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Chemical composition of apoplastic transport barriers in relation to radial hydraulic conductivity of corn roots (*Zea mays* L.)

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Abstract. The hydraulic conductivity of roots (Lp_r) of 6- to 8-d-old maize seedlings has been related to the chemical composition of apoplastic transport barriers in the endodermis and hypodermis (exodermis), and to the hydraulic conductivity of root cortical cells. Roots were cultivated in two different ways. When grown in aeroponic culture, they developed an exodermis (Casparian band in the hypodermal layer), which was missing in roots from hydroponics. The development of Casparian bands and suberin lamellae was observed by staining with berberin-aniline-blue and Sudan-III. The compositions of suberin and lignin were analyzed quantitatively and qualitatively after depolymerization (BF_3/methanol-transesterification, thioacidolysis) using gas chromatography/mass spectrometry. Root Lp_r was measured using the root pressure probe, and the hydraulic conductivity of cortical cells (Lp) using the cell pressure probe. Roots from the two cultivation methods differed significantly in (i) the Lp_r evaluated from hydrostatic relaxations (factor of 1.5), and (ii) the amounts of lignin and aliphatic suberin in the hypodermal layer of the apical root zone. Aliphatic suberin is thought to be the major reason for the hydrophobic properties of apoplastic barriers and for their relatively low permeability to water. No differences were found in the amounts of suberin in the hypodermal layers of basal root zones and in the endodermal layer. In order to verify that changes in root Lp_r were not caused by changes in hydraulic conductivity at the membrane level, cell Lp was measured as well. No differences were found in the Lp values of cells from roots cultivated by the two different methods. It was concluded that changes in the hydraulic conductivity of the apoplastic rather than of the cell-to-cell path were causing the observed changes in root Lp_r.

Key words: Apoplastic barrier – Endodermis – Exodermis – Hydraulic conductivity – Lignin – Suberin – *Zea* (root, water transport)

Introduction

It has been known for a long time that water uptake by roots is a variable process. It depends on the structure and anatomy of roots which, in turn, is affected by environmental factors such as drought, salinity, anoxia, temperature and heavy metals (Azaizeh et al. 1992; Birner and Steudle 1993; North and Nobel 1995, 1996; Peyrano et al. 1997; Schreiber et al. 1999). These factors or stresses have a considerable impact on the hydraulic conductivity (water permeability) of roots (Lp_r). On the other hand, the intensity and nature of the forces set up between soil solution and root xylem are important (Steudle et al. 1987; Steudle and Frensch 1989; Steudle and Peterson 1998; Steudle 1999). It is known that root Lp_r increases with increasing demand from the shoot (increasing tensions developed in root xylem), i.e. the force/flow relations of roots will usually be non-linear (Brewig 1937; Fiscus 1975; Weatherley 1982; Rüdinger et al. 1994). In the presence of gradients of hydrostatic pressure, Lp_r is usually much higher than in the presence of osmotic gradients which play a role at low or absent transpiration. In other words, water flow caused by 1 bar of osmotic pressure would be substantially smaller than that caused by 1 bar of hydrostatic pressure.

There is some discussion as to whether the apoplast or the parallel cell-to-cell path dominate during water uptake (Steudle and Frensch 1996; Steudle 1997; Steudle and Peterson 1998; Tyerman et al. 1999). In the apoplast, there are no membranes and flow through

Abbreviations and symbols: A (A_r) = cell (root) surface area; d_{cell} = cell diameter; ε = cell elastic modulus; ECW = isolated endodermal cell walls; l_{cell} = cell length; Lp (Lp_r) = hydraulic conductivity of cell (root); π^i (π^i_o) = (stationary) osmotic pressure of the cell; P (P_o) = (stationary) cell turgor pressure; P_r = root pressure; π^o_o = osmotic pressure of the medium; RHCW = isolated rhizodermal and hypodermal cell walls; $T^w_{1/2}$ ($T^w_{1/2r}$) = half-time of cell (root) pressure relaxation; V = cell volume; V_s = water volume of root pressure probe and attached root during measurements

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the porous wall material is only driven by hydrostatic pressure. Along the cell-to-cell path, water moves across a series of membranes, and osmotic gradients play a role in addition to hydrostatic. In the apoplast, the existence of hydrophobic barriers in the exo- and endodermis is crucial (Casparian bands, suberin lamellae). In the membranes of the cell-to-cell path, aquaporins or water channels largely mediate water flow (Steudle and Henzler 1995; Maurel 1997; Steudle 1997, 1999; Tyerman et al. 1999). Both pathways interact with each other. The separation of the contribution of the pathways requires the measurement of water flows at the cell and tissue (organ) level, e.g. by using different pressure probes (Steudle 1993). Depending on the developmental state of the root, the contribution of the pathways to overall root Lp_r will vary. The system is fairly complex and, perhaps, best described by the composite transport model which accounts for the complex structure of roots (Steudle 1997, 1999; Steudle and Frensch 1996; Steudle and Peterson 1998; Tyerman et al. 1999). According to the model, a two-stage type of limitation or regulation of water flow (root Lp_r) has been proposed. A coarse regulation is achieved by the fact that the contribution of apoplastic water flow (cell wall Lp) around protoplasts increases with increasing tensions set up during transpiration. A fine regulation would be maintained by the action of aquaporins or water channels in the parallel cell-to-cell path which has a relatively high hydraulic resistance. Coarse regulation would be important in normal situations of unstressed plants allowing for an adjustment of root Lp_r and water flow in response to the demands from the shoot. Fine regulation, on the other hand, would dominate in stressed roots, when the apoplast is largely blocked by apolastic barriers. Fine regulation by water channels would either allow water uptake in the presence of transpiration (water channels open) or would prevent water losses to a dry soil in its absence (channels closed). It is known that the activity of aquaporins is under metabolic control (Johansson et al. 1996). During the adaptation of plants to water shortage in the soil, the two-stage model would be very effective. So, the mechanisms by which roots regulate water uptake are quite different from those which regulate water losses from the shoot. In the shoot, the cuticle is a barrier which is nearly impermeable to water, and stomata (triggered by different environmental factors) control water flow. In the root, such a 'simple' mechanism is not possible because (i) ions have to be taken up besides the water and (ii) water losses have to be prevented under certain conditions, when uptake is not possible. Apoplastic barriers play an important role during the optimization of root hydraulics.

In the past few years, considerable progress has been made in the structural and anatomical analysis of apolastic barriers in roots, namely, in investigating the development of Casparian bands in the hypodermis (a hypodermal layer with a Casparian band is called the exodermis, see Peterson 1997; Enstone and Peterson 1997, 1998; Steudle and Peterson 1998). There is evidence that, besides the endodermis, the exodermis plays an important role in controlling the uptake of water and solutes across roots (Zimmermann and Steudle 1998; Freundl et al. 1999). Techniques have been developed to isolate the non-cellulosic compounds in cell walls (lignin, suberin, proteins) and to analyze this material after depolymerization and subsequent gas-chromatographic (GC/FID, GC/FTIR) and mass spectrometeric analysis (GC/MS; Zeier and Schreiber 1997, 1998; Schreiber et al. 1999; Zeier et al. 1999). The techniques allow the separation of endodermal cell wall components from those of the hypodermis. A chemical analysis of apoplastic barriers requires amounts of only 0.5 mg, which is sufficient to follow changes in the amount and composition of the material during root development. Changes caused by the environment such as high salinity and heavy-metal concentration in the soil solution have been monitored (Schreiber et al. 1999).

In the present paper, we are concerned with the role of apoplastic transport barriers in young maize roots. When grown hydroponically, roots do not develop Casparian bands in the hypodermis, which they do when grown in mist culture (aeroponics; Zimmermann and Steudle 1998). We compare differences in root hydraulic properties with changes in the deposition of hydrophobic barriers and their composition using the techniques just mentioned. Detailed measurements of radial water flows at the level of entire roots (root pressure probe) and individual cortical cells (cell pressure probe) have been related to detailed chemical analyses. We show a clear relation between decreases in root Lp_r and increases in the amounts of suberin in roots grown aeroponically. According to the results, aliphatic suberin is the material which provides most of the impermeability of Casparian bands and suberin lamellae to water. Compared with the effects of treatments on apoplastic barriers, there were no effects induced by the treatments along the cell-to-cell path, at least in the cortex.

Materials and methods

Plant material and treatments. Maize (*Zea mays* L. cv Helix; Kleinwanzlebener Saatzucht AG, Kleinwanzleben, Germany) seeds were germinated for 3–4 d in the dark on filter paper wetted with 0.1 mM $CaCl_2$. Half of the seedlings were transferred to an aeroponic (mist) culture system consisting of a polyvinyl chloride box of 1 m × 1 m × 1 m and an air humidifier (Zimmermann and Steudle 1998). The other half was grown in hydroponic culture. Plastic tanks (10 l) with aerated nutrient solution were used. During both treatments, the same growth chamber (day/night rhythm: 14/10 h; 20/17 °C) and the same nutrient solution were used (composition: 0.7 mM K_2SO_4, 0.1 mM KCl, 2 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 0.1 mM KH_2PO_4, 1 μM H_3BO_3, 0.5 μM $MnSO_4$, 0.5 μM $ZnSO_4$, 0.2 μM $CuSO_4$, 0.01 μM $(NH_4)_6Mo_7O_{24}$, and 200 μM Fe-EDTA). Plants employed in the experiments had an age (including germination) of 6–8 d (root pressure probe) or 8 d (all other experiments; histochemical and chemical analysis).

Anatomy. The anatomy of roots from both culture methods was observed in cross-sections. Freehand cross-sections from the main roots of 8-d-old maize plants were stained for 1 h with 0.1% berberine hemisulfate and for another hour with 0.5% aniline blue (w/v, Brundrett et al. 1988). Sections were made at different distances from the root tip (20, 40, 60, 80, 100, 150, 200, 250 mm),

stained and viewed under an epifluorescence microscope using an ultraviolet filter set after staining (excitation filter BP 365, dichroitic mirror FT 395, barrier filter LP 397; Zeiss, Oberkochen, Germany). Photographs were taken on Kodak Elite 200 ASA films. Alternatively, roots were fixed in a phosphate-buffered saline (pH 7.4) solution of formaldehyde (3.7%; w/w) for several weeks. They were then placed on a specimen holder using embedding medium (Microm, Walldorf, Germany) and frozen to −70 °C. At −25 °C, cross-sections of 10–20 μm were cut using a cryo-mycrotome (Cryostat H 500 M, Microm,Walldorf, Germany) at different distances from the root tip (80, 150, 200, 250 mm) and transferred to glass slides. Sudan-III-staining was performed according to Gerlach (1984) using a saturated solution of Sudan III (Merck) dissolved in an ethanol/water mixture (1:1; v/v). Specimens were covered by a few drops of the Sudan III solution, heated to 70 °C for 10 min and cleared with glycerol/water (1:1; v/v). Sections were viewed under bright-field illumination and recorded with a video camera (Sony XC 003) and an image analyser (Intas, Göttingen). The length of the main root (245–375 mm) was determined with a ruler. Diameters (0.65–1.1 mm) were measured with a caliper.

Isolation of cell walls from maize roots. Cell walls from different parts of the roots were isolated enzymatically as described previously (Schreiber et al. 1994). Roots were separated into two halves. The younger half (zone I) had no visible lateral roots. It ranged from the root tip to the middle of the root (up to 122–187 mm). The older half (zone II) ranged from the middle of the root where laterals started to emerge, to the root base. Roots from each zone were cut into segments of 30 mm and incubated separately in an enzymatic solution containing cellulase (Onozuka, R-10; Serva) and pectinase (Macerozyme R-10; Serva). Cell walls from different root tissues which resisted the enzymatic attack were separated mechanically after several days. Central cylinders consisting of the endodermis (endodermal cell walls = ECW) and enclosed xylem vessels were pulled out from the hypodermal cylinder. Since both rhizodermal and attached hypodermal cell walls resisted the enzymatic attack and did not separate from each other, a cell wall fraction called RHCW (isolated rhizodermal and hypodermal cell walls) was isolated. Central cylinders were separated into ECW and xylem vessels. The ECW were collected for further analysis. Isolated cell wall material was washed with borate buffer (10 mM, pH 9) and deionized water, dried and stored for further use.

Depolymerization and chromatographic analyses of isolated cell walls. The ECW and RHCW were extracted using chloroform/methanol (1:1; v/v), dried again and depolymerized using chemical degradation methods specific for the detection of suberin and lignin, as described in detail by Zeier and Schreiber (1997, 1998). Chloroform/methanol extracts were used for analysis without further purification. After the transesterification of isolated cell wall material, suberin was analyzed according to Kolattukudy and Agrawal (1974). Monomers released after suberin depolymerization were separated into two fractions, i.e. (i) aliphatic suberin consisting of the linear long-chain aliphatic compounds with chain lengths varying between C_{16} and C_{28} and (ii) aromatic suberin containing phenolic compounds such as coumaric and ferulic acids (Zeier et al. 1999). Thioacidolysis was used for the detection of lignin (Lapierre et al. 1991). Gas chromatography and mass spectrometry were used for the characterization of the chloroform/methanol extracts and of suberin and lignin (Zeier and Schreiber 1997, 1998). Prior to injection, samples were derivatized by BSTFA (N,N-bis-trimethylsilyltrifluoroacetamide; Machery-Nagel, Düren, Germany) to convert free hydroxyl and carboxyl groups to their respective trimethylsilyl esters and ethers. Qualitative sample analyses were performed by gas chromatography (HP 5890 Series II gas chromatograph; Hewlett-Packard) combined with a quadrupole mass selective detector (HP 5971 A mass selective detector; Hewlett-Packard). Quantitative sample analyses were carried out on an HP 5890 Series II gas chromatograph (Hewlett-Packard), equipped with a flame ionization detector (FID).

Reproducibility. In the chemical analyses, root cell walls from at least 20 individual plants were isolated. They were combined to obtain a representative sample. If not stated otherwise, results of the gas-chromatographic analyses represent means of three replicates with standard deviations. With the exception of ECW isolated from zone II of roots grown aeroponically, it was not possible to obtain sufficient amounts of wall material of ECW for repetitions in the suberin analyses. For ECW, lignin amounts could not be determined at all for the same reason.

Root pressure-probe experiments. Root pressure-probe experiments were performed as described previously (Steudle 1993; Steudle et al. 1993). Briefly, the excised root segment was tightly connected to a root pressure probe using a cylindrical silicone seal which was prepared from liquid silicone material (Xantopren; Bayer, Leverkusen, Germany). End segments had a length of 108–169 mm and varied in diameter between 0.65 and 1.1 mm. Inner diameters of seals were adapted to diameters of individual root segments and adjusted by a screw. Seals were water-tight even at pressures of several bars, but did not interrupt water flow in the xylem. After each experiment, the proper function of seals was tested by cutting off the root at the seal and controlling the decrease in the time constants of pressure relaxations. When root xylem remained open, there was a drastic decrease in half-times after the cut. Otherwise, the experiment was discarded. Root segments fixed to the probe were bathed in nutrient solution which circulated along the roots to avoid problems with unstirred layers. Root pressures (P_r in MPa) were measured with a pressure transducer and recorded on a strip-chart recorder. Usually, stable root pressures developed within 1–3 h. Hydrostatic and osmotic relaxations were performed by either changing the xylem pressure (moving the metal rod in the probe) or the osmotic pressure of the medium. Transient changes in pressure were followed which allowed root Lp_r to be calculated from half-times of pressure relaxations ($T^w_{1/2r}$) according to (Steudle 1993):

$$\frac{\ln(2)}{T^w_{1/2r}} = A_r \frac{\Delta P_r}{\Delta V_s} Lp_r \qquad \text{(Eq. 1)}$$

where $\Delta P_r/\Delta V_s$ (in MPa · m^{-3}) is the elastic coefficient of the measuring system; V_s denotes the water volume of the system, and A_r the effective surface area of the root. The ratio $\Delta P_r/\Delta V_S$ was measured by inducing step changes in the volume by moving the metal rod and recording the resulting changes in root pressure (ΔP_r). The effective root surface area A_r of the root segment was calculated from its length and diameter, subtracting an apical part of 15–20 mm, where the tracheary elements of protoxylem, early metaxylem and late metaxylem were immature (Peterson and Steudle 1993). Test solutions used in osmotic experiments contained 20–40 mM NaCl (≈40–80 mosmol kg^{-1}, which is equivalent to osmotic pressures of 0.1–0.2 MPa) in addition to the nutrients of the medium.

Cell pressure probe experiments. The cell pressure probe was used to measure turgor (P) and hydraulic conductivity (Lp) of root cortical cells (Azaizeh et al. 1992; Steudle 1993). A glass microcapillary with an outer tip diameter of 4–8 μm was filled with silicone oil (type AS4; Wacker, München, Germany) and attached to the oil-filled pressure chamber of the probe which contained an electronic pressure transducer. A micromanipulator allowed careful insertion of the tip into an individual cortical cell by moving the probe. When the tip was introduced into a cell, a meniscus formed between cell sap and silicone oil in the microcapillary. By measuring the depth of insertion of the tip into the cortex, the location of punctured cells in the cortex could be determined. When a stationary turgor pressure (P_o) was recorded, the hydraulic parameters of the cell were determined (cell elastic modulus ε and half-time $T^w_{1/2}$). The cell elastic modulus (ε in MPa) was measured by instantaneously changing the cell volume (ΔV) and recording the resulting changes in cell turgor pressure (ΔP). The elastic modulus (ε) was calculated from (V = cell volume):

$$\varepsilon = V \frac{\Delta P}{\Delta V} \quad . \qquad \text{(Eq. 2)}$$

Cell volumes were estimated from cross-sections and longitudinal sections assuming a cylindrical shape of cells ($V = \pi \cdot (d_{cell}/2)^2 \cdot l_{cell}$; l_{cell} = cell length; d_{cell} = cell diameter). Using a micrometer screw, a metal rod could be moved backward and forward to change the position of the meniscus. Hydrostatic relaxations were performed by moving the meniscus to a new position and keeping it constant while recording the pressure relaxation of the cell. Using the half-time of the relaxation ($T^w_{1/2}$), Lp was calculated using the following equation (Azaizeh et al. 1992):

$$Lp = V \cdot \frac{\ln(2)}{A \cdot T^w_{1/2} \cdot (\varepsilon + \pi^i_o)} \quad , \qquad \text{(Eq. 3)}$$

where A denotes the cell surface area. The osmotic pressure of the cell (π^i_o) was calculated from P_o and from the osmotic pressure of the medium (π^o_o), since:

$$P_o = \pi^i_o - \pi^o_o \, . \qquad \text{(Eq. 4)}$$

Cells were located at distances of 40–160 mm from the root tip and 44–380 μm from the root surface.

Results

Anatomy. Roots of 8-d-old maize plants usually had a length of 240–375 mm. A primary endodermis with a Casparian band in the radial cell walls developed at distances of 20–40 mm from the root tip in roots grown aeroponically (Fig. 1B,G) and matured into the secondary state at distances between 60 and 80 mm (Fig. 1I). Likewise, roots grown hydroponically developed a primary endodermis between 20 and 40 mm behind the root tip (Fig. 1A,C). It matured into the secondary state between 80 and 120 mm (Fig. 1E). In the roots studied, the endodermis never developed into the tertiary state. Roots grown hydroponically did not develop a Casparian band in the hypodermis (Fig. 1A). However, roots grown aeroponically did develop a Casparian band at distances from 40 to 60 mm from the root tip (exodermis; Fig 1B). Sudan III stained complete suberin lamellae around hypodermal cells at distances of 80 mm from the root tip in roots grown aeroponically (Fig. 1H,K). The hypodermis of roots grown in hydroponic culture did not exhibit suberin lamellae up to a distance of 200 mm from the root tip (Fig. 1D).

Cell dimensions. Cell dimensions and numbers were measured using root sections from the zone between 80 and 120 mm from the root apex, i.e. in the zone where the cell pressure probe was used. In roots grown aeroponically, the mean surface area of cortical cells was $1.2 \cdot 10^{-8}$ m^2 [mean diameter of 28 ± 5 μm (± SD; n = 220 cells) and mean length of 200 ± 29 μm; n = 153 cells]. The mean surface area of cortical cells of roots grown hydroponically was $2.1 \cdot 10^{-8}$ m^2 [mean diameter of 28 ± 5 μm (n = 95 cells) and mean length of 225 ± 66 μm (n = 33 cells)].

Chemical composition of isolated cell wall samples. Detectable amounts of aliphatic suberin (Fig. 2A), aromatic suberin (Fig. 2B) and lignin (Fig. 2C) were released from ECW and RHCW isolated from maize roots. The ECW isolated from root zone I with Casparian strips showed significantly lower suberin amounts compared to root zone II where suberin lamellae developed (Fig. 2A). With the exception of RHCW of zone I, there were no differences in the aliphatic suberin contents of cell wall samples between the two treatments. By a factor of 2.4, the aliphatic suberin concentration in zone I was larger in RHCW with Casparian strips and suberin lamellae (isolated from roots cultivated aeroponically), than in RHCW lacking Casparian strips and suberin lamellae (obtained from roots cultivated hydroponically). Between the two cultivation methods, there were no differences in the concentration of aromatic suberin in the ECW and RHCW of either root zone (Fig. 2B). Lignin amounts could be determined only for RHCW (Fig. 2C). They were higher by a factor of 1.6 in zone I of roots cultivated aeroponically than in the same zone of roots from hydroponic culture. In zone II, lignin amounts in RHCW from the two cultivation methods were not statistically different. There were no dectectable amounts of wax-like substances in chloroform/methanol extracts of ECW and RHCW in either zone and cultivation method.

Qualitatively, the composition of the three different cell wall fractions (aliphatic suberin, aromatic suberin, lignin) isolated from ECW and RHCW did not differ for the two treatments (Fig. 3). Looking more closely at the substance classes and chain-length distributions of the aliphatic suberin monomers released after transesterification of RHCW from root zone I, it is obvious that they were very similar. This was also true for the ratios of the three lignin monomers [*p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units] released from RHCW after thioacidolysis (Fig. 4). The H/G/S ratios changed from root zone I to root zone II, but within the same root zone H/G/S ratios were not significantly different for the two cultivation methods (Fig. 4).

Hydraulic measurements. Stationary root pressures (P_{ro}) measured using root segments from both cultivation methods were not different. They ranged from 0.1 to 0.3 MPa. Table 1 shows root hydraulic conductivities (Lp_r), which were obtained from hydraulic and osmotic experiments on excised primary roots. Data represent average values for entire segments. For roots from both treatments, hydrostatic Lp_r was significantly larger than osmotic Lp_r. The difference between hydrostatic and osmotic Lp_r was larger in roots grown hydroponically (factor of 20) than in roots from aeroponics (factor of 10). Hydrostatic Lp_r was significantly larger (factor of 1.5) in roots from hydroponic culture than in roots from aeroponic culture (*t*-test; p = 0.05; n = 11 roots). Osmotic Lp_r did not differ between treatments.

Hydraulic conductivities measured on different root cortical cells showed a big scatter in the data from different individual cells (Fig. 5). No significant differences could be detected between treatments. However, despite the large scatter, the data indicate a somewhat higher Lp for cells from hydroponic roots [$Lp = (2.7 \pm 3.7) \times 10^{-7}$ m · s^{-1} · MPa^{-1}] than from aeroponic roots [$Lp = (1.3 \pm 1.1) \times 10^{-7}$ m · s^{-1} · MPa^{-1}]. The volumetric elastic moduli of cells (ε) ranged from ε = 0.8 to 18.3 MPa (hydroponics) and ε = 0.3 to 5.5 MPa (aeroponics). Values of Lp in Fig. 5 are

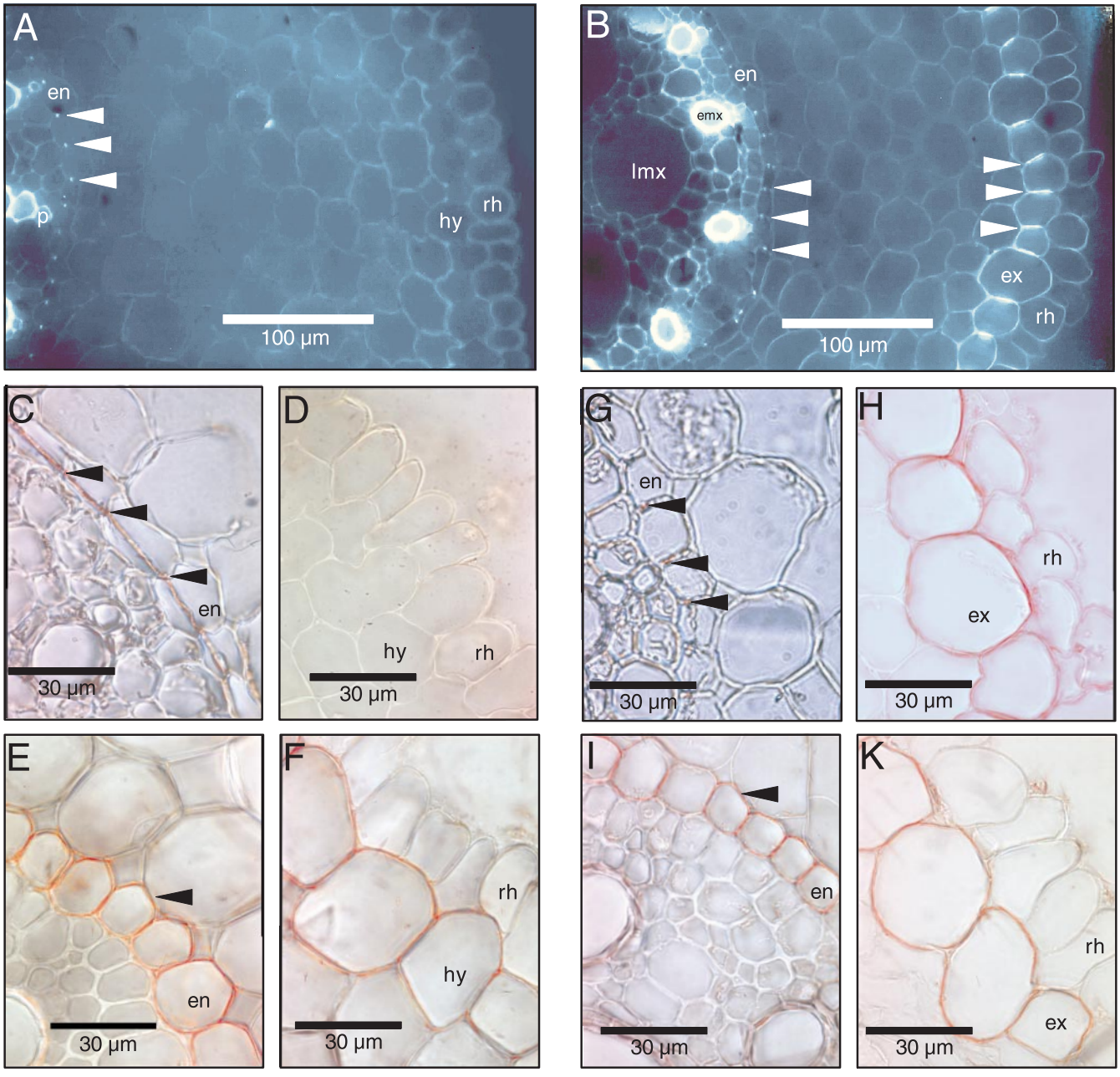

Fig. 1A–K. Cross-sections of primary roots of 8-d-old maize plants grown hydroponically (**A**,**C**,**D**,**E** and **F**) or aeroponically (**B**,**G**,**H**,**I** and **K**). **A,B** Freehand cross-sections were stained with berberine-aniline blue and viewed with UV/violet light (wavelength: 390–420 nm) under an epifluorescence microscope. Lignified vessels and Casparian bands appear bright. In **A** (hydroponic culture), the distance from the root tip was 80 mm. *rh*, rhizodermis *hy*, hypodermis without Casparian bands; *en*, endodermis (primary state) with Casparian bands (*arrowheads*); *p* mature protoxylem. In **B** (aeroponic culture), the distance from the root tip was 50 mm. e*x*, mature exodermis, (secondary state) with Casparian bands (*arrowheads*); *en*, endodermis, (primary state) with Casparian bands (*arrowheads*); *emx*, mature early metaxylem, *lmx*, immature late metaxylem. Sections **C–K** were stained with Sudan III and viewed under bright-field illumination. Suberized cell walls appear bright red. In section **C** (hydroponic culture), endodermal cell walls *en* with Casparian strips (*arrowheads*) and band plasmolysis are shown in root zone I, 150 mm behind the root tip. **D** (hydroponic culture): rhizodermal (*rh*) and hypodermal cell walls (*hy*) not stainable with Sudan III in root zone I, 150 mm behind the root tip. **E** (hydroponic culture): endodermal cell walls *en* with suberin lamellae (*arrowhead*) in root zone II, 250 mm behind the root tip. **F** (hydroponic culture): rhizodermal (*rh*) and hypodermal cell walls (*hy*) with Sudan III-stained suberin lamellae in root zone II, 250 mm behind the root tip. **G** (aeroponic culture): endodermal cell walls *en* with Casparian strips (*arrowheads*) in root zone I, 80 mm behind the root tip. **H** (aeroponic culture): rhizodermal (*rh*) and exodermal cell walls (*ex*) with Sudan III-stained suberin lamellae in root zone I, 80 mm behind the root tip. **I** (aeroponic culture): endodermal cell walls *en* with suberin lamellae (*arrowhead*) in root zone II, 200 mm behind the root tip. **K** (aeroponic culture): rhizodermal (*rh*) and exodermal cell walls (*ex*) with Sudan III-stained suberin lamellae in root zone II, 200 mm behind the root tip

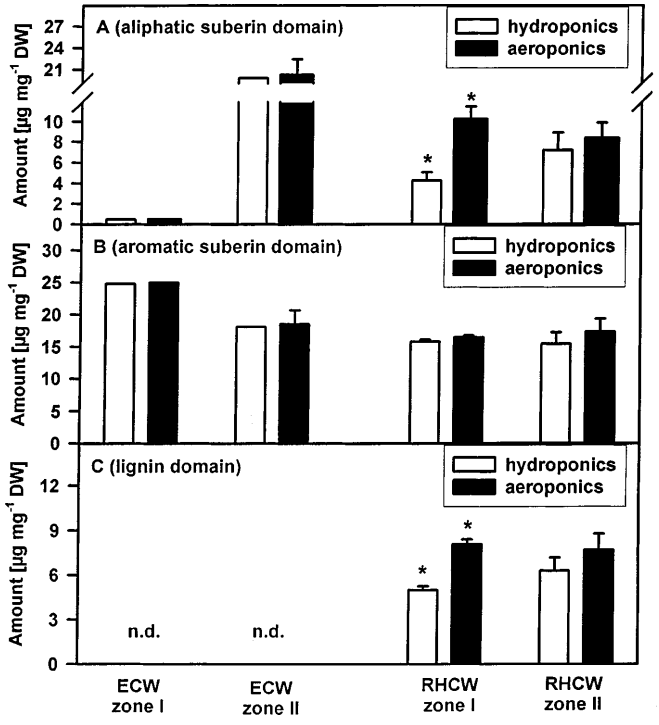

Fig. 2A–C. Concentrations of aliphatic suberin (**A**), aromatic suberin (**B**) and lignin (**C**) released from endodermal (*ECW*) and rhizodermal/hypodermal (*RHCW*) cell walls isolated from primary maize roots after transesterification with BF_3/methanol (**A**,**B**) and thioacidolysis (**C**). Roots were cultivated either in hydroponics (*white bars*) or aeroponics (*black bars*). Prior to analysis, roots were separated into a proximal root *zone I* ranging from the root tip to the middle of the root where laterals had not yet evolved, and a distal root *zone II* ranging from the middle of the root where laterals had started to emerge to the base of the root. Concentrations are given in µg cell wall polymer per mg dry weight of isolated cell wall material (ECW and RHCW). Columns with error bars represent means ± SD of three replicates. Results without error bars represent single determinations (not sufficient cell wall material for replicates). Lignin amounts of ECW could not be determined (*n.d.*) since there was only a very limited amount of isolated cell wall material. *Asterisks* indicate statistically significant differences at the 95% level (*t*-test) between values from different treatments (hydroponics or aeroponics)

given as mean ± SD; Lp was calculated from mean values or cell dimensions (diameter and length), dP/dV, $T^w_{1/2}$ and π^i according to Eq. 3 without considering propagated errors in the different independently measured variables. To evaluate the total error of a single measurement of Lp the Gaussian law of error propagation has to be applied, i.e. Zhu and Steudle (1991):

$$\frac{SD_{Lp}}{Lp} = \pm\sqrt{\left(\frac{\varepsilon-\pi^i_o}{\varepsilon+\pi^i_o}\frac{SD_{d_{cell}}}{d_{cell}}\right)^2+\left(\frac{\varepsilon}{\varepsilon+\pi^i_o}\frac{SD_{l_{cell}}}{l_{cell}}\right)^2+\left(\frac{\varepsilon}{\varepsilon+\pi^i_o}\frac{SD_{dP/dV}}{dP/dV}\right)^2+\left(\frac{SD_{T^w_{1/2}}}{T^w_{1/2}}\right)^2+\left(\frac{SD\pi^i_o}{\varepsilon+\pi^i_o}\right)^2}, \quad \text{(Eq. 5)}$$

where SD_{Lp} denotes the standard deviation of Lp that incorporates all errors due to the five independent variables ($T^w_{1/2}$, dP/dV, π^i_o, l_{cell}, d_{cell}) used to evaluate Lp. The errors for the determination of the cell diameters for hydroponic and aeroponic culture were 20% and 18%, respectively. The errors for the determination of the cell length for hydroponic and aeroponic culture were 29% and 24%, respectively. The standard deviation in the determination of $T^w_{1/2}$ was lower than 53% for cells from hydroponic culture and lower than 63% for cells from aeroponics. The standard deviation in the determination of dP/dV was lower than 39% for cells from hydroponic culture and lower than 28% for cells from aeroponics. Mean values for ε and π^i_o were ε = 7.0 MPa and π^i_o = 0.3 MPa in hydroponics, ε = 3.5 MPa and π^i_o = 0.3 MPa in aeroponics. The propagated error of Lp was calculated for each cell separately. It ranged from 36% to 63% for cells from hydroponics and between 27% and 70% for cells from aeroponics.

Discussion

Data concerning the hydraulic conductivity (cell and organ level) have been correlated for the first time with those of the chemical composition of apoplastic barriers in the exo- and endodermis of young maize roots (Casparian bands; suberin lamellae). Roots from hydroponic culture differed from those from aeroponics by (i) the existence of a Casparian band in the hypodermis (exodermis), (ii) increased amounts of suberin and lignin in the hypodermal layer, and (iii) the reduced hydraulic conductivity for radial water flow (root Lp_r). The latter effect is well known from a recent study (Zimmermann and Steudle 1998). It resembles the effects of stresses of different kinds (salinity, drought, anoxia) that promote the development of the endo- and exodermis and its suberization, and reduce the hydraulic conductivity at the cell and root level (Azaizeh et al. 1992; Cruz et al. 1992; Birner and Steudle 1993; Stasovsky and Peterson 1993; North and Nobel 1995, 1996; Zhang and Tyerman 1999). Stresses delay root growth, and Casparian bands develop earlier, which results in some kind of a 'compression' of root zones. Besides the effects on apoplastic barriers, it also is possible that aquaporin expression and activity differed between the treatments. So, the results from the present hydraulic measurements fit into a general picture known from studies of stress effects on root hydraulics.

Compared to hydroponics, there was a general trend towards higher lignin and suberin amounts in hypodermal cell walls of primary maize roots grown aeroponically (Fig. 2). Analyses of endodermal cell walls, however, showed no differences between treatments. The detailed analyses of the different substance classes,

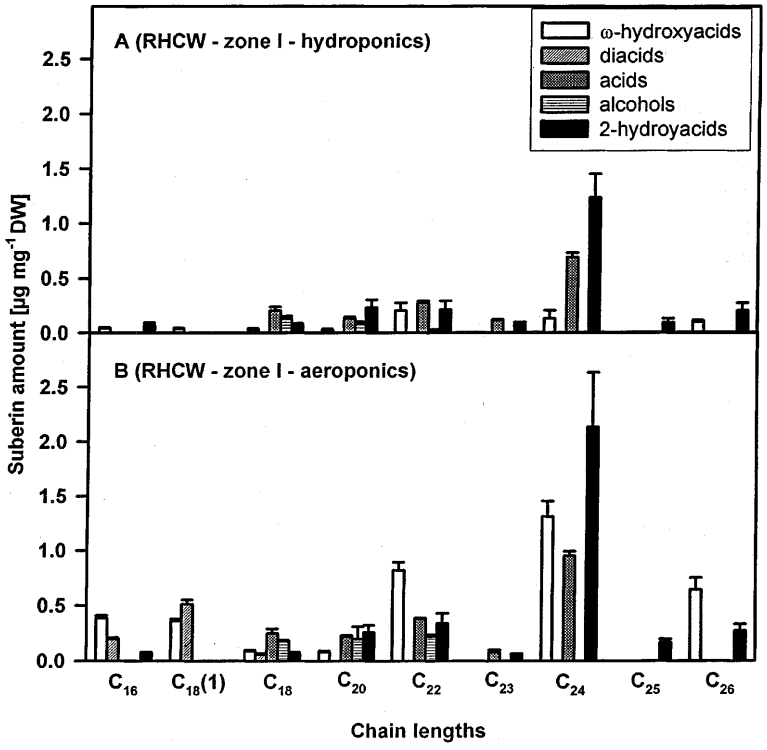

Fig. 3A,B. Substance class compositions and chain-length distributions of aliphatic suberin monomers released from RHCW isolated from primary maize roots cultivated in hydroponics (**A**) and aeroponics (**B**). Quantitive amounts of aliphatic suberin are significantly higher in RHCW cultivated aeroponically (**B**) than in those cultivated hydroponically (**A**). Qualitative compositions are similar for both treatments. Data are means ± SD of three replicates

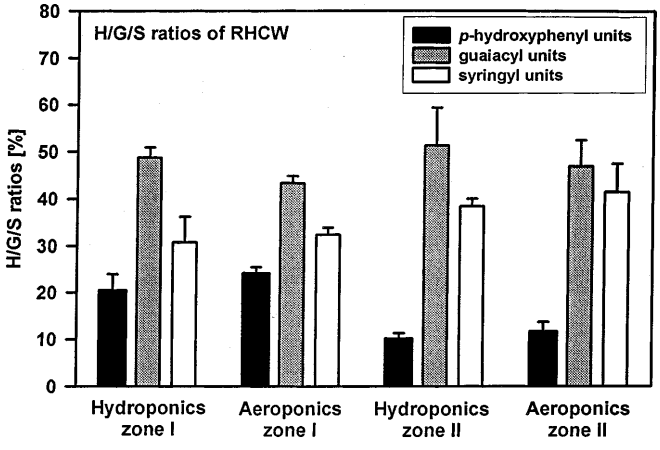

Fig. 4. Ratios of the three lignin monomers *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units released from RHCW of primary maize roots. The H/G/S ratios of the same root zone are similar and independent of the cultivation method (treatment). However, G and S units shift to higher and H units to lower values in the basal root zone II. Data are means ± SD of three replicates

the chain-length distribution of the aliphatic suberin (Fig. 3) domain, and the monomeric composition of the lignin domain (Fig. 4) revealed no major differences in the qualitative composition of the suberin and lignin domains between the two different cultivation methods. It appears that, during aeroponic culture, roots sense some kind of a stress signal at the outer cell layers of the roots, resulting in an overall increase in suberin and lignin biosynthesis. The nature of this signal is unknown. Although the roots' surroundings were saturated with water in the mist culture (water potential of zero), there may have been local fluctuations of water potential at the root surface. These may have been caused by increases in the salt concentration at the root surface (as water was rapidly taken up), or by decreases in temperature (as water evaporated from the root surface). Another possibility could be that the exposure to the gaseous surroundings caused mechanical effects because bouyancy was missing. There is some turbulence in the tanks which may have caused an additional mechanical stress. It is known that weak mechanical stimuli may induce responses in roots (e.g., hyperpolarization of the plasma membrane; Monshausen and Sievers 1998). Because media were saturated with oxygen in both treatments, it is unlikely that differences in the oxygen concentration at the root surface caused the effect.

Most interestingly, statistically significant differences between the different cultivation methods were observed for the amounts of aliphatic suberin and lignin in hypodermal cell walls in the distal half (zone I) of the roots (Fig. 2). This observation is in good accordance with anatomical and histochemical investigations of this zone from roots grown aeroponically, the results of which show Casparian bands and the early development of complete suberin lamellae in the exodermis (Fig. 1B,H,K). Thus, anatomical and histochemical observations and the chemical analysis provide clear evidence that apoplastic barriers were more pronounced in hypodermal walls of roots grown aeroponically than in those grown hydroponically.

The development of an exodermis had a substantial effect on water uptake across the root cylinder. Hydraulic conductivity (Lp_r) from hydrostatic experiments was smaller by a factor of 1.5 for root end segments

Table 1. Hydraulic conductivity of end segments (zone I) of 6-to-8-d-old maize root systems as measured with the root pressure probe. Seedlings were cultivated either in hydroponics or aeroponics. Hydrostatic relaxations were induced by moving a metal rod in the probe, which produced a hydrostatic pressure gradient between xylem and medium. During osmotic relaxations, water flows were driven by osmotic pressure gradients set up by changing the osmotic pressure of the nutrient solution (addition of 10–40 mM NaCl)

Root	Age of root (d)	Length of measured segment (mm)	Surface area of measured segment (mm^2)	Hydraulic conductivity Lp_r, as measured in hydrostatic relaxations ($m \cdot s^{-1} \cdot MPa^{-1} \cdot 10^{-8}$)	Hydraulic conductivity Lp_r, as measured in osmotic relaxations ($m \cdot s^{-1} \cdot MPa^{-1} \cdot 10^{-8}$)
Hydroponics					
Root 1	7	138	408	8.5 ± 2.0	0.23 ± 0.047
Root 2	6	108	299	22.0 ± 5.5	0.47 ± 0.033
Root 3	6	128	377	22.2 ± 6.9	0.54 ± 0.12
Root 4	6	146	341	30.2 ± 6.4	1.05 ± 0.053
Root 5	7	114	202	17.5 ± 5.9	0.99
Root 6	7	149	388	13.2 ± 3.9	1.33
Root 7	6	150	402	13.4 ± 2.6	0.52 ± 0.08
Root 8	8	150	332	11.2 ± 1.1	1.13 ± 0.23
Root 9	7	140	353	24.7 ± 4.0	0.70 ± 0.23
Root 10	7	155	439	20.6 ± 7.9	1.57 ± 0.13
Root 11	7	142	405	10.4 ± 2.2	1.41
Mean	6.7	138.2	359	17.6	0.90
SD	0.6	15.4	66	6.9	0.44
n	11	11	11	11	11
Aeroponics					
Root 2	7	141	400	17.3 ± 5.6	0.80 ± 0.0091
Root 3	8	155	419	16.4 ± 4.5	0.65 ± 0.078
Root 5	6	137	385	22.5 ± 9.9	1.35 ± 0.29
Root 6	8	160	418	12.2 ± 4.4	0.89 ± 0.11
Root 7	7	132	341	10.4 ± 3.2	0.70 ± 0.0088
Root 8	8	126	297	8.4 ± 3.2	1.04 ± 0.13
Root 9	8	158	401	4.8 ± 1.1	–
Root 10	7	169	405	7.8 ± 4.2	1.91 ± 0.07
Root 11	7	147	290	14.9 ± 9.7	1.70 ± 0.43
Root 12	7	120	317	5.1 ± 2.3	1.48 ± 0.85
Root 13	7	121	83	5.6 ± 1.6	0.62
Mean	7.3	142.4	341	11.4	1.1
SD	0.6	16.8	98	5.8	0.47
n	11	11	11	11	10

from aeroponics than for those from hydroponics. An even larger factor of 3.6 has been found in an earlier study with root systems of older maize plants (age 12–21 d; Zimmermann and Steudle 1998). Presumably, the difference is due to the fact that suberin lamellae and Casparian bands were much more developed in older than in the younger roots used here, which should result in a bigger overall effect on water uptake. Unlike the steady-state flows used in the former study to measure Lp_r, the hydrostatic pressure pulses used in the present experiments resulted in transient water flows which, presumably, were located mainly in the stele and endodermis. Therefore, due to the experimental setup, the effect of the exodermis may have been somewhat diminished.

Absolute values of Lp_r and Lp found for both treatments were similar to hydraulic conductivities obtained in earlier studies for young maize roots grown hydroponically (Zhu and Steudle 1991; Azaizeh and Steudle 1991; Birner and Steudle 1993; Steudle et al. 1993; Frensch et al. 1996). One important point is that the hydraulic conductivity of single cortical cells (Lp) was not different from the hydraulic conductivity of radial water flow across the whole root (Lp_r; hydrostatic experiments). This indicated that the contribution of apoplastic water flow was substantial, even in the endodermis (Zimmermann and Steudle 1998). In osmotic experiments, root Lp_r was much smaller than in hydrostatic experiments indicating that there was a substantial cell-to-cell transport. The findings are in line with the composite transport model proposed earlier (Steudle 1994, 1997; Steudle and Frensch 1996; Steudle and Peterson 1998).

There is an alternative to the explanation just given for the changes in Lp_r in terms of apoplastic barriers. It may be argued that the differences in Lp_r were due to a different expression of aquaporins (water channels) or changes in the activity of aquaporins in cell membranes resulting in a different cell hydraulic conductivity (Lp). Therefore, Lp was measured as well to show whether or not changes in Lp could contribute to the measured changes in Lp_r in the two different treatments. The results do not support the alternative explanation, although there was a trend (though not significant) indicating that aeroponic treatment caused a decrease in Lp. There was no difference between treatments in the osmotic root Lp_r. Under these conditions, water should largely move from cell to cell. This provides additional

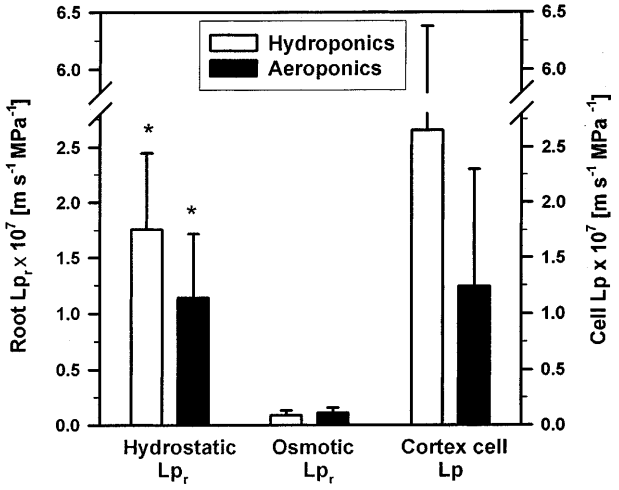

Fig. 5. Hydraulic conductivities of proximal root segments (Lp_r; zone I) and cortical cells (Lp), as measured on maize roots cultivated either hydroponically (*white bars*) or aeroponically (*black bars*). Measurements at the root level were performed using the root pressure probe. Root Lp_r of 6- to 8-d-old roots is given. Data represent means ± SD (n = 10–11 roots). Bars marked with *asterisks* indicate a statistically significant difference (95% level; *t*-test; n = 11 roots). In hydrostatic experiments, hydraulic conductivity was measured by changing the root turgor pressure with the aid of the probe. In osmotic experiments, relaxations were induced by changing the osmotic pressure of the medium. At the level of individual cells, the cell pressure probe was employed to evaluate the hydraulic conductivity of cortical cells (Lp) punctured in zone I of 8-d-old roots from hydrostatic relaxations. Means are given ±SD; n = 14–16 cells. The difference in mean cell Lp (factor of 2.1) between the two treatments was not significant (95% level; *t*-test; n = 14–16 cells)

evidence that aquaporins were not involved. At least, their effect was not significant. This, however, does not exclude the possibility that water channels may play a role under conditions of severe stress when roots are heavily suberized, as has been proposed earlier (Steudle and Peterson 1998). In roots which allow an apoplastic water flow, a substantial water uptake occurs in transpiring plants and is switched off when transpiration ceases. Under these conditions the cell-to-cell path, which may be under metabolic control, may dominate, since aquaporins can be activated by phosphorylation (Johansson et al. 1996; Tyerman et al. 1999). Considering the water flow intensities, the switching between the apoplastic path (controlled by hydrophobic barriers) and the cell-to-cell path (controlled by water channels) could provide a switching between a coarse and fine regulation of water flow (see *Introduction*; Steudle and Peterson 1998; Steudle 1999).

It is known that cell Lp varies substantially within the cortex of maize roots, but there are no clear trends for a radial gradient in cell Lp (Zhu and Steudle 1991). Recently, Henzler et al. (1999) measured diurnal rhythms in root Lp_r of lotus. The changes found (maximum of Lp_r found 5–7 h after the onset of the light phase; minimum during the night) were correlated with changes in the content of messenger RNA encoding for water channels in cell membranes. The authors concluded that there was an effect of water channels, i.e. a considerable cell-to-cell transport. However, the Lp of cortical cells did not change. This indicated that changes in Lp (expression of water-channel activity) were concentrated in the endodermis or the stele (Schäffner 1998). A similar effect cannot be completely excluded for maize. Measurements in the stele and endodermis are badly required. However, they are technically difficult and not yet available.

In conclusion, the results indicate a clear effect of apoplastic barriers induced by aeroponic culture on root water uptake (Lp_r). Additional effects caused by changes in the activity of water channels could not be demonstrated, but this point requires more detailed analyses. It appears that aliphatic suberin is the compound which largely provides the impermeability of the apoplast to water. Since this refers to suberin lamellae in the tangential cell walls besides Casparian bands, the cell-to-cell path could be affected as well. According to the composite transport model, changes in root Lp_r could be caused by a combined effect on the apoplastic and cellular path, (coarse and fine regulation). Fine regulation may be achieved by an activation of water channels as proposed earlier (Steudle and Peterson 1998; Steudle 1999). More detailed analyses of the chemical composition of apoplastic barriers and of root and cell hydraulic conductivity in different root zones and tissues are required. The hydraulic analysis should incorporate measurements of cell Lp in the stele.

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