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Water and solute permeabilities of Arabidopsis roots in relation to the amount and composition of aliphatic suberin

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Abstract

Although it is implied that suberized apoplastic barriers of roots negatively correlate with water and solute permeabilities, direct transport measurements across roots with altered amounts and compositions of aliphatic suberin are scarce. In the present study, hydroponically grown Arabidopsis wild types (Col8 and Col0) and different suberin mutants with altered amounts and/or compositions (horst, esb1-1, and esb1-2) were used to test this hypothesis. Detailed histochemical studies revealed late development of Casparian bands and suberin lamellae in the horst mutant compared with wild types and esb mutants. Suberin analysis with gas chromatography and mass spectrometry (GC-MS) showed that the horst mutant had ~33% lower amounts of aliphatic monomers than Col8 and Col0. In contrast, enhanced suberin mutants (esb1-1 and esb1-2) had twice the amount of suberin as the wild types. Correlative permeability measurements, which were carried out for the first time with a root pressure probe for Arabidopsis, revealed that the hydraulic conductivity (Lp) and NaCl permeability (Ps) of the whole root system of the horst mutant were markedly greater than in the respective wild types. This was reflected by the total amounts of aliphatic suberin determined in the roots. However, increased levels of aliphatic suberin in esb mutants failed to reduce either water or NaCl permeabilities below those of the wild types. It was concluded that the simple view and the conventional assumption that the amount of root suberin negatively correlates with permeability may not always be true. The aliphatic monomer arrangement in the suberin biopolymer and its microstructure also play a role in apoplastic barrier formation.

Key words: Aliphatic suberin, Arabidopsis, gas chromatography, hydraulic conductivity, mass spectrometry, mutants, NaCl permeability.

Introduction

Water and solute transport in roots has been shown to be variable due to (i) species specificity, with different morphologies and structures; (ii) growth conditions—that is, hydroponics, aeroponics, and soil; and (iii) different biotic and abiotic stresses (Weatherley, 1982; Kramer and Boyer, 1995; Steudle, 2000a). According to the water demand from the shoot, the roots can adapt or even regulate water flow by changing the pathways (apoplastic versus cell to cell). Evidence collected over the past decade shows that the phenomenon of variable root hydraulics is not only related to the permeability of root cell membranes to water (as it largely is for nutrient ions), but it also depends on some variability along the apoplastic passage (Steudle and Peterson, 1998). According to the composite structure of roots, the water flow across roots can be described in terms of a composite transport model which allows water to move through the membranes and/or along the apoplast depending on the resistances (Steudle, 1989, 2001).

The apoplast is defined as the extraprotoplastic (outside the plasmalemma) compartment in the plant body and comprises cell walls, intercellular spaces, and the xylem (Münch, 1930; Peterson and Cholewa, 1998). Unmodified celluloseic cell walls provide a porous network for the transport of a diverse range of substances such as water, gases, nutrient ions, and other solutes (Peterson and Cholewa, 1998; Nobel, 1999). The symplast, on the other
hand, is the continuum of the cytoplasm interconnected by plasmodesmata. Hence, the terms ‘apoplastic’ and ‘symplastic’ transport refer to movements within the two defined compartments. In addition, water and ions also move across plasma membranes in cells, termed the transmembrane pathway. To date, it is not possible to separate the symplastic and transmembrane components experimentally and so they are summarized as the ‘cell to cell pathway’ (Steudle and Frensch, 1996; Steudle, 2000a, b). Water flow through the cell to cell pathway is mainly governed by aquaporins in the plasma membrane (Maurel, 1997; Henzler et al., 1999; Tyerman et al., 1999). Because roots can adjust their hydraulic conductivity according to the water demand from the shoot, combinations and switching between pathways are an important issue (Steudle and Frensch, 1996; Steudle and Peterson, 1998; Steudle, 2000a, b).

It is well known that water and ion movement through the apoplast and membranes can be hampered by modifications in the walls of certain localized cell layers such as the endodermis and the exodermis. These modifications, also termed apoplastic barriers, are known to occur in two forms: (i) state I—the development of Casparian bands in the radial and transverse cell walls due to encrustations of suberin and lignin biopolymers in the water-filled channels of primary cell walls (Schreiber et al., 1999). The Casparian bands are generally accepted to be the main resistors against water and solute fluxes through the apoplast. (ii) State II—the deposition of suberin lamellae as sheets on the inner surface of the primary cell walls, just outside the plasmalemma, named secondary cell walls. Lüttge and Higinbotham (1979) termed the lamellae as ‘adcrusted suberin’. Unlike Casparian bands, suberin lamellae hinder the transmembrane transport of water and solutes.

Suberin is a heterogeneous biopolymer composed of aliphatic and aromatic domains (Kolattukudy, 1980; Bernards and Lewis, 1998; Schreiber et al., 1999; Franke et al., 2005). Aliphatic domains, rather than aromatic domains, impart a defined hydrophobic characteristic to the walls, providing barrier properties against water and solute transport (Schreiber et al., 1999). The aliphatic domain is a polyester polymer primarily comprised of ω-hydroxyacids, ω,ω-dicarboxylic acids (diacids; DAs), carboxylic acids, and primary alcohols as long-chain aliphatic constituents with chain lengths ranging from C_{16} to C_{32}; in Arabidopsis, it is up to C_{24} (Schreiber et al., 1999; Franke et al., 2005). In the past, it has been shown that the amount of aliphatic suberin in roots is negatively correlated with the uptake of water in corn (Zimmermann et al., 2000) and of some nutrient ions in Arabidopsis (Baxter et al., 2009). This was further supported by the study of Kotula et al. (2009a, b), in which the elevated levels of suberin in the peripheral layers of stagnantly grown rice markedly decreased the radial oxygen loss and oxygen permeability of the roots. However, a study on the direct correlation between the altered amounts and compositions of suberin and the water and solute permeabilities of roots is still lacking.

In the present study, Arabidopsis wild types and different suberin mutants with altered amounts of suberin were used to investigate whether or not the change of quantity and/or quality of hydrophobic polymers directly correlates with water and solute permeabilities. Measurements with root pressure probes carried out for the first time in Arabidopsis whole root systems revealed that the hydraulic conductivities (Lp) and solute permeabilities (P_{st}) of Arabidopsis roots were in between those of herbaceous and woody plants. Even though the horst mutant (Höfer et al., 2008), with reduced amounts of hydrophobic suberin in its roots, resulted in increased permeabilities, the esb mutants (Baxter et al., 2009), with enhanced levels of aliphatic suberin, failed to reduce the permeabilities below the wild type values. Hence, the present discovery does not agree with the conventional assumption that the amount of root suberin always negatively correlates with water and solute permeabilities, at least not for Arabidopsis.

Materials and methods

Selection of Arabidopsis cultivars

All physiological and biochemical experiments were performed using plants of the Arabidopsis thaliana ecotype Columbia (genotypes: Col8, Col0, horst, esb1-1, and esb1-2). The selection was based on altered (elevated or reduced) amounts and monomer compositions of aliphatic suberin in the apoplastic barriers of roots, which were determined in previous studies (Höfer et al., 2008; Baxter et al., 2009). The Col8 and Col0 genotypes were Columbia wild types that served as the controls. The horst mutant was a T-DNA insertion line leading to the knockout of the cytochrome P450 CYP86A1 (At5g58860), a fatty acid ω-hydroxylase which is expressed in the root endodermis and significantly contributes to suberin monomer biosynthesis (Höfer et al., 2008). When grown in soil, it was shown to contain 60% less total aliphatic suberin in the roots compared with Col8. The mutants of esb1-1 and esb1-2, also termed ionomic mutants (Lahner et al., 2003), were two independent alleles of T-DNA insertions in At2g8670, which is expressed in the root endodermis. Suberin levels of esb1-1 and esb1-2 are significantly enhanced (Baxter et al., 2009). Soil-grown esb1-1 plants were shown to have 2-fold greater amounts of suberin compared with Col0 and to reduce the accumulation of certain ions, such as Ca, Mn, and Zn, in the shoot. Even though Arabidopsis mutants with altered amounts of suberin were discovered (with respect to their wild types), as yet no studies have investigated their impact on water and solute permeabilities. For the first time, this study combined chemical analyses with transport studies of the roots of hydroponically grown Arabidopsis plants.

Plant growth

All plants were cultivated in hydroponics according to the method previously described by Tocquin et al. (2003). Three seeds were placed onto each of the seed holders (made from Eppendorf tubes, after removing the bottom part), filled with 0.65% agar, which were then inserted into the holes of a black PVC plate or lid. One PVC plate contained 14 holes. Before insertion, a piece of mesh was used to cover the bottom of each agar well or seed holder to prevent the agar coming out of the container (150 mm×370 mm×130 mm), which was then filled with the nutrient solution. The standard Arabidopsis nutrient solution contained 1.0 mM Ca(NO_{3})_{2}, 0.13 mM NH_{4}H_{2}PO_{4}, 5.1 mM KNO_{3}, 0.50 mM MgSO_{4}, 0.03 mM NaOH, 0.02 mM EDTA, 0.02 mM FeSO_{4}, 9.6 μM H_{2}BO_{3}, 2.0 μM MnCl_{2}, 0.3 μM ZnSO_{4}, 0.2 μM CuSO_{4}, 0.1 μM MoO_{3}, 0.08 μM Co(NO_{3})_{2}, and 29 μM NH_{4}NO_{3}. The osmotic concentration was 10 mOsmol kg^{-1}, which was equivalent to an osmotic pressure of 0.025 MPa. The culture medium was replaced each week. The hydroponics culture was maintained at
a temperature of 25 °C with a 16 h light (120 μmol m⁻² s⁻¹) and 8 h dark cycle. The relative humidity was ~40–50%. The plants used in the experiments were grown for 3–4 weeks, and the root lengths are given in Table 1 for better comparison.

**Histochemical studies to detect apoplastic barriers in the roots**

In order to detect the development of Casparian bands along the root endodermis, total roots were stained for 2 h with 0.1% (w/v) berberine hemisulphate and for a further hour with 0.5% (w/v) aniline blue (Brundrett et al., 1988). The roots were then viewed under an epifluorescence microscope using an ultraviolet (UV) filter set (excitation filter BP 365, dichroic mirror FT 395, barrier filter LP 397; Zeiss, Oberkochen, Germany). In order to detect the suberin lamellae in the endodermis, cross-sections were made at distances of 10, 20, 30, and 40 mm from the root apex using a cryostat microtome (Microm HM 500M, Microm International, Walldorf, Germany). The cross-sections were then stained with lipophilic Sudan Red 7B and viewed under white light. Whole roots were stained with lipophilic fluorochrome fluorol yellow 088 in order to detect the root periderm (Brundrett et al., 1991). The stained periderm could be seen by yellow fluorescence under UV light.

**Analysis of suberin in roots of Arabidopsis**

In order to analyse suberin, the roots which were used to measure water and solute permeabilities (see below) were incubated in an enzymatic solution containing 1% (v/v) cellulase (Celluclast, Novo Nordisk, Denmark) and 1% (v/v) pectinase (Trenolin, Erbsloh, Germany) in citric buffer (0.01 M) at pH 3.0 for 3–4 weeks. After this period, the walls, which were not modified by apoplastic barriers such as suberin or lignin, were digested away. Modified walls, such as those of the endodermis, periderm, and stelar xylem vessels, resisted digestion treatment. The digested root samples were washed in borate buffer (0.01 M; pH 9.2) for 3 d, replacing the solution every day, followed by three washes with deionized water. Then, the samples were thoroughly extracted in a mixture of chloroform and methanol (1:1; v/v) for 3 d, replacing the solution several times. The resulting cell wall material was dried and stored over silica gel for subsequent chemical analyses.

To depolymerize the suberin, the dried root samples were transesterified using a mixture of methanol/boron trifluoride (MeOH/BF₃; Fufka, Germany) at 70 °C for 16 h, as described by Zeier and Schreiber (1998). The released methyl esters of the suberin monomers were derivatized using 20 μl of pyridine and 20 μl of BSTFA (N,N-bis(trimethylsilyl)trifluoroacetamide; Machery-Nagel, Düren, Germany) at 70 °C for 40 min. Derivatization converted the free carboxyl and hydroxyl groups to their trimethylsilyl (TMS) esters and ethers, respectively. The TMS derivatives were analysed using gas chromatography (GC) and mass spectrometry (MS; Agilent Technologies, Böblingen, Germany). The released monomers were quantified by GC and flame ionization detection (Agilent Technologies), referring to an internal standard (20 μg of n-dotriacontane). The results of the suberin analyses were related to the unit surface area of the roots. Five to six replicates were used for each cultivar.

**Preparation of root systems for the root pressure probe and measurement of water and solute permeabilities**

Three-week-old plants were removed from the seed holders and any agar remaining on the roots was gently washed away with distilled water without damaging the roots. In order to obtain a lateral root-free zone with a length of 10–15 mm, laterals which emerged at the basal part of the main root were shaved with a double-edged razor blade. The shaved roots were again transferred to the hydroponic culture and left for 1 week to heal the wounds. Shaving of the laterals enabled the root system to be fixed to the pressure probe successfully.

The whole root system of the *Arabidopsis* plants grown for 4 weeks was excised close to its base and tightly fixed to a root pressure probe using cylindrical silicon seals (Xantopren; Bayer, Leverkusen, Germany). Great care was taken when handling the roots not to damage them. Stable root pressure (*P*ᵣ) developed within 1–2 h after fixing the roots to the pressure probe. In the hydrostatic experiments, water flows were induced by moving the meniscus either forwards to increase the pressure in the system (exosmotic water flow) or backwards to reverse the flow (endosmotic water flow). As described by Hose et al. (2000), hydrostatic relaxation curves are composed of two exponential phases brought about by different rates of changes of *P*ᵣ over time; the initial rapid phase covers ~80% of the entire pressure (volume) and is followed by a slow reversible phase related to the concentration polarization effects at the endodermis (Steudle and Fresch, 1989; Knipfer et al., 2007). In the present study, the initial phase of hydrostatic relaxation (see Fig. 1A) was used to measure *k*ᵣ1/2 and the hydrostatic hydraulic conductivity (*Lp₀w*) was calculated from the rate constant of water exchange between the root xylem and the medium (*k*ᵣ) (Steudle et al., 1987; Steudle, 1989).

\[
kᵣ = \frac{\ln(2)}{rᵣ^{1/2}} = \frac{Lp₀w \times Aᵣ \times β}{Vᵣ},
\]

where *rᵣ* is the half-time of water exchange between the root xylem and the medium. The elastic coefficient (β) is the corresponding pressure change in relation to the change in volume of the measuring system (*ΔPᵣ/ΔVᵣ*). It was determined by inducing stepwise changes of volume (*ΔVᵣ*) with the aid of a movable metal rod and by measuring the resulting changes in root pressure (*ΔPᵣ*; Steudle and Jeschke, 1983). Finally, *Aᵣ* is the surface area of the conductive part of the root, which is ~98% of the total surface area.

In the osmotic experiments (see Fig. 1B), the original nutrient solution was rapidly exchanged with a medium containing 25 mM NaCl (~50 mOsmol/kg; measured with a freezing point osmometer), which is a permeating solute. The external root medium was rapidly bubbled with air to aerate the system as well as to minimize the external unstirred layer effect. The water phase of the biphasic osmotic root pressure relaxation yielded *k*ᵣ, from which the osmotic hydraulic conductivity of the root (*Lp₀w*) was calculated, using Equation 1.

Permeability coefficients of the roots to the solutes (*P*ᵣ) were determined by the rate constant of solute exchange (*k*ᵣ; second phase in biphasic relaxations), as given by Steudle et al. (1987):

\[
kᵣ = \frac{\ln(2)}{rᵣ^{1/2}} = \frac{Aᵣ \times Pᵣ \times Vᵣ}{Vᵣ},
\]

where *rᵣ* is the half-time of solute exchange and *Vᵣ* is the volume of functional xylem in the root system, which is ~5% from the total root volume, as measured in the cross-sections. Root reflection coefficients (*rₛ*) for NaCl (permeating solute) were calculated from the following equation:

### Table 1. Comparison of root lengths, surface areas, and steady-state root pressures of different Arabidopsis genotypes of the Columbia ecotype grown in hydroponic culture for 4 weeks

| Genotype | Root length (cm) | Root surface area (cm²) | Root pressure (MPa) |
|----------|------------------|-------------------------|---------------------|
| harst    | 14.9±4.0         | 48.6±20.3               | 0.11±0.03           |
| Col8     | 16.2±3.4         | 67.4±27.2               | 0.15±0.05           |
| Col0     | 16.8±2.2         | 68.8±0.99               | 0.18±0.06           |
| esb1-1   | 18.7±3.3         | 70.8±20.6               | 0.09±0.02           |
| esb1-2   | 19.6±4.5         | 86.1±21.8               | 0.08±0.02           |

Values are means ±SD of eight roots.
where $\Delta P_r$ is the maximum change in root pressure caused by changes in the osmotic pressure of the medium ($\Delta \pi_c = RT \cdot C_s$; $R =$ universal gas constant, $T =$ absolute temperature, $C_s =$ concentration of solute ‘$s$’ in the medium), and $t_{\text{min}}$ is the time required to reach the minimum root pressure.

After each measurement, the proper function of the seal was confirmed by cutting off the root at the seal and checking the decrease in the time constant of pressure relaxations. When the xylem of the root remained open, there was a drastic decrease in the half-times (by at least one order of magnitude) or an increase in hydraulic conductance after the cut. If not, the experiment was discarded. After the measurements the root system was removed from the pressure probe in order to measure the surface area. The roots were scanned using a scanner (Canon Deutschland GmbH, Krefeld, Germany) and the Photoshop 7 program. The surface areas were calculated assuming that the roots were cylindrical.

**Measurements of the apoplastic bypass flow of the tracer dye, PTS**

An excised *Arabidopsis* root system was fixed to a pressure chamber as described by Zimmermann and Steudle (1998). The root base was tightly connected to the steel chamber with a flexible rubber material (Terostat, Germany) in order to apply pneumatic pressure to the root medium. The root medium contained the apoplastic tracer PTS (trisodium 3-hydroxy-5,8,10-pyrenetrisulphonate) at a concentration of 0.01% (w/v), which was not toxic and was dissolved in the nutrient solution. The pressure in the chamber was raised to 0.1 MPa (1 bar) and the exuded xylem sap from the cut surface of the root was collected with the aid of a syringe for 1 h. For a few experiments, 2–3 laterals were removed from the root systems with a razor blade and the experiment was repeated. The concentration of PTS in the root medium and in the exuded xylem sap was measured using a fluorescence spectrometer at excitation wavelength 405 nm and emission wavelength 515 nm (Shimadzu, Deutschland GmbH, Duisburg, Germany). The spectrofluorometer was calibrated using five different concentrations of PTS. In the range of concentrations between $10^{-5}$% and $10^{-7}$% PTS (w/v), emission increased linearly with increasing concentration.

**Statistical analysis**

Data on suberin analysis, water and solute permeabilities were analysed using analysis of variance (ANOVA) and the means were compared using the Least Significant Difference (LSD) test at the $P = 0.05$ level. The analytical software Statistix was used to perform all statistical tests.
**Results**

*Plant morphology*

The *horst* mutant had relatively shorter roots than those of the wild types, whereas the *esb* mutants had the longest roots (Table 1). The size of surface area of the roots followed the same pattern as root length.

*Root anatomy: development of Casparian bands, suberin lamellae, and periderm*

Casparian bands in the endodermis were detected as typical wavy walls with a yellowish green fluorescence after staining with berberin-aniline blue. In the *horst* mutant, the Casparian bands first appeared as faint green wavy bands as far as 15–20 mm from the root tip (Fig. 2A). In contrast, Casparian bands in *Col8* and *esb*1-1 developed as close as 5 mm from the root tip, and both these genotypes had stained bands with intense yellowish green fluorescence at 8 mm (Fig. 2B, C). However, the intensity of fluorescence was greater in the *esb* mutant than in *Col8*. The lipophilic suberin lamellae were detected as red stains in the cell walls after staining with Sudan red. In the *horst* mutant, no endodermal suberin lamellae were detected at 10 mm from the tip (Fig. 2D), but there was a complete ring at ~40 mm (Fig. 2E). In *Col8*, only a few cells had suberin lamellae at 10 mm (Fig. 2F), but they appeared as a complete ring in the endoderm at 20 mm (Fig. 2G). Unlike the other genotypes, in the *esb* mutants, complete suberin lamellae were detected as close as 9 mm from the root tip (Fig. 2H). The periderm was indicated by a yellowish green fluorescence after staining the roots with fluorochrome Fluorol yellow 088. In all of the genotypes, the periderm only appeared at the very basal parts of the roots. The stained periderm was visible as a net in *Col8* (Fig. 2I).

*Chemical composition of suberin in Arabidopsis roots*

The chemical composition of suberin was determined for hydroponically grown *Arabidopsis* roots after using them for the root pressure probe measurements (see below). Trans-esterification of enzymatically digested cell wall materials with BF3/MeOH released monomers of aliphatic suberin. The released aromatic suberin was found in traces (data not shown) compared with the aliphatics. In regard to the unit surface area, the total content of aliphatic suberin in the *horst* mutant was ~33% lower than that in the wild types, whereas *esb*1-1 and *esb*1-2 mutants had 2-fold greater amounts than their wild types ($F_{6,32}=157.5; \ P<0.001$; Table 2). A comparative study showed that the total contents of aliphatic suberin in the roots of *Arabidopsis* and crop plants were within the same range (Table 2).

![Fig. 2](https://academic.oup.com/jxb/article-abstract/62/6/1961/595134)
Table 2. Total amounts of aliphatic suberin (μg cm⁻²) in Arabidopsis roots measured by gas chromatography and mass spectrometry

Total aliphatic suberin released after transesterification of apoplastic barriers with BF₃/methanol in different genotypes of Arabidopsis roots grown in hydroponic culture for 4 weeks. Data represent means ±SE of five replicates. Different letters indicate significant differences at the P < 0.05 level (data from this study only). Data in the literature are given for a comparison between Arabidopsis and other plant species (suberin in either the endodermis or peripheral layers, i.e. hypodermis).

| Plant species | Total aliphatic suberin (μg cm⁻²) | Reference |
|---------------|----------------------------------|-----------|
| Arabidopsis   |                                  | This study|
| horst         | 1.02±0.08 a                      |           |
| Col8          | 1.50±0.07 b                      |           |
| Col0          | 1.40±0.08 b                      |           |
| esb1-1        | 3.20±0.08 c                      |           |
| esb1-2        | 2.90±0.05 d                      |           |
| Peripheral cell layers (μg cm⁻²) | Endodermis (μg cm⁻²) |
| Cicer arietinum | 0.4                                | 22.4      | Zeier et al. (1999) |
| Zea mays (Helix) | 0.5–2.4                        | 0.6–1.2     | Hartmann (2002) |
| Ricinus communis | 2.8–10.6                        | 22.5      | Zeier et al. (1999) |
| Pluim sativum  | 3.3                                | 7.5       | Zeier et al. (1999) |
| Oryza sativa   | 4.5–9.5                           | 9.6–17.2  | Effinger (2002) |

A detailed study of the monomer composition in Arabidopsis root suberin revealed that it was primarily composed of fatty acids, ω-hydroxy acids, DAs, 2-hydroxy acids and alcohols, which were also the prominent substance classes of soil-grown Arabidopsis plants (Fig. 3A; Höfer et al., 2008; Baxter et al., 2009). In all genotypes, fatty acids, ω-hydroxy acids, and DAs were the prominent components, representing >75% of the total. When the suberin monomers were compared, in general, esb1-1 and esb1-2 had the greatest amounts of almost all components except 2-hydroxy acids, and horst had the least (F₆,32=213 at P < 0.001 for fatty acids; F₆,32=99 at P < 0.001 for alcohols; F₆,32=88 at P < 0.001 for ω-hydroxy acids; F₆,32=221 at P < 0.001 for DAs; Fig. 3A). The wild types, Col8 and Col0, had significantly greater amounts of these monomers than either the wild types (Col8 and Col0) or the horst mutant (P < 0.05).

Further analysis of the individual aliphatic monomer components of suberin revealed that C₂₂ fatty acids, C₁₆ and C₁₈(1) DAs, and C₁₆, C₁₈(1), and C₂₂ ω-hydroxy acids were the prominent components in all Arabidopsis genotypes studied here (Fig. 3B). The esb1-1 and esb1-2 mutants had greater amounts of these monomers than either the wild types (Col8 and Col0) or the horst mutant; in some cases these were several times greater (t-test; P < 0.05; Fig. 3B). Comparing horst with its wild types, a remarkable reduction of monomers was observed, mainly in C₁₆, C₁₈(1), and C₂₂ DAs and ω-hydroxy acids (t-test; P < 0.05; Fig. 3B).

Hydraulic conductivity of the roots

When connected to the root pressure probe the root systems took 1–2 h to generate steady-state, positive root pressures. Stable pressures varied according to the individual root systems and the means ranged between 0.1 MPa and 0.2 MPa (Table 1). Hydrostatic hydraulic conductivity (Lₚₒₛ) was calculated from pressure relaxation curves resulting from the induced water flows (Fig. 1A). The Lₚₒₛ of horst was almost three times greater than that of the other Arabidopsis genotypes studied here (F₆,38=72.1; P < 0.001; Table 3), demonstrating that reduced suberization resulted in increased Lₚₒₛ. However, the Lₚₒₛ values of all the other genotypes were the same (P < 0.05), indicating that elevated amounts of suberin in esb1-1 and esb1-2 failed to reduce the water permeability below that of their wild types.

Osmotic hydraulic conductivity (Lₚₒₛ) was calculated from the water phase of biphasic osmotic relaxations (Fig. 1B). The Lₚₒₛ was the same for all Arabidopsis genotypes without any significant differences, except for horst, which was ~2-fold greater (F₆,38=23.9; P < 0.001; Table 3). However, there was a trend towards the esb mutants having lower Lₚₒₛ values than their wild types. With the exception of horst, the Lₚₒₛ/Lₚₒₛ ratios of the other genotypes were around unity, indicating a relatively greater cell to cell water flow than apoplastic water flow. The ratio of 1.7 for horst was significantly greater than unity, indicating a relatively greater apoplastic water flow (Table 3). These results are in line with previous studies where Arabidopsis Lₚ, was measured using other techniques (see Javot et al., 2003; Postaire et al., 2010). However, they differed markedly from other crop species such as maize, beans, and cucumber, but were similar to rice (Table 3).

Apoplastic bypass flow of the fluorescent tracer, PTS

PTS is a mobile fluorescent tracer that is confined to the apoplast and not able to cross a healthy plasma membrane (Moon et al., 1986). When measured with the pressure chamber using a positive pressure 0.1 MPa, the concentration of PTS in the xylem exudate (percentage of the root medium), which denotes the apoplastic bypass flow in Arabidopsis roots, was apparently small (Table 4). The bypass flow of PTS did not depend on the amount of suberin in the roots (P < 0.05; Table 4). In general, regardless of the genotype, roots of all Arabidopsis plants formed tight barriers against PTS, exhibiting absolute apoplastic bypass flows of <0.25%. When two or three laterals were excised from the intact root systems the bypass flows of PTS increased 10- to 20-fold (Table 4).
Once the roots had achieved steady-state pressures, the osmotic pressure of the medium was changed—that is, by adding 25 mM NaCl (0.125 MPa of osmotic pressure) or replacing it with the nutrient solution. Hypertonic conditions caused an efflux of water, whereas hypotonic conditions caused an influx. However, there was a reverse influx (efflux) of solutes into (and out of) the root, which was denoted as the solute phase (second phase of the biphasic relaxations; Fig. 1B). Following a minimum (or maximum) in pressure, the root pressure tended to return to baseline. Obviously, the roots of all of the genotypes of Arabidopsis studied failed to reach the original root pressure in the solute phase precisely, demonstrating a possible active pumping of NaCl out of the root and an inhibition of the plasma membrane ion pumps by NaCl (Fig. 1B). The permeability coefficient ($P_{sr}$) and reflection coefficient ($r_{sr}$) of the solutes were determined from rate constants of the solute phases. The $P_{sr}$ of horst was ~2-fold greater than that of the wild types ($F_{6,30}=11.4; P < 0.001$; Table 5), possibly due to the late or patchy development of apoplastic barriers. The higher $P_{sr}$ of horst correlated with the greater $L_p$ for this mutant. In contrast, the $P_{sr}$ values of Arabidopsis with those of other plant species, rice and maize were found to have greater values; however, these values were smaller than those of Phaseolus (Table 5).

The passive selectivity of the whole root system as a single tissue for NaCl, which is termed the reflection coefficient ($\sigma_{sr}$), ranged from 0.3 to 0.4 (Table 4). The esb1-1 and esb1-2 mutants had significantly greater $\sigma_{sr}$ values than horst, whereas the $\sigma_{sr}$ values of the wild types were intermediate ($F_{6,40}=2.2; P < 0.05$; Table 5). In comparison, the $\sigma_{sr}$ values of Arabidopsis for NaCl were greater than those for rice, but lower than those for maize and Phaseolus (Table 5). The lower values of $\sigma_{sr}$ for horst correlated with the higher values of $L_p$ and $P_{sr}$, indicating that the barrier in horst...
tended to be more ‘leaky’ than those in the wild types and esb mutants (see Discussion).

**Correlation between total amounts of root suberin versus permeabilities**

In Fig. 4, the total amounts of *Arabidopsis* root suberin are plotted against the permeabilities. Hydrostatic (*Lp*<sub>hy</sub>) and osmotic (*Lp*<sub>os</sub>) hydraulic conductivities did not decrease linearly with increasing amounts of aliphatic suberin in the roots. *Horst* showed the lowest amount of total suberin in the roots, but it had the greatest water permeabilities. Even though the esb mutants had almost double the amount of suberin compared with their wild types, the reduction in *Lp*<sub>hy</sub> was not that striking (Fig. 4A). However, there was a better negative correlation between total suberin and *Lp*<sub>os</sub> (Fig. 4B).

When comparing solute permeability (*P*<sub>s</sub>) with total root suberin, the mutant *horst* obviously had the highest *P*<sub>s</sub> and exhibited the lowest amounts of aliphatics. In contrast, the esb mutants had the lowest *P*<sub>s</sub> and the greatest amounts of aliphatics (Fig. 4C). Even though the esb mutants had a 2-fold greater amount of suberin than the wild types, this did not result in a 2-fold decrease in the *P*<sub>s</sub> and deviated from the predicted linear negative correlation.

**Discussion**

To the best of the authors’ knowledge, this is the first study in which the permeability of roots for water and solutes was related to the amount and composition of suberin using *Arabidopsis* wild types and different suberin mutants. *Arabidopsis* proved to be an ideal species for testing apoplastic permeabilities due to a suberized endodermis in the primary stage, which is then replaced by a suberized periderm during secondary growth. Primarily and notably, *Arabidopsis* is currently the most available plant species for suberin mutants, with either increased or decreased suberin amounts compared with the wild types. The apoplastic barriers of the roots, composed of aromatic and aliphatic suberin and of lignin, are known to show a negative

### Table 3. Hydraulic conductivity (*Lp*) of *Arabidopsis* root systems

Root *Lp* was determined using a root pressure probe at room temperature. Values are means ± SE (*n*=8–10 roots). Different letters within the same column indicate significant differences at the *P* < 0.05 level (data from this study only). Data in the literature are given for a comparison between *Arabidopsis* and other plant species, which were grown in hydroponics.

| Plant species (whole root systems) | *Lp* (10<sup>-6</sup> m s<sup>-1</sup> MPa<sup>-1</sup>) | Reference |
|-----------------------------------|-------------------------------|-----------|
|                                    | Hydrostatic *Lp* (*Lp*<sub>hy</sub>) | Osmotic *Lp* (*Lp*<sub>os</sub>) | Ratio: *Lp*<sub>hy</sub>/Lp<sub>os</sub> |
| *Arabidopsis*                      |                               |           |                        |
| *horst*                           | 9.5±1.1<sup>a</sup>           | 5.7±1.0<sup>a</sup> | 1.71±0.15<sup>a</sup> | This study |
| Col-8                             | 3.8±0.2<sup>b</sup>           | 3.5±0.1<sup>b</sup> | 1.12±0.06<sup>b</sup> |          |
| Col-0                             | 3.7±0.3<sup>b</sup>           | 3.6±0.4<sup>b</sup> | 1.04±0.07<sup>b</sup> |          |
| esb1-1                            | 3.3±0.2<sup>b</sup>           | 3.2±0.2<sup>b</sup> | 1.07±0.04<sup>b</sup> |          |
| esb1-2                            | 3.1±0.3<sup>b</sup>           | 2.8±0.3<sup>b</sup> | 1.09±0.03<sup>b</sup> |          |
| *Arabidopsis*                     |                               |           |                        |
| Wild type                         | 9.0±0.3                        | 3.0        |                        | Javot et al. (2003) |
| PIP 2-1 mutant                    | 8.5±0.3                        | 2.5        |                        |          |
| PIP 2-2 mutant                    | 8.5±0.4                        | 2.5        |                        |          |
| Phaseolus vulgaris                | 30.0                           | 0.56       | 54                    | Newman (1973); Fiscus (1986) |
| Corn cv. Helix                    | 26±15                          | 4.6±2.2    | 5.6                   | Zimmermann and Steudle (1998) |
| Hydroponics                       | 7.3±2.6                        | 3.2±1.7    | 2.3                   | Ranathunge et al. (2003) |
| Aeroponics                        | 2.8±1.3                        | 2.4±1.1    | 1.1                   |          |

### Table 4. PTS (trisodium-3-hydroxy-5,8,10-pyrenetrisulphonate) bypass flow of *Arabidopsis* root systems

Root PTS uptake was determined using a pressure chamber at room temperature. Values are means ± SE (*n*=8–10 roots). Different letters within the same column indicate significant differences at the *P* < 0.05 level (data from this study only). Data in the literature are given for a comparison between *Arabidopsis* and other plant species, which were grown in hydroponics.

| Plant species | PTS concentration in xylem sap (given as % of PTS in medium) | Reference |
|---------------|---------------------------------------------------------------|-----------|
| *Arabidopsis* |                                                               |           |
| *horst*       | 0.23±0.05<sup>a</sup>                                         | This study |
| Col-8         | 0.18±0.09<sup>a</sup>                                          |          |
| esb1-1        | 0.21±0.03<sup>a</sup>                                          |          |
| Injured roots |                                                               |           |
| *horst*       | 2.47                                                          |          |
| Col-8         | 2.75                                                          |          |
| esb1-1        | 4.30                                                          |          |
| Corn (Zea mays)| 0.33±0.29                                                     | Zimmermann and Steudle (1998) |
| Rice cv. IR26 | 0.05–0.1<sup>a</sup>                                           | Yeo et al. (1987) |
| Rice cv. IR36 | 0.63                                                           | Yadav et al. (1996) |
| Rice cv. IR36 | 0.72±0.26                                                     | Garcia et al. (1997) |
| Wheat cv. Punjab | 0.06±0.005                                                   | Garcia et al. (1997) |

<sup>a</sup> Measured by elution technique.
correlation to water and solute permeabilities (Steudle and Peterson, 1998; Zimmermann et al., 2000; Schreiber et al., 2005). However, these conclusions were made on the basis of studies in which the plants were grown under different growth conditions or exposed to different abiotic stresses. For the first time, this study showed direct transport measurements with respect to altered amounts and compositions of suberin in roots from plants grown under the same conditions.

Unlike previous studies, the present study determined the amount of Arabidopsis root suberin per unit surface area, which is indeed more applicable when correlated to transport properties. Estimation of the surface area is time consuming and labour intensive. The total root suberin is made up from aliphatics and aromatics, but the latter were present as traces compared with the former (data not shown; see also Franke et al., 2005). Aliphatic suberin can be found in the walls of the endodermis (in young roots with a primary growth) and of the periderm (in basal zones with a secondary growth) in Arabidopsis roots. However, it has been shown that the amount of peridermal suberin is greater than the amount of endodermal suberin in total (Höfer et al., 2008). ω-Hydroxy acids, DAs, and primary carboxylic acids were the most abundant suberin monomers released from the Arabidopsis cell wall samples, which were also discovered in previous studies on Arabidopsis and other plant species (Schreiber et al., 1999, 2007; Franke et al., 2005).

A comparison of the different genotypes demonstrated that Col8 and Col0 had an ~1.5 times greater amount of total aliphatics than horst (Table 2). This is in agreement with Höfer et al. (2008) and Li et al. (2007), although these authors grew the plants in soil and determined the amount of suberin per unit dry weight rather than by the root surface area. Irrespective of growth conditions and the determined units, the trend of the suberin increment was the same, although the absolute values were not comparable. A detailed study of the suberin composition showed that the major reduction appeared in C_{16} and C_{18} ω-hydroxy acids and DAs of horst plants (Fig. 3A, B). When comparing the enhanced suberin mutants (esb) with their wild types, the total amount of aliphatic suberin was found to be greater by 2-fold (Table 2), which also agrees with the discovery of Baxter et al. (2009). Nevertheless, the absolute amounts were not comparable due to different growth conditions and expressed units. A detailed suberin study demonstrated that the typical suberin monomers, such as ω-hydroxy acids, DAs, and carboxylic acids, were strikingly higher in the esb mutants (Fig. 3A, B), as also found by Baxter et al. (2009).

The present study may have underestimated the total amount of aliphatic suberin by referring to the amounts in the roots’ outer surface area rather than the amount in the endodermal area. Apparently, the number of fine roots with primary growth (with or without a suberized endodermis) outnumbered the roots with secondary growth (only with a periderm), which was found to be ~5% of the whole root system. Hence, the given suberin amounts in this study might be the lower limits.

It was necessary to consider the possible sources of errors or artefacts before accepting that the measured water and solute permeabilities were real. (i) Great care was taken when handling the roots and fixing them to the pressure probes without exposing them to stresses or bending in order to avoid injuries or physical defects. (ii) At the end of each experiment, the roots were excised at the seal and checked for proper tightness. If the xylem remained open, the root pressure dropped down to zero immediately. In addition, roots with a fully opened xylem showed a drastic decrease in half-times of water exchange (by at least one order of magnitude). This proved that the axial hydraulic resistance at the seal was negligible compared with the radial hydraulic resistance. If this was not the case, the experiments were discarded. (iii) It is well documented that unstirred layers may underestimate the $L_{p,x}$ rather than the $L_{p,hy}$ (Dainty, 1963; Steudle and Tyerman, 1983; Knipfer et al., 2007). In order to minimize the thickness of the external unstirred layers, the medium was vigorously mixed with an air stone during the osmotic experiment. However, great care was taken to avoid too much shaking or turbulence, which would have resulted in damage to the fine roots.

According to the composite transport model, water flows can be apoplastic, cell to cell, or both, depending on the
Development in Col8 and esb mutants, in which the bands appeared as close as 5 mm from the tip (see Fig. 2B, C). This also suggests that the extra deposition of suberin in the developed bands of the esb mutants could not effectively reduce hydrostatic water flow through the apoplast (Fig. 4). This finding may be related to the way in which suberin is clogged into the walls as discussed by Hose et al. (2001). When aliphatic suberin intensely fills the pores of cell walls, small amounts may be sufficient to make an efficient barrier against both water and polar solutes. In this case, a surplus of suberin added to the cell walls would not further reduce $L_p$ or $P_s$.

Comparison of the present results with earlier data obtained from a pressure chamber showed that the absolute values of $L_{p_{hy}}$ of Arabidopsis were within the same range (Javot et al., 2003; Table 3). Hence, irrespective of the technique used (either a root pressure probe or a pressure chamber), the water permeability data in the present study and previous studies are comparable. Unfortunately, the recent measurements by Postaire et al. (2010) were not comparable with the present data due to the different units used for $L_p$.

Unlike the Casparian bands, suberin lamellae are always deposited on all walls of the endodermis, except in passage cells (Peterson and Enstone, 1996; Peterson and Cholewa, 1998). The deposited hydrophobic lamellae surround the protoplast and restrict the flow through the plasmalemma. The osmotic hydraulic conductivity ($L_{p_{os}}$), in which water mainly moves through the membranes across the root, was found to be greater by a factor of 1.6 in the horst mutant than in the wild types (Table 3). This striking difference can be explained in terms of the late development of suberin lamellae further up from the root tip (20–30 mm) in horst compared with the wild types, in which the development of lamellae appeared at ~10 mm from the root tip (see Fig. 2H). It was also evident that the wild types had a greater number of passage cells in the endodermis of younger root zones. This lack of a reduction in $L_{p_{os}}$ in the esb mutants might have been due to the occurrence of pits or pores in the endodermal lamellae, as recently found in onions by Waduwara et al. (2008), allowing water to flow through the water channels (aquaporins) of cell membranes. Therefore, the method of pore closure in the cell walls by aliphatic suberin is an important factor for making tight barriers against water. This hypothesis is supported by experimental results showing that lamellae development did not markedly elevate the hydraulic resistance in barley (Sanderson, 1983), maize (Clarkson et al., 1987), and in the peripheral layers of rice (Ranathunge et al., 2003).

The ratio between $L_{p_{hy}}$ and $L_{p_{os}}$ predicts the prominent pathway for water, either cell to cell or apoplastic, which are parallel and simply additive to the overall flow. If the ratio is around one, as was found for Arabidopsis, the

![Fig. 4. Variation of hydrostatic hydraulic conductivity, $L_{p_{hy}}$ (A), osmotic hydraulic conductivity, $L_{p_{os}}$ (B), and solute permeability, $P_s$ (C) in relation to the changes in total amount of aliphatic suberin in the roots of different Arabidopsis genotypes. The non-linear relationship between permeabilities and suberin quantities revealed that the amount of aliphatics does not directly correlate with the transport properties of roots.](https://academic.oup.com/jxb/article-abstract/62/6/1961/595134)
contribution of the cell to cell pathway is relatively greater than that of the apoplastic pathway for the overall water flow. In *horst*, the ratio was significantly greater than unity but still many times smaller than in other herbaceous species such as maize and *Phaseolus vulgaris* (Fiscus, 1986; Steudle and Peterson, 1998; Zimmermann and Steudle, 1998), indicating a relatively insignificant apoplastic flow. As found for *Arabidopsis*, in *Phaseolus coccineus* and barley, the ratio was around or even lower than unity because of a high membrane permeability (Steudle and Jeschke, 1983; Steudle and Brinckmann, 1989) due to the greater water flow through the aquaporins. Previously, many studies showed that osmotic water permeability was typically lower than the hydrostatic hydraulic conductivity due to greater apoplastic water flow (Steudle and Frensch, 1989; Cruz et al., 1992; Steudle et al., 1993; Rüdinger et al., 1994; Steudle and Peterson, 1998). The lower osmotic permeability in many plant species is due to the greater number of cell layers in the root cylinder, including the endodermis, where water has to cross at least two membranes in each cell. Since the cells are arranged in series, the membrane resistances for the overall radial water flow are additive. In primary *Arabidopsis* roots, water molecules only have to cross 3–4 root cell layers in order to reach the stele. Indeed, this number is strikingly small compared with the number of cell layers in other roots. The relatively greater $L_{p_w}$ in *Arabidopsis* can be explained in terms of a lower membrane resistance due to a few cell layers in the cross-sectional area and abundant plasma membrane aquaporins, such as *PIP2;2-1* and *PIP2;2-2* (Javot et al., 2003). As proposed by Schäffner (1998), aquaporins might be concentrated in the passage cells of the endodermis, the rate-limiting layer in *Arabidopsis* for water flow. Hachez et al. (2006) also showed expression of plasma membrane aquaporins in the endodermis and exodermis, stele, epidermis, and mid-cortex of maize roots. However, it should be taken into account that once the lamellae develop, surrounding and masking the plasmalemma in the endodermis, the contribution of aquaporins to the overall water flow should be reduced.

During the apoplastic tracer flux measurements with PTS, the roots were handled gently and great care was taken to avoid injuring the roots, which could have led to artefacts. The positive pressure applied to the medium resulted in a solvent drag of PTS through the apoplasm of roots with the bulk/viscous flow of water. Unlike for water, the selectivity of the root for PTS was striking. Irrespective of the amounts and composition of root suberin, and of the genotype, the apoplastic bypass flow of PTS, which denotes the amount of tracer that has moved into the xylem by bypassing the Casparian bands in the endodermis, was markedly low (Table 4). Removal of 2–3 lateral roots strikingly increased the PTS concentration in the xylem sap due to the greater solvent drag of the tracer through open xylem vessels driven by hydrostatic pressure gradients. The bypass flows were within the same range as found for many other species studied earlier (see Table 4). However, the amount of PTS dragged by water through the apoplast, crossing the suberized barriers in *Arabidopsis*, does not quantitatively imply the bypass flux of water due to its (i) greater physical size compared with water; (ii) the three negative charges which can be repelled by the fixed negative charges of the cell walls; and (iii) the 30 times greater molecular weight compared with water. Hence, PTS markedly underestimated the flux of water through the apoplast, as earlier explained by many other authors (Peterson et al., 1981; Moon et al., 1986; Zimmermann and Steudle, 1998). It was concluded that any attempt to correlate the bypass flow of PTS to water is doomed to be a failure. However, it may be a good indicator of the bypass flow of NaCl and other solutes.

Similar to water, the NaCl permeability ($P_{sr}$) of the *horst* mutant was substantially greater than for the wild types and *esb* mutants. However, an increment of the aliphatic suberin in the *esb* mutants failed to decrease the $P_{sr}$ of roots for NaCl below those of the wild types, which is somewhat different from the previous findings of Baxter et al. (2009). These authors found that increased levels of suberin in *esb1-1* and *esb1-2* decreased the accumulation of Ca, Mn, and Zn, but slightly increased the accumulation of Na in the shoot. These slight differences in Na transport in the present and previous studies may be due to differences in the experimental design and growth conditions. Baxter and colleagues grew plants in the soil and studied the accumulation of different elements in the shoots for about a week, which showed long-term effects. In contrast, the data from the present study show the selectivity of Na" in the roots of hydroponically grown plants in short-term experiments. In addition, the present study took direct permeability measurements for NaCl in the roots. Assuming no or negligible amounts of solutes moved into the root through the suberized periderm in very basal zones, which is reasonable, it is suggested that the extra deposition of suberin in the primary roots had no negative effect on NaCl permeability. In contrast, a reduction of suberin in *horst* made the wall barriers more porous and resulted in a greater solute permeability. The higher values of $L_{p_w}$ and $P_{sr}$ in the *horst* mutant indicate that the movement of water and NaCl was apoplastic to some extent, despite the existence of apoplastic barriers. In comparison with other plant species, the $P_{sr}$ of NaCl in *Arabidopsis* root systems is smaller than in corn or rice but greater than in *Phaseolus* species (see Table 5).

Reflection coefficients ($\sigma_{sr}$) refer to the passive selectivity of the whole root tissue for solutes (including membranes and the apoplast) which is inversely related to the $P_{sr}$. It is assumed that $\sigma_{sr}$ values for solutes in unmodified primary cell walls are zero or very close to zero (Steudle, 1996). However, modified cell walls, such as the endodermis and periderm in *Arabidopsis* roots, strive against the movement of solutes and increase the $\sigma_{sr}$. Generally, values of $\sigma_{sr}$ range from 0 to 1, where 0 means no selection of solutes by the tissue and 1 means no solute flows ($P_{sr} = 0$), or they are totally blocked. For the first time, this study presents the $\sigma_{sr}$ and $P_{sr}$ values of *Arabidopsis* roots, which could not be determined in previous studies using the pressure chamber technique (Javot et al., 2003; Boursiac et al., 2005; Postaire et al., 2010). The measured $\sigma_{sr}$ values for NaCl ranged from
0.3 to 0.4. The lowest was found in horst, which nicely correlated with the highest $P_{sr}$. The esb mutants had a greater selectivity for NaCl than horst, but they were not significantly different from those of the wild types (Table 5). The $\sigma_{sr}$ of Arabidopsis for NaCl was lower than those of maize and Phaseolus spp., but it was greater than that of rice (Table 5). Rice is known to have a greater apoplastic bypass flow of $Na^{+}$, leading to its low salt resistance (Yeo et al., 1987; Krishnamurthy et al., 2009). A $L_{ph}/L_{po}$ of unity would suggest a $\sigma_{sr}$ close to 1. However, in a composite transport system, $\sigma_{sr}$ could be low despite a low $L_p$ and $P_{sr}$, as was also found for woody species (Steudle and Heydt, 1997). In the root, a low $\sigma_{sr}$ is due to the fact that two parallel pathways (apoplastic and cell to cell) contribute to the overall water flow. Under these conditions, the overall $\sigma_{sr}$ would be a weighted mean of the $\sigma_{sr}$ values of both pathways. Both pathways would contribute to the overall $\sigma_{sr}$ according to their hydraulic conductance (Steudle, 1992). Therefore, the overall $\sigma_{sr}$ values in Arabidopsis were fairly small and similar to those of woody species, but greater than in rice and smaller than in other herbaceous species.

In conclusion, for the first time, this study provides direct information on the correlation between root permeabilities with changes in aliphatic suberin amounts and composition in Arabidopsis roots. Variations in suberin amounts in the roots did not tightly correlate with water and solute permeabilities. Reduced amounts of aliphatic suberin resulted in increased permeabilities in horst due to the late deposition of Casparian bands and suberin lamellae as patchy structures. It may also have been due to the presence of more pores in the barriers because of the lack of the required amounts of suberin to make them tight and compact. However, elevated levels of aliphatics in the esb mutants failed to reduce the permeabilities compared with the wild types. Obviously, the extra deposition of hydrophobic suberin into fully developed apoplastic barriers did not necessarily strengthen them further. These findings imply that some caution is required when correlating suberin amounts with the permeability of roots. The simple view that the quantity of hydrophobic suberin negatively correlates with permeability may not always be true. The monomer arrangements in the suberin biopolymer, as well as its microstructure, also have to be considered.

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References

Baxter I, Hosmani PS, Rus A, Lahner B, Borevitz JO, Muthukumar B, Mickelbart MV, Schreiber L, Franke RB, Salt DE. 2009. Root suberin forms an extracellular barrier that affects water relations and mineral nutrition in Arabidopsis. PLoS Genetics 5, e1000492.

Bernards MA, Lewis NG. 1998. The macromolecular aromatic domain in suberized tissue: a changing paradigm. Phytochemistry 47, 915–933.

Boursiac Y, Chen S, Luu DT, Sorieu M, van den Dries N, Maurel C. 2005. Early effects of salinity on water transport in Arabidopsis roots. Molecular and cellular features of aquaporin expression. Plant Physiology 139, 790–805.

Brundrett MC, Enstone DE, Peterson CA. 1988. A berberine-aniline blue fluorescent staining procedure for suberin, lignin and callose in plant tissues. Protoplasma 146, 133–142.

Brundrett MC, Kendrick B, Peterson CA. 1991. Efficient lipid staining in plant material with Sudan red 7b or fluorescent yellow 088 in polyethylene glycol-glycerol. Biotechnic and Histochemistry 66, 111–116.

Clarkson DT, Robards AW, Stephens JE, Stark M. 1987. Suberin lamellae in the hypodermis of maize (Zea mays) roots; development and factors affecting the permeability of hypodermal layers. Plant, Cell and Environment 10, 83–93.

Cruz RT, Jordan WR, Drew MC. 1992. Structural changes and associated reduction of hydraulic conductance in roots of Sorghum bicolor L. following exposure to water deficit. Plant Physiology 99, 203–212.

Dainty J. 1963. Water relations of plant cells. Advances in Botanical Research 1, 276–329.

Effinger N. 2002. Apoplastische Barrieren in den Wurzeln von Reis und Mais: chemische Zusammensetzung und Einfluss auf die radiale hydraulische Leitfähigkeit. Diploma thesis. Germany: University of Würzburg.

Fiscus EL. 1986. Diurnal changes in volume and solute transport coefficients of Phaseolus roots. Plant Physiology 80, 752–759.

Franke R, Briesen I, Wojciechowski T, Faust A, Yephremov A, Nawrath C, Schreiber L. 2005. Apoplastic polyesters in Arabidopsis surface tissues—a typical suberin and a particular cutin. Phytochemistry 66, 2643–2658.

Garcia A, Rizzo CA, Ud-Din J, Bartos SL, Senadhira D, Flowers TJ, Yeo AR. 1997. Sodium and potassium transport to the xylem are inherited independently in rice, and the mechanism of sodium:potassium selectivity differs between rice and wheat. Plant, Cell and Environment 20, 1167–1174.

Hachez C, Moshelion M, Zelazny E, Cavez D, Chaumont F. 2006. Localization and quantification of plasma membrane aquaporin expression in maize primary root: a clue to understand their role as cellular plumbors. Plant Molecular Biology 62, 305–323.
Hartmann K. 2002. *Struktur, Funktion und chemische Zusammensetzung suberinisierter Transportbarrieren im Apoplasten Höherer Pflanzen* PhD thesis, University of Würzburg, Germany.

Henzler T, Waterhouse RN, Smyth AJ, Carvajal M, Cooke DT, Schäffner AR, Steudle E, Clarkson DT. 1999. Diurnal variations in hydraulic conductivity and root pressure can be correlated with the expression of putative aquaporins in the roots of *Lotus japonicus*. *Planta* 210, 50–60.

Hose E, Clarkson DT, Steudle E, Schreiber L, Hartung W. 2001. The exodermis: a variable apoplastic barrier. *Journal of Experimental Botany* 52, 2245–2264.

Hose E, Steudle E, Hartung W. 2000. Abscisic acid and hydraulic conductivity of maize roots: a study using cell- and root-pressure probes. *Planta* 211, 874–882.

Höfer R, Briesen I, Beck M, Pinot F, Schreiber L, Franke R. 2008. The *Arabidopsis* cytochrome P450 CYP86A1 encodes a fatty acid omega-hydroxylase involved in suberin monomer biosynthesis. *Journal of Experimental Botany* 59, 2347–2360.

Javot H, Lauvergeart V, Santoni V, Martin-Laurent F, Guclu J, Vinh J, Heyes J, Franck KL, Schäffner AR, Bouchez D. 2003. Role of a single aquaporin isoform in root water uptake. *The Plant Cell* 15, 509–522.

Knipfer T, Das D, Steudle E. 2007. During measurements of root hydraulic resistance with pressure probes, the contribution of unstirred layers is minimized in the pressure relaxation mode: comparison with pressure clamp and high-pressure flowmeter. *Plant, Cell and Environment* 30, 845–860.

Kolattukudy PE. 1980. Biopolymesters of plants: cutin and suberin. *Science* 208, 990–1000.

Kotula L, Ranathunge K, Schreiber L, Steudle E. 2009a. Functional and chemical comparison of apoplastic barriers to radial oxygen loss in roots of *Oryza sativa* L. grown in aerated or deoxygenated solution. *Journal of Experimental Botany* 60, 2155–2167.

Kotula L, Ranathunge K, Steudle E. 2009b. Apoplastic barriers effectively block oxygen permeability across outer cell layers of rice roots under deoxygenated conditions: roles of apoplastic pores and of respiration. *New Phytologist* 184, 909–917.

Kramer PJ, Boyer JS. 1995. *Water relations of plants and soil*. Orlando: Academic Press.

Krishnamurthy P, Ranathunge K, Franke R, Prakash HS, Schreiber L, Mathew MK. 2009. The role of apoplastic transport barriers in salt tolerance of rice (*Oryza sativa* L.). *Planta* 230, 119–134.

Lahner B, Gong JM, Mahmoudian M, Smith EL, Abid KB. 2003. Genomic scale profiling of nutrient and trace elements in *Arabidopsis thaliana*. *Nature Biotechnology* 21, 1215–1221.

Li Y, Beisson F, Koo AJK, Molina I, Pollard M, Ohrogge J. 2007. Identification of acyctransferases required for cutin biosynthesis and production of cutin with suberin like monomers. *Proceedings of the National Academy of Sciences, USA* 104, 18339–18344.

Lütte U, Higinbotham N. 1979. *Transport in plants*. Berlin: Springer-Verlag.

Maurel C. 1997. Aquaporins and water permeability of plant membranes. *Annual Review of Plant Physiology and Plant Molecular Biology* 48, 399–429.

Miyamoto N, Steudle E, Hirasawa T, Lafitte R. 2001. Hydraulic conductivity of rice roots. *Journal of Experimental Botany* 52, 1835–1846.

Moon GJ, Clough BF, Peterson CA, Allaway WG. 1986. Apoplastic and symplastic pathways in *Avicennia marina* (Forsk.) Verh. roots by fluorescent racer dyes. *Australian Journal of Plant Physiology* 13, 637–648.

Münch E. 1930. *Die Stoffbewegungen in der Pflanze*. Fischer, Jena.

Newman El. 1973. Permeability to water of five herbaceous species. *New Phytologist* 72, 547–555.

Nobel PS. 1999. *Physicochemical and environmental plant physiology*. San Diego: Academic Press Inc.

Peterson CA, Cholewa E. 1998. Structural modifications of the apoplast and their potential impact on ion uptake. *Zeitschrift Pflanzenernährung und Bodenkunde* 161, 521–531.

Peterson CA, Emanuel ME, Humphreys GB. 1981. Pathway of movement of apoplastic dye tracers through the endodermis at the site of secondary root formation in corn (*Zea mays*) and broad bean (*Vicia faba*). *Canadian Journal of Botany* 59, 618–625.

Peterson CA, Enstone DE. 1996. Functions of passage cells in the endodermis and exodermis of roots. *Physiologia Plantarum* 97, 592–598.

Postaire O, Tournaire-Roux C, Grondin A, Boursiac Y, Morillon R, Schäffner AR, Maurel C. 2010. A PIP1 aquaporin contributes to hydrostatic pressure-induced water transport in both the root rosette of *Arabidopsis*. *Plant Physiology* 152, 1418–1430.

Ranathunge K, Steudle E, Lafitte R. 2003. Control of water uptake by rice (*Oryza sativa* L.): role of the outer part of the root. *Planta* 217, 193–205.

Rüdinger M, Hallgren SW, Steudle E, Schulze ED. 1994. Hydraulic and osmotic properties of spruce roots. *Journal of Experimental Botany* 45, 1413–1425.

Sanderson J. 1983. Water uptake by different regions of the barely root. Pathways of radial flow in relation to development of the endodermis. *Journal of Experimental Botany* 34, 240–253.

Schäffner AR. 1998. Aquaporin function, structure, and expression: are there more surprises to surface in water relations? *Planta* 204, 131–139.

Schreiber L, Franke R, Hartmann K. 2007. Chemical composition of apoplastic transport barriers in roots: quantification of suberin depositions in endodermal and hypodermal root cell walls. In: Sattelmacher B, Horst WJ, eds. *The apoplast of higher plants: compartment of storage, transport and reactions. The significance of the apoplast for the mineral nutrition of higher plants*. Heidelberg: Springer, 109–117.

Schreiber L, Franke R, Hartmann K, Ranathunge K, Steudle E. 2005. 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). *Journal of Experimental Botany* 56, 1427–1436.

Schreiber L, Hartmann K, Skrabs M, Zeier J. 1999. Apoplastic barriers in roots: chemical composition of endodermal and hypodermal cell walls. *Journal of Experimental Botany* 50, 1267–1280.
Steudle E. 1989. The regulation of plant water at the cell, tissue, and organ level: roles of active processes and compartmentation. In: Schulze E-D, ed. Flux control in biological systems. From the enzyme to the population and ecosystem level. New York: Academic Press Inc, 237–299.

Steudle E. 1992. The biophysics of plant water: compartmentation, coupling with metabolic processes, and flow of water in plant roots. In: Somero CN, Osmond CB, Bolis CL, eds. Water and life. Comparative analysis of water relationships at the organismic; cellular; and molecular levels. Berlin: Springer-Verlag, 173–204.

Steudle E. 2000a. Water uptake by roots: effects of water deficits. Journal of Experimental Botany 51, 1531–1542.

Steudle E. 2000b. Water uptake by plant roots: an integration of views. Plant and Soil 226, 45–56.

Steudle E. 2001. The cohesion–tension mechanism and the acquisition of water by plant roots. Annual Review of Plant Physiology and Plant Molecular Biology 52, 847–875.

Steudle E, Brinckmann E. 1989. The osmometer model of the root: water and solute relations of Phaseolus coccineus. Botanica Acta 102, 85–95.

Steudle E, Frensch J. 1989. Osmotic responses of maize roots. Planta 177, 281–295.

Steudle E, Frensch J. 1996. Water transport in roots: role of the apoplast. Plant and Soil 187, 67–79.

Steudle E, Heydt H. 1997. Water transport across tree roots. In: Rennenberg H, Eschrich W, Ziegler H, eds. Trees—contributions to modern tree physiology. Leiden, The Netherlands: Backhuys Publishers, 239–255.

Steudle E, Jeschke WD. 1983. Water transport in barley roots. Planta 158, 237–248.

Steudle E, Murrmann M, Peterson CA. 1993. Transport of water and solutes across maize roots modified by puncturing the endodermis. Further evidence for the composite transport model of the root. Plant Physiology 103, 335–349.

Steudle E, Oren R, Schulze ED. 1987. Water transport in maize roots. Plant Physiology 84, 1220–1232.

Steudle E, Peterson CA. 1998. How does water get through roots? Journal of Experimental Botany 49, 775–788.

Steudle E, Tyerman SD. 1983. Determination of permeability coefficients, reflection coefficients, and hydraulic conductivity of Chara corallina using the pressure probe: effects of solute concentrations. Journal of Membrane Biology 75, 85–96.

Tocquin P, Corbesier L, Havelange A, Pieltain A, Kurten E, Bernier G, Perilleux C. 2003. A novel high efficiency, low maintenance, hydroponic system for synchronous growth and flowering of Arabidopsis thaliana. BMC Plant Biology 3, 1–10.

Tyerman SD, Bohnert HJ, Maurel C, Steudle E, Smith JAC. 1999. Plant aquaporins. Their molecular biology, biophysics and significance for plant water relations. Journal of Experimental Botany 50, 1055–1071.

Waduwara CI, Walcott SE, Peterson CA. 2008. Suberin lamellae of the onion root endodermis: their pattern of development and continuity. Canadian Journal of Botany 86, 623–632.

Weatherley PE. 1982. Water uptake and flow into roots. In: Lange OL, Nobel PS, Osmond CB, Zeigler H, eds. Encyclopedia of plant physiology, vol. 12B. Berlin: Springer Verlag, 79–109.

Yadav R, Flowers TJ, Yeo AR. 1996. The involvement of the transpirational bypass flow in sodium uptake by high and low-sodium-transporting lines of rice developed through intravarietal selection. Plant, Cell and Environment 19, 329–336.

Yeo AR, Yeo ME, Flowers TJ. 1987. The contribution of an apoplastic pathway to sodium uptake by rice roots in saline conditions. Journal of Experimental Botany 38, 1141–1153.

Zeier J, Ruel K, Ryser U, Schreiber L. 1999. Chemical analysis and immunolocalization of lignin and suberin in the endodermis and hypodermis/rhizodermis of developing maize (Zea mays L.) roots. Planta 209, 1–12.

Zeier J, Schreiber L. 1998. Comparative investigation of primary and tertiary endodermal cell walls isolated from the roots of five monocotyledonous species: chemical composition in relation to fine structure. Planta 206, 349–361.

Zimmermann HM, Hartmann KD, Schreiber L, Steudle E. 2000. Chemical composition of apoplastic transport barriers in relation to radial hydraulic conductivity of corn roots (Zea mays L.). Planta 210, 302–311.

Zimmermann HM, Steudle E. 1998. Aporplastic transport across young maize roots: effect of the exodermis. Planta 206, 7–19.