The chemical composition of suberin in apoplastic barriers affects radial hydraulic conductivity differently in the roots of rice (Oryza sativa L. cv. IR64) and corn (Zea mays L. cv. Helix)

Lukas Schreiber1,*, Rochus Franke1, Klaus-Dieter Hartmann1, Kosala Ranathunge2 and Ernst Steudle2

1 Institut für Zelluläre und Molekulare Botanik, Universität Bonn, Kirschallee 1, D-53115 Bonn, Germany
2 Lehrstuhl Pflanzenökologie, Universität Bayreuth, Universitätsstraße 30, D-95440 Bayreuth, Germany

Received 21 October 2004; Accepted 23 February 2005

Abstract
Apoplastic transport barriers in the roots of rice (Oryza sativa L. cv. IR64) and corn (Zea mays L. cv. Helix) were isolated enzymatically. Following chemical degradation (monomerization, derivatization), the amounts of aliphatic and aromatic suberin monomers were analysed quantitatively by gas chromatography and mass spectrometry. In corn, suberin was determined for isolated endodermal (ECW) and rhizo-hypodermal (RHCW) cell walls. In rice, the strong lignification of the central cylinder (CC), did not allow the isolation of endodermal cell walls. Similarly, exodermal walls could not be separated from the rhizodermal and sclerenchyma cell layers. Suberin analyses of ECW and RHCW of rice, thus, refer to either the entire CC or to the entire outer part of the root (OPR), the latter lacking the inner cortical cell layer. In both species, aromatic suberin was mainly composed of coumaric and ferulic acids. Aliphatic suberin monomers released from rice and corn belonged to five substance classes: primary fatty acids, primary alcohols, diacids, \( \omega \)-hydroxy fatty acids, and 2-hydroxy fatty acids, with \( \omega \)-hydroxy fatty acids being the most prominent substance class. Qualitative composition of aliphatic suberin of rice was different from that of corn; (i) it was much less diverse, and (ii) besides monomers with chain lengths of C16, a second maximum of C28 was evident. In corn, C24 monomers represented the most prominent class of chain lengths. When suberin quantities were related to surface areas of the respective tissues of interest (hypodermis and/or exodermis and endodermis), exodermal cell walls of rice contained, on average, six-times more aliphatic suberin than those of corn. In endodermal cell walls, amounts were 34 times greater in rice than in corn. Significantly higher amounts of suberin detected in the apoplastic barriers of rice corresponded with a substantially lower root hydraulic conductivity \( (L_p) \) compared with corn, when water flow was driven by hydrostatic pressure gradients across the apoplast. As the OPR of rice is highly porous and permeable to water, it is argued that this holds true only for the endodermis. The results imply that some caution is required when discussing the role of suberin in terms of an efficient transport barrier for water. The simple view that only the quantity of suberin present is important, may not hold. A more detailed consideration of both the chemical nature of suberins and of the microstructure of deposits is required, i.e. how suberins impregnate wall pores.

Key words: Apoplast, cell wall, endodermis, hydraulic conductivity, suberin, water transport.

Introduction
The hydraulic properties of roots largely vary between species (Kramer and Boyer, 1995) and are strongly affected by environmental conditions which result in changes in root anatomy and morphology (Steudle and Peterson, 1998). To
explain variable water uptake, a composite transport model has been set up, which includes physical as well as physiological elements (Steudle, 2000, 2001). According to the model, there are three parallel pathways of water uptake from the soil solution into the central cylinder of the root: (i) the apoplastic pathway around protoplasts, (ii) the symplastic pathway through plasmodesmata, and (iii) the transcellular pathway with the water molecules moving from one living cell to the next by crossing two plasma membranes and part of the apoplast at each cell layer. The symplastic and transcellular components together are usually considered as the cell-to-cell path, since it is, to date, not possible to separate them experimentally. Along the cell-to-cell path, water transport may be regulated in terms of an expression and/or activation of aquaporins and development of suberin lamellae (Peterson and Cholewa, 1998; Tyerman et al., 1999). The hydraulic conductivity of the apoplastic path may be decreased by the deposition of suberin in Casparian bands in the cell wall (Zimmermann et al., 2000).

Chemically, apoplastic barriers of roots are depositions of the biopolymers lignin and suberin within the cell wall matrix, which may occlude wall pores previously filled with water (Schreiber et al., 1999). Among the different substances, aliphatic suberin rather than lignin or aromatic suberin has the most pronounced effect on the barrier function of biopolymers (Schreiber et al., 1999). In the endodermis of primary roots, the deposition of apoplastic barriers first occurs when Casparian bands form in the primary state of endodermis (for a review see Ma and Peterson, 2003). Later, many species form suberin lamellae at the inner tangential surfaces of endodermal cells with the exception of the passage cells; this constitutes the secondary developmental state of endodermis. Frequently, apoplastic barriers are found in the hypodermis of roots (Hose et al., 2001). Either suberin lamellae are deposited at the inner surfaces of hypodermal cell walls, or Casparian bands are formed prior to the deposition of lamellae. A hypodermis with Casparian bands is called an exodermis (Peterson and Perumalla, 1984).

Environmental factors such as drought salt stress and growth conditions intensify the formation of apoplastic barriers in roots (Radin and Matthews, 1989; North and Nobel, 1994; Reinhardt and Rost, 1995). For example, cultivation of corn seedlings in an aeroponic system induced the formation of an exodermis in primary roots which was not expressed in hydroponic culture (Zimmermann et al., 2000). In turn, water uptake was substantially reduced. Hence, ‘root hydraulics’ are efficiently regulated by modifying the hydraulic resistance along the apoplastic and cell-to-cell pathways by the deposition of the hydrophobic biopolymer suberin.

When rice is grown in paddy fields, one would expect that its hydraulics would not limit water uptake. However, this is not so. In rapidly transpiring shoots of field-grown rice, a water shortage has been observed, even when roots were exposed to a medium fully saturated with water (Hirasawa et al., 1992, 1996). The explanation for this observation is that radial water transport across rice roots is limiting, and that high demands for water from the shoot were not met by the supply from the root. This hypothesis is supported by the fact that there are pronounced apoplastic transport barriers in rice roots, i.e. well-developed exodermal and endodermal cell layers, in addition to a lignified layer of sclerenchyma cells at the inner side of the exodermis (Ranathunge et al., 2003). Not surprisingly, the measured overall radial hydraulic conductivity of rice roots was significantly lower than that of roots of other cereals such as corn (Miyamoto et al., 2001).

This investigation aimed to provide more detailed information on the structural reasons for the differences between the hydraulics of roots of rice and corn. The chemical composition of endodermal and exodermal cell walls of corn roots has been characterized in great detail (Zeier et al., 1999). However, to date, nothing is known about the chemical composition of the apoplastic barriers in rice roots. Corn was selected for comparison because (i) it is a closely related crop species, but cultivated under completely different conditions; (ii) corn roots also exhibit depositions of suberin in endodermal and hypodermal cell walls despite having a different root anatomy from that of rice (Zeier et al., 1999); and (iii) it has been shown that environmentally-induced changes in the composition of apoplastic barriers influence radial hydraulic conductivity of water in roots (Zimmermann et al., 2000). Chemical analyses were related to changes in the hydraulic conductivity in both species to establish whether the lower hydraulic conductivity of rice roots can be explained by differences in the density and/or chemical composition of apoplastic barriers.

**Materials and methods**

**Plant materials and cultivation**

Seeds of rice (*Oryza sativa* L. cv. IR64; International Rice Research Institute, Manila, Philippines) were germinated for 5 d on wet filter paper in the light at 27 °C. Cultivation of seedlings was continued for another 35 d in climatic chambers on an aerated hydroponic culture system with 12 h light (500 µmol m<sup>−2</sup> s<sup>−1</sup> of PAR) and temperatures varying between 27 °C during the day and 22 °C at night. The nutrient solution was replaced every week. It contained 0.09 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.05 mM KH<sub>2</sub>PO<sub>4</sub>, 0.05 mM KNO<sub>3</sub>, 0.03 mM K<sub>2</sub>SO<sub>4</sub>, 0.06 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 0.07 mM MgSO<sub>4</sub>, and 0.11 mM Fe-EDTA as macronutrients and 4.6 µM H<sub>3</sub>BO<sub>3</sub>, 1.8 µM MnSO<sub>4</sub>, 0.3 µM ZnSO<sub>4</sub>, and 0.3 µM CuSO<sub>4</sub> as micronutrients.

Seeds of corn (*Zea mays* L. cv. Helix; Kleinwanzleben Saatzucht AG, Kleinwanzleben, Germany) were germinated for 5 d on wet filter paper in the dark. Cultivation was continued for another 7 d in climatic chambers on an aerated hydroponic culture system (14 h light; PAR: 500–600 µmol m<sup>−2</sup> s<sup>−1</sup>) and day/night temperatures of 20/17 °C. The nutrient solution contained 0.7 mM K<sub>2</sub>SO<sub>4</sub>, 0.1 mM KCl, 2 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 0.5 mM MgSO<sub>4</sub>, 0.1 mM KH<sub>2</sub>PO<sub>4</sub>, and 0.2...
Fe-EDTA mM as macronutrients and 1 μM H$_3$BO$_4$, 0.5 μM MnSO$_4$, 0.5 μM ZnSO$_4$, 0.2 μM CuSO$_4$, and 0.01 μM (NH$_4$)$_6$Mo$_7$O$_{24}$ as micronutrients.

Roots of 12-d-old corn plants had an average length of 0.26 m, whereas those of 40-d-old rice plants were 0.45 m long. For chemical analysis, roots of both species were divided into two zones. In younger root zones (zone I), laterals were not yet present (corn) or were limited in number (rice). In older root zones (zone II), both species had well-developed laterals. Average lengths of zone I in corn and rice roots were 0.11 m and 0.06 m, respectively. Average lengths of zone II of corn and rice roots were 0.15 m and 0.39 m, respectively.

**Light microscopy**

Freehand cross-sections were cut at different distances from the root tip for both species. To examine the developmental stages of rice endodermis, cross-sections were taken 30, 50, 100, and 200 mm from the root tip for both species. To examine the developmental stages of rice endodermis, cross-sections were taken 50, 60, 100, and 10 μl of BSTFA (N,N-bis-trimethylsilyl trifluoroacetamide; Machery-Nagel, Düren, Germany). This procedure converted free carboxy- and hydroxy groups to their trimethylsilyl (TMS) esters and ethers, respectively.

TMS-derivatives were analysed by means of gas chromatography (GC) and mass spectroscopy (MS). Released monomers were quantified by gas chromatography and flame ionization detection (GC-FID; HP 5890 Series II, Hewlett-Packard, Palo Alto, California, USA) using the HPChemStation software (Hewlett-Packard) and referring to an internal standard (20 μg dotriacontane). Monomers were identified using gas chromatography connected with a quadrupole mass selective detector (HP 5971A, Hewlett-Packard).

Results of suberin analyses were either expressed on the basis of total dry weight of original isolates used for the depolymerization or to the surface area of layers where apoplastic barriers are located (exodermis, hypodermis, and endodermis, respectively). Surface areas were calculated from root lengths and diameters obtained by light microscopy of root cross-sections. For 10 individual root samples, correlations between sample dry weights and corresponding surface areas were worked out, whereby the weights of wall preparations (ranging between 100 μg and 1 mg; see above) were precisely determined using a microbalance with an accuracy of 1 μg (Sartorius Microwaage, Göttingen, Germany). Using the ratios between dry weights of wall preparations and surface areas, the amounts of suberin obtained by chemical analysis could be related to surface area.

**Cell wall isolation and preparation**

20–50 g fresh weight (FW) of material from either zones I or II of roots of rice and corn were incubated separately in enzymatic solutions of cellulase (Onozuka R-10, Serva) and pectinase (Macer-ozyme R-10, Serva) in citric buffer (0.01 M) adjusted to pH 3.0. After several days, cell walls which had resisted the enzymatic attack could be sampled under a binocular microscope using forceps. By pulling, central cylinders of corn roots could be separated from cortical sleeves, and endodermal cell walls (ECW) enclosing xylem vessels could successfully be isolated from the rest of the stele (Zeier et al., 1999). Rhizodermal and hypodermal cell walls (RHCW) of corn roots could not be separated and so were always isolated and analysed together. Isolated wall samples were washed in borate buffer (0.01 M, pH 9.2), dried, and stored over silica gel.

As the cell walls of the central cylinder of rice including the endodermis as the outermost cell layer completely resisted enzymatic attack, suberin analyses of the endodermis of rice roots therefore had to be carried out for entire isolated central cylinders (CC). The central cortex of rice roots was composed of aerenchyma, which separated the stele from four cell layers at the root periphery: rhizodermis, exodermis, sclerenchyma, and one layer of unmodified cortical cells. These four cell layers are termed the outer part of roots (OPR: Ranathunge et al., 2003). During enzymatic treatment, only walls of the innermost unmodified cortical layer of the OPR could be digested away. Hence, wall preparations used for analyses still contained the walls of rhizodermal, exodermal, and sclerenchymatous cells. With the exception of the innermost cortical layer, they were identical to those of the outer part of roots (OPR) used in transport studies by Ranathunge et al. (2003). Cell wall preparations of rice were washed and stored as described above.

**Suberin analysis**

Prior to transesterification, cell wall preparations were thoroughly extracted for 16 h using a mixture (1:1, v:v) of chloroform (99% purity; Roth, Karlsruhe, Germany) and methanol (99% purity; Roth) at 40 °C. For suberin depolymerization, the resulting extracted samples of known dry weight (between 1 and 10 mg) were trans-esterified as described by Zeier and Schreiber (1998) using a mixture of methanol/boron trifluoride (MeOH/BF$_3$; Fluka), according to the procedure of Kolattukudy and Agrawal (1974). Released suberin monomers were derivatized for 40 min at 70 °C using a 1:1 mixture of 10 μl dry pyridine (GC-grade, Merck, Darmstadt, Germany) and 10 μl of BSTFA (N,N-bis-trimethylsilyl trifluoroacetamide; Machery-Nagel, Düren, Germany). This procedure converted free carboxy- and hydroxy groups to their trimethylsilyl (TMS) esters and ethers, respectively.

Results of suberin analyses were either expressed on the basis of total dry weight of original isolates used for the depolymerization or to the surface area of layers where apoplastic barriers are located (exodermis, hypodermis, and endodermis, respectively). Surface areas were calculated from root lengths and diameters obtained by light microscopy of root cross-sections. For 10 individual root samples, correlations between sample dry weights and corresponding surface areas were worked out, whereby the weights of wall preparations (ranging between 100 μg and 1 mg; see above) were precisely determined using a microbalance with an accuracy of 1 μg (Sartorius Microwaage, Göttingen, Germany). Using the ratios between dry weights of wall preparations and surface areas, the amounts of suberin obtained by chemical analysis could be related to surface area.

**Root-pressure probe experiments**

Root pressure probe experiments were performed as described previously for corn and rice (Zimmermann et al., 2000; Miyamoto et al., 2001). Excised root segments were tightly connected to a root pressure probe using a cylindrical silicone seal prepared from liquid silicone material (Xantopren, Bayer, Leverkusen, Germany). The segments of corn roots used were between 75 and 120 mm in length and had diameters between 0.65 and 1.1 mm. End segments of rice roots were 150–200 mm long with a diameter of 0.8–1.2 mm. In corn, stable root pressures were normally obtained after 1–3 h, whereas in rice between 5 h and 12 h were required (Miyamoto et al., 2001). Root segments fixed to the probe were bathed in nutrient solution circulated along the roots to avoid problems with unstirred layers. Hydrostatic and osmotic relaxations were performed by changing either xylem pressure (moving the metal rod in the probe) or the osmotic pressure of the external medium. Test solutions used in osmotic experiments contained 20–40 mM NaCl (∼40–80 mOsmol kg$^{-1}$ of osmotic concentration, which is equivalent to osmotic pressures of 0.1–0.2 MPa) in addition to the nutrients within the medium. Transient changes of pressure were followed. Root hydraulic conductivity ($L_p$) was calculated according to equation 1 using the half-time of water exchange ($T_{1/2}$) or the rate constant $k_w$ (Steudle et al., 1987):

$$k_w = \ln(2) / T_{1/2}$$

$$A_k = \frac{\Delta P}{\Delta V}$$

$A_k$ is the effective surface area of the root investigated and $\Delta P/\Delta V$, (in MPa m$^{-3}$) is the elastic coefficient of the measuring system. $\Delta P/\Delta V$ was measured by inducing step-changes in the volume and recording the resulting changes in root pressure ($\Delta P$). Responses in root pressure to changes in osmotic pressure were biphasic, with a rapid water phase (efflux or influx) followed by a slower solute phase. At the end of each experiment, the proper functioning of the mounted root was tested by cutting off the root at the seal and checking the decrease of the time constants of pressure relaxations.
When root xylem remained open during fixation to the probe, there was a drastic decrease in $L_{p,r}$ after excision. If not, the data from the measurements were discarded.

Statistics

Suberin analyses and root pressure probe measurements were made for the same set of plants cultivated at the same time under identical conditions. The radial hydraulic conductivity of rice and corn roots was determined for six replicates of each species. Hydraulic conductivities measured for the roots of this set of rice plants have already been reported elsewhere (Ranathunge et al., 2003), whereas those for corn roots are presented here for the first time. The suberin composition of isolated cell wall samples of corn (ECW and RHCW) and rice (CC and OPR) were determined by analysing three replicates, each consisting of roots sampled from at least 20–30 individual plants. Results are given as means ± SD. To check for statistical significance, t-tests were conducted between pairs of means.

Results

In rice roots, endodermal suberin lamellae first appeared 30 mm behind the root tip (Fig. 1A) and all endodermal cells had suberin lamellae at 50 mm; 5–8 passage cells were present. At 100 mm, endodermal cells were characterized by U-shaped tertiary cell wall depositions, and there were, on average, 5–8 passage cells still (Fig. 1B). At 200 mm behind the root tip, no passage cells were observed in the endodermis (Fig. 1C). Exodermal suberin lamellae started to develop 30 mm behind the root tip in rice and were fully developed at about 60 mm behind the tip (Fig. 1D). In the root endodermis of corn, there were no endodermal suberin lamellae at a distance of 50 mm behind the root tip (Fig. 1E); these first became apparent 60 mm behind the root tip and were fully developed at distances of between 100 and 150 mm (Fig. 1F). On average, the endodermis had 3–4 passage cells at 100 mm and this number decreased to 2–3 at 150 mm behind the root tip (for passage cells also see the Introduction). Suberin lamellae in the root hypodermis of corn first appeared at 60 mm behind the root tip (Fig. 1G), but the deposition of suberin lamellae in the hypodermis was not complete even at a distance of 120 mm behind the root tip (Fig. 1H).

The average lengths of zone I of corn and rice roots were 0.11 m and 0.06 m, respectively, and the average lengths of zone II of corn and rice roots were 0.15 m and 0.39 m, respectively. In isolated OPR of zone II of rice, the chain length distributions of aliphatic suberin monomers belonging to the five detected substance classes ranged from $C_{16}$ to $C_{30}$ (Fig. 2A). Chain lengths of $C_{16}$ and $C_{28}$ were the most abundant in rice suberin, but chain lengths $C_{22}$ and $C_{24}$ could not be detected. As in the OPR of zone II, similar qualitative suberin compositions were also found for the CC of root zone II and the OPR and CC of root zone I of rice (data not shown). Chain length distribution of the five substance classes in the RHCW in zone II of corn roots ranged from $C_{16}$ to $C_{26}$, with $C_{24}$ being the most abundant chain length (Fig. 2B). A qualitative pattern of suberin composition similar to that shown in Fig. 2B was found for the ECW of root zone II and for RHCW and ECW of root zone I of corn (data not shown).

When comparing zones I and II of rice, there was no pronounced trend of an increase in aliphatic suberin (Fig. 3A), $\omega$-Hydroxy fatty acids formed the most prominent substance class of rice root suberin (Fig. 3A). In corn, greater amounts of aliphatic suberin were observed in zone II than in zone I (Fig. 3B). Similar to rice, $\omega$-hydroxy fatty acids represented the most prominent class of aliphatic suberin in corn roots (Fig. 3B).

When comparing the quantities of aliphatic suberin released from the OPR and CC of both zones of rice roots to the dry weight of isolated cell wall material, no differences between root zones were found. However, in corn roots, the quantity of suberin present was much greater in zone II than in zone I (Fig. 4A). Suberin amounts in zone I of corn were lower than in either zone of rice. However, suberin amounts in root zone II of corn were much higher than in rice (Fig. 4A). However, when suberin amounts are related to root surface area, a completely different picture emerged, as aliphatic suberin contents were, on average, 6-fold and 34-fold greater in both zones of rice roots than in corn (OPR versus RHCW and CC versus ECW, respectively; Fig. 4B). In both species, there was a slight trend for the amounts of aliphatic suberin to increase between root zone I and II (Fig. 4B).

In both species, aromatic suberin was basically composed of coumaric and ferulic acids. When expressed relative to the dry weight of the isolated wall material, the amounts of aromatic suberin were similar in the OPR and CC of both root zones of rice (Fig. 5A). The quantities of aromatic suberin of corn were 3–4-fold lower than in rice (Fig. 5A). When the quantity of aromatic suberin was expressed per unit root surface area, much higher differences were apparent between rice and corn. The amount of aromatic suberin in rice OPR was, on average, 80-fold greater than in corn RHCW and 50-fold greater when comparing rice CC with corn ECW (Fig. 5B). The reason for this difference is that dry weights were larger in rice than in corn, but root surface areas were similar. However, reference to surface area instead of dry weight is physiologically relevant, since suberin is supposed to form an apoplastic transport barrier. In both species, contents of aromatic suberin were greater in root zone II as in zone I (Fig. 5A).

The radial hydraulic conductivity of the distal segments of corn roots ($L_{p,r}$) was 10 times greater when measured in the presence of a hydrostatic pressure gradient compared with an osmotic pressure gradient (Table 1). The radial hydraulic conductivity of end segments of rice roots ($L_{p,r}$) was 3.4 times greater when measured in the presence of a hydrostatic pressure gradient compared with an osmotic pressure gradient (Table 2). $L_{p,r}$ of rice roots measured in the presence of a hydrostatic pressure gradient was 2.6-fold
lower than in corn roots (significant at the 95%-level; Fig. 6). In the presence of an osmotic pressure gradient, however, the radial hydraulic conductivities of the two species were not significantly different (t-test; Fig. 6).

**Discussion**

According to the composite transport model of the root, water transport in the apoplast can be distinguished from
water transport along the cell-to-cell path by the type of pressure gradient applied. In the presence of an osmotic pressure gradient, water flow is largely restricted to the cell-to-cell rather than the apoplastic path around protoplasts. In the presence of hydrostatic pressure gradients, there will be an apoplastic water flow in addition to the cell-to-cell component. There was no difference in the osmotic \( L_{p-o} \) between rice and corn roots, but there was a pronounced difference when comparing hydrostatic \( L_{p-h} \), which was 2.6-fold smaller in rice than in corn (Fig. 6). This suggested differences in the apoplastic component resulting from apoplastic barriers.

Differences in the formation and/or structure of apoplastic barriers between the roots of these two species support this view, as indicated by the light microscopy and histochemistry results obtained. The data from this study show that the exodermis of rice was already fully developed at 30–60 mm from the root tip (including a suberin lamella). In hydroponically grown corn, an exodermis is missing (Zimmermann et al., 2000) and suberin lamellae started to be deposited in the hypodermis relatively far from the root tip (around 60 mm), and were still patchy in mature root zones (around 120 mm). Furthermore, apoplastic barriers in the rice endodermis (Casparian bands and a suberin lamella) developed much earlier than in corn. However, compared with corn, the rice endodermis retained a greater number of passage cells without developing suberin lamellae in mature parts of the root.

Roots of the two species showed differences in both the qualitative chemical composition of suberin and the total amount of these compounds. It is known that the biopolymer suberin forms most of the barrier against water transport, and that the aliphatic domain should be more efficient than the aromatic (Schönherr, 1982; Vogt et al., 1983). Therefore, analyses were restricted to aliphatic suberin, although it has been shown that apoplastic barriers in roots contain significant amounts of other biopolymers such as lignin, carbohydrates, and cell wall proteins (Schreiber et al., 1999). Comparing the qualitative composition of aliphatic suberins of rice and corn, showed that rice is much less diverse. All five substance classes (primary fatty acids, primary alcohols, diacids, \( \omega \)-hydroxy fatty acids, and 2-hydroxy fatty acids) could only be detected at lower chain lengths between \( C_{16} \) and \( C_{20} \). The chain lengths \( C_{22} \) and \( C_{24} \) were completely missing and higher chain lengths from \( C_{26} \) to \( C_{30} \) were represented only by \( \omega \)-hydroxy fatty acids (Fig. 2A). In corn, chain lengths of the monomers continuously increased from \( C_{16} \) to \( C_{26} \). Each chain length was formed by at least two, and in most cases three or more substance classes, leading to suberin with a larger variation in terms of its component monomers. Although there are obvious differences in the qualitative composition of suberin between the two species, it is difficult to deduce functional differences between both types of suberin in terms of barrier properties. However,
Based on the lower diversity of monomers and especially on the longer chain lengths formed by only one substance class, there was speculation that rice suberin is more hydrophobic than that of corn.

When proceeding from the younger (zone I) to the older part of the root (zone II), a pronounced developmental gradient was evident in the hypodermis and endodermis of corn (Zeier et al., 1999). In all five substance classes, the amounts of monomers significantly increased between 3-fold and 15-fold in corn depending on the root zone and substance class (Fig. 3B). In rice, the gradients in suberin development were smaller (Fig. 3A), suggesting that the amounts of suberin required for the formation of the apoplastic barriers were already fully deposited in zone I of rice roots, and there was no need for further deposition of suberin in zone II (Fig. 3A). It is concluded that, unlike corn, there were already well-suberized apoplastic arrays in the younger parts of rice roots as was also evident from the microscopic investigations. In corn roots, however, there was a pronounced developmental gradient along the root, which was also evident from the microscopic investigations (Fig. 1). For rice, this may represent an adaptation to the flooded habitat, in which it normally grows. Comparing the total amounts of all monomers added (Fig. 4A), the developmental gradient of aliphatic suberin along corn roots is evident compared with rice.

### Table 1. Hydraulic conductivity of end segments of 12-d-old corn roots (Zea mays L. cv. Helix) measured with the root pressure probe

Plants were cultivated in aerated hydroponics, and hydrostatic pressure gradients between the xylem and the medium were induced by hydrostatic relaxations. Osmotic water flow was induced by adding NaCl to the external nutrient solution.

<table>
<thead>
<tr>
<th>Root</th>
<th>Surface area of measured corn root segments (mm²)</th>
<th>Hydraulic conductivity $L_p$, as measured in hydrostatic relaxations (m s⁻¹ MPa⁻¹ × 10⁻⁵)</th>
<th>Hydraulic conductivity $L_p$, as measured in osmotic relaxations (m s⁻¹ MPa⁻¹ × 10⁻⁵)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>350</td>
<td>9.9</td>
<td>1.1</td>
</tr>
<tr>
<td>2</td>
<td>390</td>
<td>4.7</td>
<td>0.27</td>
</tr>
<tr>
<td>3</td>
<td>310</td>
<td>8.6</td>
<td>1.4</td>
</tr>
<tr>
<td>4</td>
<td>380</td>
<td>6.5</td>
<td>0.31</td>
</tr>
<tr>
<td>5</td>
<td>320</td>
<td>15</td>
<td>1.0</td>
</tr>
<tr>
<td>6</td>
<td>280</td>
<td>1.3</td>
<td>1.5</td>
</tr>
<tr>
<td>Mean</td>
<td>339</td>
<td>9.5</td>
<td>0.93</td>
</tr>
<tr>
<td>SD</td>
<td>42</td>
<td>3.7</td>
<td>0.52</td>
</tr>
</tbody>
</table>
However, this was not the case for the aromatic suberin monomers detected (Fig. 5A).

From both light microscopy and histochemistry it is evident that most of the aliphatic suberin monomers released from the different isolated cell wall samples are likely to have been located in the apoplastic barriers of the endo- and hypo- or exodermis of both species. Only with corn endodermis did isolated cell wall samples (ECW) really represent the suberized apoplastic barrier of interest. This was not the case with corn hypodermis since isolated cell wall material was composed of hypodermal and rhizodermal cell walls (RHCW), although microscopy and histochemistry clearly showed that the aliphatic suberin was mostly deposited in the hypodermal cell walls (Fig. 1).

For rice, this argument becomes even more important, since suberin in the exodermis had to be analysed using isolated cell wall samples composed of three cell layers (OPR). Finally, the endodermal suberin could only be analysed using complete central cylinders (CC) due to the fact that lignified walls resisted enzymatic degradation in rice.

Consequently, the amounts of suberin detected will necessarily underestimate the actual suberin content in the barrier, when they are related to cell wall isolates containing additional cell wall material, leading to increased dry weights. The data presented in Figs 4A and 5A were therefore replotted as the quantity of suberin amounts per unit surface area of the relevant tissues of interest (exodermis, hypodermis, and endodermis). Physiologically, expression of suberin content per surface area makes more sense when comparing different species.

The pronounced difference between rice and corn was apparent when suberin content was expressed per unit surface area. The quantity of aliphatic suberin in rice exodermis was, on average, 6-times higher than in corn hypodermis and it was, on average, 35-fold greater in rice endodermis than in corn endodermis (Fig. 4B). In both species, there was a slight trend for the amount of aliphatic suberin to increase along the roots from zone I to zone II.

For aromatic suberin, even larger differences were obtained when the values were expressed per unit of root surface area. The amounts of aromatic suberin were, on average, 50–80-times greater in the apoplastic barriers of rice than in corn (Fig. 5B). However, in contrast to aliphatic suberin, the quantification of aromatic suberin may have been overestimated. It is known that aromatics (ferulic and coumaric acids) are also covalently linked to normal non-lignified or suberized cell walls in grasses (Chabbert et al., 1994). This is also evident from the pronounced autofluorescence of all cell walls in corn and rice roots, indicating the existence of aromatic compounds in nearly all cell walls. Nevertheless, the pronounced difference in quantities of aliphatic suberin in apoplastic transport barriers in roots

Table 2. Hydraulic conductivity of end segments of 30-d-old rice roots (Oryza sativa L. cv. IR64) measured with the root pressure probe

Plants were cultivated in aerated hydroponics, and hydrostatic pressure gradients between the xylem and the medium were induced by hydrostatic relaxations. Osmotic water flow was induced by adding NaCl to the external nutrient solution.

<table>
<thead>
<tr>
<th>Root</th>
<th>Surface area of measured corn root segments (mm²)</th>
<th>Hydraulic conductivity ( L_{pr} ) as measured in hydrostatic relaxations (m s⁻¹ MPa⁻¹ x 10⁻⁸)</th>
<th>Hydraulic conductivity ( L_{pr} ) as measured in osmotic relaxations (m s⁻¹ MPa⁻¹ x 10⁻⁸)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>438</td>
<td>3.3</td>
<td>0.9</td>
</tr>
<tr>
<td>2</td>
<td>535</td>
<td>3.6</td>
<td>0.9</td>
</tr>
<tr>
<td>3</td>
<td>273</td>
<td>3.0</td>
<td>0.7</td>
</tr>
<tr>
<td>4</td>
<td>326</td>
<td>4.4</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>374</td>
<td>4.0</td>
<td>2.0</td>
</tr>
<tr>
<td>6</td>
<td>531</td>
<td>3.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Mean</td>
<td>413</td>
<td>3.7</td>
<td>1.1</td>
</tr>
<tr>
<td>SD</td>
<td>108</td>
<td>0.6</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Fig. 6. Hydraulic conductivities \( L_{pr} \) measured by using hydrostatic (hydrostatic \( L_{pr} \)) and osmotic (osmotic \( L_{pr} \)) pressure gradients with the end-segments of rice (Oryza sativa L. cv. IR64) and corn roots (Zea mays L. cv. Helix). Data for rice are from Ranathunge et al. (2003) while those for corn were taken from Table 1. In the hydrostatic experiments, hydraulic conductivity was measured by changing the root turgor pressure with the aid of the root pressure probe. In the osmotic experiments, relaxations were induced by changing the osmotic pressure of the external medium. Means with standard deviations (\( n = 6 \) roots). Asterisks indicate a statistically significant difference at the 95% confidence level (t-test).
could help to explain why hydrostatic $L_p$ was significantly lower in rice than in corn, whereas osmotic $L_p$, did not differ significantly between species.

The simple conclusion that water permeability would be reduced as the amount of suberin is increasing is hard to justify according to recent results for the hydraulic permeability of the outer part of rice roots ($L_{OPT}$). The OPR of rice contains larger amounts of suberin than corn but is, nevertheless, highly permeable to water. It was shown that $L_{OPT}$ was larger by a factor of 30 than the overall $L_p$ of intact rice roots, which contained both an exo- and endodermis arranged in series (Ranathunge et al., 2003). Clogging of the apoplastic pores of the OPR by small ink particles and/or copper-ferrocyanide precipitates substantially decreased the apoplastic water flow (Ranathunge et al., 2004, 2005). The effect was even more pronounced when insoluble salts were precipitated in the OPR (Ranathunge et al., 2005). These results suggest that wall pores within the OPR were still open despite containing large amounts of suberin. In rice, there is even an apoplastic bypass-flow of ions (Yeo et al., 1987; Yadev et al., 1996). It is concluded that, at least for rice, any conclusion drawn from suberin contents regarding the water permeability of the cells and tissues involved is premature.

Schreiber et al. (2004) described a comparable situation for the suberized periderm of potato. Wound periderm of potato, containing about 60% of the quantity of suberin detected in native periderm, had water permeabilities which were about 100-fold higher. This is difficult to explain in terms of suberin content. In wound periderm, the major function of suberin is related to pathogen defence rather than protection of living tissue from desiccation. In a similar way, it could be argued that strong suberin depositions in rice OPR are required to protect roots grown in paddy fields from pathogen attack. However, it is more likely that suberin deposition prevents losses of oxygen from the aerenchyma, as indicated by the measurement of changes in the permeability coefficient of oxygen along developing rice roots (L Kotula and E Steudle, unpublished results). Thus, analyses of suberin depositions in apoplastic barriers and their detailed chemical structure are a necessary, but not sufficient, prerequisite to explain the observed changes in water permeability, since the precise molecular and topographical deposition of suberin in root cell walls must also be determined as the latter determines the reduction of porosity and permeability of roots. In order to make barriers really water-tight, suberin should fill all wall pores (intermicrofibrillar spaces), i.e. it must completely impregnate the wall material. In fact, hydrophobic aliphatic suberin may have problems in filling pores consisting of a rather hydrophilic material such as cellulose. To date, virtually nothing is known about the microstructure of apoplastic barriers. Histochemistry does not help much here. Techniques with a high spatial resolution are required to resolve porosity in arrays containing apoplastic suberin depositions, such as the precipitation techniques mentioned above or molecular techniques (i.e. suberin-specific antibodies).

In summary, the results show that the deposition of suberins in root cell walls do not allow straightforward conclusions to be drawn regarding the degree of inhibition of water and ion transport. Comparison of the roots of rice and corn indicates that the main hydraulic resistances were located at the endodermis, which contained 35-times more suberin in rice than in corn. Future work relating the radial hydraulic conductivity of rice and corn roots to the existence of apoplastic transport barriers badly requires answers to the following questions: (i) to what extent does the hypodermis of corn roots contribute to the overall resistance of the radial water flow and is it similar or completely different from rice? (ii) what is the exact nature of the local deposition of suberin in apoplastic barriers, and are there techniques available which offer the necessary spatial resolution? (iii) to what extent are additional structural biopolymers such as lignin or cell wall proteins import constituents of apoplastic barriers in rice roots? This information is required for a better understanding of the structure and the function of suberized apoplastic barriers in roots.

Acknowledgements

We thank Professor Carol A Peterson, University of Waterloo, Canada for reading and discussing the manuscript. The authors gratefully acknowledge financial support by the Deutsche Forschungsgemeinschaft (DFG) within the Schwerpunktprogramm ‘Apoplast’ to Lukas Schreiber and Ernst Steudle (SCHR 506/5-2 and Ste 319/3-3).

References


