SUPPLEMENTARY FILE S1 – EQUATIONS FOR CALCULATION OF HYDRAULIC AND ASSOCIATED PARAMETERS

(see also Knipfer and Fricke, 2010)

Root pressure probe experiments

Halftimes ($T_{1/2}$) of hydrostatic and osmotic pressure relaxations were used for calculation of root hydraulic conductance ($L_r$, in m$^3$ s$^{-1}$ MPa$^{-1}$) (Eqn 1). Hydraulic conductance was related to the total surface area of root(s) ($A_r$) to calculate hydraulic conductivity ($L_{pr}$, in m s$^{-1}$ MPa$^{-1}$) (Eqn 2).

$$L_r = \frac{\ln 2}{T_{1/2} \beta}$$  \hspace{1cm} (Eqn 1)

$$L_{pr} = \frac{\ln 2}{T_{1/2} \beta A_r}$$  \hspace{1cm} (Eqn 2)

The elastic modulus of the measuring system ($\beta$; 3.7 and 3.0 x 10$^9$ MPa m$^{-3}$ for seminal and adventitious roots, respectively) was determined by inducing step changes of volume and measuring the resulting changes of root pressure.

Vacuum perfusion experiments

Root hydraulic conductance and conductivity was determined as water uptake from the root medium, which was measured as gravimetric loss in weight of nutrient solution. Water uptake was driven osmotically (exudation) or occurred in response to applied hydrostatic partial vacuums ($\Delta P_v$). Prior to application of partial vacuum, the osmotic flow rate, $Q_r^{os}$, was measured from the linear part of the flow versus time plot. The driving force ($\Delta C$) for the osmotic water flow was the difference in osmotic pressure ($\Delta \pi$, in MPa) between the root
medium \((RT \times C_{\text{medium}})\) and xylem \((RT \times C_{\text{xylem}})\). Water loss from the beaker containing nutrient solution due to evaporation, \(Q_{\text{eva}}\), was negligible and was corrected for according to:

\[
Q_{r}^{\text{os}} - Q_{\text{eva}} = L_p \cdot A_r \cdot (RT \cdot \Delta C) = L_r \cdot (RT \cdot \Delta C) \quad \text{(Eqn 3)}
\]

The hydrostatic flow rate, \(Q_{r}^{\text{by}}\), in response to partial vacuum was determined from the linear part of the flow \textit{versus} time plot after \(Q_{\text{eva}}\) and \(Q_{r}^{\text{os}}\) were subtracted. \(L_p\) and \(L_r\) was related to measured \(Q_{r}^{\text{by}}\) as follows,

\[
Q_{r}^{\text{by}} - (Q_{\text{eva}} + Q_{r}^{\text{os}}) = L_p \cdot A_r \cdot \Delta P_v = L_r \cdot \Delta P_v \quad \text{(Eqn 4)}
\]

Root exudation

Root water uptake was measured as osmotically-driven water flow into a glass capillary attached to the cut (basal) end of a root or root system. The rise of xylem sap in the capillary was recorded at time intervals of 5 min over one hour, and \(Q_{r}^{\text{os}}\) was calculated from the linear part of the flow \textit{versus} time plot. Osmotic root hydraulic parameters were calculated according to \textit{Eqn 3}.

Hydraulics of entire root system

A vacuum-set up similar to the one used for individual roots was used. The individual hydraulic conductance of the root system \((L_R)\), root/shoot junction \((L_{R/S})\), and mesocotyl \((L_{\text{meso}})\) was calculated as follows,

\[
\frac{1}{L} = \frac{1}{L_R} + \frac{1}{L_{R/S}} + \frac{1}{L_{\text{meso}}} = R_R + R_{R/S} + R_{\text{meso}} = R \quad \text{(Eqn 5)}
\]

The corresponding resistances, \(R\) (s MPa m\(^{-3}\)), are the inverse of \(L\).
Root surface area

The surface area of roots, \( A_r \), was determined after each hydraulic experiment by measuring the length (\( l \)) and radius (\( r \)) of the main axis of roots and the number (\( n_{lateral} \)), length (\( l_{lateral} \)), and radius (\( r_{lateral} \)) of lateral roots. Treating the root as a cylinder, \( A_r \) was calculated as follows:

\[
A_r = (2\pi \cdot l \cdot r^2) + n_{lateral} \cdot (2\pi \cdot l_{lateral} \cdot r_{lateral}^2)
\]

(Eqn 6)

Transpiration and whole-plant hydraulics

Transpiration rates (\( T_{plant} \), m\(^3\) s\(^{-1}\)) of entire plants were determined gravimetrically in the growth chamber. The hydraulic conductance of the leaf, \( L_{Leaf} = \frac{1}{R_{Leaf}} \) (m\(^3\) s\(^{-1}\) MPa\(^{-1}\)) and of the whole-plant, \( L_{Plant} = \frac{1}{R_{Plant}} \) (m\(^3\) s\(^{-1}\) MPa\(^{-1}\)), were calculated according to:

\[
L_{Plant} = \frac{1}{R_{Plant}} = \frac{T_{Plant}}{\Delta \Psi_{Medium-Air}},
\]

(Eqn 7)

and

\[
L_{Leaf} = \frac{1}{R_{Leaf}} = \frac{T_{Plant}}{\Delta \Psi_{Leaf-Air}},
\]

(Eqn 8)

Determination of conductance was based on water potential gradients given in Supplementary Table S1. \( \Delta \Psi_{Leaf-Air} \) of Eqn 8 was calculated according to:

\[
\Delta \Psi_{Leaf-Air} = \Psi_{air} - \Psi_{xylem} = \frac{RT}{V_w} \cdot \ln\left(\frac{e}{100}\right) - \left[ P_{cell} - RT \cdot C_{cell} \right]
\]

(Eqn 9)

where \( T \) is the temperature in the climate chamber during the day (\( T = 294 \) K) and night (\( T = 289 \) K), \( V_w \) is the molar volume of water (in m\(^3\) mol\(^{-1}\) at 20\(^\circ\)C), and \( RH \) is the relative humidity (70%). This results in \( \Psi_{air} \) (day) being -48.28 MPa and \( \Psi_{air} \) (night) being -47.46 MPa. An upper estimate of \( \Psi_{xylem} \) was made by determining the water potential of epidermal cells (\( \Psi_{cell} = P_{cell} - RT \times C_{cell} \)) of the transpiring blade of leaf three of plants in the growth chamber, assuming that leaf xylem and epidermis are in good hydraulic connection. Turgor pressure (\( P_{cell} \)) of cells was determined with a cell-pressure probe for plants in the
growth chamber. Osmolality (mosmol kg\(^{-1}\)) was determined for bulk leaf extract, which was obtained through a centrifugation technique, using picolitre osmometry (Fricke and Peters, 2002) and converted into osmotic pressure (40.75 mosmol kg\(^{-1}\) corresponds to 0.1 MPa osmotic pressure at room temperature). Osmolality of bulk leaf extract is a close approximation of that of epidermal cells (Fricke 2004). Turgor pressure was 0.80 ± 0.06 MPa and 1.12 ± 0.21 MPa (average ± SD of 10 cell analyses) and osmotic pressure was 1.06 ± 0.24 MPa and 1.24 ± 0.29 MPa in epidermal cells of leaf three and two, respectively, leaf two being the main transpiring surface of plants (see also Supplementary Table S1). As a result, the water potential of leaf two and three was -0.26 and -0.12 MPa, respectively, during the day, and these figures represented most negative estimates of \(\Psi_{xylem}\) in these leaves. The difference in water potential between leaf internal air spaces and ambient air, \(\Delta \Psi_{\text{leaf}}\), was -48.16 MPa in the light- and -47.34 MPa in the dark-period. (It is likely that leaf water potential in the dark was close to 0 MPa and that \(\Delta \Psi_{\text{leaf}}\) was by 0.12-0.26 MPa more negative; this would have resulted in less than 1% error in the calculation of conductance). Based on measurements of osmolality of the medium (mean of 15 mosmol kg\(^{-1}\)), and \(P = 0\) MPa, \(\Psi_{\text{medium}}\) averaged 0.04 MPa which resulted in \(\Delta \Psi_{\text{medium-air}}\) of -48.32 MPa (Day) and -47.50 MPa (Night).

Transport resistances along barley plants

Barley plants were modelled as an electric circuit (Fig. 1B, main manuscript). Hydraulic resistances, whether at root or cell level, are not necessarily constant, but can be affected by environmental stress or plant developmental stage (e.g. Bramley et al., 2009; Murai-Hatano et al., 2008). Therefore, the hydraulic parameters listed below can be viewed as variables. However, since we know neither the causal nor quantitative relationship between parameters
and variables, and since we grew and analysed plants under well-defined conditions, we did not include this variability in the model below but treated parameters as constants.

The hydraulic transport resistance of the whole-plant, $R_{\text{Plant}}$, was determined from the difference in water potential between air and root medium ($\Delta \Psi_{\text{Medium-Air}} = \Psi_{\text{air}} - \Psi_{\text{medium}}$, driving force) and the overall water flow through the plant, $F_{\text{plant}}$. The individual transport resistances at the root level ($R_R$), root/shoot junction ($R_{R/S}$), and shoot level ($R_S$), were determined by knowing their individual corresponding driving forces, i.e. $\Delta \Psi_{\text{Medium-Root}}$, $\Delta \Psi_{\text{Root-Shoot}}$, and $\Delta \Psi_{\text{Leaf-Air}}$, and their corresponding water flows, i.e. $F_R$, $F_{R/S}$ and $F_S$.

Transport resistances were related to their driving forces and flows as follows,

$$\frac{1}{R_{\text{Plant}}} = \frac{F_{\text{plant}}}{\Delta \Psi_{\text{Medium-Air}}} = \frac{1}{R_R} + \frac{1}{R_{R/S}} + \frac{1}{R_S} = \frac{F_R}{\Delta \Psi_{\text{Medium-Root}}} + \frac{F_{R/S}}{\Delta \Psi_{\text{Root-Shoot}}} + \frac{F_S}{\Delta \Psi_{\text{Leaf-Air}}} \quad \text{(Eqn 10)}$$

Seminal (SR) and adventitious (AR) roots were treated as parallel arranged hydraulic resistances (Fig. 1C, main manuscript). The hydraulic resistance of the entire root-system, $R_R$, was calculated from the hydraulic resistance, $R_{SR}$ and $R_{AR}$, of individual roots and the number per plant of roots ($n_{SR}$ and $n_{AR}$) as follows,

$$\frac{1}{R_R} = \left( \frac{1}{R_{SR}} \cdot n_{SR} \right) + \left( \frac{1}{R_{AR}} \cdot n_{AR} \right) \quad \text{(Eqn 11)}$$

In individual roots, axial and radial hydraulic resistances, $R_{\text{axial}}$ and $R_{\text{radial}}$, where treated as serial resistances (Fig. 1D, main manuscript). $R_{\text{radial}}$ was separated into two parallel resistances, one representing the apoplastic ($R_{\text{radial}}^{\text{hy}}$) and one representing the cell-to-cell pathway ($R_{\text{radial}}^{\text{os}}$). Axial and radial hydraulic resistances of seminal and adventitious roots were related as follows to the overall hydraulic resistance, $R_{SR}$ and $R_{AR}$ of an individual root,

$$R_{SR} \cdot R_{AR} = R_{\text{axial}} \left( \frac{1}{R_{\text{radial}}^{\text{hy}}} + \frac{1}{R_{\text{radial}}^{\text{os}}} \right)^{-1} \quad \text{(Eqn 12)}$$
Dilution of xylem sap (exudate) osmolality in dependence of transpirational water flow

The rate of osmotic driven water flow depends on the osmotic gradient between root medium and root xylem. Hence, osmotic water flow and gradients as measured during root exudation were representative for a non-transpiring plant. When water flow was increasing as a result of transpiration, solutes in the root xylem became diluted. This could lead to a reduction in the osmotic driving force (Passioura, 1984; Munns and Passioura, 1984). Hence, the gradient in (osmotic pressure) osmolality (∆OP$_{os}$) between root xylem (OP$_{xylem}$) and root medium (OP$_{medium}$), which was used for the calculation of root osmotic hydraulic conductance and conductivity had to be corrected for increasing transpirational flow rates of the plant (Q$_{Plant-x}$), according to Eqn 13:

$$\Delta OP_{os-x} = OP_{os-x} - OP_{medium} = \frac{Q_{os-0}}{Q_{Plant-x}} \cdot OP_{xylem-0} - OP_{medium} \quad (\text{Eqn 13})$$

By knowing the osmotic flow rate (Q$_{os-0}$) and root xylem osmolality (OP$_{xylem-x}$) at zero transpiration, as measured during root exudation, the gradient in osmolality (∆OP$_{os-x}$) could be calculated for a given transpirational water flow (Q$_{Plant-x}$). The dilution factor of OP$_{os-x}$ was given by the ratio of Q$_{os-0}$/Q$_{Plant-x}$. Calculations of OP$_{xylem-x}$ were in agreement with measurements of the reduction of xylem osmolality in barley plants in relation to increasing transpirational water flow by Munns and Passioura (1984). The simulated relationship is shown in Figure 6A (main Manuscript).

The decrease of osmotic flow rates (Q$_{os-x}$) with increasing Q$_{Plant-x}$ was calculated for the mean osmotic hydraulic conductance as presented in Table 3 (main Manuscript), according to Eqn 14:

$$Q_{os-x} = \frac{\Delta OP_{os-x}}{\Delta OP_{os-0}} \cdot Q_{os-0} = \frac{\Delta OP_{os-x}}{OP_{xylem-0} - OP_{medium}} \cdot (L_{os} \cdot \Delta P_{os}) \cdot n_{roots} \quad (\text{Eqn 14})$$
The hydrostatic driving force of the root-system ($\Delta P_{hy-x}$) which was required to compensate for $Q_{plant-x}$ was calculated according to Eqn. 15, depending on the reduction of $Q_{os-x}$. $\Delta P_{hy-x}$ was calculated for the mean root hydrostatic conductance of Tab. 3 (main Manuscript),

$$\Delta P_{hy-x} = \frac{Q_{hy-x}}{L_{hy} \cdot n_{roots}} = \frac{Q_{Plant-x} - Q_{os-x}}{L_{hy} \cdot n_{roots}}. \quad (Eqn \ 15)$$

Units:

OP = osmolality (mosmol kg$^{-1}$)

$Q$ = Volume flow rate (m$^3$ s$^{-1}$)

$P$ = Pressure (MPa)

$L$ = conductance (m$^3$ s$^{-1}$ MPa$^{-1}$)

Leaf permeance and night-time transpiration

Transpiration rates and their corresponding fluxes (day, $8.9 \times 10^{-4}$ mols$^{-1}$m$^{-2}$; night, $1.1 \times 10^{-4}$ mols$^{-1}$m$^{-2}$) as determined in the present study were compared with permeance values ($p$) of the cuticle ($2.5 \times 10^{-4}$ m s$^{-1}$ when the total rather than projected leaf area is used as reference system; Richardson et al., 2007) and stomata ($= 34 \times 10^{-4}$ ms$^{-1}$). Day and night-time fluxes were related to permeance values according to,

$$p = \frac{f}{\Delta c_{wv}} \quad (Eqn \ 16)$$

where $f$ is the flux in mols$^{-1}$m$^{-2}$, and $\Delta c_{wv}$ is the difference of the saturation concentration of water vapour in mol m$^{-3}$ (Nobel 1991, p. 419, 549). $\Delta c_{wv}$ was estimated from the difference of $c_{wv}$ between the leaf (hum. $\approx 99.9\%$) and the air (hum. $\approx 70\%$). During the day at 21°C, $c_{wv}(\text{leaf})$ was 1.017 mol m$^{-3}$ and $c_{wv}(\text{air})$ was 0.713 mol m$^{-3}$ resulting in a $\Delta c_{wv}(\text{day})$ of 0.304 mol m$^{-3}$. During the night at 15°C, $c_{wv}(\text{leaf})$ was 0.712 mol m$^{-3}$ and $c_{wv}(\text{air})$ was 0.499 mol m$^{-3}$.
resulting in a $\Delta c_{\text{wv}}(\text{night})$ of 0.213 mol m$^{-3}$. According to Eqn. 16, this calculates to a $p(\text{day})$ of $33 \times 10^{-4}$ ms$^{-1}$ and a $p(\text{night})$ of $5.2 \times 10^{-4}$ ms$^{-1}$.
**Supplementary Table S1**

**Table S1.** Calculation of leaf water potential ($\Psi_{\text{leaf}}$) and of the difference in water potential between leaf tissues and atmosphere ($\Delta \Psi_{\text{leaf-air}}$), and medium and atmosphere ($\Delta \Psi_{\text{medium-air}}$). The water potential gradient $\Delta \Psi_{\text{medium-air}}$ was used for calculation of plant hydraulic conductance. Water potential of the root medium was determined from osmolality of medium (see below). Leaf water potential was calculated as the difference in turgor of epidermal cells (as determined with the cell pressure probe in the growth chamber for transpiring plants) and osmolality of bulk leaf extract (as determined by picolitre osmometry). It has previously been shown that osmolality of bulk leaf extracts and epidermal cell saps is similar (Fricke *et al.*, 1994, Fricke and Peters, 2002). Osmolality was converted into osmotic pressure by using a conversion of 40.75 mosmol kg$^{-1}$ of osmolality being equivalent to 0.1 MPa of osmotic pressure. The vapour pressure deficit and water potential of the air was calculated using an ambient (growth chamber) relative humidity of 70 % and a day and night temperature of 294 K and 289 K, respectively. Analyses were carried out on the mature leaf two - the main transpiring leaf of barley plants analysed - and on the emerged portion of the developing leaf three. All measurements are given in MPa; results for turgor, osmotic pressure and leaf water potential are means ± SD of (n) leaf analyses. For details of calculations, see Supplementary file S1.
|     | Leaf cell turgor | Leaf osmotic pressure | Air water potential, day/night | Medium water potential | Difference in water potential | Difference in water potential |
|-----|------------------|-----------------------|-------------------------------|------------------------|------------------------------|------------------------------|
| 2   | 1.12 ± 0.21(6)   | 1.24 ± 0.29(7)        | -48.28 / -0.12                | 0.04 ± 0.01 (12)      | 48.32 /                      | 48.16 /                      |
| 3   | 0.80 ± 0.06(4)   | 1.06 ± 0.24(7)        | -48.28 / -0.26                | 0.04 ± 0.01 (12)      | 48.32 /                      | 48.02 /                      |
**Supplementary Figure S1**

**A**

![Graph A showing root pressure response to root excision over several hours to days.](image)

**B**

![Graph B showing root pressure over days.](image)

**Figure 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. (A) A 14-d old barley plant, which had six seminal roots and little developed adventitious roots, was placed in nutrient solution. One seminal root was fixed from the tip end (cut at 5 cm from the tip) to a root pressure probe, such that it was still connected to the remaining barley plant, with five intact seminal...
roots. Root pressure gradually increased and reached a stable value of 0.24 MPa after 1 h. Shortly afterwards, the remaining five seminal roots were cut open, one by one, in the root hair region (arrows). After all seminal roots had been cut the remaining root pressure was 0.1 MPa. (B) When the setup used in (A) was left over a period of two days, root pressure gradually recovered to a new value after 18 h (0.13 MPa), dropped subsequently, and then increased again to about 0.13 MPa.
Supplementary Figure S2

**Figure S2.** Cross-section of an adventitious root of hydroponically-grown barley, highlighting a dense layer of root hairs covering the root surface. A dense layer of root hairs covers the surface of roots. Hairs appear much more abundant than on the cross-section of an adventitious root shown in Fig. 3E of the main manuscript. This is partly because the present section was much thicker (1-2 mm compared to about 0.1 mm) and partly because the root was 1-2 d advanced in development. The picture was taken with a fluorescence stereomicroscope (M165FC, LEICA, Wetzlar, Germany), and the barley plants was 16 d old. The picture is a colour-overlay of the auto fluorescence of the root using an UV- and GFP-filter; scale bar, 225 µm.
Figure S3. Comparison of (A) immature and (B) mature central metaxylem (cMX) in seminal roots of hydroponically-grown barley. Free-hand cross-sections were taken 5-10 mm from the root tip (A) and 20-30 mm from the root tip (B). Section were stained for 30 min with 0.1% berberine-hemisulfate and counterstained for 1-3 min with 0.5% toluidine blue as used for detection of Casparian bands and 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 with strongly lignified cell walls (bright blue yellowish signal) were classified as mature (Brundrett et al., 1988; Bramley et al., 2009). Scale bar = 55 µm.
Figure 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. Measured halftimes of the initial water exit phase were around 14.6 s ($T_{1/2}^{os}$) and that of the second solute (and associated water) uptake phase were around 320 s ($T_{1/2}^{solute}$). Since NaCl is a permeant solute, this could have resulted in a “real” halftime of the initial phase ($T_{1/2}^{os*}$) which is smaller than the halftime of 14.6 s used for calculation of osmotic root hydraulic conductivity. According to a two-compartment analyses, using $T_{1/2}^{os}$ and $T_{1/2}^{solute}$ we can determine $T_{1/2}^{os*}$ of the corrected initial response curve ($P(t)_{initial}^{*} = P(t)_{initial} - P(t)_{solute}$, see Eqns 1 and 2), without the contribution of the second phase. The measured bi-phasic exponential response curve for root pressure ($P$) and a maximum pressure drop after addition of NaCl ($\Delta P_{solute}$) is represented by a exponential initial phase (green, $P(t)_{initial}$, Eqn 1) and a second
solute phase (red, $P(t)^{\text{solute}}$, Eqn 2) which reflects only water uptake associated with solute uptake (red line):

$$P(t)^{\text{initial}} = \Delta P_{\text{solute}} \cdot e^{-\frac{\ln 2}{T_{1/2}} t} \quad \text{(Eqn 1)}$$

$$P(t)^{\text{solute}} = \Delta P_{\text{solute}} \cdot (1 - e^{-\frac{\ln 2}{T_{1/2}^{\text{solute}} t}}) \quad \text{(Eqn 2)}$$

As can be seen, correction for the solute uptake phase does not alter the originally determined half-time (and osmotic hydraulic conductivity) of the rapid water exit phase.