Aquaporin-facilitated water uptake in barley (*Hordeum vulgare* L.) roots

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Supplementary File S1

Methodological details of hydraulic measurements

Root exudation experiments

For measurements of individual roots, seminal and adventitious roots were excised at the base under water and inserted at their base about 10 mm into a glass capillary (ϕ = 0.55 mm) and sealed to the capillary with a drop of super glue. The rise of xylem sap in the capillary was recorded at time intervals of 5 min for one hour. Exudate was stored under liquid paraffin in microcentrifuge tubes in the freezer, and analysed within days for osmolality using picolitre osmometry (Fricke and Peters, 2002). Osmotic flow rates (Q_r^{os}) were calculated from the linear part of the flow *versus* time plots. The driving force was the difference in osmotic pressure ($\Delta\pi$, MPa) between root xylem (π_{xylem}) and medium (π_{medium}) multiplied by the corresponding reflection coefficient for solutes (σ = 1.0; Knipfer and Fricke, 2010a). Root hydraulic conductivity was calculated as follows:

$$Lp_r = \frac{Q_r^{os}}{A_r \cdot \sigma \cdot (\pi_{xylem} - \pi_{medium})}. \qquad \text{(Eqn 1)}$$

Treating the root as a cylinder, the total surface area of root(s) (A_r) was determined 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, when present:

$$A_r = (2\pi \cdot l \cdot r) + n_{lateral} \cdot (2\pi \cdot l_{lateral} \cdot r_{lateral}) \qquad \text{(Eqn 2)}$$

The surface area A_r of the entire root system was the sum of A_rs of individual roots.

Cell pressure probe experiments

Hydraulic analyses of cortical cells were performed with the cell pressure probe on seminal and adventitious roots of intact plants. Roots were bathed in nutrient solution used for growing plants. When cortex cells were punctured with the silicon oil filled glass capillary of the cell pressure probe a meniscus formed between cell sap and silicon oil. By turning the micrometer screw with the aid of a motor, the meniscus was pushed back to the root surface and kept there at a constant position. When an equilibrium cell turgor pressure had been maintained for 1-3 min, hydrostatic pressure relaxations were induced through rapid pressure pulses ($\Delta P \approx 0.15$ MPa). The resulting half-times ($T_{1/2c}$) of pressure relaxations were used to calculate cell hydraulic conductivity (Lp_c) (Eqn 3). For each cell, between 4-10 pressure relaxations were induced.

$$Lp_c = \frac{V}{A} \cdot \frac{\ln(2)}{T_{1/2c}} \cdot \frac{1}{(\varepsilon_c + \pi_c)} \qquad \text{(Eqn 3)}$$

where V = cell volume, A = cell surface area, , π_c = osmotic pressure of cell sap (≈ cell turgor pressure), and ε_c = cell elastic modulus. Cell dimensions (length and diameter) of the 1st to 4th cortical cell layer were determined from free hand cross- and longitudinal-sections, using a calibrated stereomicroscope (40x magnification). For the calculation of ε_c, known changes in cell volume (ΔV) were induced by moving the cell sap/silicon oil meniscus between two reference points along the microcapillary. The microcapillary was calibrated through separate experiments by expelling water contained between the two reference points into liquid paraffin and calculating the volume of water droplet from its diameter. Cell elastic modulus was calculated from ΔV and associated changes in pressure (ΔP) according to:

$$\varepsilon = V \cdot \frac{\Delta P}{\Delta V} \qquad \text{(Eqn 4)}$$

ΔV ranged between 7 x 10^{-14} to 18 x 10^{-14} m^3 depending on the capillaries used. When $T_{1/2c}$ was in the sub-second range, the measured cell elastic modulus (ε_c) was corrected for fast water flow, and the corrected ε_c* used for calculating Lp_c (Volkov *et al.*, 2007):

$$\varepsilon_c^* = \varepsilon_c \cdot \frac{\ln(2) \cdot t \cdot T_{1/2c}^{-1}}{1 - \exp^{-\ln(2) \cdot t \cdot T_{1/2c}^{-1}}} \qquad \text{(Eqn 5)}$$

Transpiration measurements

Transpiration rate was determined gravimetrically for intact plants in the growth chamber. Single barley plants were fixed in a measuring cylinder filled with nutrient solution and placed on a balance (CP323, Sartorius, Göttingen, Germany). Changes in weight of the cylinder were recorded at 2 min intervals using computer software (sartoCollect 1.0, Sartorius, Göttingen, Germany). Transpirational water loss (m^3) was equivalent to the weight loss (g) recorded, minus the weight loss which was due to evaporational water loss from the solution surface of the measuring cylinder. The latter was determined trough separate experiments and accounted for less than 5 % of total water loss. At the end of each transpiration measurement, leaf surface area was determined. Transpiration rates were expressed in $m^3\ m^{-2}\ s^{-1}$ by relating volume flow rates ($m^3\ s^{-1}$) to total leaf surface area (m^2, sum of upper and lower leaf surface area).

Supplementary Table S1

Table S1. Summary of a selection of studies, which examined the effect of mercury chloride ($HgCl_2$) as an aquaporin (AQP) inhibitor on cell-, root-, and whole-plant-hydraulics. Recovery was measured using a reducing agent (e.g. β-mercapthoethanol, ME). ABA = Abscisic acid, g_s = stomata conductance, J_{vr} = root water flux, Lp_r = root hydraulic conductivity, Lp_c = cell hydraulic conductivity, P_f = osmotic water permeability, T_r, transpiration rate. Please note that while this is a comprehensive list, it may not be an exhaustive list.

Study	**Species**	**$HgCl_2$-treated plant organ or cell**	**Control parameter measured**	**$HgCl_2$-treated, % of control (conc.)**	**Recovery, % of control (Agent, conc.)**	**Conclusions and additional information obtained during $HgCl_2$ treatment**
Maggio and Joly, 1995	Tomato (*Solanum lycopersicum*)	Root-system	Lp_r = $4.6x10^{-7}$ m s^{-1} MPa^{-1}	43% (500 μM)	80% (β-ME, 60 mM)	Inhibition of AQP mediated transmembrane water flow; no effect on K^+ concentration of xylem exudate.
Tazawa *et al*., 1997	Barley (*Hordeum vulgare*)	Individual root	Lp_r = 0.027 pm s^{-1} Pa^{-1}	8-17% (100 μM)	60% (β-ME, 10 mM)	Barley roots highly Hg^{2+}-sensitive; AQPs provide most conductive pathway of osmotic water transport.
Wan and Zwiazek, 1999	Aspen (*Populus tremuloides*)	Root-system	J_{vr}=$1.8x10^{-8}$ m^3 m^{-2} s^{-1}, g_s = 23 mmol m^{-2} s^{-1}	47% (0.1 mM), 30% (0.1 mM)	91% (β-ME, 50 mM), 48% (β-ME, 50 mM)	Intact, Hg^{2+} treated roots trigger stomatal closure, most likely via ABA; g_s not affected by Hg^{2+} with excised roots.
Zhang and Tyerman, 1999	Wheat (*Triticum aestivum*)	Root cortical cells	Lp_c = $5.6x10^{-7}$ m s^{-1} MPa^{-1}	25% (100 μM)	60% (β-ME, 5 mM)	Inhibition of AQPs of wheat cortex cells; $HgCl_2$ causes depolarization of membrane; no effect

						of K^+ channel blocker TEA on Lp_c.
Hukin *et al*., 2002	Maize (*Zea mays*)	Root cortical cells	Lpc = $1.8x10^{-7}$ m s^{-1} MPa^{-1}	40% (20 μM)	56% (β-ME, 5 mM)	Lp_c in root elongation zone unaffected, but reduced in older root regions.
Hachez *et al*., 2006	Maize (*Zea mays*)	Isolated root protoplasts	P_f = 10.5 μm s^{-1}	33% (250 μM)	- (not tested)	Reduction in P_f by Hg^{2+} in root-hair zone; high P_f related to increased *ZmPIP* expression.
Beaudette *et al*., 2007	Pea (*Pisum sativum*)	Individual root	Lp_r = 790 μl min^{-1} MPa^{-1}, T_r = n.d	50% (1-100 μM), 53-100% (1-100 μM)	- (not tested), - (not tested)	Changes in Lp_r correlated with *PsPIP2*s expression; AQP-blockage by Hg^{2+} accompanied by increase in *PsPIP2;1* expression; no relation between Hg^{2+}-treated roots and reduction in T_r caused by ABA.
Bramley *et al*., 2009	Wheat (*Triticum aestivum*)	Root-system, Individual root, Root cortical cells	Lp_r = $7x10^{-8}$ m s^{-1} MPa^{-1}, Lp_r = $12x10^{-8}$ m s^{-1} MPa^{-1}, Lp_c = $1.7x10^{-6}$ m s^{-1} MPa^{-1}	70% (50 μM), 50% (50 μM), 23% (50 μM)	- (not tested), - (not tested), - (not tested)	$HgCl_2$ treatments demonstrate localised influences of AQPs on root hydraulics (root *versus* cell level); Lp_c largely controlled by AQPs.
SUMMARY	Several species	Individual root cells (protoplasts) to intact roots-systems.	Root level (Lp_r), Cell level (Lp_c)	8-50% (1-500 μM) 23-40% (20-100 μM)	60-91% (β-ME, 10-60 mM), 56-60% (β-ME, 5 mM)	AQPs mediate transmembrane water flow; changes in Lp can correlate with PIP expression; stomata closure after $HgCl_2$ treatment of roots may be triggered by ABA; depolarization of membrane potential does not alter xylem K^+ concentration; inhibition of K^+ channels does not affect Lp.

Supplementary Figure S1

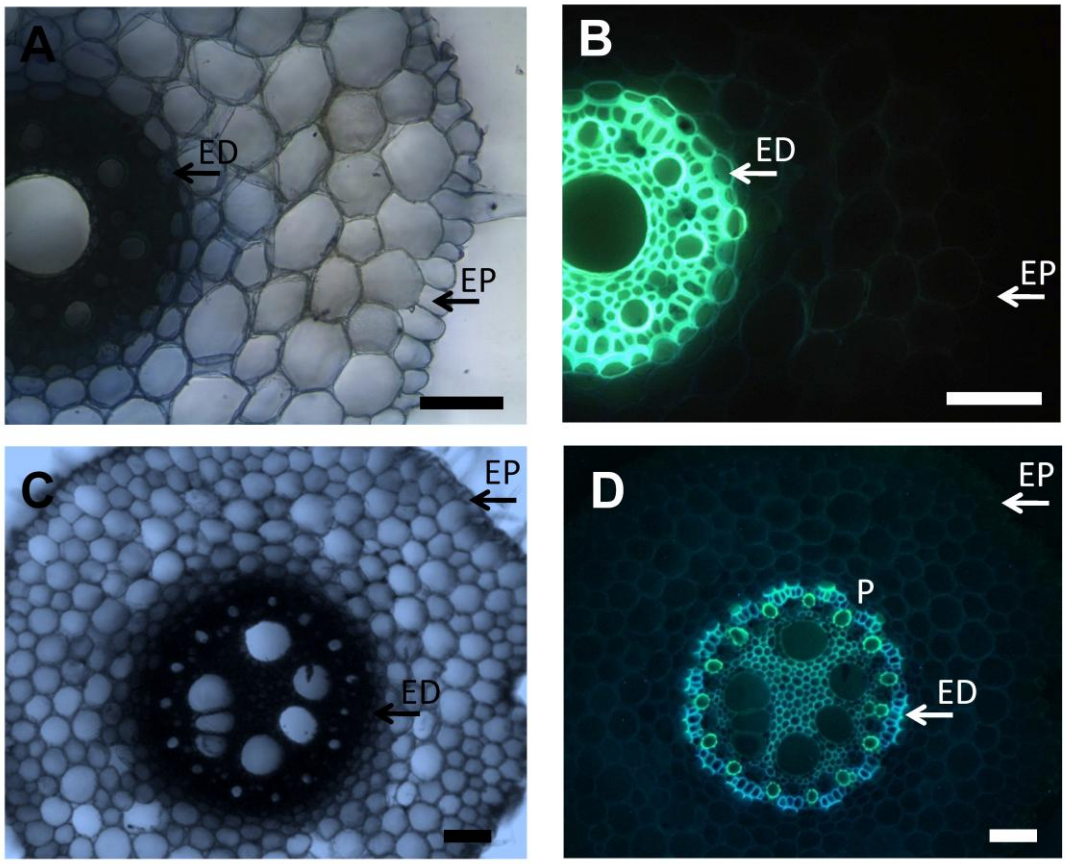

Figure S1. Cross-sections of seminal and adventitious roots of hydroponically grown barley plants highlighting the absence of an exodermis. Free-hand cross-sections were taken 1-2 cm below the root-shoot junction corresponding to the most mature root region. Sections were stained with berberine-hemisulfate and counterstained with toluidine blue and viewed under (A,C) bright light and (B,D) fluorescence light (excitation wavelength 390-420 nm) to visualize Casparian bands and the developmental state of endo- and exodermis (Knipfer and Fricke, 2011). The endodermis (ED) of (A,B) seminal and (C,D) adventitious roots shows asymmetrically thickened cell walls (state III of endodermis development including Casparian bands; Knipfer and Fricke, 2011), except in passage cells (P) opposite of xylem pools of adventitious roots. In seminal and adventitious roots an exodermis did not develop, which is usually located beneath the epidermis (EP). Scale bar (A,B) = 55 µm, (C,D) = 75 µm.