of Savitzky and Golay (1964). Derivatives were used here to remove uninformative variances (baseline shift) due to physical scattering effects and to better extract chemical variances from spectral data. The last step was to apply a statistical tool named principal component analysis (Massart et al., 1998) on preprocessed spectra. Such tools are generally used to discover particular structures in multivariate spectral data sets and more precisely here to determine if different spectral contributions exist between plant lines.

Sequence data from this article can be found in the GeoBank/EMBL data libraries under accession numbers 814853 for the MUR2 gene, 816556 for the MUR3 gene, 835035 for the KOR1 gene, 829376 for the RSW1 gene, 822105 for the QUA1 gene, 844160 for the QUA2 gene, 818076 for the ARAD1 gene, and 824749 for the EFC1 gene.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Bright-field microscopy corresponding to Figure 3.

Supplemental Figure S2. Bright-field microscopy corresponding to Figure 4.

Supplemental Figure S3. Bright-field microscopy corresponding to Figure 5.

Supplemental Figure S4. Bright-field microscopy corresponding to Figure 6.

Supplemental Figure S5. Controls with omission of primary antibody: border-line cells were stained with the secondary antibody FITC-conjugated anti-mouse IgG for the wild type (A) and the qua1-1 mutant (B).

Supplemental Figure S6. Controls with omission of primary antibody: border-like cells were stained with the secondary antibody FITC-conjugated anti-rat IgG for the wild type (A) and the qua1-1 mutant (B).

Supplemental Table S1. Summary of immunolocalization of polysaccharide and arabinogalactan-protein epitopes in cell wall of border-like cells and secreted mucilage.

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LITERATURE CITED


