REVIEW ARTICLE

Apoplastic barriers in roots: chemical composition of endodermal and hypodermal cell walls

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Received 3 February 1999; Accepted 28 April 1999

Abstract

Based on the characterization of the chemical composition of endodermal and hypodermal cell walls isolated from seven monocotyledonous and three dicotyledonous plant species, a model of the composition of apoplastic barriers in roots is proposed. Depending on the species, endodermal and hypodermal cell walls of roots contained varying amounts of the biopolymers suberin, lignin, cell wall proteins, and carbohydrates. Although analysis of the chemical composition of these apoplastic barriers of roots is now possible, it is pointed out that conclusions from these data concerning the functional properties of these cell walls can not easily be drawn. However, in analogy to suberized periderms it is argued that the suberin should play a role in establishing an apoplastic transport barrier in roots, albeit not a perfect barrier. Furthermore, due to the combined occurrence of suberin, lignin and cell wall proteins it is argued that endodermal and hypodermal cell walls also have an important function as barriers towards pathogens. Finally, it is pointed out that additional experimental approaches combining the investigation of transport properties and of the chemical composition of apoplastic transport barriers in roots are necessary before the function of endodermal and hypodermal cell walls in roots can be fully understood.

Key words: Apoplast, Casparian strip, endodermis, hypodermis, lignin, root, transport, suberin.

Introduction

The vegetative body of terrestrial higher plants is composed of the three organs roots, stems and leaves. Whereas the stems connect above- and below-ground plant organs, leaves and roots are designed to interact with the environ-
and transverse endodermal cell walls are impregnated with lipophilic and aromatic substances (Casparian strips) (Fig. 1a), which are thought to block the apoplastic continuum between cortex and central cylinder for water and dissolved solutes. With certain species, normally when the rhizodermis is shed and lateral roots emerge, the secondary developmental stage of the endodermis is formed. It is characterized by the uniform deposition of a thin, lipophilic suberin lamella to the inner surface of radial and tangential cell walls of endodermal cells (Fig. 1b, c). Finally, there may be a tertiary developmental stage with heavy U-shaped cell wall deposition on the inner tangential and the radial cell walls of the endodermal cells (Fig. 1d, e).

Due to its peculiar cell wall differentiation and ultrastructural features, the primary endodermis is regarded as the main apoplastic transport barrier for the passive uptake of water and dissolved ions from the soil solution into the xylem vessels located in the central cylinder of the root (van Fleet, 1961). It is argued that water and ions, which have passively travelled from the soil solution through the cell walls of the root cortex to the endodermis, must pass through the protoplast of the living endodermal cell in order to gain access to the central cylinder of the root (Clarkson and Robards, 1975). In this way, root selectivity allowing the separation between nutrients and harmful substance is thought to be accomplished (Marschner, 1995). From electron microscopy, it is well known that the plasma membrane is always tightly attached to the radial cell wall in the region of the Casparian strips (Bonnett, 1968; Karahara and Shibaoka, 1992). When cells with Casparian strips are plasmolyzed, the protoplast will not be detached from that region of the radial walls where the Casparian strips are developed (Bonnett, 1968; Enstone and Peterson, 1997). All these observation taken together strongly indicate that apoplastic transport in plant roots is blocked when they form Casparian strips.

However, there is also good evidence that the hypodermis at the root surface might form an important barrier towards passive apoplastic diffusion in roots (Clarkson, 1991). Generally, hypodermal cell walls are also incrusted with lipophilic and aromatic compounds (Peterson, 1998). Furthermore, in response to certain environmental stimuli, there can be the formation of Casparian strips in the hypodermis as they occur in the primary developmental stage of the endodermis (Reinhardt and Rost, 1995; Enstone and Peterson, 1998). Following the terminology of Peterson, a hypodermis with Casparian strips should be called exodermis (Perumalla and Peterson, 1986). Thus, Casparian strips always form a characteristic feature in primary endodermal cell walls, whereas they can be developed in hypodermal cell walls in reaction to certain environmental influences.

Looking at the literature, there is a lot of work dealing with the function of endo- and hypodermis as apoplastic barriers towards the passive diffusion of fluorescent dyes, ions, stable isotopes, and radioactive tracers (Nagahashi et al., 1974; du Pont and Leonard, 1977; Sanderson, 1983; Moon et al., 1986; Clarkson et al., 1978, 1987; Peterson et al., 1993). From this work there is convincing evidence that the endodermis and to a certain degree also the hypodermis can form barriers for the apoplastic transport (Peterson and Cholewa, 1998; Steudle and Peterson, 1998). In view of these data, there are surprisingly few reports dealing with the chemical composition of endodermal and hypodermal cell walls. To understand and evaluate the function of apoplastic transport barriers fully, their chemical structure should be taken into account and integrated into models dealing with water and ion uptake of roots. For this reason, the following review will (1) summarize recent results obtained in this laboratory on the chemical composition of apoplastic transport barriers of roots and (2) as far as is possible relate this structural information to root functional properties.

**Experimental approach**

Since its first description (Caspary, 1858, 1866) there have been many attempts to identify the chemical nature of the cell wall deposition in Casparian strips. The basic approach in earlier times was light microscopy in connection with histochemistry, giving indirect evidence for the chemical nature of Casparian strips (von Guttenberg, 1968; Wilson and Peterson, 1983). Summarizing all this former work, a somewhat contradicting picture was established which indicated that cell wall depositions in Casparian strips are characterized by lipophilic compounds (normally assumed to be suberin) and/or aromatic compounds (normally assumed to be lignin). However, with the exception of a very limited number of approaches (Espelie and Kolattukudy, 1979; Pozuelo et al., 1984), a direct characterization and thorough investigation of the chemical composition of Casparian strips by modern analytical tools has not been carried out.

Therefore, an experimental approach for the characterization of endodermal and hypodermal cell walls was developed which consisted of three consecutive steps: (1) enzymatic isolation of the cell wall material of interest, (2) chemical degradation and (3) qualitative and quantitative analysis of the monomer composition released from the isolated cell wall polymers (Fig. 2).

**Enzymatic isolation**

Since endodermal and hypodermal cell walls contain additional substances which are different from ordinary primary cell walls, they resist an enzymatic treatment with cell wall-degrading enzymes like cellulase and...
Fig. 1. Endodermal maturation of a 10-d-old corn (*Zea mays* L.) plant. Cross-sections were taken at five different positions (4, 12, 20, 28, and 36 cm from the tip) along a primary root 40 cm long. Sections were examined in the light microscope after staining with the red lipophilic dye Sudan III. (a) Primary endodermis with Casparian strips (white arrows). (b) Transition from the primary (white arrows) to the secondary stage of development with a suberin lamella. (c) Complete secondary stage of development with a suberin lamella in each endodermal cell. (d) Beginning of the tertiary developmental stage with U-shaped secondary cell walls deposited on the suberin lamella. (e) Advanced tertiary developmental stage of the endodermis with increased U-shaped depostions of secondary cell wall material.
Fig. 2. Scheme illustrating the experimental approach applied for the analysis of endodermal and hypodermal cell walls isolated from the roots of various plant species. (SEM: Scanning Electron Microscopy; TEM: Transmission Electron Microscopy; LM: Light Microscopy; FM: Fluorescence Microscopy; CHN: Elemental Analysis; FTIR: Fourier Transform Infrared Spectroscopy; GC: Gas Chromatography; FID: Flame Ionization Detector; MS: Mass Spectroscopy; HPLC: High Performance Liquid Chromatography).

pectinase (Robards et al., 1976; Karahara and Shibaoka, 1992; Schreiber et al., 1994). As a result, endodermal and hypodermal cell walls are separated and can be purified. The fine network of endodermal (Fig. 3a) and hypodermal cell walls which is obtained can be subjected to the second step of chemical degradation. Generally, cell wall samples isolated from the endodermis are pure (Fig. 3a, b).

However, isolated cell walls from the root surface, which are called hypodermal for reasons of simplicity (including rhizodermal cell walls in several cases), contained additional cell material (Zeier, 1998). With plants cultivated in hydroculture, the rhizodermis survives significantly longer compared to plants grown in soil and it also resists the enzymatic degradation to a certain degree. Thus, with the species Pism sativum L., Cicer arietinum L., Ricinus communis L., Allium cepa L., and Zea mays L. hypodermal cell walls together with rhizodermal cell walls formed the sample of isolated cell wall material. In the case of other monocotyledonous species, roots were sampled from soil-grown plants in which one to three additional layers of dead cells (brachysclereids) were located outside the hypodermis, these also resisted enzymatic digestion. Thus, with the species Clivia miniata Reg., Agapanthus africanus (L.) Hoffm., Aspidistra elatior Bl., and Iris germanica L., isolated cell walls were derived from the hypodermis and of the brachysclereids. Finally, a multilayered hypodermis was also isolated from aerial roots of Monstera deliciosa Liebm.

Chemical degradation
Since there was convincing evidence from literature that suberin and/or lignin form important constituents of endodermal and hypodermal cell walls (Espelie and Kolattukudy, 1979; Wilson and Peterson, 1983; Pozuelo et al., 1984), depolymerization methods were applied, normally used for the degradation of suberin (Kolattukudy and Agrawal, 1974) and lignin (Lapierre et al., 1991). In addition to these biopolymers, it was reasonable to assume that isolated cell wall samples would still contain carbohydrates which were protected from cellulase and pectinase attack due to the presence of suberin and lignin. Therefore, monomeric carbohydrate
composition was also measured after acidic hydrolysis (Blakeney et al., 1983). Finally, there was evidence from the literature that endodermal and hypodermal cell walls contained nitrogen, indicating the occurrence of structural cell wall proteins (Pristley and North, 1922; Schreiber et al., 1994). Thus, as a fourth possible group of substances occurring in apoplastic transport barriers in roots, the amino acid composition after acid hydrolysis of the proteins was also investigated.

Chromatographic analysis

After depolymerization by the respective degradation methods, sample preparation from the reaction mixtures for lignin, suberin and carbohydrate analyses generally included extraction with organic solvents, concentration of the samples by solvent evaporation, derivatization of alcoholic and carboxylic groups and, finally, analysis by gas chromatography (Fig. 2; Schreiber, 1996; Zeier and Schreiber, 1997, 1998). Three different detection systems were applied during gas chromatography. Quantitative analysis was accomplished using a gas chromatograph coupled to a flame ionization detector (GC/FID); qualitative analysis was carried out with a gas chromatograph coupled to a mass selective detector (GC/MS); in rare cases, the identification of certain functional groups was supported by analysing the samples on a gas chromatograph coupled to a Fourier transform infrared detector (GC/FTIR). Amino acids were characterized by HPLC after derivatization using ninhydrine.

Detected biopolymers

Suberin

A large number of suberin monomers varying in chain length and functionality normally occurred in isolated endo- and hypodermal cell wall samples after BF$_3$/MeOH transesterification (Fig. 4). In general, five dominant substance classes of suberin monomers, could be detected in most of the isolated cell wall samples; these were $\omega$-hydroxyacids, 1, $\omega$-diacids, primary carboxylic acids, primary alcohols, and 2-hydroxyfatty acids (Fig. 4). In most samples, the dominating monomers were the C$_{18}$-unsaturated $\omega$-hydroxyaromatic acid (18-hydroxy-1,18-dioic acid) and 1, $\omega$-dicarboxylic acid (octadec-9-ene-1,18-dioic acid), which have been described as characteristic suberin markers (Bernards and Lewis, 1998). Thus, aliphatic suberin monomers in endo- and hypodermal cell walls were generally chemically the same as suberin occurring in above-ground and below-ground periderms of plants (Kolattukudy, 1980).

FTIR-spectroscopy of isolated endodermal cell walls of Cicer arietinum revealed that suberin-characteristic absorption bands at 2920 cm$^{-1}$ and 2851 cm$^{-1}$, which represent methylene groups occurring in linear aliphatic suberin monomers, completely disappeared after treatment of the samples with BF$_3$/MeOH (Zeier, 1998). This provides good evidence that suberin can completely be degraded and thus is quantitatively recorded by the technique used. In certain species significant amounts of aromatics, especially coumaric and ferulic acids, in addition to the aliphatic suberin monomers, were released after transesterification. This fraction will be called the aromatic suberin domain, in order to differentiate it from the non-esterified, aromatic lignin domain described in the next paragraph.

Recently it was pointed out by Bernards and Lewis (1998) that the aromatic suberin domain of wound periderm from potato is different from that of lignin, since it is composed of (poly)hydroxycinnamates like feruloyltryptamine. In consensus with this view, thioacidolysis of potato wound periderm released only traces of 'lignin-specific' trithioethylated monomers (Negrel et al., 1996). However, as will be shown below, characteristic lignin monomers were always released from isolated endo- and hypodermal cell walls from roots. Furthermore, feruloyltyramine, which has been found as a characteristic aromatic compound in suberized wound periderm of potato, was not detected in our samples. Thus, there are obviously clear differences in the composition of the aromatic domain between suberized potato wound periderm and suberized endodermal and hypodermal cell walls in roots.

Lignin

Trithioethylated guaiacyl and syringyl units, which were derived from the lignin precursors coniferyl and syringyl alcohol, respectively, were the most prominent peaks in

![Fig. 4. Gas chromatogram of suberin monomers obtained after transesterification of endodermal cell walls in their tertiary development in stage isolated from Zea mays L. roots. As representative suberin monomers the two C$_{18}$-unsaturated compounds $\omega$-hydroxyaromatic acid (18-hydroxy-octadec-9-enoic acid) and 1, $\omega$-dicarboxylic acid (octadec-9-ene-1,18-dioic acid) are marked (dark arrows). To give an example of one of the five different substance classes of suberin monomers occurring in the endodermal of corn roots ($\omega$-hydroxyacids, 1, $\omega$-diacids, primary carboxylic acids, primary alcohols, and 2-hydroxyfatty acids) the homologous series of the $\omega$-hydroxyfatty acids ranging from C$_{16}$ to C$_{28}$ is also marked (light arrows).](https://academic.oup.com/jxb/article-abstract/50/337/1267/530648)
all the isolated cell wall samples (Fig. 5). Trithioethylated p-hydroxyphenyl units, derived from the lignin precursor coumaryl alcohol were found in lower amounts and definitely not in all isolated cell wall samples. Trithioethylated lignin monomers always appeared as split peaks since they occur as erythro- and threo-diasteromers. These trithioethylated lignin monomers are the best evidence for the occurrence of lignin, since the basic C₆C₃-structure of the lignin monomers is preserved. However, a series of further degradation products of lower molecular weight with a partial degradation of the C₃-side chain were also detected. Additionally, small amounts of lignin dimers were detected after desulphurization of the lignin monomers (Lapière et al., 1991). Taking into account the highly condensed structure of the lignin polymer, with many different bonds besides the β-O-4-bonds preferentially cleaved by thioacidolysis, there must be lignin oligomers larger than dimers after thioacidolysis, which can not be analysed by the GC-methods applied here due to technical limitations. Therefore, it can be stated that total lignin amounts are underestimated after analysis by the degradation procedure. A quantitative comparison of lignin detected by thioacidolysis with total lignin after applying the acetylbromide method (Johnson et al., 1961), measuring lignin content spectroscopically from absorption at 280 nm, revealed that thioacidolysis gives 25% of the total lignin content (Tables 1, 2).

![Fig. 5. Gas chromatogram of lignin monomers obtained after thioacidolysis of endodermal cell walls in their tertiary developmental stage isolated from Zea mays L. roots. As representative lignin degradation products in large amounts, trithioethylated guaiacyl (G) and syringyl units (S) and, in lower amounts, trithioethylated p-hydroxyphenyl units (H) can be detected. The peaks of the lignin monomers are always split since each of the three trithioethylated degradation products occur as erythro- and threo-diasteromers. Peaks marked with asterisks represent carbohydrates which are also partially released during thioacidolysis.](https://academic.oup.com/jxb/article-abstract/50/337/1267/530648)

**Amino acids**

Different amounts of the amino acids were released from isolated cell walls after acidic hydrolysis with concentrated HCl depending on the species and the developmental stage of the sample. The most abundant amino acids were hydroxyproline (6–17%), serine (6–11%), lysine (4–7%), glycine (9–25%), proline (3–8%), and valine (5–8%), all of which are characteristic constituents of structural cell wall proteins (Cassab and Varner, 1988; Showalter, 1993).

**Carbohydrates**

After acidic hydrolysis with concentrated H₂SO₄ monomeric carbohydrates generally appeared as the most prominent group of compounds, which were detected in isolated cell wall samples from the endodermis and hypodermis of roots (Tables 1, 2). Glucose (a hexose) and xylose and arabinose (pentoses) were the dominating monomers (Tables 1, 2).

**Chemical composition of endodermal and hypodermal cell walls**

Isolated endodermal cell walls of the mono- and dicotyledonous species examined were all composed of suberin, lignin, carbohydrates, and cell wall proteins (Zeier and Schreiber, 1997, 1998; Zeier et al., 1999). However, the relative amounts of the different biopolymers varied considerably with endodermal developmental stage (Table 1). Isolated Casparian strips (primary endodermis) were strongly lignified and also showed high carbohydrate and protein contents. Lignin contents were higher in the Casparian strips of the monocotyledonous species Monstera deliciosa and Clivia miniata than in the dicotyledonous species Pisum sativum (Table 1). The primary endodermis also released detectable amounts of aliphatic suberin monomers, but the amounts were approximately one order of magnitude lower than the total lignin contents. The dominating pentose released from Casparian strips by acid hydrolysis was arabinose (Ara). This sugar, together with the released amino acids hydroxyproline (Hyp), proline, serine (Ser), lysine, glycine, and valine, indicate the presence of typical cell wall glycoproteins like extensins. Ser-(Hyp)₄ represents a common pentapeptide sequence in extensins and Ara is bound glycosidically to the hydroxyl groups of Hyp resulting in Hyp-(Ara)₃ or Hyp-(Ara)₄ patterns (Showalter, 1993). The secondary endodermis is characterized by the deposition of a distinct lamella onto the radial and inner tangential endodermal walls. It can be stained with lipophilic dyes like Sudan III (Fig. 1c). A markedly different chemical composition occurred in the secondary endodermis in comparison to Casparian strips. Most strikingly, the aliphatic suberin content increased and was
approximately one order of magnitude higher than the content in the Casparian strips (Table 1). In parallel to the suberin deposition, the carbohydrate contents of the secondary endodermal cell walls were reduced. Again, the high amino acid release together with arabinose as the dominating pentose indicated the presence of high amounts of cell wall proteins. The presence of cell wall proteins in the secondary endodermis of *Cicer arietinum* was additionally confirmed by immunolocalization experiments using antibodies against extensins and proline-rich glycoproteins (Zeier, 1998). Finally, the lignin contents in the secondary stage of development lamellae were lower than in Casparian strips.

Tertiary endodermal cell walls were characterized by the deposition of lignified, non-suberized carbohydrate cell walls onto the stage II suberin lamella (Table 1). Consequently, when expressed on a dry weight basis, lower suberin contents were measured in the tertiary wall isolates of *Agapanthus africanus* and *Aspidistra elatior*, when compared to the species owing roots with a stage II endodermis. This reduction of the dry weight-related suberin content was even more pronounced in endodermal isolates of *Allium cepa* and *Iris germanica*, which showed additional tertiary thickening of the inner tangential endodermal walls. The tertiary deposits were composed of lignin and carbohydrates and showed a reduced protein content (Table 1). The lignin was similar to that of the Casparian strip of the monocotyledonous species with respect to the ratios of guaiacyl/syringyl distributions. Both, Casparian strip lignin and the lignin of the tertiary deposits was rich in guaiacyl and syringyl monomers with approximately

### Table 1. Chemical composition of endodermal cell walls isolated from six monocotyledonous (*Monstera deliciosa* Liebm., *Clivia miniata* Reg., *Agapanthus africanus* (L.) Hoffmgs. *Aspidistra elatior* Bl., *Iris germanica* L. and *Allium cepa* L.) and three dicotyledonous (*Pisum sativum* L., *Cicer arietinum* L. and *Ricinus communis* L.) species

<table>
<thead>
<tr>
<th>Developmental stage of the endodermis</th>
<th>Species</th>
<th>Aliphatic suberin</th>
<th>Aromatic suberin</th>
<th>Lignin</th>
<th>Amino acids</th>
<th>Carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Casparian strip)</td>
<td><em>M. deliciosa</em></td>
<td>0.4</td>
<td>0.1</td>
<td>6.5 (26)</td>
<td>13.7</td>
<td>49.8 (Ara)</td>
</tr>
<tr>
<td></td>
<td><em>C. miniata</em></td>
<td>1.2</td>
<td>0.2</td>
<td>5.2 (21)</td>
<td>12.7</td>
<td>42.1 (Ara)</td>
</tr>
<tr>
<td></td>
<td><em>P. sativum</em></td>
<td>2.5</td>
<td>—</td>
<td>2.7 (11)</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>II (suberin lamella)</td>
<td><em>C. arietinum</em></td>
<td>19.5</td>
<td>—</td>
<td>2.1 (8)</td>
<td>8.2</td>
<td>13.7 (Ara)</td>
</tr>
<tr>
<td></td>
<td><em>R. communis</em></td>
<td>9.8</td>
<td>0.7</td>
<td>0.8 (3)</td>
<td>20.4</td>
<td>19.3 (Ara)</td>
</tr>
<tr>
<td>III (secondary cell wall deposits)*</td>
<td><em>A. africanus</em></td>
<td>3.0</td>
<td>0.3</td>
<td>3.9 (16)</td>
<td>2.6</td>
<td>58.2 (Xyl)</td>
</tr>
<tr>
<td></td>
<td><em>A. elatior</em></td>
<td>2.5</td>
<td>0.3</td>
<td>4.2 (17)</td>
<td>5.5</td>
<td>39.2 (Xyl)</td>
</tr>
<tr>
<td>III (secondary cell wall deposits)*</td>
<td><em>A. cepa</em></td>
<td>1.8</td>
<td>0.3</td>
<td>3.8 (15)</td>
<td>2.3</td>
<td>62.5 (Xyl)</td>
</tr>
<tr>
<td></td>
<td><em>I. germanica</em></td>
<td>1.6</td>
<td>2.0</td>
<td>4.7 (19)</td>
<td>0.9</td>
<td>61.6 (Xyl)</td>
</tr>
</tbody>
</table>

*Only radial walls with secondary cell wall deposits.

*U-shape secondary cell wall deposits to the radial and inner tangential cell walls.

*Numbers in brackets give lignin amounts determined by the acetyl bromide method.

*Most prominent sugar monomers are given in brackets (Ara = arabinose, Xyl = xylose).

*Not detectable.

*Not determined.

### Table 2. Chemical composition of hypodermal cell wall isolates from six monocotyledonous (*Monstera deliciosa* Liebm., *Clivia miniata* Reg., *Agapanthus africanus* (L.) Hoffmggs. *Aspidistra elatior* Bl., *Iris germanica* L. and *Allium cepa* L.) and three dicotyledonous (*Pisum sativum* L., *Cicer arietinum* L. and *Ricinus communis* L.) species

<table>
<thead>
<tr>
<th>Type of hypodermal isolate</th>
<th>Species</th>
<th>Aliphatic suberin</th>
<th>Aromatic suberin</th>
<th>Lignin</th>
<th>Amino acids</th>
<th>Carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multilayered*</td>
<td><em>M. deliciosa</em></td>
<td>5.0</td>
<td>0.3</td>
<td>8.8 (35)</td>
<td>2.4</td>
<td>20.1 (Xyl)</td>
</tr>
<tr>
<td>Multilayered*</td>
<td><em>C. miniata</em></td>
<td>0.7</td>
<td>5.1</td>
<td>2.7 (11)</td>
<td>3.3</td>
<td>n.d.</td>
</tr>
<tr>
<td>Hypodermal/rhizodermal*</td>
<td><em>P. sativum</em></td>
<td>1.3</td>
<td>—</td>
<td>0.4 (2)</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Hypodermal/rhizodermal*</td>
<td><em>C. arietinum</em></td>
<td>0.5</td>
<td>—</td>
<td>1.1 (4)</td>
<td>17.7</td>
<td>32.6 (Ara)</td>
</tr>
<tr>
<td>Hypodermal/rhizodermal*</td>
<td><em>R. communis</em></td>
<td>4.7</td>
<td>0.4</td>
<td>0.7 (3)</td>
<td>16.3</td>
<td>29.9 (Ara)</td>
</tr>
<tr>
<td>Multilayered*</td>
<td><em>A. africanus</em></td>
<td>2.4</td>
<td>0.1</td>
<td>4.1 (16)</td>
<td>1.9</td>
<td>50.9 (Xyl)</td>
</tr>
<tr>
<td>Multilayered*</td>
<td><em>A. elatior</em></td>
<td>1.1</td>
<td>2.2</td>
<td>5.0 (20)</td>
<td>4.1</td>
<td>n.d.</td>
</tr>
<tr>
<td>Hypodermal/rhizodermal*</td>
<td><em>A. cepa</em></td>
<td>2.3</td>
<td>1.1</td>
<td>3.8 (15)</td>
<td>4.5</td>
<td>n.d.</td>
</tr>
<tr>
<td>Multilayered*</td>
<td><em>I. germanica</em></td>
<td>4.2</td>
<td>0.4</td>
<td>2.5 (10)</td>
<td>1.9</td>
<td>27.2 (Xyl)</td>
</tr>
</tbody>
</table>

*Several layers of hypodermal cells, which were stained by Sudan III.

*One layer of cells stained by Sudan III and additional autofluorescent cell layers.

*Hypodermal and rhizodermal cell walls.

*Numbers in brackets give lignin amounts determined by the acetyl bromide method.

*Most prominent sugar monomers are given in brackets (Ara = arabinose, Xyl = xylose).

*Not detectable.

*Not determined.
equal contents of both monomers in most species. \( p \)-Hydroxyphenyl units were nearly absent (Zeier and Schreiber, 1997, 1998). In comparison, the lignin composition of the dicotyledonous endodermal cell walls (primary isolates of *Pisum sativum* and secondary isolates of *Cicer arietinum* and *Ricinus communis*) were clearly enriched in \( p \)-hydroxyphenyl- and guaiacyl units indicating a lower methoxyl content at the aromatic core (Zeier et al., 1999a). The carbohydrate composition of the tertiary deposits was characterized by high cellulose and xylene contents. The latter was in contrast to the carbohydrate composition of the stage I and II endodermis indicating differences in the hemicellulose fraction and confirming again the low glycoprotein content of the stage III deposits due to low amounts of arabinose. In general, lignified secondary cell walls involved in mechanical support are known to have high xylene contents (Sitte et al., 1998), confirming the view that the tertiary endodermal cell wall deposits contribute to mechanical stabilization.

Hypodermal cell walls were composed of suberin, lignin, carbohydrates, and wall proteins (Table 2). With the exception of *Allium cepa*, monocotyledonous hypodermal isolates derived from mature roots contained multiple cell layers (brachysclereids) with at least one layer staining positively with Sudan III (Zeier, 1998). According to the data from this study, the dry weight-related aliphatic suberin content was higher in species with a multiple hypodermis where several cell layers where stained with Sudan III (e.g. *Monstera deliciosa* and *Iris germanica*), compared to species with only one staining layer and several other autofluorescent but unstaining layers like *Clivia miniata*, *Aspidistra elatior* and *Agapanthus africanus* (Table 2). In all hypodermal isolates lignin was detected (Table 2).

Looking at the composition of hypodermal isolates in more detail, some characteristic features of the chemical nature were obvious. All species contained clearly detectable amounts of \( p \)-hydroxyphenyl units in their hypodermal lignin (Zeier and Schreiber, 1997, 1998; Zeier et al., 1999a) and most species possessed ester-linked aromatics (here called ‘aromatic suberin’) like \( p \)-coumaric and ferulic acids in their cell walls (Table 2). This should render hypodermal cell walls recalcitrant to biodegradation (Monties and Calet, 1992; Nicholson and Hammerschmidt, 1992) and, therefore, could represent a molecular adaptation of the outermost root tissues being in direct contact with the rhizosphere. The enriched \( p \)-hydroxyphenyl content of hypodermal lignin might be explained further by mechanical stress experienced while the root is moving through the soil. It is well known that mechanical stress increases the \( p \)-hydroxyphenyl content of lignins in the case of compression-wood of horizontally growing trees (Campbell and Sederhoff, 1996). This interpretation was confirmed by experiments comparing the hypodermal lignin of *Zea mays* roots growing in soil and in hydroponics in which the former showed a clearly higher \( p \)-hydroxyphenyl content than the latter (Zeier, 1998).

The hypodermal composition of the dicotyledonous species examined differed markedly from that of the monocotyledonous hypodermal nature (Table 2). Dicotyledonous hypodermal isolates unlike their monocotyledonous counterparts, contained high amounts of cell wall proteins and were only slightly lignified. With the exception of *Ricinus communis* they also contained only low amounts of suberin. Since cross-linked cell wall proteins also render the walls more resistant to micro-organisms, these findings might reflect different strategies of preventing pathogen invasion between mono- and dicotyledonous roots. Furthermore, suberized barriers in the roots of the dicotyledonous species examined seem to be predominantly in the endodermis and not in the outer root parts. This interpretation is nicely supported by the observation that the apoplastic dye berberine can easily enter the root cortex of dicotyledonous species, where it accumulates in front of the endodermis (Aloni et al., 1998). However, with the monocotyledonous species, the entrance of berberine in the root cortex was very limited and it tended to accumulate outside the hypodermal cell wall.

### Chemical composition of endodermal and hypodermal cell walls isolated from *Zea mays*

On the basis of the chemical composition of endodermal and hypodermal cell walls of the nine different species presented above, each of which is in a distinct developmental stage, it is not possible to compare the different developmental stages of endodermal and hypodermal cell walls directly, since quite different species were investigated. In order to compare the ontogenetic development of the endodermis and the hypodermis, work with primary roots of *Zea mays* cultivated in hydroculture was continued. Going from the tip to the base of the root, allows one to follow the ontogenetic development of apoplastic root barriers within a single species (Fig. 1). The maturational of the endodermis through the primary, secondary and tertiary developmental stages were followed simultane-ously (Zeier et al., 1999b).

Primary roots of 10-d-old maize plants, which were between 35–45 cm, were divided into five zones of equal lengths varying between 7–9 cm. Endodermal and hypodermal cell walls were separately isolated from each zone and analysed for the occurrence of suberin and lignin. The endodermis of zone I (0–8 cm; Fig. 1a) was exclusively within the primary developmental stage, with Casparian strips in the radial cell walls. A transition from the primary to the secondary developmental stage, charac-terized by the suberin lamella deposited on the inner cell wall surface, occurred over the length of several cm within
zone II (8–16 cm; Fig. 1b). The secondary developmental stage was fully developed in zone III (16–24 cm; Fig. 1c) and a further maturation of the tertiary developmental stage with the characteristic U-shaped cell wall thickening of the endodermis happened in zones IV (24–32 cm; Fig. 1d) and V (32–40 cm; Fig. 1e).

Dry weight-related aliphatic suberin contents of the endodermis constantly increased from zone I to zone II with the highest value in zone III, where the suberin lamella was completely developed (Figs 1c, 6a). Then, suberin amounts decreased again from zone III to zone V when the maturation of the tertiary developmental stage occurred (Figs 1c, 6a). This somewhat puzzling trend in the suberin amounts, first increasing and then decreasing again, is easily explained by the fact that the data are given in the units $\mu g$ mg$^{-1}$ dry weight. With increasing tertiary cell wall depositions, the dry weight of the isolated endodermis increases; thus, the relative amount of suberin decreases although its absolute amount need not change. In order to overcome this problem, the absolute amounts of suberin have been related to root length and it can easily be seen that suberin amounts constantly increase from zone I over zone II to zone III, where the suberin lamella is completely developed (Fig. 6b). From zone III over zone IV to zone V, suberin amounts stayed on a plateau (Fig. 6b). These results in the chemical composition of the endodermis nicely reflected the picture obtained from anatomical analysis using histochemical techniques (Fig. 1). In contrast to the amount of suberin, endodermal lignin contents related to root length increased from zone I to zone V (Fig. 6b).

Looking more closely at the detailed chemical composition of the endodermal suberin, of *Zea mays*, there was a clear developmental trend (Zeier *et al.*, 1999b). The suberin in the endodermal cell walls with Casparian strips was characterized by a relatively large fraction of monofunctional carboxylic acids and the highest chain length of the suberin monomers observed was C$_{24}$. Over zone II to zone III, when suberin lamellae developed, a change in the chemistry of endodermal suberin could be observed. The o-hydroxyfatty acids, described as characteristic markers of suberin, were the most prominent class of compounds. In addition, the chain length distribution of the suberin monomers constantly increased, reaching from C$_{16}$ to C$_{28}$ in the mature suberin of zone III where complete suberin lamellae had developed. Further changes in the suberin composition in zones IV and V did not occur. Lignin composition of the Casparian strips was characterized by relatively low amounts of $p$-hydroxyphenyl units, which increased in zones II to III reflecting a higher $p$-hydroxyphenyl content in the suberin lamella. With increasing tertiary cell wall depositions in zones IV and V, the $p$-hydroxyphenyl content decreased again and lignin, rich in syringyl units was deposited. The lamella lignin obviously differed from the lignin of the Casparian strip and the tertiary deposits by an enrichment in $p$-hydroxyphenyl units.

Fig. 6. Quantities of suberin and lignin released from endodermal and hypodermal/rhizodermal cell walls isolated from primary roots of 10-d-old corn (*Zea mays* L.) plants. Amounts are expressed on the basis of isolated endodermal walls dry weight (a) and to root length (b). Suberin and lignin in isolated hypodermal/rhizodermal cell walls related to dry weight (c) and root length (d).

Environmental effects on the apoplastic barriers of *Zea mays*

In order to investigate the effect of certain environmental stress factors on the suberin and lignin composition of...
apoplastic barriers in roots, *Zea mays* was grown in a hydroculture solution containing 100 μM CdCl₂ (heavy metal stress), 100 mM NaCl (salt stress) and 300 g kg⁻¹ polyethylene glycol (osmotic stress) (Zeier, 1998). After 6 d, primary roots were sampled and endodermal and hypodermal cell walls were isolated separately. In these experiments the tedious and extremely time-consuming differentiation among the different root zones, as described above for the length-dependent characterization of the apoplastic root barriers of *Zea mays*, was not carried out.

There are many indications in the literature that plants react towards different environmental factors (drought and salinity) at the level of their apoplastic barriers in roots (North and Nobel, 1995; Perumalla and Peterson, 1986; Reinhardt and Rost, 1995). A clear reaction of *Zea mays* towards the different stress factors was observed when amounts of suberin and lignin were related to root length. A 3-fold increase in endodermal suberin was observed as a reaction towards Cd-stress, and a 1.5-fold increase in NaCl- and PEG-treated roots. For hypodermal suberin, a 1.5-fold increase in amount was induced by Cd- and NaCl-stress, whereas the PEG-treatment increased suberin amounts by a factor of 3. There was also an average increase of lignin by a factor of 2. It was generally observed that the different stress factors led to a severe reduction in root lengths and that larger parts of the root were in the secondary and tertiary developmental stage of the endodermis. Furthermore, larger parts of the hypodermis were suberized. However, the qualitative chemical composition and substance class distributions of endodermal and hypodermal suberin and lignin of stressed plants was rarely affected and it did not differ from these of control plants.

It has been reported that the hypodermis of some species also develops a Casparian strip and a suberin lamella quite similar to the endodermis when plants are grown in humidified air (aeroponic cultivation) instead of using a liquid hydroculture solution (Clarkson et al., 1987). It is argued that an aeroponic cultivation more resembles natural conditions within the soil, which would contain air-filled spaces with a high humidity. Thus, under these growth conditions, certain environmental stimuli induce the formation of an exodermis. In a first approach, suberin compositions in the hypodermis and the endodermis of 8-d-old *Zea mays* roots grown under hydroponic and aeroponic conditions were again compared. Endodermal and hypodermal walls of the younger parts of the roots where no laterals had developed were isolated and analysed separately from the older parts of the roots. The results showed clearly that suberin amounts in the exodermis in the younger parts of aeroponically grown roots were nearly three times higher than those of the hypodermis of hydropponically grown roots (Zimmermann et al., 1999). No differences in suberin contents of the exodermis and the hypodermis of older roots where laterals had developed were detected. Furthermore, there were no effects on the suberin amounts in the endodermis between aeroponic and hydroponic cultivation.

**Functions of root apoplastic barriers**

It is important to state that it is not possible to predict the exact function of a certain cell wall from an analysis of its chemical composition. Nevertheless, from the occurrence or absence of certain substances some careful qualitative conclusions concerning the function of these cell walls can be drawn. With regard to the transport properties of root apoplastic barriers towards water and dissolved compounds, suberin will definitively be the most interesting and relevant biopolymer. There are many reports on the efficient barrier properties of suberin towards water from the above-ground stem (Schönherr and Ziegler, 1980) and the subterranean storage organs of several plant species (Soliday et al., 1978, 1979; Vogt et al., 1983). The question arises to what extent endodermal and hypodermal suberin can be compared to the suberin of these species. Lignin is always formed as a biopolymer which reinforces the strength of cell walls (Lewis and Yamamoto, 1990). According to our knowledge, there are no indications that lignin is a good barrier for the diffusion of water and dissolved substances. This immediately raises the question ‘why does lignin always occur in combination with suberin in endodermal and hypodermal cell walls?’ The answer probably lies in the fact that both polymers fulfill multiple functions in the plants, besides their function as a transport barrier in the case of suberin and as a cell wall reinforcing material in the case of lignin. In the following sections (1) these results on the chemical composition in terms of transport properties of these cell walls will be tentatively interpreted, (2) some simple model calculations estimating the transport properties of suberized cell walls will be presented and (3) the additional function of these root cell walls as important barriers towards microorganisms will be discussed.

**Transport properties of endodermal and hypodermal cell walls**

The fact that suberin is always present in the state I, II and III endodermis and in all investigated hypodermal cell walls indicates that these cell walls will form an apoplastic transport barrier to a certain extent. In the extreme case of aerial roots of *Monstera deliciosa* (which are directly exposed to the low water potential of the air) the hypodermis contained higher amounts of suberin by one order of magnitude (Table 2) compared to the endodermis (Table 1; Zeier and Schreiber, 1998). The
hypodermis obviously must prevent an excessive loss of water from the root cortex. In soil-grown roots of these species the hypodermis contains only a 2- to 3-fold higher suberin amount than does the endodermis.

The more important difference between the hypodermis of *Monstera deliciosa* and those of other monocotyledonous species is the presence of typical waxes in the former (Zeier, 1998). It is well known that the wax-free suberin and cutin polymer itself is a very ineffective transport barrier towards water and dissolved substances (Schönherr, 1982), but the deposition of waxes to the polymer normally decreases its permeability by orders of magnitude (Soliday *et al*., 1979; Vogt *et al*., 1983; Schönherr and Riederer, 1989). For example, it has been shown that the periderm of freshly harvested potatoes has a fairly high water permeability which rapidly decreases within 5 d by a factor of 10 upon storage of the tuber in air (Vogt *et al*., 1983). This was interpreted as an incorporation of newly synthesized waxes into the suberin polymer.

This comparison with the suberin from potato periderm indicates that the suberin of most of the soil-grown roots which have been investigated up to now will have lower barrier properties, since normally no or only traces of wax-like substances were detected. This view is supported by recent findings, which showed that even abscisic acid is able to cross the endodermal barrier in the apoplast to a certain extent (Freundl *et al*., 1998). The fact that environmental stress such as a low water potential in air results in an adaptation by increasing the barrier properties, indicates that plants are able to adapt to changing environmental conditions. As long as plants are growing in well-watered soil or in hydroculture, there is obviously no pressure to establish a good barrier towards water permeability. A certain kind of stress obviously forms the cultivation under aeroponic conditions, which leads to a 3-fold increase in absolute suberin amounts in the hypodermis. This increased suberin amount clearly affected radial root transport properties, since a 2- to 3-fold decrease in root hydraulic and osmotic conductivity was observed with aeroponically grown *Zea mays* roots compared to roots grown in hydroponic solution (Zimmermann and Steudle, 1998).

Experiments with further stress factors indicated again the roots showed adaptations on the level of their apoplastic cell wall barriers. The general increase of lignin and suberin amounts in endodermal and hypodermal cell walls of *Zea mays* can partially be related to decreased root growth and continued maturation of the stressed roots. From light microscopic investigations, it is evident that larger parts of the stressed roots of *Zea mays* were in their final endodermal and hypodermal developmental stage, which is also described in the literature (Clarkson *et al*., 1987). However, in the case of Cd it is obviously the endodermis which is most affected (with 3-fold higher amounts of suberin compared to the control). This could be interpreted as a response to prevent the transport of Cd to the shoot by keeping it outside the central cylinder. With the PEG-treatment, which basically simulates water shortage in the medium, a 3-fold increase in the suberization of the hypodermis was observed. If a situation of a decreased water supply to roots occurs under natural conditions, it is a reasonable reaction of the root to increase the barrier properties of the hypodermis in order to protect the cortex from an increased water loss. With NaCl, where the suberization of the endodermis and the hypodermis is increased, the roots simultaneously have to prevent an uptake of NaCl to the shoot and an increased water loss to the external soil solution due to increased pressures.

Looking more closely at the development of the endodermis and the hypodermis in primary roots of *Zea mays*, it is obvious that absolute suberin amounts were always higher in the hypodermal cell walls compared to the endodermal cell walls. However, this does not allow the conclusion that the hypodermis necessarily represents the main barrier towards an apoplastic transport in *Zea mays*, without having a close look at the root anatomy. It is well established that under favoured growth conditions the hypodermis matures later and develops much more asynchronously compared to the endodermis (Perumalla and Peterson, 1986). For example, the light microscopic investigation of the roots used in our study (Zeier *et al*., 1999b) indicated that the complete formation of suberin lamellae in all hypodermal cells was only visible in zone V, whereas suberin lamellae in the endodermis started to form in zone II and were completely developed in all endodermal cells in zone III. Furthermore, Caspianar strips are always formed in all endodermal cells, whereas their development in hypodermal cells is strongly influenced by environmental conditions. Thus, although there were higher amounts of suberin detected by the analytical tools in this study, it must be assumed that the endodermis forms the more perfect barrier with much more synchronous development due to its faster maturation.

In case of this view it is interesting to compare these results with a detailed study of the radial and longitudinal transport of water and ions in hydroponically grown roots of *Zea mays* (Frensch *et al*., 1996). It was shown that reflection coefficients were low close to the root tip and solute permeability was high. Here Caspianar strips are formed and their incomplete differentiation coupled with a certain suberin composition might be responsible for this observation. However, in the region where complete Caspianar strips were formed (zones I and II), reflection coefficients increased and solute permeability decreased, whereas root hydraulic conductivity was still high. This might be due to semipermeable properties of Caspianar strips allowing water to pass more easily than...
solute and/or to lower barrier properties of the biomembranes of endodermal cells towards water compared to solutes and/or to lower barrier properties of the biomembranes of endodermal cells towards water compared to solutes. The latter could be due to the occurrence of aquaporins, which have been shown to be preferentially located in endodermal cells (Schäffer, 1998). Finally in the root zones III to V, when a complete suberin lamella has developed, the endodermis as a whole forms the main barrier towards the radial transport of water. A suberin lamella surrounding the complete protoplast will interfere with water flow across the biomembrane and thus, at this developmental stage, symplastic transport across the endodermis will be favoured. However, the discussion presented is exclusively related to roots cultivated in hydroculture and the situation can change when aeroponically grown roots are investigated (Zimmermann and Steudle, 1998).

Model calculations

As stated above, there are no direct methods to measure barrier properties of suberized endodermal and hypodermal cell walls directly. However, a direct measurement of water permeability has been carried out with suberized potato periderm (Vogt et al., 1983). Since the chemical composition of the potato suberin (Kolattukudy and Agrawal, 1974) is not very different from hypodermal and endodermal suberin, it is a reasonable assumption that transport properties will be similar. Thus, it is possible to obtain a rough estimation of the apoplastic transport properties of endodermal and hypodermal cell walls towards water.

Permeances of potato periderm were in the order of $1-3 \times 10^{-10}$ m s$^{-1}$ (Vogt et al., 1983), which is comparable to water permeabilities of most cuticles, located at the leaf/air interface (Schreiber and Riederer, 1996). The amount of suberin per area in potato periderm is $50 \mu g \text{cm}^{-2}$ (own determinations). In order to relate the suberin amount of the corn root to that of potato periderm, the average suberin amount of $500 \text{ng cm}^{-1}$ per root length in zone III, where a complete suberin lamella is developed, must be related to the respective surface area of the endodermal cell walls. With an average length of a tangential endodermal cell wall in zone III of 20 $\mu$m and an average number of 70 endodermal cells in this root zone, the surface area of the endodermal cylinder amounts to $0.14 \text{cm}^2 \text{cm}^{-1}$ root length. Thus, approximately $3.6 \mu g \text{cm}^{-2}$ suberin are deposited in the endodermal cell layer, which is nearly 14 times lower than the amount in the multilayered potato periderm.

The same calculation can be done for root zone I, where suberin is present only in Casparian strips. It has been calculated (Steudle et al., 1993) that the apoplastic area in primary maize roots, formed by the radial cell walls carrying the Casparian strips is about 12% of the total surface area of the endodermal cell walls. With a suberin amount of $30 \text{ng cm}^{-1}$ root length and an apoplastic surface area of approximately $0.014 \text{cm}^2 \text{cm}^{-1}$ root length, a total suberin amount of $2.5 \mu g \text{cm}^{-2}$ apoplastic root surface is obtained, which is about 24 times lower than the suberin amount in the potato periderm.

These calculations indicate that apoplastic barrier properties of the endodermis towards water can not be very pronounced compared to potato. Recent experimental findings showed also that the apoplastic barrier of the endodermis towards water and dissolved substances was by no means perfect (Peterson et al., 1993; Freundl et al., 1998). It simply must be concluded that the apoplastic barrier in the primary endodermis (Casparian strips) is still permeable for water to a certain degree. Furthermore, it must be pointed out that the potato tuber periderm is composed of about six suberized cell layers arranged in series (Vogt et al., 1983). Thus a single layer of cells in the periderm of potato should have an average suberin amount of only $8 \mu g \text{cm}^{-2}$, a value which is in the same order of magnitude as the suberin amount in the endodermis. Thus, it can be concluded that an efficient barrier of suberized cells towards water needs the serial arrangement of several layers of suberized cells in combination with the deposition of waxes. However, it must be added that the situation for dissolved solutes may be quite different. A heterogeneous and complex cell wall like the Casparian strip, composed of carbohydrates, structural proteins, lignin and suberin will carry positive and negative charges. Thus, it will form a charged barrier strongly reducing the penetration rate of charged molecules like ions, whereas small uncharged water molecules might diffuse across this barrier more readily.

Barriers towards microbial attack

As already pointed out above, endodermal and hypodermal walls have to fulfil multiple functions other than their role as apoplastic transport barriers, for example, to provide a barrier to the attack of pathogenic microorganisms. The complicated mixture of suberin, lignin and structural cell wall proteins is a good indication of this additional barrier property towards microorganisms. Generally, the wound reaction of plants towards a fungal or bacterial infection includes the deposition of a very resistant mechanical barrier called wound periderm (Nicholson and Hammerschmidt, 1992). This wound periderm contains very much the same components, such as the polymers suberin and lignin, in addition to increased amounts of structural cell wall polymers. It has been reported that amounts of extensin can be increased during a plant/pathogen interaction in order to reinforce the cell walls and prevent pathogens from further invasion (Borg-Olivier and Monties, 1993). Thus, from this point of view endodermal and hypodermal cell walls can be regarded as kind of preformed wound periderm, which has exactly to conduct this task (Peterson, 1998).
Conclusions and outlook

It has been shown for 10 different plant species that endodermal and hypodermal cell walls forming apoplastic barriers of roots are composed of suberin, lignin, carbohydrates, and structural cell wall proteins. Thus, it is now possible to analyse the qualitative and quantitative composition of apoplastic barriers in the roots from different mono- and dicotyledonous species. Unfortunately, such investigations do not necessarily allow simple conclusions concerning their functions to be reached. In comparison to the suberized periderm of potato tubers, the properties of suberized endodermal cell walls as apoplastic transport barriers were estimated and it was concluded that endodermal and hypodermal cell walls should form less perfect barriers towards water than suberized potato periderm. Structural and functional similarities of apoplastic barriers of roots to wound periderm formed by plants as a defence reaction towards pathogens are highlighted.

However, the many questions still remaining will form the basis for future experimental approaches. In order to estimate more directly the function of suberin as an apoplastic transport barrier, a combined analysis of the chemical composition of apoplastic barriers and of radial transport properties of control plants versus plants adapted to certain environmental influences should be carried out. Additionally, a biochemical and/or molecular biological approach in combination with cell wall analysis and radial transport measurements should be chosen in future, to analyse the effect of reduced or altered suberin contents in the apoplastic barriers of roots on radial transport properties. Furthermore, the nature and function of the relatively abundant structural cell wall proteins in endodermal and hypodermal walls still remains to be elucidated. Since the experimental techniques needed for the analysis of these questions are essentially available, the near future will hopefully bring conclusive new information on the structure and function of root apoplastic barriers.

Acknowledgements

The authors are indebted to Professor R. Guggenheim (Labor für Rasterlektrokemikoskopie, Universität Basel, Switzerland) for offering the opportunity for the transmission electron microscopic investigations. We gratefully acknowledge financial support by the Deutsche Forschungsgemeinschaft.

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