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RESEARCH PAPER

Water uptake by seminal and adventitious roots in relation to whole-plant water flow in barley (*Hordeum vulgare* L.)

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Abstract

Prior to an assessment of the role of aquaporins in root water uptake, the main path of water movement in different types of root and driving forces during day and night need to be known. In the present study on hydroponically grown barley (*Hordeum vulgare* L.) the two main root types of 14- to 17-d-old plants were analysed for hydraulic conductivity in dependence of the main driving force (hydrostatic, osmotic). Seminal roots contributed 92% and adventitious roots 8% to plant water uptake. The lower contribution of adventitious compared with seminal roots was associated with a smaller surface area and number of roots per plant and a lower axial hydraulic conductance, and occurred despite a less-developed endodermis. The radial hydraulic conductivity of the two types of root was similar and depended little on the prevailing driving force, suggesting that water uptake occurred along a pathway that involved crossing of membrane(s). Exudation experiments showed that osmotic forces were sufficient to support night-time transpiration, yet transpiration experiments and cuticle permeance data questioned the significance of osmotic forces. During the day, 90% of water uptake was driven by a tension of about –0.15 MPa.

Key words: Aquaporins, barley (*Hordeum vulgare*), cuticle, exudation, hydraulic conductivity, pressure probe, root water uptake, night-time transpiration.

Introduction

Plants appear in all shapes and sizes, yet in physical terms they are variable hydraulic conductors that use a naturally occurring gradient in the energy content of water (water potential) between root environment (soil, hydroponics) and shoot environment (atmosphere) to drive the uptake of water and dissolved mineral nutrients (Fig. 1A). Hydraulic resistances as they occur at the root and shoot level can limit the flow of water through the plant, analogous to Ohm's Law (van den Honert, 1948; Landsberg and Fowkes, 1978; Frensch, 1997). The main hydraulic barrier to water uptake by roots is the radial transport path, between root epidermis and xylem, rather than the axial path along xylem conduits (Frensch and Steudle, 1989; Steudle and Peterson, 1998). The radial resistance to water flow can be divided into an apoplastic (cell wall, middle lamella, and intercellular air space) and a cell-to-cell (through plasmodesmata and across membranes) component (see Fig. 1A, D), the latter involving aquaporins.

The contribution of water flow through aquaporins to root water uptake depends on water crossing aquaporin-containing membranes along the radial path. The main path of water movement may differ in response to environment, root developmental stage, prevailing driving force (osmotic, hydrostatic), day/night, or changes in root anatomy induced by stress (Steudle and Jeschke, 1983; for review see Steudle and Peterson, 1998; Steudle, 2000; Maurel *et al.*, 2010; Murai-Hatano *et al.*, 2008). Therefore, prior to a detailed molecular study of the role of individual aquaporin isoforms in the control of root water uptake (for review see Maurel *et al.*, 2008), it is necessary to analyse the contribution of different types of root to water uptake, in dependence of the main radial path of water movement and prevailing driving force. Root hydraulic properties can change with the magnitude of water flow induced across roots (e.g. Passioura and Munns, 1984). Therefore, integrating different measurements and scaling up to whole

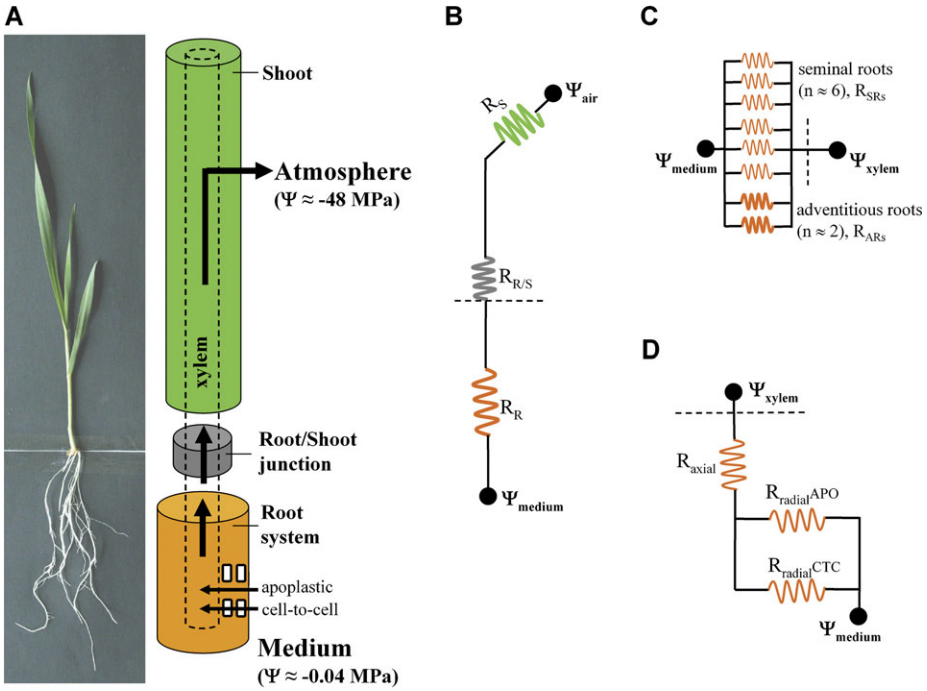

Fig. 1. Hydraulic resistances along the soil–plant–atmosphere continuum. (A) The picture shows a 15-d-old barley plant as analysed in the present study. Water flow through the plant is driven by a difference in water potential, $\Delta\Psi$, between root medium (approximately –0.04 MPa) and atmosphere (approximately –48 MPa at 70% relative humidity and 21 °C). Radial water uptake into roots can occur along an apoplastic and a cell-to-cell path, the latter involving aquaporins. Water is transported axially along the xylem and may encounter resistances within the root system, at the root–shoot junction, or within the shoot (leaf). In the shoot, radial flow of water and exit into the atmosphere can be limited by the radial flow path or the conductance at the exit point (stomata, cuticle). (B–D) Major hydraulic resistances arranged analogously to an electrical circuit. (B) Resistance of the root system (R_R), the root–shoot junction ($R_{R/S}$), and the shoot (R_S). (C) Within the root system, seminal and adventitious roots are treated as hydraulic resistances arranged in parallel (R_{SRs} and R_{ARs}, respectively). (D) In each root, axial and radial hydraulic resistance are treated as being arranged in series (R_{axial}, R_{radial}); the radial resistance (R_{radial}) is divided into two resistances arranged in parallel, an apoplastic (R^{APO}_{radial}), and a cell-to-cell resistance (R^{CTC}_{radial}).

plants is needed for a comprehensive analysis of hydraulic properties. There exists a range of methods to analyse the hydraulic behaviour of roots. Among the most commonly used techniques are the root pressure probe, exudation, and vacuum perfusion. These techniques involve different experimental set-ups, can apply different driving forces, and induce different flow rates across roots. Since we do not know how root hydraulics are affected by these differences and which technique provides a 'true' reflection of root hydraulic properties, these techniques should be used in combination. This, however, has not been done.

Barley plants were analysed when they were 14–17 d old. At this age, plants have two types of roots: seminal and adventitious. Seminal roots contain an elaborate system of lateral roots and are developed further compared with adventitious roots, which only possess a well-developed main root axis (Hacket, 1967, 1969). The thicker (in diameter) adventitious roots have more cortical cell layers and contain more central metaxylem vessels, of larger diameter, than seminal roots. The difference in developmental state between seminal and adventitious roots is expected to affect the functioning and development of the endodermis as the osmotic barrier for radial water and solute transport (Steudle and Peterson, 1998; Schreiber *et al.*, 1999; Enstone *et al.*, 2003), and impact on the radial and axial hydraulic properties of the two types of root.

The experimental strategy of the present study was to use the root pressure probe, exudation, and vacuum perfusion techniques to determine the hydraulic conductance and, together with determination of root surface area, the conductivity of seminal and adventitious roots of barley in response to osmotic and hydrostatic gradients. Together with determination of axial hydraulic conductivity, this allowed us to calculate radial in addition to osmotic hydraulic conductivity. From the average number of seminal and adventitious roots per plant and water flow rates determined on individual roots the water flow (uptake) rate of the entire root system could be calculated and compared with experimentally determined values. Through exudation experiments, we determined directly osmotic forces during the day and night. Taking into consideration that osmotic gradients decrease (dilution of xylem sap) with increasing transpiration rates (Munns and Passioura, 1984), which we determined gravimetrically during day and night, we could calculate the contribution of osmotically driven water uptake during day- and night-time transpiration. Vacuum perfusion experiments provided a relationship between hydrostatic forces and water flow rates. This

relationship could be used, by subtraction, to calculate from whole-plant transpiration rates and osmotic root water uptake the hydrostatic forces required to support transpiration rates. Finally, root excision experiments were carried out, where day- and night-time transpiration rates were measured in response to a reduction in the total number of roots (and root surface), or causing major solute leaks from the stele, or bypassing any radial resistance to water uptake. By modelling the plant as an electrical circuit (van den Honert, 1948) (Fig. 1B–D) these data allowed us to (i) determine the main transport resistances along the plant (root system, root–shoot junction, shoot); (ii) quantify the contribution of seminal and adventitious roots to root water uptake; (iii) relate hydraulic properties and flow rates determined on individual roots to those determined on entire root systems; (iv) conclude on the magnitude of osmotic and hydrostatic forces required to drive root water uptake during the day and night; and (v) assess the potential contribution of water flow along a pathway involving membranes (and possibly aquaporins) to root water uptake.

Materials and methods

To keep the volume of the manuscript at a minimum, all calculations are detailed in Supplementary File S1.

Plant material and growth conditions

Barley (*Hordeum vulgare* L. cv. Golf, Svalöf Weibull AB, Svalöf, Sweden) plants were grown on modified half-strength Hoagland solution in a growth chamber as described previously (Fricke and Peters, 2002). Plants grew at a day/night length of 16/8 h and temperature of 21/15 °C. Relative humidity was 70% and photosynthetically active radiation at the level of the developing leaf 3 was 300–400 μmol m^{-2} s^{-1}. Plants were analysed when they were 14–17 d old. At this developmental stage, plants had two fully expanded leaves (leaves 1 and 2). The main developing leaf was leaf 3, and leaf 4 started to emerge from the sheaths of older leaves when plants were 16–17 d old.

The first major roots that appeared, after radicle formation, during germination of barley seedlings were seminal roots. Barley plants had between six and seven seminal roots. Adventitious roots, which differ in morphology and anatomy from seminal roots (see Figs. 2, 3) started to appear when plants were 11–13 d old (see also Esau, 1965).

Barley plants were analysed when they were 14–17 d old and not when they were at more advanced growth stages, since the root system became more complex and difficult to handle (tangled lateral roots), which made it more likely that damage to roots and formation of leaks (hydraulic, solute) would occur (Miller, 1987). Also, results could be related to data on leaf growth, which have all been obtained on 14- to 17-day-old plants (e.g. Fricke *et al.*, 1997, 2006; Fricke, 2002; Fricke and Peters, 2002).

Root growth, anatomy, and surface area

To obtain information about growth of roots during the developmental window when hydraulic properties were determined, fresh weight and length of roots were measured daily when plants were 14–17 d old. Three independent batches of plants with 12 plants each were analysed.

Root anatomy was studied on free-hand cross-sections that were made from different root developmental regions, as specified in text and figure legends. Sections were observed with a Leica microscope (DM IL; Leica, Wetzlar, Germany) and captured with a digital camera (DFC300 FX; Leica, Wetzlar, Germany). For analyses of general root anatomy, e.g. detection of lignified cell walls (bright blue signal) or polysaccharides rich in carboxy groups (turquoise/pink signal), sections were stained with 0.5% Toluidine Blue for 1 min and viewed under bright light (O'Brien *et al.*, 1964). For the detection of Casparian bands (bright yellow signal), sections were stained for 30 min with 0.1% berberine hemisulfate and counterstained for 1–3 min with 0.5% Toluidine Blue. Sections were viewed under fluorescence light using a UV/violet filter with an excitation wavelength of 390–420 nm (Brundrett *et al.*, 1988; Hachez *et al.*, 2006; Bramley *et al.*, 2009). Mature xylem vessels, having highly lignified walls (bright blue/yellowish signal), were identified with the same method. Suberin and lipid deposits (intense red signal) were visualized by staining sections with Sudan Red 7B (or Sudan III) for 2.5 h (Brundrett *et al.*, 1991).

Surface area of roots was determined after each hydraulic experiment by measuring the length and radius of the main axis of roots and the number, length, and diameter of lateral roots. Surface area was calculated by treating roots as cylinders.

Root hydraulic measurements

Hydraulic properties of roots were analysed when plants were 14–17 d old. This made it possible to carry out replicate analyses on each batch of plants. The alternative, to collect entire sets of data for each specific age (e.g. 14 d, 15 d), would have required a very high number of replicate analyses and restricted the parallel application of a range of techniques.

Root pressure probe, vacuum perfusion, and root exudation were used to determine hydraulic properties of roots, as detailed previously (Knipfer and Fricke, 2010). Analyses were carried out in the laboratory. Hydraulic measurements resulted in values of conductance [unit: m^3 (of water flow) s^{-1} MPa^{-1} (of driving force)]. Conductance was either related to root surface area (m^2) to calculate conductivity (m s^{-1} MPa^{-1}) or was converted into resistance (inverse of conductance, s MPa m^{-3}). The calculation of hydraulic parameters is detailed in Supplementary File S1 (see also Knipfer and Fricke, 2010).

Root pressure probe experiments. Individual roots were excised close (1–2 cm) to their base, fixed to the probe, and bathed in the same medium in which the plant had been grown. The medium was circulated to minimize external unstirred layer effects (see Fig. 3 in Knipfer and Fricke, 2010, for comparison of data for stagnant and circulated media). When a stable root pressure was reached (0.5–2h) pressure relaxations were induced, either by imposing a hydrostatic pressure pulse (±0.05 MPa, hydrostatic relaxations) or by adding 25 mM NaCl to the root medium (osmotic relaxations). Half-times of pressure relaxations ($T_{1/2}$) were used for calculation of root hydraulic conductivity. Possible internal unstirred layers, which can occur at the cortex-facing side of the endodermis during diffusion of solutes through the root cylinder (Tyree *et al.*, 2005), were considered as part of the overall root hydraulic resistance, whereas internal unstirred layers on the stele-facing side of the endodermis were negligible (Knipfer and Fricke, 2010), in contrast to studies on corn (Knipfer *et al.*, 2007). Biphasic osmotic pressure relaxations consisted of an initial water exit phase and a second much slower water uptake phase caused by solute uptake (see also Knipfer and Fricke, 2010). When taking into account that a permeant solute (NaCl) was used during osmotic experiments, the initial water exit phase might have been dampened by the slow solute, and associated water uptake phase. However, for a typical half-time of the fast initial water exit and slower solute uptake phase of 14 s and 320 s, respectively, the underestimation of the half-time of the fast water exit phase (and osmotic water permeability) was <1% (see also Supplementary Fig. S4).

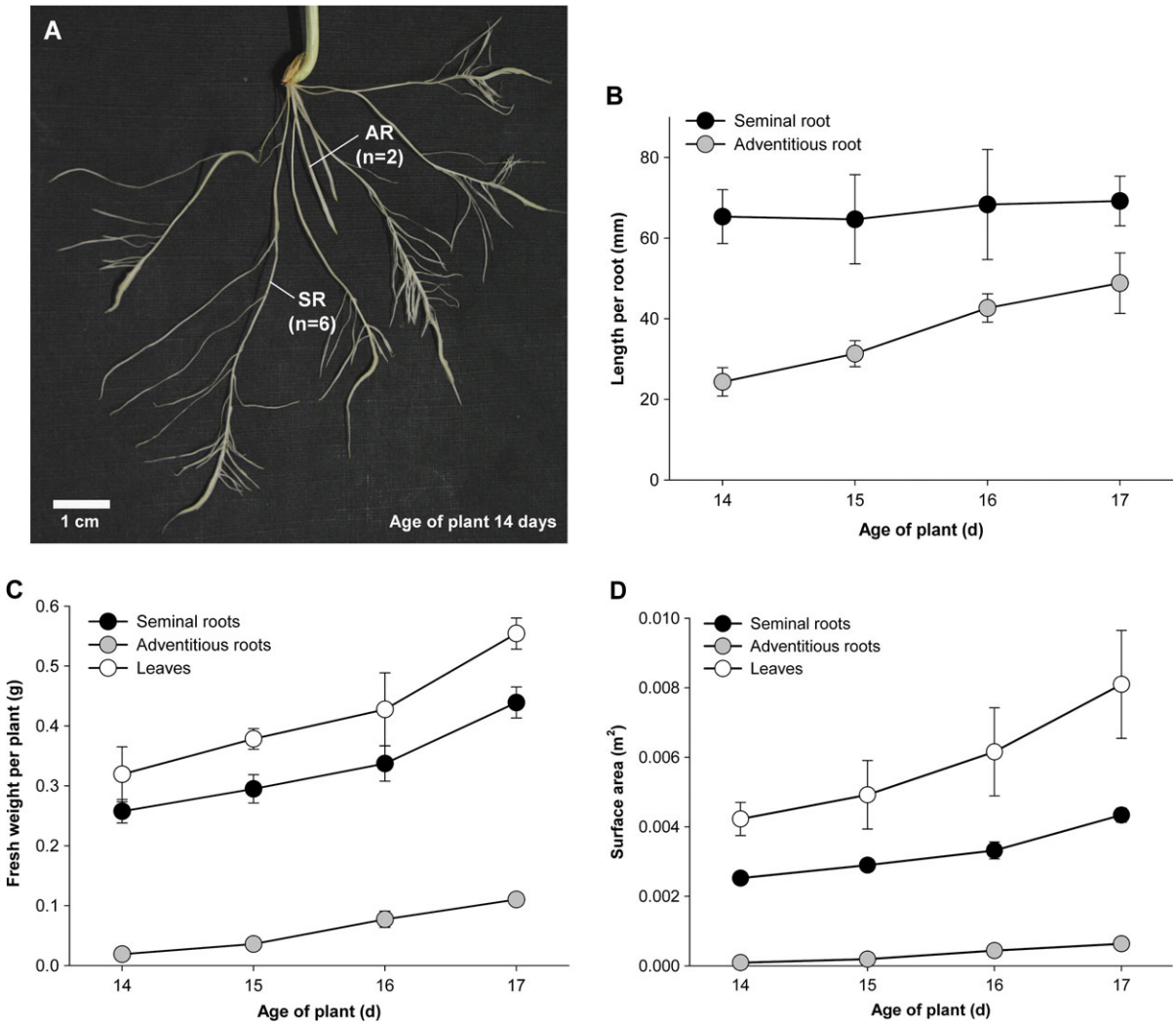

Fig. 2. Root and leaf growth in 14- to 17-d-old barley plants. (A) Typical root system showing (n=6) seminal roots (SR) and (n=2) adventitious roots (AR). (B) Average length of the main axis of individual seminal and adventitious roots. (C) Total fresh weight per plant of the entire set of seminal and adventitious roots and of leaves 1–3. (D) Total surface area per plant of the entire set of seminal and adventitious roots and of leaves 1–3. Results are means±SD (error bars) of values from (n=)12 plants, from three batches of plants. Where error bars seem to be absent, they are smaller than the symbol size. The surface area of roots was derived from an independently determined relationship between root fresh weight and surface area [seminal roots, n=17, fresh weight (g) ~9.96×area (m^2)+0.0054, r^2=0.75; adventitious roots, n=7, fresh weight (g) ~168.4×area (m^2)+0.0033, r^2=0.88].

Axial root hydraulic conductivity and xylem development was determined by successively cutting back roots from the tip (Frensch and Steudle, 1989). After each cut half-times were measured again. Measured changes in half-time were directly proportional to changes in root hydraulic resistance. A rapid decrease in half-time at a certain position along the root was an indication that the main conducting (meta)xylem was mature and fully functional (Frensch and Steudle, 1989). Axial root hydraulic conductivity was calculated from half-times determined for the basal 2 cm root segment.

Vacuum perfusion. A root was fixed to a glass capillary and supported in such a way that water uptake could be measured as gravimetric loss in weight of nutrient solution. Osmotically driven water uptake was measured prior to hydrostatically driven water uptake (application of partial vacuum using a vacuum pump). Water flow was used to calculate hydraulic conductance and conductivity. Axial hydraulic conductance and conductivity was determined after roots had been excised 2 cm below the root base.

Root exudation. An individual root or an entire root system was attached with the excised root base or excised mesocotyl, respectively, to a glass capillary (diameter 0.5 mm and 1.5 mm, respectively). The rise of xylem sap in the capillary was measured at time intervals of 5 min over a period of 1 h (individual roots) or at intervals of 15 min over a period of 2 h (entire root system). Exudate volume was measured after each interval using the capillary diameter and increase in height of the exudate column, when individual roots were analysed; or exudate volume was determined by weighing the collected exudate on a balance (1 g = 1 ml; CP323P; Sartorius, Göttingen, Germany), when entire root systems were analysed, which resulted in exudate volumes that exceeded the capacity of capillaries. The osmotic flow rate was calculated from the linear part of the flow against time plot. The osmotic driving force for

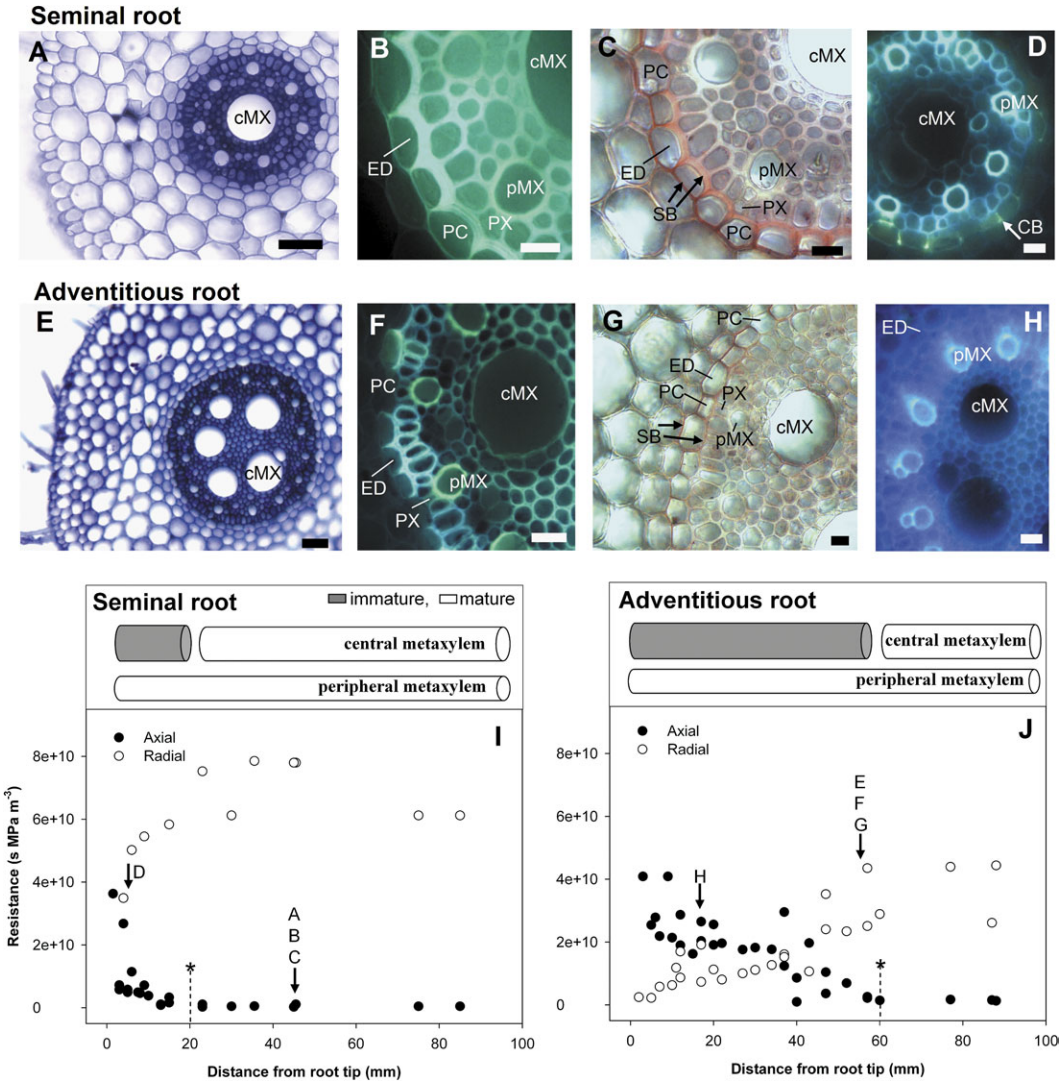

Fig. 3. Anatomy and xylem development of seminal and adventitious roots of barley. (A–D) Cross-sections of seminal roots taken at 40–60 mm (A–C, lateral root zone) and 5–10 mm (D, tip region) from the tip. (A–C) Seminal roots have typically one central (cMX) and eight peripheral metaxylem (pMX) vessels, the latter located close to the much smaller protoxylem vessels (PX). The stelar region is highly lignified and metaxylem vessels are mature. The endodermis (ED) is mature and shows asymmetrically thickened cell walls, which is typical of state III of endodermis development (Enstone *et al.*, 2003), in all cells including passage cells (PC). Wall depositions of suberin (SB, arrow) can be detected in all cells of the endodermis. (D) Close to the root tip only peripheral but not central metaxylem vessels are mature. Casparian bands can be detected (CB, arrow). (E–H) Cross-sections of adventitious roots taken at 40–60 mm (E–G, root hair region) and 15–20 mm (H, tip region) from the root tip. (E–G) Adventitious roots have typically 5–7 central and 14 peripheral metaxylem vessels. The stelar region shows some lignification. The endodermis shows secondary wall thickening except in passage cells. There are fewer suberin depositions in adventitious compared with seminal roots and depositions are lacking from some passage cells. Central metaxylem appears less mature than in seminal roots. (H) Closer to the root tip, only peripheral but not central metaxylem vessels appear mature. Sections shown in (A) and (E) were stained with Toluidine Blue and viewed under bright light; sections in (B) and (F) were stained with berberine hemisulfate and counterstained with Toluidine Blue and viewed under fluorescence light (390–420 nm) to visualize Casparian bands and xylem development (Brundrett *et al.*, 1988). Sections in (C) and (G) were stained with Sudan Red 7B and viewed under bright light to visualize depositions of suberin (Brundrett *et al.*, 1991). (I–J) Root pressure probe analyses of axial and radial hydraulic resistance (inverse of conductance) along (I) seminal and (J) adventitious roots. The axial hydraulic resistance was experimentally determined from half-times obtained through root pressure probe experiments where roots were cut back successively and in between measurements (data points) from the tip (see Frensch and Steudle, 1989). The radial resistance was calculated as the difference between the overall root resistance and the axial resistance for a particular location. (I) In seminal roots, the axial resistance decreases to very low values beyond 20 mm from the tip, whereas the radial resistance increases. This shows that central metaxylem vessels become fully mature and the endodermis fully developed at ~20 mm (as indicated by asterisk). (J) In adventitious roots, changes in axial and radial resistance (and corresponding changes in metaxylem and endodermis development) occur up to 60 mm from the tip (asterisk). Results are pooled from three root analyses each, and the location of cross-sections shown in A–H is indicated. Scale bars: (A) 55 μm, (B–D) 15 μm, (E) 75 μm, and (F–H) 25 μm.

water uptake was calculated from the difference in osmolality between root medium and exudate. Exudation measurements, as all other hydraulic analyses, were typically carried out 5–9 h into the photoperiod. For individual roots, exudation was also measured 3–5 h into the dark period.

To determine the hydraulic conductance of the root–shoot junction, a vacuum-perfusion set-up similar to the one for analyses of individual roots was used. The shoot of a barley plant was excised under water 1–2 cm above the seed, at the mesocotyl. The remaining segment of the mesocotyl with the root system attached was inserted into a water-filled glass capillary (diameter 1.5 mm). The mesocotyl was sealed with a cylindrical silicone seal in the same way as during root pressure probe experiments. A partial vacuum was applied (–0.02 MPa) to the open end of the capillary. Water flow was measured gravimetrically as for individual roots. It took ~30 min for water flow to increase linearly with time. This gave the hydraulic conductance of the combined root system, root–shoot junction, and mesocotyl portion. Roots were subsequently cut off right below (mesocotyl plus root–shoot junction) and above (mesocotyl) the seed and the corresponding flow rate measured after each cut.

Transpiration and whole-plant hydraulics

The rate of transpirational water loss of entire plants was determined gravimetrically in the growth chamber. Single barley plants were placed in a measuring cylinder, which was filled with nutrient solution and placed on a balance (CP323P). Changes in weight were recorded every 2 min over a complete day/night period using computer software (sartoCollect 1.0; Sartorius, Göttingen, Germany). Rates of transpirational water loss were corrected for evaporational water loss from the solution surface of the measuring cylinder. The latter was determined in separate experiments (under identical growth chamber settings) and accounted for <1% and for 9% of water loss recorded during the day and night period, respectively (not shown).

The hydraulic conductance and resistance of the whole plant was calculated from the transpirational water flux rate and the difference in water potential between root medium and atmosphere ($\Psi_{medium-air}$ was –48.32 MPa during the day and –47.50 MPa during the night; Supplementary Table S1). By modelling the plant as an electrical circuit (Fig. 1B), the transport resistance at the root level, root–shoot junction, and shoot was determined. Seminal and adventitious roots were treated as parallel-arranged hydraulic resistances (Fig. 1C). The hydraulic resistance of the entire root system was calculated from the hydraulic resistance of individual roots and the number of roots. In individual roots, axial and radial hydraulic resistances were treated as serial resistances (Fig. 1D). The radial resistance was divided into two parallel resistances, one representing the apoplastic and one representing the cell-to-cell pathway.

Root excision experiments

To test the significance of an intact, complete root system for transpirational water loss and leaf growth, a set of experiments was carried out on 13- to 17-d-old barley plants, in which a specified number (see text and figure legends) of roots was cut off close to their base (~2 cm from the root–shoot junction). Roots were positioned in such a way that the cut was either submerged in nutrient solution or above the nutrient solution, in which case the cut was sealed with Vaseline to prevent air from entering xylem vessels. In an additional type of experiment, the tip region of seminal roots was cut off, in the root hair region (~2 cm from the root tip), and the cut was kept submerged in nutrient solution. Transpirational water loss of plants prepared in such a way was determined gravimetrically for one day/night cycle, as described above. For the determination of leaf growth, the length of the developing leaf 3 was measured with a ruler in the morning and afternoon, over several days, and the increment in length per measurement interval was used to calculate leaf elongation velocity (mm h^{-1}).

Osmolality measurements

Osmolality of root exudate and medium was determined by picolitre osmometry as described previously for cell and bulk leaf extracts (Tomos *et al.*, 1994; Fricke and Peters, 2002). Samples were analysed immediately following collection or were stored beneath a layer of liquid paraffin (to minimize evaporation) in 0.2 ml centrifuge tubes at 4 °C for up to 3 d.

Experimentally determined and calculated values

Some data were determined experimentally, while others were calculated. Hydraulic conductance and conductivity were calculated from experimentally determined values of flow rates, or half-times of water exchange, driving forces, and root surface areas. Transpiration rates were determined experimentally and the flow component driven through osmotic and hydrostatic gradients was calculated, as was the contribution of root types to plant water uptake. Axial hydraulic resistance along roots was determined experimentally and used to calculate radial hydraulic resistance. The hydraulic resistance of the shoot (between root–shoot junction and air) was calculated from experimentally determined values of whole-plant, root system, and root–shoot junction resistance. Any growth-related data (fresh weight, length) were determined experimentally.

Results

Root growth and xylem development

Seminal roots appeared during germination, in their final number, whereas adventitious roots appeared when plants were 11–13 d old. When plants were 14–17 d old—the developmental stage at which they were analysed—there were six to seven seminal roots and two to four stem-borne adventitious roots per plant. Adventitious roots had a well-developed root hair region, but in contrast to seminal roots, no lateral roots at the developmental stage analysed (Fig. 2A; see also Hacket and Bartlett, 1971). The length of seminal roots ranged from 47 to 105 mm (72 roots analysed) and averaged 65±7 and 69±6 mm when plants were 14 d and 17 d old, respectively (Fig. 2B). Some of the variation in seminal root length might have been due to the primary root being longer than the other seminal roots. In comparison, the length of adventitious roots ranged from 7 to 75 mm (36 roots analysed) and averaged 24±4 and 49±8 mm at 14 d and 17 d, respectively (Fig. 2B). Between days 14 and 17 of plant growth, fresh weight and surface area of seminal roots increased largely as the result of growth of lateral roots. In contrast, in adventitious roots, increases were due to elongation of the main root axis (Fig. 2C, D). The increase per plant in fresh weight and surface area of roots occurred parallel to an increase in fresh weight and area of leaves, particularly for seminal roots at the time leaf 4 started to emerge (days 16–17, Fig. 2D). The average surface area of an individual seminal root ($5.2\pm1.2\times10^{-4}$ m^2) was 3-fold larger than that of an adventitious root ($1.6\pm0.6\times10^{-4}$ m^2).

A detailed study on the structure of the barley root system in terms of branching pattern and root dimensions

has been given by Hackett (1967, 1969) and Hackett and Bartlett (1971). Seminal roots had a mean diameter of 509±82 μm, between four and five cortical cell layers, and typically one large central and eight smaller and circularly arranged peripheral metaxylem vessels (Fig. 3). The peripheral metaxylem vessels were early metaxylem—being fully functional during early stages of development of a root segment—whereas the central vessel was late metaxylem—being the last of the xylem elements to become fully functional (at the root hair zone and towards the root base; Heimisch, 1951; Esau, 1965). The average diameter of central metaxylem vessels was 48 μm at the tip and 53 μm at the base region of seminal roots; the average diameter of peripheral metaxylem vessels was 17 μm (tip) and 14 μm (base; see Fig. 3A, E, Table 1). Compared with seminal roots, adventitious roots were 1.5- to 2-fold thicker (mean diameter 968±82 μm as compared with 509±82 μm), had seven to eight cortical cell layers, and ~14 peripheral and 6 central metaxylem vessels (Fig. 3A, E). The diameter of vessels was larger than in seminal roots, with diameters averaging 53 μm and 76 μm for central and 19 μm and 24 μm for peripheral metaxylem at the tip and base, respectively (Fig. 3, see also Table 1). Stelar cells were less lignified in adventitious as compared with seminal roots. The endodermis of the mature region of seminal roots was entirely in its tertiary state, with secondary wall thickening and strong suberization throughout (Fig. 3 B, C). In contrast, the endodermis of adventitious roots had passage cells which lacked secondary wall thickenings and suberin (Fig. 3 F, G).

Closer to the tip of both types of root, central metaxylem vessels were not lignified and could be classified as immature as compared with peripheral metaxylem vessels, which had highly lignified walls (Fig. 3D, H; Brundrett *et al.*, 1988; Bramley *et al.*, 2009; see also Supplementary Fig. S3). Casparian bands could be detected in seminal but not in adventitious roots in the region close to the tip (see Fig. 3D, H). Casparian bands appeared during root development prior to the formation of additional wall depositions in the endodermis (compare above).

Judging from cross-sections, which were taken at 40–50 mm from the root tip, it appeared that the large central metaxylem vessels were developed further, and possibly more conductive, in seminal compared with adventitious roots. This was supported through root pressure probe analyses in which the half-time of water exchange between root medium and xylem was first measured for intact roots and then measured while successively cutting back roots from the tip (see also Frensch and Steudle, 1989). Data on half-times and whole-root conductance could be used to construct spatial profiles of axial and radial resistance (inverse of conductance) along the main axis of roots (see also Frensch and Steudle, 1989) (Fig. 3I, J). A large decrease in axial resistance (faster water flow), which reflected movement of water through a cut-open mature xylem vessel, was observed in seminal roots at ~20 mm from the tip; in contrast, in adventitious roots, this decrease was not observed until ~60 mm from the tip (Fig. 3I, J; see asterisk). Parallel to the decrease in axial resistance from the tip towards the base of roots, radial hydraulic resistance increased, reaching final high values at ~20 mm and 60 mm from the tip in seminal and adventitious roots, respectively (Fig. 3I, J).

When axial hydraulic conductance of xylem was estimated for the most mature root region based on metaxylem dimensions, using Hagen–Poiseuille's law, the axial conductance was by one to two orders of magnitude larger in adventitious compared with seminal roots (Table 1).

Root hydraulic properties

The force that drove water uptake into roots and that had to be known to calculate hydraulic conductance could be determined directly in root pressure probe experiments from the magnitude of induced changes in root pressure (through hydrostatic or osmotic means); similarly, during vacuum

Table 1. Xylem dimensions and predicted axial conductance of seminal and adventitious roots of 14- to 17-d-old barley plants

Dimensions and number of mature xylem vessels were used to calculate axial xylem conductance [(m^3 s^{-1} MPa^{-1})×10^{-12}] for a 20 mm root segment by applying Hagen–Poiseuille's law. Seminal (more advanced) and adventitious roots differed in developmental stage and architecture, and the root base corresponded to the lateral-root zone in seminal and the root-hair zone in adventitious roots. Values are given as mean±SD of five (seminal) and three (adventitious) root analyses; the range of values is given in brackets '(min–max)'.

Root	Root zone	Distance from tip, mm	Peripheral metaxylem		Central metaxylem		Calculated axial hydraulic conductance (m^3 s^{-1} MPa^{-1})×10^{-12}	
			Number of vessels	Diameter of vessels, μm	Number of vessels	Diameter of vessels, μm	Peripheral metaxylem	Central metaxylem
Seminal	Tip	5–10	8±1	17±2[a]	1±0	48±18[a]	820 (497–1280)	not mature
	Base	50–70	8±1	14±2[a]	1±0	53±10[a]	377 (203–643)	9723 (3817–19357)
Adventitious	Tip	5–10	14±0	19±4[a,b]	6±1	53±12[a]	1430 (660–5700])	not mature
	Base	50–70	14±1	24±2[b]	6±1	76±4[b]	5700 (4020–7850)	245300 (197980–301150)

Statistical significance of difference in diameter of metaxylem vessels between type of root and root zone is indicated by different superscripts ($P \leq 0.05$, Student's *t*-test).

perfusion the size of the applied vacuum gave the driving force for hydrostatically induced water flow. For osmotically induced flow prior to and during application of vacuum and during exudation experiments, the difference in osmolality between medium and xylem had to be known. Since both experiments effectively measured the flow rate of exudate from an excised root (system), the osmotic driving force was determined for the exudation set-up and applied to both types of experiment. The osmotic driving force in individually analysed roots was determined during the day and night periods. The driving force was twice as high in adventitious compared with seminal roots (0.164 MPa as compared with 0.086 MPa). The driving force was lowest (0.034 MPa) when entire root systems were analysed (Table 2).

The reason for the much lower exudate osmolality of entire root systems is not known to us. Entire root systems contained, in contrast to individually measured roots, the very base of roots, the root–shoot junction, and the very base of shoot. The combined volume of these tissues was at least one order of magnitude smaller than that of exudate collected, which rules out any dilution effect through either extrusion of water or uptake of solutes by these tissues. Also, if anything, the exudate flow (m^3 s^{-1}) obtained on entire root systems was slightly lower, not larger, than that calculated for a seminal root system using data obtained through exudation analyses of individual seminal roots. This rules out flow-dependent dilution of xylem solutes (Munns and Passioura, 1984) in exudates collected from entire root systems. The only alternative explanation that we have is that there might have been a wound effect, which caused the shoot base, through some signal, to impact on radial solute uptake and xylem loading in roots. The generally small standard deviation for replicate analyses makes the possibility of an experimental artefact unlikely.

Vacuum perfusion provided reproducible results only for seminal but not for adventitious roots (not shown). We do not know why adventitious roots were not suitable for this set-up, but our most likely explanation is that roots were difficult to fix (with super glue) (in)to glass capillaries and that there may have been bypass flow of liquid between the outer surface of the root and inner surface of the capillary in a non-reproducible manner. Therefore, results from vacuum perfusion experiments are shown only for seminal roots.

Table 2. Osmotic forces driving root water uptake

The driving force was calculated as the difference in osmolality between root exudate and medium. Exudates were collected as part of exudation experiments; media were collected in parallel. Results are means±SD of seven or eight (day) and four (night period) root analyses.

Root	Day or night	Osmolality, mosmol kg^{-1}		Driving force (mosmol kg^{-1}) [MPa]
		Root medium	Exudate	
Seminal	Day	14±4	$49±6^{a}$	$(35±10)^{a}$ [0.086±0.025]
	Night	17±8	$78±24^{b}$	$(61±18)^{b}$ [0.150±0.044]
Adventitious	Day	14±4	$81±7^{b,c}$	$(67±7)^{b,c}$ [0.164±0.017]
	Night	16±9	$60±12^{b,d}$	$(44±17)^{a,b}$ [0.108±0.042]
Entire root system	Day	17±2	$31±3^{e}$	$(14±4)^{d}$ [0.034±0.010]

Statistical significance of difference in exudate osmolality or in driving force between day and night and between types of root analysed is indicated by different superscripts ($P \leqslant 0.05$, Student's t-test).

Root hydraulic conductance determined with the root pressure probe in hydrostatic experiments was four times higher in seminal compared with adventitious roots (65×10^{-10} compared with 15×10^{-10} m^3 s^{-1} MPa^{-1}, Table 3). This was largely due to a difference in surface area between the two types of root in these experiments ($5.19±0.92\times10^{-4}$ m^2 and $1.53±0.22\times10^{-4}$ m^2, in seminal and adventitious roots). As a result, hydraulic conductivity (conductance per unit surface area) was comparable between seminal and adventitious roots (Table 3). When hydrostatic hydraulic conductance of seminal roots was determined through vacuum perfusion, values were in the same range, though almost 50% higher compared with values obtained with the root pressure probe. Vacuum perfusion data reflected steady state water flow whereas root pressure probe data were obtained from transient water flow. Any formation of unstirred layers and interference with measurements (lowering hydraulic conductance) would have been expected to be larger during vacuum perfusion. However, this was clearly not the case.

Hydraulic conductance obtained through osmotic experiments ranged from 27×10^{-12} to 119×10^{-12} m^3 s^{-1} MPa^{-1} in seminal and from 6.2×10^{-12} to 10×10^{-12} m^3 s^{-1} MPa^{-1} in adventitious roots. Osmotic hydraulic conductivity was within the same range in the two types of root, with a tendency towards higher values in seminal roots (Table 3).

Values of root conductance and conductivity obtained through the three methods included a radial and an axial component. To determine the radial component, the axial component had to be known. The axial component was determined for 20 mm long root segments close to the root base, where conducting metaxylem vessels were most mature and axial hydraulic conductance highest. The axial hydraulic conductance close to the root base, as determined with the root pressure probe, was almost 20 times higher in seminal compared with adventitious roots; axial conductivity was six times higher in seminal roots (Tables 4, 5). The radial conductance was orders of magnitude lower than the axial conductance, particularly in seminal roots (Tables 4, 5). Osmotic and hydrostatic experiments gave values for conductance that were within the same range. The same applied to conductivity.

Root-system and whole-plant hydraulics

Average values of hydraulic conductance determined on individual roots (Table 3) were used to calculate the hydraulic conductance of the entire set of roots (seminal, adventitious) and these values could then be used to calculate the hydraulic conductance of the entire root system of a plant. The entire set of seminal roots conducted,

Table 3. Hydraulic conductance [(m^3 s^{-1} MPa^{-1})×10^{-12}] and conductivity [(m s^{-1} MPa^{-1})×10^{-8}] of individual seminal and adventitious roots of hydroponically grown barley plants

Values for each method are given as means ± SD of three roots; the range of values is given in brackets '(min–max)', together with the overall mean.

Hydraulic parameter	Root	Hydrostatically induced water flow			Osmotically induced water flow			
		Root pressure probe	Vacuum perfusion	Mean (range)	Root pressure probe	Vacuum perfusion	Exudation	Mean (range)
Conductance	Seminal	65±30[a,b,c]	94±17[a,b]	80 (65–94)	27±11[de]	119±52[b]	61±11[a,b]	69 (27–119)
	Adventitious	15±6.8[d]	–	15	10±6.8[d,f]	–	6.2±1.8[f]	8.1 (6.2–10)
Conductivity	Seminal	13±2.6[a,b]	20±3.6[d]	16.5 (13–20)	5.4±2.0[c]	25±10[b,d]	12±1.8[a]	14 (5.4–25)
	Adventitious	10±5.1[a,b,c]	–	10	6.3±3.4[a,c]	–	5.1±0.5[c]	5.7 (5.1–6.3)

Statistical significance of difference in conductance or conductivity between types of root, experimental approach, and hydrostatically and osmotically induced water flow is indicated by different superscripts ($P \leq 0.05$, Student's *t*-test). '–', values obtained for adventitious roots through the vacuum-perfusion set-up suffered from high variation between replicate analyses, suspect to artefacts, and were not considered.

Table 4. Axial and radial hydraulic conductance [(m^3 s^{-1} MPa^{-1})×10^{-12}] of seminal and adventitious roots of hydroponically grown barley plants

Axial conductance was measured for a 20 mm long root segment near the root base (most mature root tissue); radial conductance was calculated as the difference between the overall hydraulic conductance of a root and its axial conductance. Values for each experiment are given as means±SD of three roots; the range of values is given in brackets '(min–max), together with the overall mean.

Conductance	Root	Hydrostatically induced water flow			Osmotically induced water flow			
		Root pressure probe	Vacuum perfusion	Mean (range)	Root pressure probe	Vacuum perfusion	Exudation	Mean (range)
Axial	Seminal	2980±1720[a]	21100±3330[b]	12040 (2980–21100)	–	–	–	–
	Adventitious	158±205[c]	–	158	–	–	–	–
Radial	Seminal	67±3.7[a]	94±17[b]	81 (67–94)	28±11[c]	120±52[a,b,d]	63±11[ad]	70 (28–120)
	Adventitious	24±16[c,e]	–	24	12±6.7[c,e]	–	6.5±1.9[e]	9.3 (6.5–1 2)

Statistical significance of difference in axial, or in radial conductance between types of root and experimental approach is indicated by different superscripts ($P \leq 0.05$, Student's *t*-test); '–', not measured, or not applicable.

Table 5. Axial and radial hydraulic conductivity [(m s^{-1} MPa^{-1})×10^{-8}] of seminal and adventitious roots of hydroponically grown barley plants

Conductivity was calculated by relating values of conductance shown in Table 4 to root surface area. The surface area ranged from 4.9 to 5.5×10^{-4} m^2 in seminal and 1.2 to 1.5×10^{-4} m^2 in adventitious roots. Conductivity is given as means±SD of three root analyses; the range of values is given in brackets '(min–max)', together with the overall mean.

Conductivity	Root	Hydrostatically induced water flow			Osmotically induced water flow			
		Root pressure probe	Vacuum perfusion	Mean (range)	Root pressure probe	Vacuum perfusion	Exudation	Mean (range)
Axial	Seminal	548±266[a]	4480±790[b]	2514 (548–4480)	–	–	–	–
	Adventitious	93±112[c]	–	93	–	–	–	–
Radial	Seminal	13.2±3.0[a,b,c]	20.0±3.7[b]	16.6 (13.2–20)	5.4±2.0[e]	25.3 ±10.3[a,b]	11.8±1.8[a,f]	14.1 (5.4–25.3)
	Adventitious	16.0±11.7[a,b,c]	–	16.0	7.3 ±3.4[c,e,f]	–	5.4±0.6[d]	6.3 (5.4–7.3)

Statistical significance of difference in axial, or in radial conductance between types of root and experimental approach is indicated by different superscripts ($P \leq 0.05$, Student's *t*-test); '–', not measured, or not applicable.

on average, 4.5×10^{-10} and 5.2×10^{-10} m^3 water s^{-1} MPa^{-1} in osmotic and hydrostatic experiments, respectively. This compared with rates of 0.24×10^{-10} and 0.45×10^{-10} m^3 water s^{-1} MPa^{-1}, respectively, in adventitious roots and resulted in an entire root system conductance of 4.7×10^{-10} and 5.7×10^{-10} m^3 water s^{-1} MPa^{-1}, respectively (Fig. 4). In comparison, the hydraulic conductance determined through exudation experiments (osmotic driving force) involving entire root systems averaged 7.8×10^{-10} m^3 s^{-1} MPa^{-1} (Fig. 4).

Based on data from osmotic experiments, seminal roots contributed between 85% and 98% (average 92%) and adventitious roots between 15% and 2% (average 8%) to plant water uptake. When the driving force was hydrostatic figures were within the same range, 90–93% (average 92%) and 10–7% (average 8%), respectively (Fig. 4). The major

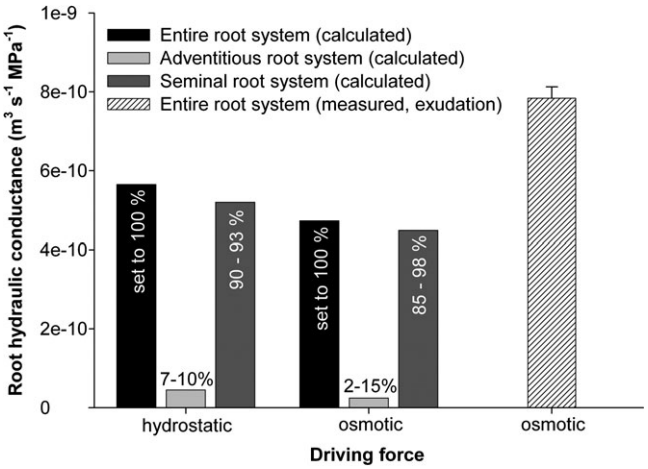

Fig. 4. Contribution of seminal and adventitious roots to water uptake in 14- to 17-d-old barley plants in dependence on the driving force (osmotic, hydrostatic). Hydraulic conductance was determined through osmotic and hydrostatic experiments for individual seminal and adventitious roots (see Table 3). The average values of these experiments were then used to calculate the hydraulic conductance of a typical seminal and a typical adventitious root system of a barley plant, containing 6–7 (average 6.5) seminal and 2–4 (average 3) adventitious roots, respectively. The sum of the two gave the conductance of the entire root system of a plant. Percentage figures give the contribution of the conductance of the seminal and adventitious root system to the conductance of the entire root system of a barley plant. The range of conductance values, as calculated from the range of means given in Table 3 was as follows (unit: $m^3\ s^{-1}\ MPa^{-1}\times10^{-10}$): hydrostatic force, seminal roots, 4.2–6.1; adventitious roots, 0.45 (results from only one analytical method used); entire root system of plant, 4.7–6.6; osmotic force, seminal roots, 1.8–7.7; adventitious roots, 0.18–0.30; entire root system of plant, 2.0–8.0. Also shown is an experimentally (exudation) determined osmotic hydraulic conductance for an entire barley root system; average and SD (error bars) of four independent root analyses.

type of root supplying water to 14- to 17-d-old barley plants was the seminal root, irrespective of the driving force.

To relate root hydraulic conductance to whole-plant water flow, transpirational water loss was measured continuously throughout the day and night periods for undisturbed plants in the growth chamber (Fig. 5A). Plants transpired water at an average rate of 8.8×10^{-11} $m^3\ s^{-1}$ during the day and 1.0×10^{-11} $m^3\ s^{-1}$ during the night (Fig. 5A). The driving force for whole-plant water flow between root medium and air differed little between day and night (Supplementary Table S1). Transpiration rates and driving forces calculated to a plant hydraulic conductance of 1.8×10^{-12} $m^3\ s^{-1}\ MPa^{-1}$ during the day and 2.1×10^{-13} $m^3\ s^{-1}\ MPa^{-1}$ during the night (Fig. 5B).

Apart from the root and shoot, the root–shoot junction may present a hydraulic bottleneck to plant water flow (Martre *et al.*, 2001), and this could affect the distribution of water potential gradient between root and shoot, which drives water uptake. Therefore, experiments were conducted in which water flow was measured prior to and following removal of the root shoot junction by applying a partial vacuum of –20 kPa. By modelling plants as an electrical circuit, conductance was converted into resistance. By far the largest resistance to water flow was located between leaf xylem and air ($5.79\pm1.51\times10^{11}$ s MPa m^{-3}, range: 3.5–8.4×10^{11} s MPa m^{-3}). The resistance of the root system ($2.30\pm1.50\times10^{8}$ s MPa m^{-3}, range: 1.3–4.9×10^{8} s MPa m^{-3}) was three orders of magnitude smaller, and the resistance of the root–shoot junction ($1.25\pm2.46\times10^{6}$ s MPa m^{-3}, range: 0.007–4.9×10^{6} s MPa m^{-3}) even smaller, by a factor of 100 (means±SD of four experiments). Based on these data it could be assumed that the water potential of xylem differed little between leaf and root.

Osmotic and hydrostatic forces driving plant water flow

Data on root hydraulic conductance and whole-plant water flow (transpiration) made it possible to calculate the gradients in water potential required to drive root water uptake. Furthermore, since root conductance was known for osmotic and hydrostatic forces, the contribution of each to driving water uptake could be calculated. Vacuum perfusion provided reproducible results only for seminal roots. For this reason, and because seminal roots contributed 92% of root water uptake, only seminal roots were considered. The average osmotic hydraulic conductance, obtained through analyses of individual roots, of a seminal root system was 4.5×10^{-10} $m^3\ s^{-1}\ MPa^{-1}$. In comparison, osmotic hydraulic conductance determined through exudation experiments for entire root systems was 7.8×10^{-10} $m^3\ s^{-1}\ MPa^{-1}$ (see Fig. 4). This would mean that a driving force of between 0.11 MPa and 0.20 MPa between root medium and xylem was required to sustain a transpirational water flow of 8.8×10^{-11} $m^3\ s^{-1}$ during the day. The water potential of the root medium was –0.04 MPa and the water potential of the root xylem, which should have been close to that of the leaf xylem, would have had to be between –0.15 MPa and –0.24 MPa to establish a gradient of 0.11–0.20 MPa. We did not determine the water potential of leaf xylem, since this would have involved considerable manipulation of plants and altered transpiration rates. Instead, we used the cell pressure probe, together with picolitre osmometry (see Supplementary Table S1) to determine the water potential of leaf epidermal cells, which should be close to that of the xylem, of leaves 2 and 3 (main transpiring surfaces) of transpiring plants in the growth chamber. Epidermal water potential was –0.12 MPa (leaf 2) and –0.26 MPa (leaf 3). This covered the range of water potential required to drive daytime water uptake rates.

The next question to address was whether the water potential gradient driving root water uptake consisted mainly of a hydrostatic (tension) or osmotic component. Exudation experiments on individual seminal roots carried out during the day gave an average osmotic gradient of 0.086 MPa between root medium (–0.034 MPa) and root xylem (–0.120 MPa, Table 2). Using this gradient and the average osmotic conductance of seminal roots (Table 3), we

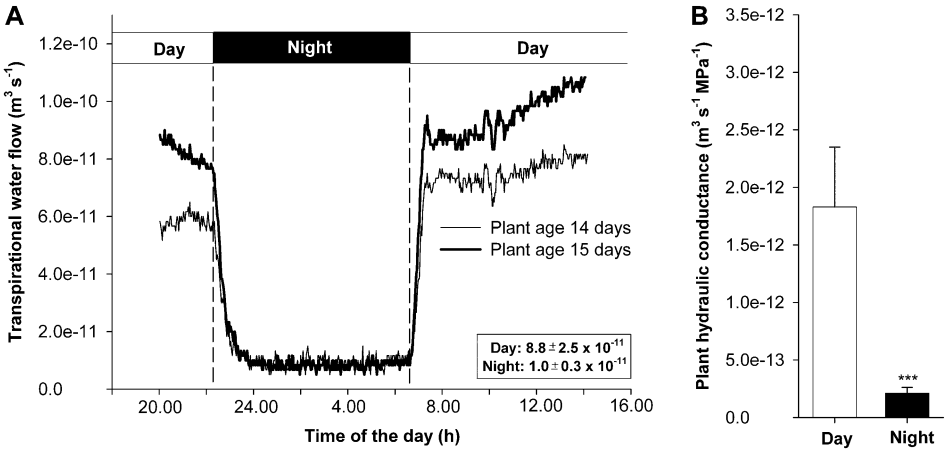

Fig. 5. Day- and night-time transpiration, and whole-plant hydraulic conductance of hydroponically grown barley plants. (A) Typical trace of gravimetrically determined transpiration of two barley plants (14 d and 15 d old at the start of measurement). Average (± SD) day and night transpiration rates of four plants are shown in the insert. (B) Whole-plant hydraulic conductance during the day and night; means±SD of four plants (***, $P<0.001$, Student's *t*-test).

calculated an osmotic flow of 3.86×10^{-11} m^3 s^{-1} for an entire seminal root system. Whole-plant transpiration rate was >2-fold higher. Since xylem osmolality decreases with increasing flow rate (see Munns and Passioura, 1984; Passioura, 1984; Miller, 1985a; Munns, 1985), we had to account for this dilution effect (Fig. 6A, for details of calculations see Supplementary File S1). At flow rates similar to those encountered during daytime transpiration, osmotically driven water uptake accounted for ~10% of root water uptake. The remaining 90% of water uptake was driven through a tension of about –0.15 MPa (Fig. 6).

In comparison, exudation rates measured during the night period on individual seminal roots resulted in a calculated water uptake rate of the entire seminal root system of $3.6\pm1.8\times10^{-11}$ m^3 s^{-1} (means±SD of four experiments, not shown). This was 3.6 times the rate of night-time transpiration of barley plants and meant that osmotic gradients measured between root medium and exudate (0.15 MPa, see Table 2) did not have to be corrected for dilution and could have been sufficient to drive root water uptake during the night in intact plants.

Transpiration in plants with reduced root systems

To assess the significance for root water uptake and plant transpiration of an intact and complete root system, where no major leaks prevent osmotic forces from building up or cause a bypass of radial hydraulic resistance, experiments were conducted on barley plants in which part of the root or root system had been removed through excision, and transpiration measured continuously through a day/night cycle (Fig. 7A). When ~90% of root biomass was removed near the base of the roots, such that the open cut end of the main root axis extended into the nutrient solution, daytime transpirational water loss (per leaf surface area) decreased on average by 70%, from 1.83×10^{-8} (intact plants) to 0.55×10^{-8} m^3 m^{-2} s^{-1} (Fig. 7B). Night-time transpirational water loss was reduced from 0.20×10^{-8} (intact plants) to 0.09×10^{-8} m^3 m^{-2} s^{-1}, a reduction of 55%. In comparison, plants that had three or five from a total of six seminal roots cut at their base, in such a way that the cut ends were not extending into the nutrient solution but sealed with Vaseline, transpired 1.17 and 0.19×10^{-8} m^3 m^{-2} s^{-1} during the day and night, respectively (reductions of 36% and 5%). Plants that had the tips of all seminal roots cut off at the root hair region (mature xylem) showed a 17% reduction in day and 10% reduction in night-time transpiration.

Removal of five of six seminal roots (cut end above nutrient solution, sealed with Vaseline) slowed down leaf 3 elongation and growth, and decreased its final length but did not delay its development. Leaf 3 was the main growing leaf in plants subjected to root excision (Fig. 7C). Removal of three of six seminal roots (cut end in nutrient solution) affected the growth of leaf 3 only slightly.

Discussion

Hydraulics and water uptake of barley roots

There exists much debate as to the role of aquaporins in root water uptake (e.g. Steudle, 2000; Javot and Maurel, 2003; Vandeleur *et al.*, 2005; Katsuhara *et al.*, 2008; Maurel *et al.*, 2008). Molecular studies that involve plants with altered expression levels of particular aquaporin isoforms may appear currently as 'the choice' yet they often suffer from redundancy among aquaporin family members or from secondary effects of transformation events (e.g. Schüssler *et al.*, 2008 and studies cited therein); they also assume that those root types or root zones studied are actually important for whole-plant water uptake and transpiration flow. Aquaporins can only be involved in regulation of root water uptake if at least one membrane is crossed along the flow path from root medium to xylem. This appears to be the case for barley, as recently concluded on theoretical grounds and determination of root reflection coefficients (Knipfer and Fricke, 2010; but see also Steudle and Jeschke, 1983). The more membranes are crossed along the radial path, the potentially more important aquaporins

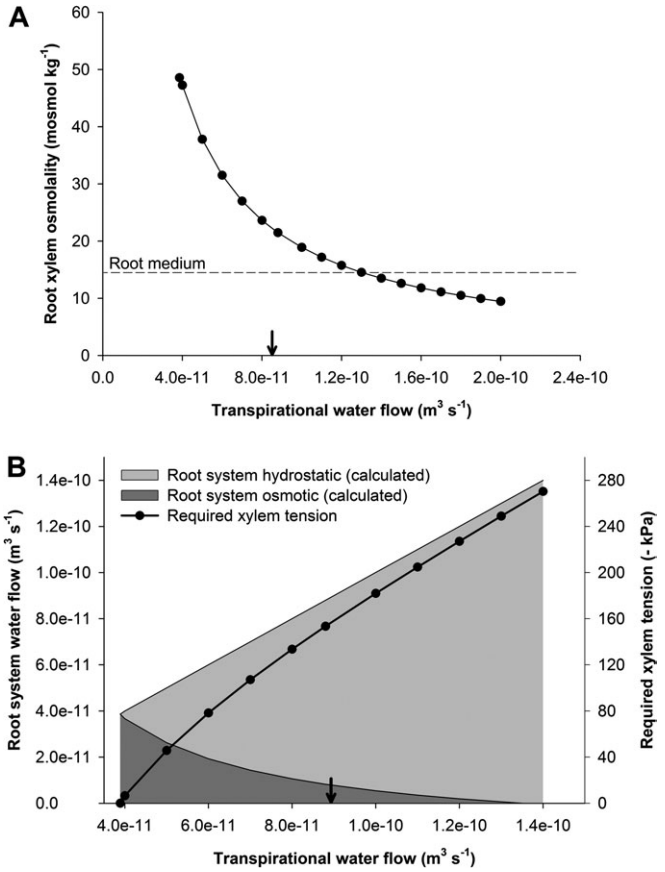

Fig. 6. Calculation of osmotic and hydrostatic forces required to drive root water uptake during the day in transpiring barley plants. (A) Osmolality of exudate of seminal roots was determined as part of exudation experiments. Since the exudate flow rate was ~40% of the rate of transpirational water loss, and since xylem solute concentrations decrease with increasing flow rate (e.g. Munns and Passioura, 1984), a simulation was carried out in which xylem osmolality was calculated in dependence of flow rate (for details, see Equation 13, Supplementary File S1). (B) Using the relationship shown in (A) a water flow driven through osmotic forces (Root system osmotic) was calculated in dependence of transpirational water flow. The difference between transpirational water flow and osmotically driven flow is water flow driven by a tension (Root system hydrostatic). Based on the linear relationship between hydrostatic flow rate and applied tension, as previously determined through vacuum perfusion experiments (Knipfer and Fricke, 2010), this allowed us to calculate the tension required to drive daytime water uptake—in addition to water uptake driven through osmotic gradients. For a transpirational water flow of 8.8×10^{-11} m^3 s^{-1} as measured in the growth chamber for barley plants during the day (see arrow), osmotic forces drove 10% while hydrostatic forces (tension of about –150 kPa) drove the remaining 90% of root water uptake.

become in facilitating water uptake. Osmotic and hydrostatic forces affect the gradient in water potential that drives water uptake along a membranous pathway. If radial water uptake per unit hydrostatic force is significantly higher than that per unit osmotic force, the apoplast should contribute substantially to the flow path.

The present study shows that by far the largest resistance to water movement along the root medium–plant–atmosphere continuum in 14- to 17-d-old hydroponically grown barley is contained within leaves, most likely at the interface between leaf tissues and atmosphere. Notably, the root–shoot junction presents negligible resistance to water flow. The main root type involved in water uptake is the seminal root. The lower contribution to water uptake of adventitious roots is due to a smaller number per plant and surface area per root but not to differences in hydraulic properties between adventitious and seminal roots.

Hydrostatic and osmotic radial conductivity are comparable in seminal roots, with averages differing by only 18%. This suggests that water moves mainly along a membranous path between root medium and xylem. While osmotic forces—in isolated root systems—are more than sufficient to account for water uptake supporting night-time transpiration, tensions are needed to drive 90% of daytime water uptake in transpiring plants. Still, osmotic forces drive 10% of daytime water uptake and are not as negligible as implied by the composite model of water transport in roots (Steudle and Peterson, 1998). The tensions required during daytime transpiration (–0.15 MPa) are rather small and, through lowering of xylem water potential, can drive water uptake along a membranous path. There is neither the need for a low resistance apoplast path nor the need for large tensions to support daytime transpiration in a herbaceous, annual plant such as barley. This leaves open the possibility that up to 100% of root water uptake is controlled through aquaporin function. Root excision experiments suggest that such a control is highly adaptive, and future experiments, in which aquaporin inhibitors such as $HgCl_2$ and H_2O_2 or treatments such as anoxia and acidosis are applied to roots (Tournaire-Roux *et al.*, 2003; Ehlert *et al.*, 2009), will show whether this is really the case.

It is an often reported phenomenon in the study of root and whole-plant hydraulics that the measured water flow rate does not increase linearly with the applied force but levels off at higher forces. This can lead to the (erroneous) assumption that hydraulic conductivity changes in a flow-dependent manner. Passioura and Munns (1984) observed for barley that this non-linearity only applied to plants grown and tested in hydroponics, but not to plants grown and tested in soil or sand culture. The authors suggested that filling of intercellular air spaces could explain some of the difference in hydraulic behaviour of plants. Rather than applying a tension, as in the present study, of –0.02 to –0.08 MPa, the authors (as others) applied an external pressure in the range 0.25–0.8 MPa to the root substrate/medium. The much lower tension applied in the present study most likely explains why water flow rate increased linearly with the applied force (for a relationship, see Knipfer and Fricke, 2010). As the present data show, there should be no need to apply tensions or external pressures larger than (–)0.15 MPa in the study of transpiring barley plants (for maize, see Miller, 1985b), effectively avoiding the riddle of non-linear behaviour of flow.

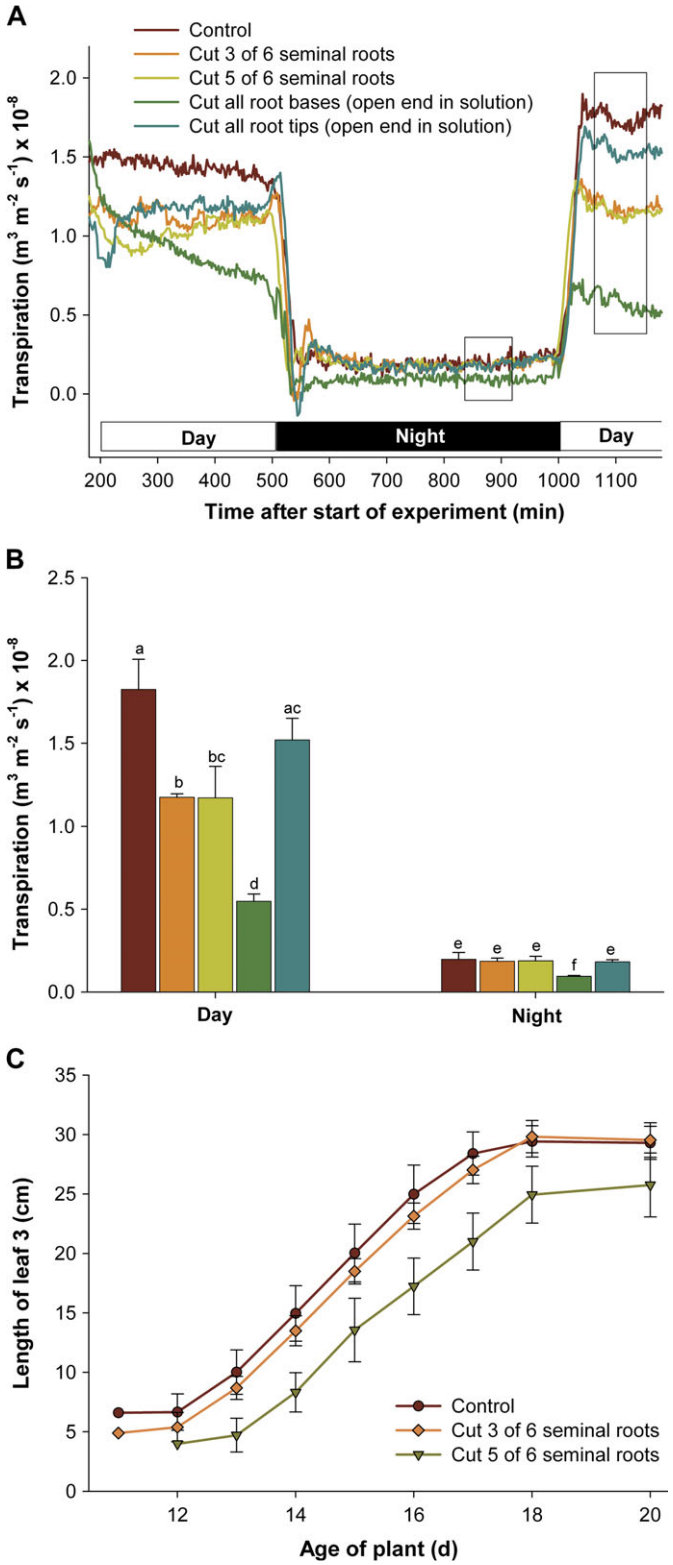

Fig. 7. Day- and night-time transpiration, and leaf growth in hydroponically grown barley plants with (partially) excised root system. Barley plants had an intact seminal root system (control); or had three or five of six seminal roots removed close to the root base, just above the nutrient solution (the cut was sealed with Vaseline); or all six roots removed close to the base, with the cut end extending ~2 cm into the nutrient solution; or had the tip 2 cm of all six seminal roots removed, with the cut end extending into the nutrient solution. Only seminal roots were manipulated, since adventitious roots were developed little and contributed little to water uptake in control plants (compare with Fig. 4). (A) Continuous recordings of transpiration. Transpirational water loss was related to total leaf area as determined at the end of experiments. The part of traces that is boxed in was used to calculate average transpiration rates during the day and night shown in (B) [means±SD of five (control) and three (treatments) plant analyses]. (C) Growth of leaf 3 in plants with an intact root system (control) and in plants that had three or five of six seminal roots excised; means±SD of 12 plant analyses from three batches of plants. In (B) statistically significant differences between transpiration rates are indicated by different letters ($P<0.05$, Student's t-test).

The slightly higher radial hydraulic conductivity obtained for seminal roots through vacuum perfusion as compared with the root pressure probe technique may result from an artefact: when a cut root is fixed to a glass capillary and vacuum is applied, the tension acts on xylem elements in the stele as in an intact transpiring plant, but it acts also on the cortex, effectively bypassing a large portion of the radial resistance to water uptake. Also, application of a tension can be expected to have similar effects on the flooding of intercellular air spaces in the root cortex as application of an external pressure in the root medium through a pressure chamber (Passioura and Munns, 1984). This will effectively increase the contact area between root (internal) surface and nutrient solution and result in a higher water flow (see also Tazawa *et al.*, 1997). In adventitious roots, the almost 2-fold difference between hydrostatically and osmotically driven water flow most likely results from root hairs forming a dense layer apposed to the root surface (Supplementary Fig. S2). This increases unstirred layers (Knipfer *et al.*, 2007), makes mixing of root medium less complete, and results in a lower-than-predicted driving force during osmotic experiments (see also Knipfer and Fricke, 2010). An alternative—or additional—explanation is that water transport through plasmodesmata of endodermal cells driven by hydrostatic gradients (Pickard, 2003) contributes to a slightly higher conductivity in hydrostatic compared with osmotic experiments. Pressure gating of plasmodesmata has been proposed to explain some of the hydraulic behaviour of barley roots (Passioura and Munns, 1984) and has also been demonstrated experimentally for leaf trichomes (Oparka and Prior, 1992).

Root hydraulic properties in relation to root development

Root development affects hydraulic parameters in different ways. Adventitious roots, which are not as fully developed as seminal roots along their main axis, in particular with respect to central metaxylem development, have an axial hydraulic conductance that is several orders of magnitude lower than predicted from anatomical data and measured for seminal roots. As a result, axial hydraulic conductance has some impact on the supply role with water of adventitious but not of seminal roots. The ratio of axial to

radial hydraulic conductance in adventitious roots ranges from 6.6 to 24, and axial conductance limits water transport by up to 13% [1/(6.6+1)]. As adventitious roots grow longer and plants grow older, the proportion of mature central metaxylem will increase and axial resistance decrease.

Radial hydraulic conductivity is similar in seminal and adventitious roots. The endodermis of seminal roots is developed further, particularly with respect to suberin depositions. If a purely apoplastic path contributes to radial water uptake as proposed by the composite model of water transport (Steudle and Peterson, 1998; Steudle, 2000) one would expect seminal roots to have a much lower radial hydraulic conductivity than adventitious roots. This is not the case and further supports the idea that the contribution to root water uptake of a purely apoplastic pathway bypassing endodermal membranes is insignificant in barley (Knipfer and Fricke, 2010) and other grasses studied (Munns, 1985; Garcia *et al.*, 1997; Läuchli *et al.*, 2008).

Night-time transpiration in barley

Night-time transpiration has been explained through incomplete closure of stomata (Caird *et al.*, 2007), but for example Rawson and Clarke (1988) conclude for wheat that cuticular water loss accounts for 13–50% of night-time water loss. The difficulty in distinguishing between the two major leaf conductance mechanisms, stomata and cuticle, is that it is not possible to experimentally verify that a stomata is 100% closed (if it ever is) or to remove 100% of the cuticle. Transpiration rates measured here during the day and night can be converted into their corresponding fluxes (day, 8.8×10^{-4} mol s^{-1} m^{-2}; night, 1.1×10^{-4} mol s^{-1} m^{-2}) and used to calculate leaf permeance (for calculations, see Supplementary File S1). A leaf permeance of 3.3×10^{-3} m s^{-1} for the day and 5.2×10^{-4} m s^{-1} for the night is calculated. The day value differs by only 3% from previously determined stomatal conductivity (3.4×10^{-3} m s^{-1}; Fricke *et al.*, 2004); the night value is about twice as high as previously determined cuticle permeance (2.5×10^{-4} m s^{-1} when the total rather than projected leaf area is used as reference system; Richardson *et al.*, 2007). These data suggest that 50% of the water that is lost through transpiration during the night is lost through the cuticle and that this causes a tension in the xylem which drives root water uptake. A figure of 50% is supported through experiments in which barley plants have all seminal roots cut at the base, with the cut end extending into the nutrient solution. These plants transpire water during the night at about half the rate observed for intact plants. However, despite having the main axis of all roots cut open, plants maintain some positive root pressure (Supplementary Fig. S1), and it cannot be excluded that this residual root pressure facilitates the residual 50% of water uptake.

Root excision: highly adaptable and reproducible

Root excision experiments resulted in some surprising observations, highlighting the adaptability of plants. The effect of excision on plant transpiration rate was remarkably reproducible between plant batches as shown by small standard deviations of means (see Fig. 7B). This points to tight control of transpirational water flow irrespective of the available root mass, at least over the experimental period studied. Night-time transpiration was affected neither by removing five out of six seminal roots, nor by cutting the tips of the main axis of all seminal roots. Cuts effectively caused a large leak, which should have made it impossible for xylem osmolality and root pressure to build up and, similar to exudation experiments on excised root systems, drive water uptake during the night. Clearly, plants were able to maintain night-time water flow rates. Either, the cuts sealed with time, as suggested by the existence of some root pressure (see Supplementary Fig. S1) or xylem tension drove much more water uptake than the 50% suggested by cuticle permeance data. The initial effect on transpiration of cutting roots was similar, regardless of how many (three or five) roots were cut and whether roots had their tips intact or removed. As long as plants had one remaining seminal root, they seemed to cope with both large leaks and greatly reduced root surface area, and entered the night period with transpiration rates very similar to those of undisturbed plants. Since the water potential gradient between root medium and atmosphere, which drives transpirational water flow, was not or little affected by root excision, maintenance of transpiration rates shows that stomatal conductance was not affected through root excision. Instead, larger tensions in the xylem or larger radial conductance in the remaining root tissues must have facilitated maintenance of transpiration. The latter points to compensatory mechanisms at the root level (Vysotskaya *et al.*, 2004), and prime molecular targets for such a mechanism are aquaporins.

Plants that had three or five seminal roots cut transpired at the same rates on the day following the excision event, yet during subsequent days, leaf growth rate and final leaf length was affected mainly in plants that had five roots excised. Two explanations come to mind. First, a shortage of solute supply may force a reduction in cell and leaf elongation rate (see next paragraph). Secondly, the root to shoot ratio is a well-regulated size in plants, which is under tight hormonal control. The latter may 'force' a reduction in shoot growth on plants that have five roots excised.

The transpiration stream provides mineral nutrients to the shoot. Together with solutes imported along the phloem into growing leaf tissues, these minerals are required to enable growing barley leaf cells to maintain osmolality during cell elongation (Fricke and Flowers, 1998). It can be calculated (not shown) from published values of water content and osmolality of growing leaf tissues of barley, from leaf growth rates (Fricke and Peters, 2002), and from daytime transpiration rates reported here that the total xylem concentration of solutes needed to meet the demand of growing leaf cells is ~3–4 mM (see also Wegner and Zimmermann, 2009), irrespective of any demand from mature leaf tissue (Fricke *et al.*, 1994). The xylem concentration in the one remaining seminal root of plants

subjected to root excision would have had to be the same, yet to compensate for the loss of the remaining five roots, the uptake rates of solutes per root surface area would have had to be six times as high as in non-disturbed plants (since that one remaining root facilitated almost as much water uptake as an intact root system). This is unlikely to have imposed a limitation on leaf growth, since the xylem concentration simulated here for transpiring conditions exceeds 20 mM (Fig. 6A).

Transpiration in those plants that had the tips of the main axis of all seminal roots cut off was almost as high as in uninjured plants the day following excision. Different root regions must have taken over the role of the missing tips, both in terms of water supply and in terms of communication between shoot and root. Candidates for such a 'substitute' role would be lateral roots, with their growing tip regions.

Given the present observations, one may ask: why do barley plants actually have six or seven, rather than one or three seminal roots? It can be predicted that the decrease in leaf growth observed in plants with one remaining seminal root affects leaf area expansion and photosynthetic yield in a cumulative and exponential way in the long term and severely compromises plant growth. Transpirational water loss in a natural environment exceeds water loss in a growth chamber and may not be sustainable by barley plants with three roots. Most importantly, barley plants grown in hydroponics receive mineral nutrients almost 'on a plate' in a highly convective medium with no diffusion barriers, whereas roots in a natural setting need to explore a soil environment that can be patchy in its mineral nutrient distribution and pose major diffusion barriers.

Supplementary data

The following data are available at *JXB* online.

Supplementary File S1. Detailed description of calculations of hydraulic and associated (e.g. root surface area) parameters.

Supplementary Table S1. Summary of measurements of water relations of leaf epidermal cells and calculation of the driving force for transpirational water loss during the day and night period.

Supplementary Fig. S1. Response of root pressure to root excision, as measured with the root pressure probe, in barley, over a period of several hours to days.

Supplementary Fig. S2. Cross-section of an adventitious root of hydroponically grown barley, highlighting a dense layer of root hairs covering the root surface.

Supplementary Fig. S3. Comparison of immature and mature central metaxylem in seminal roots of hydroponically grown barley

Supplementary Fig. S4. Two-compartment analyses of a typical osmotic pressure relaxation, which was induced with NaCl and measured with the root pressure probe, for a seminal root of barley.

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References

Bramley H, Turner NC, Turner DW, Tyerman SD. 2009. Roles of morphology, anatomy, and aquaporins in determining contrasting hydraulic behavior of roots. *Plant Physiology* **150,** 348–364.

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

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

Caird MA, Richards JH, Donovan LA. 2007. Nighttime stomatal conductance and transpiration in C_3 and C_4 plants. *Plant Physiology* **143,** 4–10.

Ehlert C, Maurel C, Tardieu F, Simonneau T. 2009. Aquaporin-mediated reduction in maize root hydraulic conductivity impacts cell turgor and leaf elongation even without changing transpiration. *Plant Physiology* **150,** 1093–1104.

Enstone DE, Peterson CA, Ma F. 2003. Root endodermis and exodermis: structure, function, and responses to environment. *Journal of Plant Growth Regulation* **21,** 335–351.

Esau K. 1965. *Plant anatomy*, 2nd edition. New York: Wiley and Sons, Inc.

Frensch J. 1997. Primary response of root and leaf elongation to water deficits in the atmosphere and soil solution. *Journal of Experimental Botany* **48,** 985–999.

Frensch J, Steudle E. 1989. Axial and radial hydraulic resistance to roots of maize (*Zea mays L.*) *Plant Physiology* **91,** 719–726.

Fricke W. 2002. Biophysical limitation of cell elongation in cereal leaves. *Annals of Botany* **90,** 157–167.

Fricke W, Akhiyarova G, Veselov D, Kudoyarova G. 2004. Rapid and tissue-specific changes in ABA and in growth rate in response to salinity in barley leaves. *Journal of Experimental Botany* **55,** 1115–1123.

Fricke W, Akhiyarova G, Wei W, *et al*. 2006. The short-term growth response to salt of the developing barley leaf. *Journal of Experimental Botany* **57,** 1079–1095.

Fricke W, Flowers TJ. 1998. Control of leaf cell elongation in barley. Generation rates of osmotic pressure and turgor, and growth-associated water potential gradients. *Planta* **206,** 53–65.

Fricke W, Leigh RA, Tomos AD. 1994. Epidermal solute concentrations and osmolality in barley leaves studied at the single-cell level. Changes along the leaf blade, during leaf aging and NaCl stress. *Planta* **192,** 317–323.

Fricke W, McDonald AJS, Mattson-Djos L. 1997. Why do leaves and leaf cells of N-limited barley elongate at reduced rates? *Planta* **202,** 522–530.

Fricke W, Peters WS. 2002. The biophysics of leaf growth in salt-stressed barley: a study at the cell level. *Plant Physiology* **129,** 1–15.

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

Hachez C, Moshelion M, Zelazny E, Cavez D, Chaumont F. 2006. Localisation and quantification of plasma membrane aquaporin expression in maize primary roots: a clue to understand their role as cellular plumbers. *Plant Molecular Biology* **62,** 305–323.

Hacket C. 1967. A study of the root system of barley. I. Effects of nutrition on two varieties. *New Phytologist* **67,** 287–299.

Hacket C. 1969. A study of the root system of barley. II. Relationships between root dimensions and nutrient uptake. *New Phytologist* **68,** 1023–1030.

Hacket C, Bartlett BO. 1971. A study of the root system of barley. III. Branching pattern. *New Phytologist* **70,** 409–413.

Heimisch C. 1951. Development of vascular tissues in barley roots. *American Journal of Botany* **38,** 523–537.

Javot H, Maurel C. 2003. The role of aquaporins in root water uptake. *Annals of Botany* **90,** 301–313.

Katsuhara M, Hanba Y, Shiratake K, Maeshima M. 2008. Expanding roles of plant aquaporins in plasma membranes and cell organelles. *Functional Plant Biology* **35,** 1–14.

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

Knipfer T, Fricke W. 2010. Root pressure and a solute reflection coefficient close to unity exclude a purely apoplastic pathway of radial water transport in barley (*Hordeum vulgare*). *New Phytologist* **187,** 159–170.

Landsberg JJ, Fowkes ND. 1978. Water movement through plant roots. *Annals of Botany* **42,** 493–508.

Läuchli A, James RA, Munns R, Huang C, McCully M. 2008. Cell-specific localization of Na^+ in roots of durum wheat, and possible control points for salt exclusion. *Plant, Cell and Environment* **31,** 1565–1574.

Matre P, Cochard H, Durand JL. 2001. Hydraulic architecture and water flow in growing grass tillers (*Festuca arundinacea Schreb*.). *Plant, Cell and Environment* **24,** 65–76.

Maurel C, Simonneau T, Sutka M. 2010. The significance of roots as hydraulic rheostats. *Journal of Experimental Botany* **61,** 3191–3198.

Maurel C, Verdoucq L, Luu DT, Santoni V. 2008. Plant aquaporins: membrane channels with multiple integrated functions. *Annual Review of Plant Biology* **59,** 595–624.

Miller DM. 1985*a*. Studies of root function in *Zea mays*: III. Xylem sap composition at maximum root pressure provides evidence of active transport into the xylem and a measurement of reflection coefficient of the root. *Plant Physiology* **77,** 162–167.

Miller DM. 1985*b*. Studies of root function in *Zea mays*: IV. Effects of applied pressure on the hydraulic conductivity and volume flow through the excised root. *Plant Physiology* **77,** 168–174.

Miller DM. 1987. Errors in the measurement of root pressure and exudation volume flow rate caused by damage during the transfer of unsupported roots between solutions. *Plant Physiology* **85,** 164–166.

Munns R. 1985. Na^+, K^+ and Cl^- in xylem sap flowing to shoots of NaCl-treated barley. *Journal of Experimental Botany* **36,** 1032–1042.

Munns R, Passioura JB. 1984. Hydraulic resistance of plants. III. Effects of NaCl in barley and lupin. *Australian Journal of Plant Physiology* **11,** 351–359.

Murai-Hatano M, Kuwagata T, Sakurai J, Nonami H, Ahamed A, Nagasuga K, Matsunami T, Fukushi K, Maeshima M, Okada M. 2008. Effect of low root temperature on hydraulic conductivity of rice plants and the possible role of aquaporins. *Plant Cell Physiology* **49,** 1294–1305.

O'Brien TP, Feder N, McCully ME. 1964. Polychromatic staining of plant cell walls by toluidine blue-O. *Protoplasma* **59,** 368–373.

Oparka KJ, Prior DAM. 1992. Direct evidence for pressure-generated closure of plasmodesmata. *Plant Journal* **2,** 741–750.

Passioura JB. 1984. Hydraulic resistance of plants. I. Constant or variable? *Australian Journal of Plant Physiology* **11,** 333–339.

Passioura JB, Munns R. 1984. Hydraulic resistance of plants. II. Effects of rooting medium, and time of day, in barley and lupin. *Australian Journal of Plant Physiology* **11,** 341–350.

Pickard WF. 2003. The riddle of root pressure. I. Putting Maxwell's demon to rest. *Functional Plant Biology* **30,** 121–134.

Rawson HM, Clarke JM. 1988. Nocturnal transpiration in wheat. *Australian Journal of Plant Physiology* **15,** 397–406.

Richardson A, Wojciechowski T, Franke R, Schreiber L, Kerstiens G, Jarvis M, Fricke W. 2007. Cuticular permeance in relation to wax and cutin development along the growing barley (*Hordeum vulgare*) leaf. *Planta* **225,** 1471–1481.

Schreiber L, Hartmann K, Skrabs M, Zeier J. 1999. Apoplastic barriers in roots: chemical composition of endodermal and hypodermal cell walls. *Journal of Experimental Botany* **50,** 1267–1280.

Schüssler MD, Alexandersson E, Bienert GP, Kichey T, Laursen KH, Johanson U, Kjellbom P, Schjoerring JK, Jahn TP. 2008. The effects of the loss of TIP1;1 and TIP1;2 aquaporins in *Arabidopsis thaliana. Plant Journal* **56,** 756–767.

Steudle E. 2000. Water uptake by plant roots: an integration of views. *Plant and Soil* **226,** 46–56.

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

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

Tazawa M, Ohkuma E, Shibasaka M, Nakashima S. 1997. Mercurial-sensitive water transport in barley roots. *Journal of Plant Research* **110,** 435–442.

Tomos AD, Hinde P, Richardson P, Pritchard J, Fricke W. 1994. Microsampling and measurements of solutes in single cells. In: Harris N,

Oparka KJ, eds. *Plant cell biology: a practical approach*. Oxford: IRL Press, 297–314.

Tournaire-Roux C, Sutka M, Javot H, Gout E, Gerbeau P, Luu DT, Bligny R, Maurel C. 2003. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. *Nature* **425,** 393–397.

Tyree MT, Koh S, Sands P. 2005. The determination of membrane transport parameters with the cell pressure probe: theory suggests that unstirred layers have significant impact. *Plant, Cell and Environment* **28,** 1475–1486.

Vandeleur R, Niemietz C, Tilbrook J, Tyerman SD. 2005. Role of aquaporins in root responses to irrigation. *Plant and Soil* **274,** 141–161.

van den Honert TH. 1948. Water transport in plants as a catenary process. *Discussions of the Faraday Society* **3,** 146–153.

Vysotskaya LB, Arkhipova TN, Timergalina LN, Dedov AV, Veselov SY, Kudoyarova GR. 2004. Effect of partial root excision on transpiration, root hydraulic conductance and leaf growth in wheat seedlings. *Plant Physiology and Biochemistry* **42,** 251–255.

Wegner LH, Zimmermann U. 2009. Hydraulic conductance and K^+ transport into the xylem depend on radial volume flow, rather than on xylem pressure, in roots of intact, transpiring maize seedlings. *New Phytologist* **181,** 361–373.