Hydraulic conductivity of rice roots

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Abstract

A pressure chamber and a root pressure probe technique have been used to measure hydraulic conductivities of rice roots (root Lp_r per m^2 of root surface area). Young plants of two rice (*Oryza sativa* L.) varieties (an upland variety, cv. Azucena and a lowland variety, cv. IR64) were grown for 31–40 d in 12 h days with 500 μmol m^{-2} s^{-1} PAR and day/night temperatures of 27 °C and 22 °C. Root Lp_r was measured under conditions of steady-state and transient water flow. Different growth conditions (hydroponic and aeroponic culture) did not cause visible differences in root anatomy in either variety. Values of root Lp_r obtained from hydraulic (hydrostatic) and osmotic water flow were of the order of 10^{-8} m s^{-1} MPa^{-1} and were similar when using the different techniques. In comparison with other herbaceous species, rice roots tended to have a higher hydraulic resistance of the roots per unit root surface area. The data suggest that the low overall hydraulic conductivity of rice roots is caused by the existence of apoplastic barriers in the outer root parts (exodermis and sclerenchymatous (fibre) tissue) and by a strongly developed endodermis rather than by the existence of aerenchyma. According to the composite transport model of the root, the ability to adapt to higher transpirational demands from the shoot should be limited for rice because there were minimal changes in root Lp_r depending on whether hydrostatic or osmotic forces were acting. It is concluded that this may be one of the reasons why rice suffers from water shortage in the shoot even in flooded fields.

Key words: Apoplastic barriers, composite transport model, hydraulic conductivity, pressure probe, root, rice, water relations.

Introduction

It is well known that the hydraulic properties of roots vary with species and environmental conditions (Brewig, 1937; Brouwer, 1954; Weatherley, 1982; Kramer and Boyer, 1995); these factors strongly influence root anatomy and morphology (Steudle and Peterson, 1998; Steudle, 2000a). Water uptake by roots can be regulated by physical and physiological processes (Steudle, 2000b, 2001). A composite transport model has been established to explain the variable permeability of roots to water (root hydraulic conductivity, Lp_r) (Steudle and Frensch, 1996; Steudle and Peterson, 1998; Steudle, 2000b). In the model, three parallel pathways for water movement in the root cylinder are considered, i.e. the apoplastic, symplastic and transcellular pathways, the latter two representing the cell-to-cell path. In the presence of apoplastic barriers (Casparian bands, suberin lamellae), the amount of water that flows along the apoplast will be limited. On the other hand, water channel (aquaporin) activity in the plasma membranes of root cells affects the cell-to-cell component of radial water flow. Water uptake (root Lp_r) usually represents the biggest resistance to the flow of liquid water in the plant. Its regulation could be the result of varying the contribution of each component. In addition, osmotic and hydrostatic pressures will cause different flow intensities through the apoplast, i.e. root Lp_r depends on the nature of driving forces. Overall, the composite transport model provides both a coarse regulation of root Lp_r (switching between the preferred path for water) that is purely physical in nature, and a fine regulation that is under metabolic control (water channel activity). The model allows for a rapid uptake of water from the soil, when the demand from the shoot is large (high tensions in root xylem; Steudle, 2001). On the other hand, when the soil is dry, the hydraulic resistance of the root will be great and may limit water losses into the dry

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soil. Hence, the model would explain the variability in root hydraulic conductivity in terms of useful adaptive mechanisms that provide regulation of water uptake.

Even for rice crops in paddy fields, where supply from the wet soil should be no problem, water shortage has been reported (Ishihara and Saito, 1987; Jiang *et al.*, 1988; Hirasawa *et al.*, 1992, 1996). Rice roots have developed apoplastic barriers such as an endo- and exodermis with Casparian bands and layers of cortical sclerenchyma with exceptionally thick, lignified walls (Morita and Abe, 1999). These may contribute to the observed problems with water uptake in this important crop. Data about root hydraulics are required to understand mechanisms of water uptake by rice roots, and to assess the extent to which root properties limit crop water use.

In this paper, basic studies on the ability of rice roots to take up water are reported and it is shown that there is little or no flexibility of Lp_r in response to environmental conditions that bring about marked changes in maize (Zimmermann and Steudle, 1998; Freundl *et al.*, 2000). Neither were there significant differences in the behaviour or the anatomy of a paddy and an upland variety. Transport properties were examined with osmotic and hydrostatic driving forces or steady-state and transient water flows, using pressure chambers and root pressure probes (Steudle, 1993). The results show that the hydraulic conductivity of rice roots is relatively low (compared with other herbaceous species) and that it does not adapt to any significant extent to the changing environmental conditions employed.

Materials and methods

Plant material

Seeds of rice (*Oryza sativa* L.: upland cultivar Azucena and lowland cultivar IR64) were germinated in the light in the climatic chamber used for plant growth (see below) on tissue soaked with tap water for 5–6 d. During this time, seedlings developed roots of up to 20 mm long and shoots of up to 35 mm tall (1st leaf emergence). The seedlings were transferred to a hydroponic culture system consisting of boxes of nutrient solution (10 l), which accommodated 24 seedlings per box. The nutrient solution contained 0.09 mM $(NH_4)_2SO_4$, 0.05 mM KH_2PO_4, 0.05 mM KNO_3, 0.03 mM K_2SO_4, 0.06 mM $Ca(NO_3)_2$, 0.07 mM $MgSO_4$, 0.11 mM Fe-EDTA, 4.6 µM H_3BO_3, 1.8 µM $MnSO_4$, 0.3 µM $ZnSO_4$, 0.3 µM $CuSO_4$ (pH = 5.5–6.0). After 2–4 d, aeration of the nutrient solution began. Nutrient solution was completely renewed every 7 d with solution adjusted to chamber temperature. Half the seedlings were transferred to an aeroponic culture system. Concentration of nutrient solution for the aeroponic system was 2-fold greater than that used for hydroponics. Using two plastic discs with slits, seedlings were fixed on top of a round polyvinyl chloride box (diameter: 0.45 m; depth 0.6 m). Roots protruded into the box through the slits. At the bottom of the box, there was an air humidifier, which continuously produced mist (Defensor, Barth&Stoecklein, Garching, Germany). Both culture systems were placed in a climatic chamber (day/night rhythm: 12/12 h;

27/22 °C, light intensity: 500 µmol m^{-2} s^{-1}). Plants used in experiments were grown for 31–40 d including the time for germination. When used, root systems of young plants of IR64 from hydroponic culture were 265–515 mm long. The overall shoot length was 325–445 mm (8th–11th leaf emergence). Plants grown in aeroponic culture had root system and shoot lengths of 320–630 mm and 290–410 mm, respectively (7th–8th leaf emergence). Young plants of Azucena grown in hydroponics developed 385–610 mm of root systems and 485–570 mm of shoots (8th–11th leaf emergence). For the plants grown in aeroponics, length of root systems and shoots was 390–720 mm and 340–440 mm, respectively (7th–8th leaf emergence). Overall, Azucena (i.e. the upland variety) grew somewhat faster in both hydroponics and aeroponics than IR64 (the lowland rice variety). Diameters of adventitious roots from hydroponics and aeroponics for IR64 were up to 1.1 mm and 0.9 mm, respectively. Those for Azucena were up to 1.3 mm and 1.1 mm, respectively. Diameters of stele from hydroponics and aeroponics for IR64 were up to 190 µm and 135 µm, respectively. Those for Azucena were up to 280 µm and 260 µm, respectively.

Root anatomy and surface area of root system

Freehand cross-sections were prepared from adventitious roots. Cross-sections were made at different distances from the root tip (10, 20, 40, 60, 80, 100, 150, 200, 250 mm). They were stained for 1.5 h with Sudan Red 7B at room temperature (Brundrett *et al.*, 1991). Sections were viewed under an optical microscope (DIALUX 22 EB, Leitz, Germany). For photographs, Kodak Elite 64 ASA film was used.

Surface areas of root systems were determined using a video camera and an image analysing software (Skye Instruments, Llandrindod Wells, UK). For better contrast, roots were stained with toluidine blue O (0.03% w/v). Surface areas of root systems were calculated from projected areas of roots that were assumed to be cylindrical in shape. The system was calibrated using metal wires of known length and diameter. For IR64, surface areas of root systems from hydroponics and aeroponics ranged between $(1.1–3.5) \times 10^{-2}$ m^2 and $(1.7–5.4) \times 10^{-2}$ m^2, respectively. Those for Azucena were $(2.2–2.7) \times 10^{-2}$ m^2 and $(1.2–2.6) \times 10^{-2}$ m^2, respectively.

Measurement of root exudation in the absence of hydrostatic pressure gradients (osmotic exudation)

Before starting these measurements, shoots were cut off using razor blades at distances of 40–70 mm from the base. All tillers except the main stem were closed using clamps. With the aid of a syringe, xylem sap exuding from the main stem was collected at the cut surface. It was collected in Eppendorf tubes and weighed (Fig. 1A). In the absence of hydrostatic pressure gradients, differences in osmotic pressure ($\Delta\pi$ in MPa) between the medium ($RT \times C^\circ$) and xylem sap ($RT \times C^i$) drove the water uptake by the root, i.e.:

$$J_{Vr} = Lp_r \sigma_{sr} \Delta\pi = Lp_r \sigma_{sr} RT(C^i - C^\circ) \qquad (1)$$

Here, σ_{sr} denotes the reflection coefficient of solutes, which was determined from experiments with the root pressure probe described below. To calculate root Lp_r, a value of $\sigma_{sr} = 0.4$ was used (see below). Osmotic concentration of the medium and of the xylem sap was measured using a freezing-point depression osmometer (Osmomat 030; Gonotec, Berlin, Germany). The osmotic pressure of nutrient solution was 0.0075 MPa.
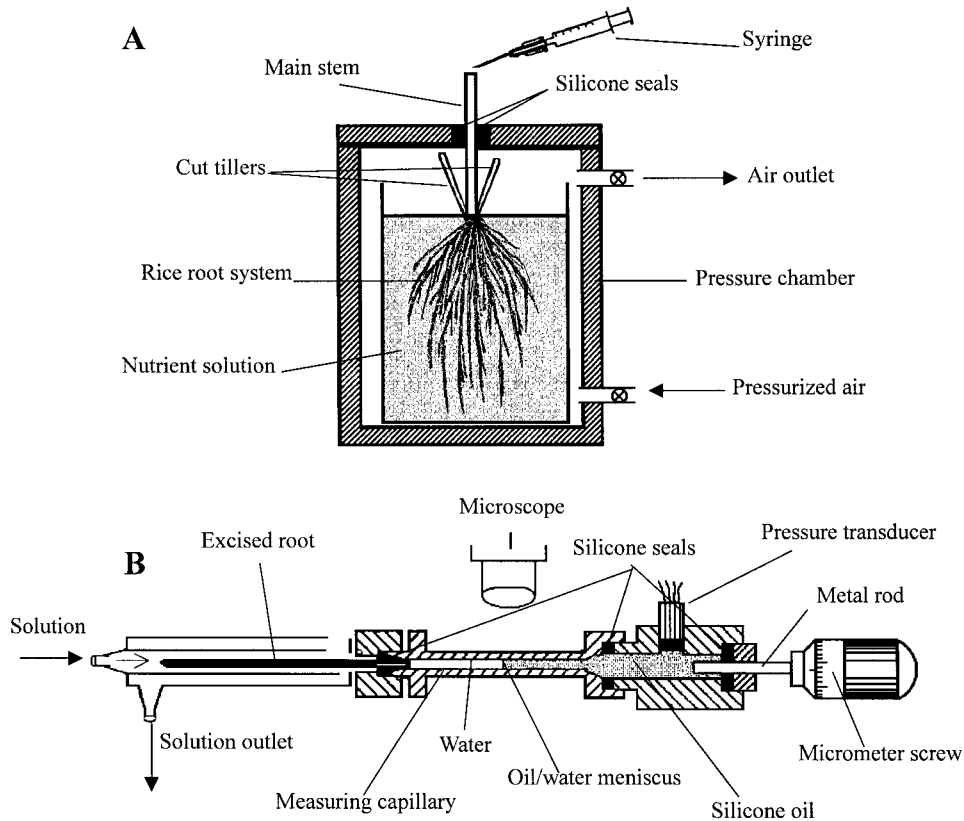
Fig. 1. Pressure chamber (A) and root pressure probe (B) for measuring water flow across root systems and individual roots of young rice plants. (A) The pressure chamber provided steady-state water flow across the roots by applying pneumatic pressure to the medium (Fig. 3). By using silicone seals, the base of the main tiller was tightly sealed to the pressure chamber. Cut ends of the remaining tillers were kept in the chamber. With the aid of a syringe, exuded xylem sap was collected in Eppendorf tubes and weighed. (B) Excised roots were connected to a root pressure probe. After steady root pressure had been built up in the system, water flow was induced by either changing the pressure in the probe with the aid of a metal rod or by changing the osmotic pressure of the medium. From the pressure/time curves obtained (Fig. 4), parameters of water and solute flow were calculated. During measurements, oil/water menisci in the measuring capillary of the root pressure probe served as points of reference.

Measurement of root exudation in the presence of hydrostatic pressure gradients

Plants used for measuring osmotic water flow were also employed to measure hydraulic conductivity of root systems in the presence of hydrostatic pressure gradients. During these measurements, roots were enclosed into a steel chamber to apply pneumatic pressure to the root medium (Fig. 1A). The base was tightly sealed using silicone seals which were prepared with a hole which had a diameter already adjusted to that of the base of main stem. The seal was cut longitudinally. For the fine adjustment of the seal, flexible rubber material (Terostat, Germany) was used, which was placed in the two halves of the seal. The pressure in the chamber was raised in steps of 0.03–0.05 MPa up to 0.35 MPa above atmospheric. Exuded xylem sap was collected as described above and weighed. For a given applied gas pressure (P_{gas} in MPa), the volume exuded from the root system (V in m^3) was plotted against time. The slopes of these relations were calculated and referred to unit surface area. This yielded the volume flow, J_{Vr} in m^3 m^{-2} s^{-1}. There was no axial water flow across the aerenchyma, because this was interrupted by the seal. Since there is a connection between vascular bundles of tillers with those of the main stem (Hanada, 1993), it was assumed that roots from tillers transport water to the main stem and that there is no difference in the resistance to water transport among roots from main stem and

tillers. A possible effect of roots emerged from tillers has been tested for in experiments where plants were grown without tillers in hydroponics. In hydrostatic experiments, the hydraulic conductivity of root systems (Lp_r in m s^{-1} MPa^{-1}) was similar in both cases. Root Lp_r was calculated from the slopes of J_{Vr} against driving forces ($P_{gas} + \sigma_{sr}\Delta\pi$) according to the following relation:

$$J_{Vr} = Lp_r(P_{gas} + \sigma_{sr}\Delta\pi) \tag{2}$$

In this equation, σ_{sr} is the root reflection coefficient for nutrient salts in the xylem which was estimated to be $\sigma_{sr} = 0.4$. This value was the upper limit of the reflection coefficient found for NaCl which was assumed to be similar to that of the salts in the xylem solution according to earlier results for maize (Steudle and Peterson, 1998). The effect of the osmotic term was not very pronounced in the range of higher values of P_{gas}. Measured values of $\Delta\pi$ were used to calculate the overall driving force in Equation (1). Alternatively, Lp_r was determined from plots of J_{Vr} against P_{gas}. Slopes of the plots were non-linear in the range of low forces, i.e. root Lp_r depended on the magnitude of water flow as found for other species (Fiscus, 1975; Rüdinger *et al.*, 1994). Lp_r was determined from the linear part of pressure/flow curves (high pressure gradients, P_{gas}), where the osmotic component of the driving force was small due to dilution effects.

Root pressure probe measurements

Root pressure probe measurements were carried out as described previously (Steudle *et al.*, 1987; Steudle and Frensch, 1989). Using a cylindrical silicone seal which was prepared from liquid silicone material (Xantopren; Bayer, Leverkusen, Germany), excised end segments of individual roots (root tips) were tightly connected to a root pressure probe (Fig. 1B). Segments had lengths of 125–235 mm and diameters of 0.7–1.2 mm for Azucena or 0.5–0.9 mm for IR64. 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 measurement, the proper function of seals was tested by cutting off the root at the seal and controlling the decrease in root presssure and the increase in hydraulic conductivity. When root xylem remained open, root pressure dropped to zero and there was a dramatic decrease in half-time after the cut (increase of hydraulic conductance). Otherwise, the experiment was discarded which happened with about one-third of the roots used. Root segments fixed to the probe were bathed in aerated nutrient solution (the same composition as that used for growth in hydroponic culture) which circulated along the roots to avoid problems with external unstirred layers. The circulation rate was high so that the medium around a root was exchanged within a fraction of a second. Root pressures (P_r in MPa) were measured with a pressure transducer and recorded. Usually, it took over 5 h to establish stable root pressures. 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 responses in pressure were followed which allowed Lp_r to be calculated from rate constants, k_{rw}, or half-times of pressure relaxations ($T_{1/2}^w$) according to the following relation (Steudle *et al.*, 1987):

$$k_{rw} = \ln(2)/T_{1/2}^w = A_r \Delta P_r / \Delta V_s Lp_r \quad (3)$$

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 is the surface area of the root. The ratio of $\Delta P_r / \Delta V_s$ was measured by inducing step changes in the volume by moving the metal rod in the probe and recording the resulting changes in root pressure (ΔP_r). Root surface area was calculated from its length and diameter. Test solutions used in osmotic experiments were prepared by adding NaCl or ethanol to tap water. Osmotic pressure of added solutes ranged between 0.12 and 0.25 MPa (50 and 100 mOsmol kg^{-1}) and 0.23 and 0.63 MPa (92 and 250 mOsmol kg^{-1}), respectively. These concentrations were not toxic to the roots as shown by a constant root pressure. Root pressure is sensitive to toxic substances (e.g. heavy metals) which affect the active pumping of nutrients to the root xylem. Responses of root pressure to changes of osmotic pressure of the medium were biphasic. There was a rapid water phase (water efflux or influx) followed by a slower solute phase (water dragged by solute diffusion). From the solute phase the permeability coefficient for given solutes (P_{sr} in m s^{-1}) was calculated:

$$k_{sr} = \ln(2)/T_{1/2}^s = A_r P_{sr} / V_x \quad (4)$$

Here, k_{sr} is the rate constant of permeation of a given solute (NaCl, ethanol) and V_x the volume of functioning (mature) xylem. From root cross-sections, V_x was estimated to be 1% of root volume. Since V_x was measured in the more basal parts of roots, the value of 1% may overestimate the real value of conducting mature xylem vessels. According to Equation (4), this would tend to overestimate P_{sr}. Root reflection coefficients

(σ_{sr}) were calculated from (Steudle *et al.*, 1987):

$$\sigma_{sr} = (P_{ro} - P_{rmin})/\Delta\pi \exp(k_{sr}t_{min}) \quad (5)$$

Here P_{ro} is the original steady-state root pressure at the time when osmotic pressure was changed. P_{rmin} is the minimum root pressure according to water efflux, $\Delta\pi$ is the change in osmotic pressure of medium and t_{min} the time required to reach the minimum.

Cutting experiments

Information on the development of xylem vessels was obtained from cutting experiments. Roots attached to the root pressure probe were successively excised from the tip step by step using a razor brade. Substantial drops in P_r were observed when mature xylem was cut and xylem sap leaked out into the medium.

Results

Root anatomy: development of adventitious roots

There were no visible differences in the development of endodermis, exodermis and aerenchyma between the two varieties and growth conditions. Therefore, photographs taken from cross-sections of adventitious roots are only shown for IR64 (the lowland variety) in Fig. 2. Aerenchyma was completely developed at the distance of 150 mm from the tip (Fig. 2A). At a distance of 40 mm from tips, roots developed a secondary endodermis, with suberin depositions on inner cell walls at the endodermis (Sudan Red staining; Fig. 2B). Passage cells were not stained. Tertiary development (cell wall thickening on suberin lamellae) of the endodermis was observed at a distance of 100 mm from the tip. The exodermis, which is a hypodermis with Casparian bands, developed at distances of 40 mm from the tip and suberin depositions were detected. With the plants used (age: 31–40 d) tertiary wall thickening in the exodermis was never seen. Highly lignified sclerenchyma tissue was found below the exodermis. At a distance of 20–40 mm from the root tip, cortical cells started to collapse and aerenchyma developed.

Steady-state hydraulic experiments

At a given P_{gas}, exuded volumes, V, increased linearly with time as shown in Fig. 3A. In Fig. 3B, water flows are plotted against both the gas pressure applied to the root systems (P_{gas}) and the overall driving force $P = P_{gas} + \sigma_{sr}\Delta\pi$. The slope was found to become steeper as P_{gas} or J_{Vr} increased. This means that Lp_r was low at low P or flow rates and increased with increasing driving force to reach a maximum value usually at pressure differences of 0.05–0.15 MPa. It can be seen that at high water flow, slopes were similar, because xylem sap was strongly diluted, and the osmotic term did not contribute much to the overall driving force in this range. Maximum Lp_r values are reported in this paper.
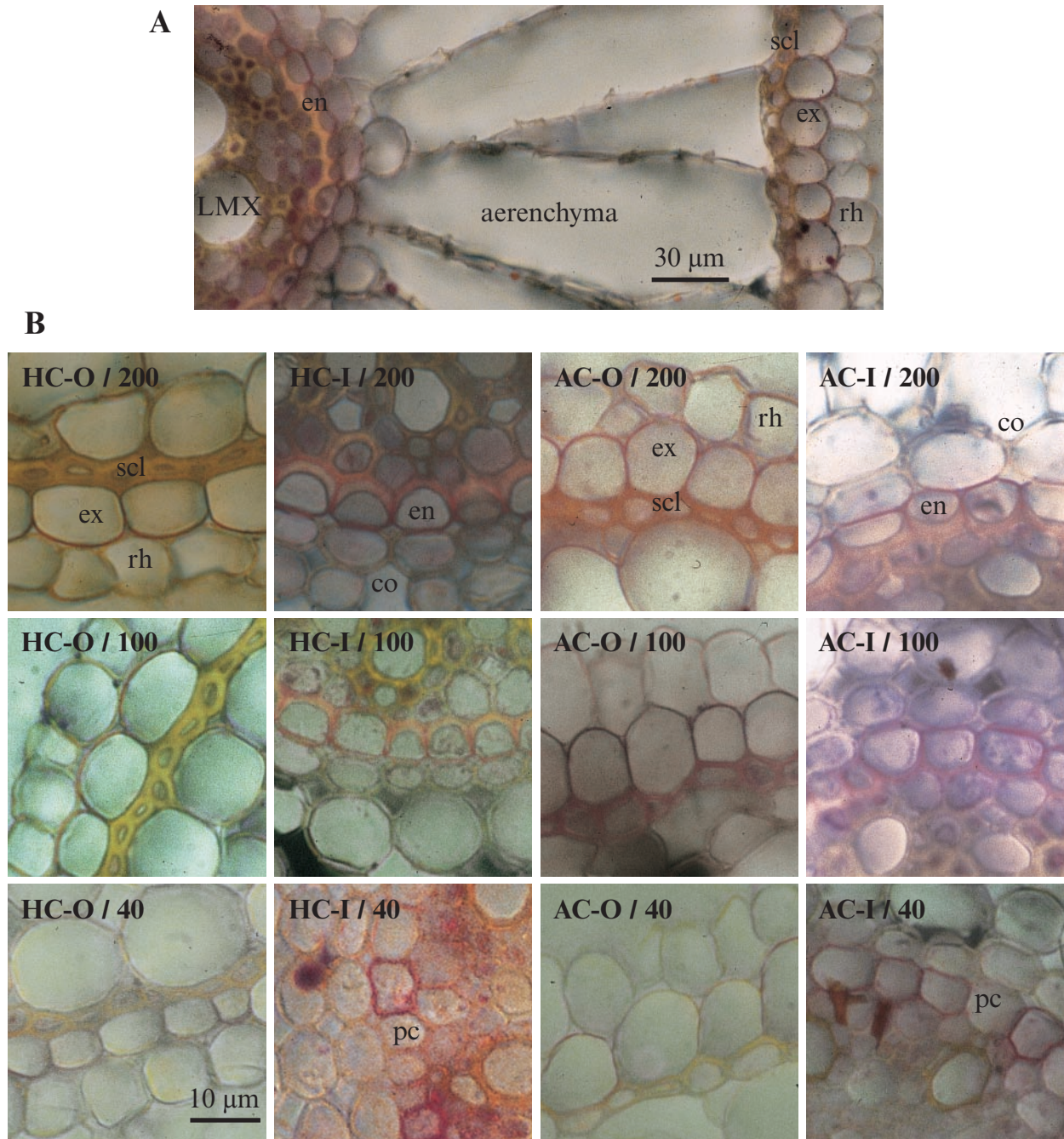
A

B

Fig. 2. Freehand cross-sections of roots of IR64 grown in hydroponic culture (HC) or aeroponic culture (AC). (A) Overview of a sector of whole root cross-section, which was taken at a distance of 200 mm from the root tip where aerenchyma was completely developed. Different symbols refer to different structures: rh, rhizodermis, ex, exodermis, scl, sclerenchyma, en, endodermis. (B) Detailed structures in the outer (denoted by O, including rhizodermis, exodermis and sclerenchyma) and inner (I, including endodermis) parts of the root are shown separately. Sections were made at distances of 200 mm, 100 mm and 40 mm from tips of an adventitious root and stained with Sudan Red 7B. Lipophilic substances such as suberin lamellae, were stained bright red. Abbreviations: co, cortex, LMX, late metaxylem, pc, passage cell. It can be seen that there was a substantial sclerenchyma developing below the exodermis. Development of exodermis and endodermis was similar for both varieties and types of cultivation.

Mean Lp_r values ($\pm$SD) of Azucena grown in hydroponics and aeroponics were $(6.3\pm3.1)\times10^{-8}$ m s^{-1} MPa^{-1} and $(7.4\pm4.2)\times10^{-8}$ m s^{-1} MPa^{-1}, respectively (Table 1). Those of IR64 were $(5.6\pm2.7)\times10^{-8}$ m s^{-1} MPa^{-1} and $(4.0\pm1.9)\times10^{-8}$ m s^{-1} MPa^{-1}, respectively. Only for IR64 grown in aeroponics Lp_r were values significantly smaller than those of Azucena grown in aeroponics (*t*-test; $\alpha=0.05$).

Osmotic exudation experiments

Osmotic exudation rate was measured at an external osmotic concentration of 0.0075 MPa. Osmotic Lp_r
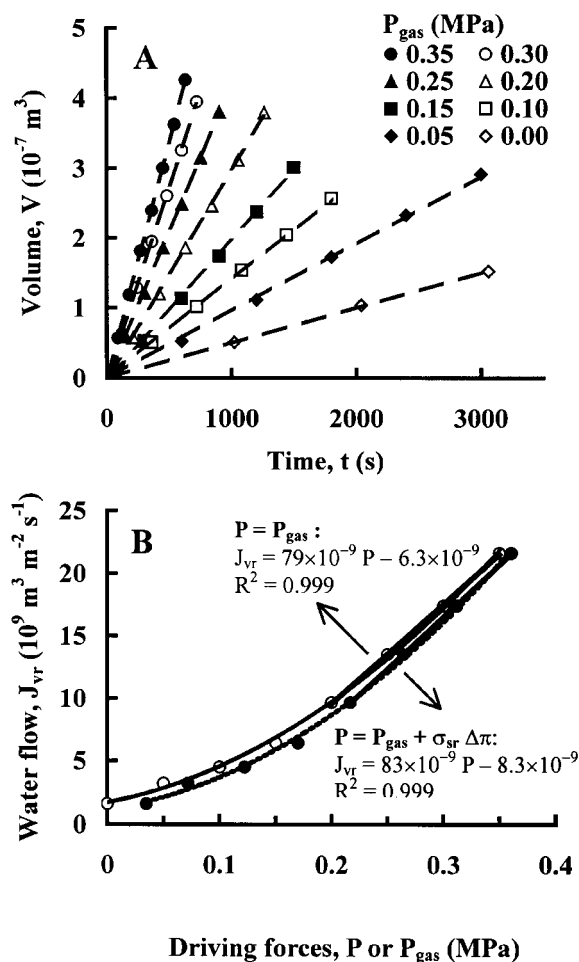
Fig. 3. Results from a typical steady-state experiment for measuring root hydraulic conductivity (Fig. 1A). (A) Water volume exuded in the presence of hydrostatic (pneumatic) pressure gradients (P_{gas}). It can be seen that the water volume increased linearly with time at a given pressure difference. (B) Steady-state water flow per unit surface area of root system (J_{Vr}) as a function of applied driving force, which is equal to either P_{gas} (open circles with solid line) or to ($P_{gas} + \sigma_{sr}\Delta\pi$) (closed circles with dotted line), calculated from the slope of the graphs shown in (A). A value of 0.4 was assumed for the reflection coefficient of the solutes present in the xylem sap. $J_{Vr}(P)$ curves were usually bent because of the dilution of xylem sap during water uptake (Fiscus' effect). Lp_r values were calculated from slopes of the linear ranges of $J_{Vr}(P)$ curves. In these ranges, osmotic pressure difference between xylem sap and medium had little effect on water flow.

was calculated from the osmotic exudation rate per unit surface area of root system (A_r) divided by the reflection coefficient ($\sigma_{sr} = 0.4$) and the osmotic pressure difference between xylem sap and medium. Osmotic Lp_r of roots of Azucena grown in hydroponics and aeroponics were $(5.5 \pm 3.7) \times 10^{-8}$ m s^{-1} MPa^{-1} and $(2.8 \pm 1.5) \times 10^{-8}$ m s^{-1} MPa^{-1}, respectively (Table 1). Those of IR64 were $(4.2 \pm 2.5) \times 10^{-8}$ m s^{-1} MPa^{-1} and $(3.6 \pm 1.3) \times 10^{-8}$ m s^{-1} MPa^{-1}, respectively. Only for Azucena grown in hydroponics, were osmotic Lp_r values significantly bigger than those of IR64 grown in aeroponics. Except for Azucena grown in aeroponics, the

values of osmotic and hydrostatic Lp_r were similar. It can be seen from Table 1 that except for Azucena grown in aeroponic culture, ratios between hydrostatic and osmotic Lp_r were not significantly different from unity, although mean values were greater than unity in all cases.

Root pressure probe measurements

5–12 h after root segments were connected to a root pressure probe, steady root pressures were established. Values varied between 0.07 and 0.40 MPa. Hydrostatic Lp_r was calculated from pressure relaxation curves that resulted from induced water flow (see Materials and methods; Fig. 4A). The values for hydroponic plants of Azucena and IR64 were $(4.7 \pm 1.0) \times 10^{-8}$ m s^{-1} MPa^{-1} and $(5.0 \pm 2.5) \times 10^{-8}$ m s^{-1} MPa^{-1}, respectively (Table 2). Those for aeroponic plants were $(3.1 \pm 1.3) \times 10^{-8}$ m s^{-1} MPa^{-1} and $(2.3 \pm 1.1) \times 10^{-8}$ m s^{-1} MPa^{-1}, respectively. From the water phase of biphasic osmotic relaxations, osmotic Lp_r was calculated (Fig. 4B). For hydroponic plants of Azucena and IR64, osmotic Lp_r were $(4.0$–$3.8) \times 10^{-8}$ m s^{-1} MPa^{-1} and $(9.2$–$10.3) \times 10^{-8}$ m s^{-1} MPa^{-1}, respectively. Those of aeroponic plants were $(2.0$–$5.4) \times 10^{-8}$ m s^{-1} MPa^{-1} and $(2.1$–$2.9) \times 10^{-8}$ m s^{-1} MPa^{-1}, respectively. The conclusion from the Lp_r values given in Table 2 is that osmotic and hydrostatic values obtained in root pressure probe experiments were statistically not different except for osmotic Lp_r for IR64 grown in hydroponics. They were almost identical to the values obtained during steady-state experiments except for osmotic Lp_r for IR64 grown in hydroponics (Table 1). The result differed markedly from results obtained earlier with maize. For this species, hydrostatic Lp_r was much bigger than the osmotic (Table 2).

As shown in Fig. 4B and C, apparent water flow reached zero after some time. The osmotic pressure of the medium was then changed and apparent water flow changed its direction according to the influx or efflux of solutes, which increased or decreased the osmotic pressure in the xylem. From rate constants of solute phases, permeability coefficients of the two solutes were determined (P_{sr} in m s^{-1}). P_{sr} values of ethanol and NaCl for both cultivars were similar (Table 3). In contrast to the water permeability, there were significant decreases of solute permeabilities of NaCl in roots of IR64 grown in aeroponics as compared with hydroponics. The value of 0.3–0.4 of the reflection coefficient (σ_{sr}) for NaCl was significantly greater than that of ethanol ($\sigma_{sr} = 0.1$), but treatment (hydroponics versus aeroponics) did not affect the absolute values of reflection coefficients. When comparing the rice data of P_{sr} and σ_{sr} with those for maize, it can be seen that in most cases, P_{sr} for rice grown in aeroponics was smaller for rice than for maize. Since the values used for V_x were probably upper limits, P_{sr} values

Table 1. *Hydraulic conductivity of root systems (*Lp_r*) of rice plants grown in hydroponic and aeroponic culture as determined in steady-state experiments (Figs 1A, 3)*

Overall shoot and root lengths and surface areas of root system (A_r) are given. Values are means $\pm$ SD (N = number of root systems). Letters following Lp_r values indicate significant differences (LSD, $P = 0.05$). Ratios between hydrostatic and osmotic root Lp_r were calculated for individual roots which were then averaged. Only for Azucena grown in aeroponics, the ratio of hydrostatic to osmotic Lp_r was significantly bigger than unity (t-test, $\alpha = 0.05$). For comparison, literature values of Lp_r of root systems of maize are given (age: 11–21 d). For this plant, the most detailed sets of data exist for both the cell and root level. It can be seen that for hydroponic maize, the hydrostatic and maximum osmotic Lp_r of maize were much bigger than the maximum values of Lp_r of rice shown here. Roots of aeroponic maize, however, exhibit similar Lp_r values. In contrast to maize, there were no big differences in Lp_r in response to different growth conditions and driving forces for rice.

Plant	N	Shoot length (m)	Length of root system (m)	A_r (10^{-2} m^2)	Lp_r (10^{-8} m^{-1} MPa^{-1})		Ratio of hydrostatic/osmotic	
					Hydrostatic	Osmotic		
Rice (*Oryza sativa*)								
Hydroponics								
Azucena	14	0.53 ± 0.02	0.55 ± 0.06	3.3 ± 0.8	6.3 ± 3.1 ab	5.5 ± 3.7 a	1.4 ± 0.7	
IR64	18	0.38 ± 0.03	0.37 ± 0.06	2.3 ± 0.6	5.6 ± 2.7 ab	4.2 ± 2.5 ab	1.8 ± 1.3	
Aeroponics								
Azucena	14	0.39 ± 0.03	0.58 ± 0.11	1.9 ± 0.5	7.4 ± 4.2 a	2.8 ± 1.5 b	3.3 ± 2.2	
IR64	14	0.34 ± 0.04	0.51 ± 0.08	2.8 ± 1.3	4.0 ± 1.9 b	3.6 ± 1.3 b	1.2 ± 0.6	
						Max Min	Max Min	
Maize [a]								
Hydroponics					26 ± 15	85 ± 60 4.6 ± 2.2	0.3 5.6	
Aeroponics					7.3 ± 2.6	26 ± 15 3.2 ± 1.7	0.3 2.3	

[a]Zimmermann and Steudle (1998). Maximum and minimum values of osmotic Lp_r are shown, which were obtained at the low and high osmotic pressure of the medium, respectively.

calculated according to Equation (4) are probably upper limits, and the differences could have been even bigger. At the same time, σ_{sr} values of rice were smaller by a factor of two than those of maize.

Cutting experiments

At the ends of root pressure probe measurements, cutting experiments were performed. P_r dropped to near zero after 40 mm of segments were cut (Fig. 5A). After each cut, hydrostatic pressure relaxations were performed. At a distance of 70 mm from tip, $T_{1/2}^w$ was decreased to about 10% of the original value for the intact segment (Fig. 5B). Hence, the hydraulic resistance of severed roots remaining at the seal was much less than that of intact root segments. This proved that during the experiments, the radial water flow across the roots rather than a resistance created at the base during sealing was measured (see above).

Discussion

As far as is known, the first detailed set of data of the hydraulic properties of rice roots is presented in this paper. Different varieties (lowland and upland) have been used which were grown under different conditions (hydroponic and aeroponic culture). Experience with other species (see reviews by Steudle and Peterson, 1998; Steudle 2000a, b, 2001) has shown that hydrostatic and osmotic driving forces would change the relative contributions of water flow via the apoplastic and cell-to-cell pathways, resulting in substantial changes in the hydraulic conductivity of roots (Lp_r). Conditions

of either steady (pressure chamber) and transient (root pressure probe) water flow were also employed. In parallel with the measurements of root Lp_r, the structure of roots was examined to see if this was different for the two varieties and if it varied when they were grown under different conditions.

The root anatomy, revealed by freehand cross-sections, did not differ for the two cultivars or for roots grown in hydro- and aeroponics. Hence it is not surprising that absolute values of root Lp_r were similar in the lowland (IR64) and upland (Azucena) varieties. The use of different driving forces did not cause significant changes of root Lp_r. In contrast to other species, there was no or little switching in root Lp_r. In maize, differences were as large as up to an order of magnitude (Frensch and Steudle, 1989; Steudle and Frensch, 1989; Zimmermann and Steudle, 1998; Steudle and Peterson, 1998). In maize, the hydrostatic root Lp_r was similar to that of the Lp of individual root cells. It was concluded that most of the water was flowing in the apoplast around protoplasts under these conditions, even in the exo- and endodermis of young roots. On the other hand, osmotic water flow was from cell to cell, and this probably explained the lower root Lp_r and higher hydraulic resistance under these conditions. For tree roots, differences between hydraulic and osmotic flow were even larger (Steudle and Heydt, 1997). In rice, one may tend to attribute the similarity between osmotic and hydraulic Lp_r to the existence of an exodermis and fibre tissue (sclerenchyma) in the outer parts of the roots which were present during all treatments and in both varieties. However, this conclusion should be made cautiously, because the walls
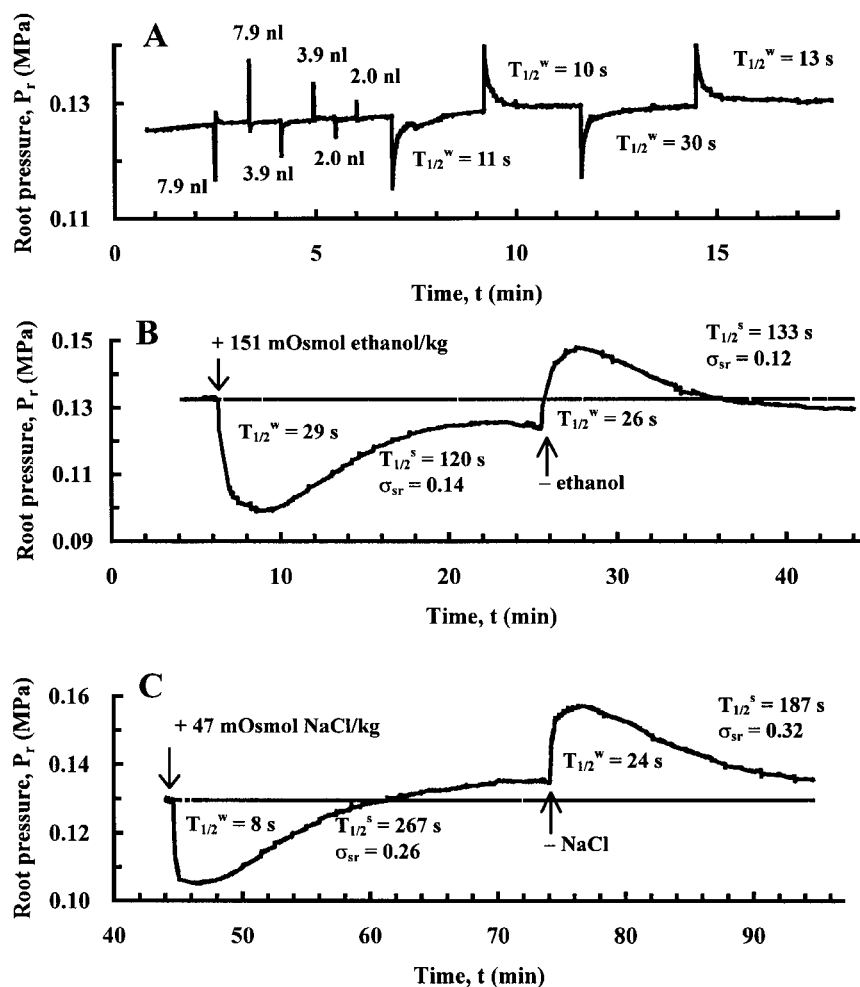
Fig. 4. Time-course of pressure during a typical experiment with the root pressure probe (Fig. 1B). The adventitious root was from Azucena grown in hydroponics for 40 d and the root segment used for the measurement had a length of 190 mm and a diameter of 1.1 mm. (A) Measurements of the elasticity of the measuring system and of hydrostatic root pressure relaxations. (B, C) Responses of root pressure to changes in osmotic pressure of the medium resulting from ethanol and NaCl. Biphasic responses consist of rapid water efflux (or influx) phases follow by solute influx (or efflux) phases, which cause a displacement of water. Due to fluctuations in root pressure ('nutrient pumping'), the base line did not stay completely constant during the entire course of an experiment (45–120 min).

of the endodermis appeared to be quite suberized and heavily lignified and may impede water flow along the apoplastic path.

There was some variability between half-times of water exchange which were between 5 and 240 s during both osmotic and hydrostatic experiments. Although this was somewhat longer than for other species, relaxations were, on average, still rather fast, considering the structure of the roots with the exodermis, sclerenchyma, aerenchyma, and endodermis as potential barriers to water flow. The hydraulic resistance of the aerenchyma may be regarded as low. According to the osmotic experiments, water may flow along the rims of living cells in the strands connecting the stele with the outer root at a rate which was similar to that found during the hydrostatic experiments. During hydrostatic relaxations, changes in water potential (pressure) were produced in the xylem. Only small water

volumes are transferred during these experiments. Because air has a high compressibility, the aerenchyma has a rather high potential storage capacity for water and should, therefore, dampen the propagation of changes in pressure in the experiments with the root pressure probe without any significant filling of the lacunae of aerenchyma. Hence, it is considered that the Lp_r measured during these experiments largely reflects that of the endodermis/stele rather than of the rest of the root. On the other hand, the root Lp_r measured during steady-state (pressure chamber) experiments included both the hydraulic resistance of the outer parts and of the stele. Since both values were similar, it may be concluded that the hydraulic resistance located in the endodermis/stele was substantial. For a definite decision of the relative contribution of outer and inner parts of roots, however, a more detailed separate measurement of the two hydraulic

Table 2. *Hydraulic conductivity (Lp$_r$) for adventitious roots of rice plants grown in hydroponic and aeroponic culture for 31–40 d, measured with the root pressure probe (Figs 1B, 4)*

Osmotic water flow was induced by changing the osmotic pressure of the medium by adding either NaCl, an osmoticum which would move predominantly in the apoplast, or ethanol, which is able to rapidly cross cell membranes (cell-to-cell passage preferred). Values shown are mean values ± SD with the number of measured roots in brackets. Ratios between hydrostatic and osmotic Lp_r were calculated for individual roots which were then averaged. Different letters following the Lp_r values show significant difference in each column (LSD, $P = 0.05$). For comparison, literature values of Lp_r of some other herbaceous species are shown as well.

Plant	Hydrostatic Lp_r (10^{-8} m MPa^{-1} s^{-1})	Osmotic Lp_r (10^{-8} m MPa^{-1} s^{-1})		Ratio of hydrostatic/ osmotic (NaCl)	Values from steady-state experiment (Table 1)/references	
		NaCl	Ethanol		Hydrostatic (10^{-8} m MPa^{-1} s^{-1})	Osmotic
Rice (*Oryza sativa*)						
Hydroponics						
Azucena	4.7 ± 1.0 (10) a	4.0 ± 2.5 (6) a	3.8 ± 3.0 (7) a	1.9 ± 1.6 (6)	6.3 ± 3.1	5.5 ± 3.7
IR64	5.0 ± 2.5 (8) a	9.2 ± 3.0 (6) b	10.3 ± 4.2 (6) b	0.7 ± 0.2 (6)	5.6 ± 2.7	4.2 ± 2.5
Aeroponics						
Azucena	3.1 ± 1.3 (8) bc	2.0 ± 1.4 (7) a	5.4 ± 4.4 (4) a	2.8 ± 2.6 (7)	7.4 ± 4.2	2.8 ± 1.5
IR64	2.3 ± 1.1 (6) bc	2.1 ± 1.8 (5) a	2.9 ± 1.9 (6) a	1.5 ± 0.8 (5)	4.0 ± 1.9	3.6 ± 1.3
Maize (*Zea mays*)						
Hydroponics	10 (90)	1.3 (34) [a]		7.7	Steudle *et al.* (1987)	
	16 (22)	2.3 (17) [b]		7.0	Steudle and Frensch (1989)	
	27 ± 13 (10)	2.2 ± 2.1 (10)		12	Steudle *et al.* (1993)	
	18 ± 7 (11)	0.9 ± 0.4 (11)		19	Zimmermann *et al.* (2000)	
Aeroponics	11 ± 6 (11)	1.1 ± 0.47 (10)		10	Zimmermann *et al.* (2000)	
Phaseolus vulgaris	30	0.56 [c]		54	Fiscus (1986), Newman (1973)	
Allium cepa	14	2 [d]		7	Melchior and Steudle (1993)	
Phaseolus coccineus	4.5 (32)	5.6 (37) [e]		0.8	Steudle and Brinckmann (1989)	
Hordeum distichon	0.3–4.3	0.3–4.3		≈1	Steudle and Jeschke (1983)	

[a] Test solutes were NaCl, ethanol, sucrose, mannitol, KNO$_3$.
[b] Test solutes were NaCl, ethanol, LiCl, KCl, KNO$_3$, NaNO$_3$, NH$_4$CO$_3$, NH$_4$NO$_3$, mannitol.
[c] Test solute was mannitol.
[d] Test solutes were KCl, NaNO$_3$, NH$_4$NO$_3$.
[e] Test solutes were NaCl, ethanol, KCl, NaNO$_3$, mannitol, methanol and urea.

Table 3. *Solute permeability (P$_{sr}$) and reflection coefficient (σ$_{sr}$) of single adventitious roots of rice plants grown either in hydroponic or in aeroponic culture for 31–40 d, measured with the root pressure probe*

Osmotic water flow was induced by changing the osmotic pressure of the medium by adding either NaCl or ethanol. Values shown are mean values ± SD with the number of measured roots in brackets. For comparison, literature values for maize and bean are shown as well. For both species, there were no significant difference in P_{sr} of NaCl and ethanol. $σ_{sr}$ values for NaCl were significantly bigger than those for ethanol (LSD, $P = 0.05$). Although there is some scatter, absolute values of solute permeabilities were similar for maize and rice, but reflection coefficients were smaller for rice.

	P_{sr} (10^{-8} m MPa^{-1})		$σ_{sr}$		References
	NaCl	Ethanol	NaCl	Ethanol	
Rice					
Hydroponics					
Azucena	0.73 ± 0.32 (6) a	0.91 ± 0.36 (7) a	0.28 ± 0.17 (6) a	0.13 ± 0.07 (7) a	
IR64	1.7 ± 1.0 (6) b	1.8 ± 1.6 (6) a	0.28 ± 0.11 (6) a	0.09 ± 0.01 (6) a	
Aeroponics					
Azucena	0.47 ± 0.34 (7) a	0.61 ± 0.25 (4) a	0.35 ± 0.10 (7) a	0.06 ± 0.02 (4) a	
IR64	0.28 ± 0.10 (5) a	0.50 ± 0.14 (6) a	0.25 ± 0.10 (5) a	0.08 ± 0.03 (6) a	
Maize	5.7 ± 3.7 (2)	4.7 ± 1.1 (2)	0.6 ± 0.3 (3)	0.3 ± 0.0 (2)	Steudle *et al.* (1987)
	1.1 ± 0.3 (5)	1.1 ± 0.6 (3)	0.5 ± 0.2 (5)	0.3 ± 0.1 (3)	Steudle and Frensch (1989)
	0.4 ± 0.1 (10)		0.6 ± 0.2 (10)		Steudle *et al.* (1993)
Phaseolus coccineus	0.02	0.6 (16)	0.59	0.3 (16)	Steudle and Brinckmann (1989)
Phaseolus vulgaris	0.1 [a]		0.9 [a]		Fiscus (1986)

[a] Measured at steady-state condition.

resistances in series would be required. Separation may be achieved by micro surgery or by puncturing the outer parts of the roots (Peterson and Steudle, 1993; Peterson *et al.*, 1993; Steudle *et al.*, 1993). However, this is technically difficult, as is the puncturing of individual root cells with the cell pressure probe to work out the cell-to-cell component of water flow. Cells are small and their walls fairly rigid.
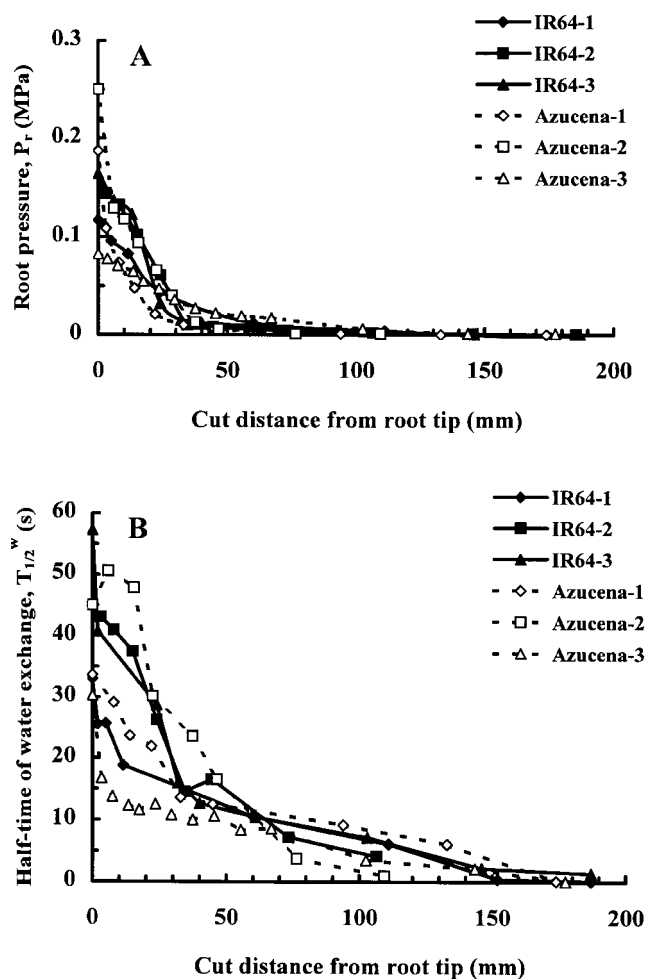
Fig. 5. Cutting experiment on six different excised adventitious roots of rice (IR64 and Azucena grown for 31–40 d in aeroponics) attached to the root pressure probe (Fig. 1B). When roots were successively cut, conducting xylem was hit and root pressure (P_r, A) and half-times ($T^w_{1/2}$, B) decreased. At a distance of 40 mm from the tip, P_r dropped to nearly zero and at a distance of 70 mm, $T^w_{1/2}$ became much smaller than that before cutting, i.e. axial water flow across the cut xylem dominated. Roots were from plants grown in aeroponics for 31–40 d.

From a comparison with other species it was found that absolute values of root Lp_r of rice were similar in some cases but were substantially smaller than those of other herbaceous roots. For example, for roots of bean (*Phaseolus coccineus*) and barley, root Lp_r was the same order as found here for rice (Steudle and Brinckmann, 1989; Steudle and Jeschke, 1983). For others such as onion, bean, and corn, Lp_r of roots from hydroponics was substantially greater than found here for rice. However, when comparing the osmotic root Lp_r of some of the other species with that of rice, values were similar. For corn roots grown in aeroponics, values were also similar (Table 2). Under these conditions, corn roots develop an exodermis which reduces Lp_r by a factor of nearly four (Zimmermann and Steudle, 1998). So, overall

the hydraulic conductivity of rice roots grown under comparable conditions appears to be substantially smaller than that of other herbs. Hydrostatic root Lp_r would be comparable to that of young tree roots such as spruce, oak, beech or pine (for data, see reviews by Steudle and Peterson, 1998; Steudle and Heydt, 1997).

It should be remembered that these data were gathered from chamber-grown young plants with high root zone aeration. For field-grown rice, root Lp_r may be even smaller than the values obtained in this study. It is known that under anaerobic conditions (such as in paddy fields), the radial loss of oxygen across rice roots is substantially decreased (Colmer *et al.*, 1998). This may be due to changes in the structure of the outer part of the root, perhaps, due to a thickening of the fibre tissue. Under these conditions, root Lp_r may also decrease; if it does there may be problems for water uptake. So, rice roots growing in stagnant anaerobic substrates would need a rather tight outer boundary to prevent oxygen losses, but this may result in a reduced capability to take up water even from a wet environment. Of course, this problem would only occur for paddy and deepwater rice. For upland rice growing in soils with high oxygen contents, an additional outer barrier may serve no purpose and be a liability for water relations of the shoot. The authors argue that the relatively low values of root Lp_r of rice are due to the anatomical structure of these roots, namely, the existence of efficient apoplastic barriers (endodermis, exodermis and lignified sclerenchyma; Morita and Abe, 1999; Clark and Harris, 1981).

Although differences were not significant, there was a tendency for the pressure-driven water flow to be larger than the osmotic. This trend is in line with the finding that there was a substantial flow of NaCl across the apoplastic barriers. However, the solute permeability (P_{sr}) measured in rice was smaller than in maize lacking an exodermis. It was larger than or similar to the salt permeability of species which have a dominating cell-to-cell flow of water (Steudle and Brinckmann, 1989). For rice, an apoplastic bypass flow of NaCl has been reported by Flowers and co-workers (Yeo *et al.*, 1987; Yadav *et al.*, 1996; Garcia *et al.*, 1997). These authors showed that NaCl as well as the apoplastic tracer PTS were dragged apoplastically into the root xylem along with the transpiration stream. The permeability studies with NaCl performed in this paper are in line with these observations, although P_{sr} may be less than in corn. In rice, the permeability of NaCl was similar to that of ethanol which should move rapidly because it also crosses cell membranes.

Experiments of the type shown in this paper need to be done on field-grown plants to verify that low values of root Lp_r are of wider significance. Usually, roots from the field are more intensely suberized than those from hydroponics or aeroponics (Stasovsky and Peterson, 1993).

If this is true for rice, hydraulic conductivities of plants from the field may be even smaller than those reported here.

In conclusion, the results show that, compared with other herbaceous species, rice roots offer a rather high hydraulic resistance to water flow. Concomitantly, the apoplastic solute (NaCl) permeability is reduced. The data indicate that effects are caused by the existence of substantial apoplastic transport barriers. Values of root Lp_r were the same when water was driven by either hydrostatic or osmotic forces and this implies that rice plants may not be able to meet higher transpirational demands from the shoot. The salient features of the structure of roots need to be clearly understood in relation to their effects on the low hydraulic conductivity. Without such knowledge much effort in breeding rice varieties that can well grow under conditions of reduced water supply in upland areas may be wasted. The results suggest that apoplastic barriers in the peripheral layers (exodermis/sclerenchyma) and in the endodermis are of great functional significance. It may be worthwhile to screen for genotypes with less developed apoplastic barriers or with barriers exhibiting a different chemical composition (Schreiber *et al.*, 1999; Zimmermann *et al.*, 2000). This approach is not discouraged by the lack of any readily observable differences in structure or root Lp_r in the two contrasting varieties used here. Perhaps an alternative approach would be to manipulate the cell-to-cell pathway so as to increase its conductivity. Current opinion suggests that this conductivity is strongly dependent on the density or activity of water channels (aquaporins) in the plasma membranes of root cells (Tyerman *et al.*, 1999; Kjellbom *et al.*, 1999). Genes that encode for plasma membrane aquaporins have been cloned and rice can be routinely transformed. It remains to be seen how much scope there can be for increasing the expression of membrane proteins which are, in wild-type plants, very abundant.

Acknowledgements

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