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Aquaporins account for variations in hydraulic conductance for metabolically active root regions of *Agave deserti* in wet, dry, and rewetted soil

G. B. NORTH[1], P. MARTRE[2,3] & P. S. NOBEL[2]

[1]Department of Biology, Occidental College, Los Angeles, California 90041, USA, [2]Department of Organismic Biology, Ecology, and Evolution, University of California, Los Angeles, California 90095–1606, USA and [3]Unite d'Agronomie, INRA – Site de Crouel, 234 Avenue du Brezet F-63039 Clermont-Ferrand, Cedex 2, France

ABSTRACT

The importance of aquaporins for root hydraulic conductance (L_P) was investigated along roots of the desert succulent *Agave deserti* in wet, dry and rewetted soil. Water channel activity was inferred from $HgCl_2$-induced reductions of L_P that were reversible by 2-mercaptoethanol. Under wet conditions, $HgCl_2$ reduced L_P for the distal root region by 50% and for the root region near the shoot base by 36% but did not affect L_P for the mid-root region. For all root regions, L_P decreased by 30–60% during 10 d in drying soil and was not further reduced by $HgCl_2$. After soil rewetting, L_P increased to pre-drying values and was again reduced by $HgCl_2$ for the distal and the basal root regions but not the mid-root region. For the distal region, water channels in the epidermis/exodermis made a disproportionately large contribution to radial hydraulic conductance of the intact segment; for the basal region, water channel activity was highest in the cortex and endodermis. The role of water channels was greatest in tissues in which cells were metabolically active both in the distal root region, where new apical growth occurs in wet soil, and in the basal region, which is the most likely root region to intercept light rainfall.

Key-words: endodermis; exodermis; hydraulic conductivity; mercury sensitivity; root water uptake; water channels; water transport.

Abbreviations: J_V, volume flux density ($m\ s^{-1}$); K_h, axial hydraulic conductivity of the xylem ($m^4\ s^{-1}\ MPa^{-1}$); L_P, root hydraulic conductance (all conductances based on the outer surface area of intact root segments; $m\ s^{-1}\ MPa^{-1}$); L_R, radial hydraulic conductance; $L_{R,EpEx}$, radial hydraulic conductance of the epidermis plus exodermis; $L_{R,-EpEx}$, radial hydraulic conductance of root segments with the epidermis and exodermis removed; $L_{R,CoEn}$, radial hydraulic conductance of the cortex plus endodermis; $L_{R,S}$, radial hydraulic conductance of the stele; Q_V, volumetric flow of water through a root segment ($m^3\ s^{-1}$).

Correspondence: Gretchen B. North. Fax: 323-341-4974; e-mail: gnorth@oxy.edu

INTRODUCTION

The relative permeability of roots to water, known as the root hydraulic conductance (L_P, also called the root hydraulic conductivity), tends to vary directly with water availability in the soil (Huang & Nobel 1994). For a number of species, including many desert succulents and rainforest cacti, decreases in L_P due to soil drying can be quickly reversed when the soil is rewetted (e.g. North & Nobel 1991, 1992, 1994). Drying-induced changes in L_P that are reversed upon rewetting and that are not accompanied by changes in root morphology or anatomy occur for a number of species, including the desert cactus *Opuntia acanthocarpa* (Martre, North & Nobel 2001) and the more mesophytic Mediterranean species *Olea oleaster* (Lo Gullo *et al*. 1998). Such flexible, short-term regulation of root water uptake appears to involve aquaporins, a family of transmembrane proteins that occur in the plasma membrane, tonoplast and other intracellular membranes and that are abundantly expressed in roots (Javot & Maurel 2002; Tyerman, Niemitz & Bramley 2002).

Water channel activity can vary with time of day (Henzler *et al*. 1999; Tsuda & Tyree 2000), with root development (Barrowclough, Peterson & Steudle 2000; North & Nobel 2000; Martre *et al*. 2001; Hukin *et al*. 2002), and in response to various stresses such as salinity (Carvajal, Martinez & Alcaraz 1999; Martinez-Ballesta, Martinez & Carvajal 2000), low nutrients (Carvajal, Cooke & Clarkson 1996; Clarkson *et al*. 2000), and drought (Martre *et al*. 2001, 2002; Siefritz *et al*. 2002). This variability is not surprising, given the diversity of aquaporins and the variety of their locations within cells and tissues. As the plasma membrane is generally much less permeable to water than is the tonoplast (Javot & Maurel 2002), the aquaporins that are more important for regulating root water uptake are likely to be proteins integral to the plasma membrane (PIPs). The expression of a variety of PIPs is generally reduced in roots during drought or osmotic stress (Smart *et al*. 2001; Katsuhara *et al*. 2002; Suga, Komatsu & Maeshima 2002),

although some studies indicate no change (Morillon & Lassalles 2002) or an increase in PIP expression (Kirch *et al.* 2000). Water channel activity can also be regulated by post-translational mechanisms, such as phosphorylation, as shown for aquaporins in the plasma membrane of leaf mesophyll cells for *Spinacia oleracea* (Johansson, Larsson & Kjellbom 1996, Johansson *et al.* 1998). Whether water channel opening is inhibited or stimulated by water stress may depend on the adaptive value of decreasing or increasing root permeability to water. For desert plants, the closure of water channels during drought would help prevent root water loss to a soil that generally has a lower water potential than does the plant.

The desert plant examined in the present study, *Agave deserti*, is a monocotyledonous, stemless rosette with essentially unbranched roots that are chiefly adventitious and nearly constant in diameter along their length. The roots arise from the base of the succulent shoot, which maintains the basal root region in a relatively well-hydrated condition even during prolonged drought. Even though the basal root region is the oldest root region, its anatomy is similar to that of the much younger, actively growing distal root region (North & Nobel 1998). L_P for the basal root region is also relatively high even after soil drying, which may be critical in the field, where light rainfall often rewets only the uppermost few centimetres of soil. Rainfall is also likely to be channelled toward the basal root region by *A. deserti*'s concave, upward-angled leaves. The ability of the roots of desert plants in general and of *A. deserti* in particular to respond to such temporal and spatial heterogeneity in soil moisture may depend on the possible closing of water channels during drying and their reopening upon rewetting.

For young roots of *A. deserti*, comparable with the distal root segments examined in the present study, L_P is reduced by treatment with $HgCl_2$, a known inhibitor of water channel activity (Javot & Maurel 2002), under wet conditions but not after 45 d of drought (North & Nobel 2000). During a shorter period of drying, such as the 10 d imposed on *A. deserti* in the present study, few anatomical changes in roots are expected, allowing a sharper focus on the role of aquaporins in regulating L_P. Specifically, three questions with respect to possible regulation of L_P in roots of *A. deserti* are addressed: (1) how do water channels respond to short-term soil drying and rewetting; (2) are water channels active along the entire length of a root, specifically, in the distal region (including the tip), at mid-root and at the base of the shoot; and (3) in what root tissues or cell layers (epidermis/exodermis, cortex/endodermis and stele) is water channel activity most important to hydraulic conductance?

To assess the presence of aquaporins as well as their importance in root water transport, L_P was measured for root segments first in water and then in $HgCl_2$. The use of $HgCl_2$ to close water channels raises a number of concerns, such as the possibility that reduction in water transport may be due to membrane damage or metabolic poisoning (Zhang & Tyerman 1999). However, preliminary experiments confirmed that an inhibitory effect on L_P for roots of *A. deserti* could be achieved with a low concentration of $HgCl_2$ (25 μM) and that such inhibition was reversible by 10 mM 2-mercaptoethanol. A further concern with this technique is that the relative impermeability of outer root tissues may preclude entry of $HgCl_2$ (Barrowclough *et al.* 2000). For a number of species, including *A. deserti*, the exodermis in older root regions tends to be more heavily suberized than in younger regions, thus possibly acting as a barrier to substances such as Hg^{2+} (Barrowclough *et al.* 2000). Removal of this layer allowed $HgCl_2$ to penetrate, and hence the mercury-sensitivity of water channels in internal root tissues could be determined. In addition, sequential tissue removal followed by measurements of L_P allowed the effect of $HgCl_2$, and, by extension, the importance of aquaporins in water transport, to be assessed radially tissue by tissue from the epidermis/exodermis to the stele at three locations along a root.

MATERIALS AND METHODS

Plant material

Four-year-old-plants of *Agave deserti* Engelm. (Agavaceae), approximately 18 cm tall and 25 cm in diameter with an average of eight unfolded leaves, were obtained from a commercial nursery. They were grown in a greenhouse at the University of California, Los Angeles in plastic trays 52 cm long × 26 cm wide × 6 cm deep that were filled with vermiculite. The plants received a mean total daily photosynthetic photon flux of 38 mol m^{-2} d^{-1} (80% of ambient solar radiation), with daily maximum/minimum air temperatures averaging 28/16 °C and daily minimum/maximum relative humidities averaging 40/70%. The soil water potential (Ψ_{soil}; MPa) in the rooting zone was maintained above –0.1 MPa by watering every other day with 0.1-strength Hoagland's solution no. 2 supplemented with micronutrients. Twenty-four plants were maintained under wet conditions for at least 45 d in the greenhouse before water was withheld to initiate soil drying for half of the plants.

The water content of the vermiculite was determined by weighing 7–10 g of soil before and after drying for 48 h in a forced-draught oven at 105 °C, and the vermiculite water potential in the rooting zone was calculated using a moisture-release curve for vermiculite (Dubrovsky, North & Nobel 1998). Under wet conditions, Ψ_{soil} was > –0.2 MPa, and at 10 d of drying it was –1.6 MPa. At 10 d of soil drying, six plants were watered so that Ψ_{soil} was increased to –0.1 MPa and then maintained at that value by watering every other day. After 45 d in wet soil, main roots arising from the stem were 300–350 mm long and averaged 3.6 mm in diameter. Three regions of main roots were examined: (1) distal (the youngest region), from the tip to 80 mm back; (2) mid-root, from 130 to 210 mm back from the tip; and (3) basal (the oldest region), 10–90 mm from the base of the succulent shoot.

Root hydraulic properties

Root hydraulic conductance (L_P, m s^{-1} MPa^{-1}) was measured on distal, mid-root and basal segments that were

about 75 mm in final exposed length (Nobel, Schulte & North 1990). Root segments about 150 mm long were gently excavated and trimmed by 50 mm from the proximal end (distal end for basal root segments) under distilled water with a razor blade. All tissues external to the stele were removed from a 15-mm length at the proximal end of the segment, and the exposed stele was then trimmed 5 mm under water and inserted into a 10-mm-length of Tygon tubing affixed to a glass capillary (inside diameter, 1.6 mm) that was half-filled with distilled water. A watertight seal between the stele and the tubing was made by inserting the tubing through a silicone gasket in a brass compression fitting. The junction between the tubing and the stele as well as the distal cut end of the mid-root and basal segments (about 5 mm) were sealed with hydrophilic vinyl polysiloxane (Reprosil; Dentsply International, Milford, DE, USA) and coated with clear nail polish. The root segment was then suspended in 200 mL of distilled water.

Water flow through the root segment was induced by applying a partial vacuum to the open end of the capillary at pressures of –40, –30, –20 and –10 kPa, regulated with a needle valve and monitored with a digital manometer (PS309; Validyne Engineering, Northridge, CA, USA). The flow rate (Q_V, $m^3\ s^{-1}$) was recorded at each pressure after the rate stabilized (in less than 10 min). L_P was calculated as the slope of the relationship between the volumetric flux density ($J_V = Q_V$ per unit root surface area, $m^3\ m^{-2}\ s^{-1}$, or $m\ s^{-1}$) and the vacuum pressure. The root surface area was calculated from root length and mean diameter.

Before measuring axial (xylem) hydraulic conductivity (K_h,$(m^3\ s^{-1})/(MPa\ m^{-1})$, or $m^4\ s^{-1}\ MPa^{-1}$), root segments were first trimmed under water at about 10 mm from the distal seal. About 1 mm at the cut end of the segment was then immersed in distilled water. Q_V was used to calculate K_h:

$$K_h = Q_V/\Delta P/\Delta x) \quad (1)$$

where the pressure difference ΔP (10 kPa) was applied along the length Δx (m) of the root segment. Measurements of Q_V were made during the first 10 s after immersion of the segment to avoid blockage of vessels by debris.

The radial hydraulic conductance (L_R, $m\ s^{-1}\ MPa^{-1}$, calculated based on the outer root surface area) along a root equals J_V at the root surface divided by the difference in water potential from the root surface to the root xylem. L_R averaged over the entire root segment was calculated by incorporating measured values of L_P and K_h together with the length (l, m) and the radius (r_{root}, m) of the root segment into a model of Landsberg & Fowkes (1978) based on leaky cable theory:

$$L_R = L_P\ \alpha/\tanh(\alpha l) \quad (2)$$

where α (m^{-1}) equals $(2\pi r_{root} L_R/K_h)^{1/2}$ which represents the length along the root xylem across which the pressure decreases by half (Landsberg & Fowkes 1978). L_R was initially set equal to L_P and gradually increased to solve Eqn 2 by iteration.

Radial hydraulic conductance of concentric root tissues

After L_P was measured for an intact root segment, the epidermis and the exodermis were removed using fine forceps under a stereomicroscope, and L_P was then measured on the stripped segment (North & Nobel 1995; Martre *et al.* 2001). Measured values of L_P and mean values of K_h for the appropriate root region were then used to calculate L_R for the stripped segment ($L_{R,\text{-EpEx}}$) using Eqn 2 and the surface area of the root segment. For another set of segments, a similar procedure was followed except that all cell layers outside the stele were removed (epidermis, exodermis, cortex and endodermis). Radial conductances are in series and are based on the outer surface area of the intact root segment in all cases (note that the same Q_V occurs across each of the concentric tissue layers). Thus, the reciprocal of L_R for an intact root segment equals the sum of the reciprocals of L_R for the epidermis plus exodermis ($L_{R,EpEx}$), the cortex plus endodermis ($L_{R,CoEn}$), and the stele ($L_{R,S}$), so

$$L_{R,EpEx} = 1/(1/L_R - 1/L_{R,\text{-EpEx}}) \quad (3)$$

For the cortex plus endodermis,

$$L_{R,CoEn} = 1/(1/L_R - 1/L_{R,EpEx} - 1/L_{R,S}) \quad (4)$$

Assessment of water channels

After measurement of L_P in water, the root segments were transferred to 25 μM $HgCl_2$ for 10 min at a pressure of –30 kPa. The pressure was then decreased to –40 kPa and L_P was measured as before. The root segments were then briefly rinsed in water, immersed in 10 mM 2-mercaptoethanol under the same conditions (10 min, –30 kPa), and L_P was measured again. To check for artifacts due to repeated measurements of L_P on the same root segment, comparable segments were repeatedly measured in water with the same protocol; L_P varied less than 3% among three such sequential measurements on four representative root segments.

Anatomy

To investigate anatomical features, root segments were sectioned with a razor blade and stained with 0.05% (w/w) toluidine blue O in water for 30 s. Sections were mounted in water and then examined with an Olympus BH2 microscope (Lake Success, NY, USA) at magnifications of 100–1000×. Sections were examined for lignin by staining with 0.5% (w/w) phloroglucinol in water followed by 20% HCl (Jensen 1962). The presence of suberin in cell walls was investigated by staining with 0.1% (w/w) Sudan red 7B in 70% ethanol; suberin lamellae appeared red, and Casparian bands were not stained. Suberin and lignin were also located in untreated sections by their autofluorescence under violet and ultraviolet light.

Statistics

Statistical analysis was done using SigmaStat 4.0 (SPSS Inc., Chicago, IL, USA). Differences in L_P due to watering treat-

ments were analysed using one-way ANOVA ($\alpha = 0.05$) followed by a Tukey's test, after verifying that the treatment effects were normally distributed with equal variance. Differences in L_P due to $HgCl_2$ treatment were analysed using paired *t*-tests or Student's *t*-test. Data are presented as means ±1 SE (n = number of measurements).

RESULTS

Root hydraulic conductance and $HgCl_2$ sensitivity of intact (non-dissected) root segments

The volume flux density (J_V) of the distal root region of *Agave deserti* for intact segments immersed in water, in 25 μM $HgCl_2$, or in 10 mM 2-mercaptoethanol (ME) following the $HgCl_2$ treatment had a linear relationship with applied pressure differences (Fig. 1). Linear relationships between J_V and pressure were also obtained for mid-root and basal root regions ($r^2 = 0.978 \pm 0.005$). Although the responses to $HgCl_2$ differed, J_V for root segments in 10 mM ME ranged from 78 to 100% of J_V in water for all root regions, averaging 83 ± 9%. The slope of these relationships was used to calculate the root hydraulic conductance (L_P).

For the distal region under wet conditions, L_P for root segments in $HgCl_2$ was 50% lower than L_P for root segments in water ($P < 0.001$; Fig. 2a). After 10 d of soil drying ($\Psi_{soil} = -1.6$ MPa), L_P for the distal region was 59% lower than under wet conditions and did not decrease further in $HgCl_2$. After 3 d of rewetting ($\Psi_{soil} = -0.1$ MPa), L_P for the distal region was restored to its value under wet conditions and was again reduced by $HgCl_2$ ($P < 0.001$).

For the mid-root region, changes in L_P measured in water in response to soil drying and rewetting were similar to the changes in the distal region (Fig. 2b). However, unlike the case for the distal region, $HgCl_2$ did not affect L_P under any of the soil moisture conditions.

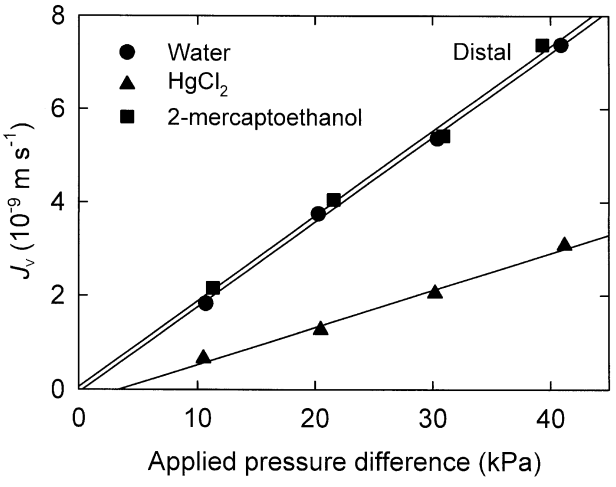

Figure 1. Relationship between J_V and the applied pressure difference for a distal root segment of *Agave deserti* under wet soil (vermiculite) conditions. J_V was measured sequentially in water, $HgCl_2$ (25 μM) and 2-mercaptoethanol (10 mM).

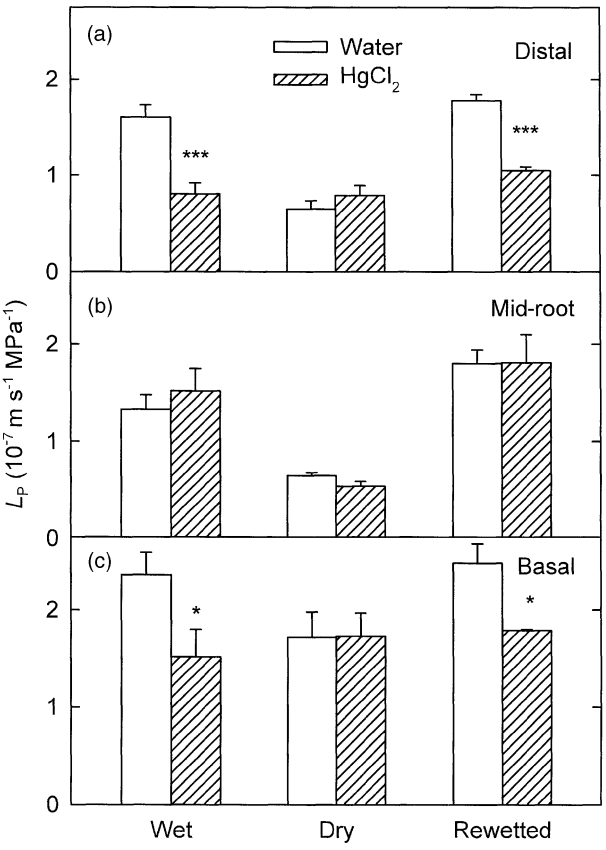

Figure 2. L_P for intact segments from the distal (a), mid-root (b) and basal (c) regions of roots of *A. deserti* measured in water (open bars) and in 25 μM $HgCl_2$ (hatched bars) in wet, dry and rewetted soil (vermiculite). Soil water potential was –0.1 MPa under wet conditions, –1.6 MPa at 10 d of soil drying and –0.1 MPa during 3 d of rewetting. Data are means +1 SE ($n = 5$ roots from different plants). Asterisks indicate significant differences due to the $HgCl_2$ treatment (paired *t*-test; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

Under wet, dry and rewetted conditions, L_P for the basal root region was higher than for distal and mid-root regions ($P < 0.05$; Fig. 2c). Similar to the case for the distal region, L_P measured in $HgCl_2$ for the basal region was lower than in water under wet soil conditions, although the reduction was only 36%. After 10 d of soil drying, L_P for the basal region was 27% lower than under wet conditions ($P < 0.05$) and did not decrease further in $HgCl_2$. After 3 d of rewetting, L_P was restored to its value under wet conditions and was reduced 28% in response to treatment with $HgCl_2$.

Changes in L_P and root growth for the distal root region in response to rewetting after soil drying were examined daily for 3 d. At 1 d of rewetting, L_P was 2.7 times higher than its value under dry conditions (day 0, Fig. 3) and was 41% lower when measured in $HgCl_2$ ($P < 0.001$). No new apical growth (as detected by root color) was apparent at 1 d. At 2 d of rewetting, L_P was 3.7 times higher than its value under dry conditions, and the reduction due to $HgCl_2$ was 50% ($P < 0.01$). New apical growth at 2 d averaged 3.8 ± 0.2 mm ($n = 4$ roots). No further increase in L_P measured in water occurred at 3 d of rewetting, although the

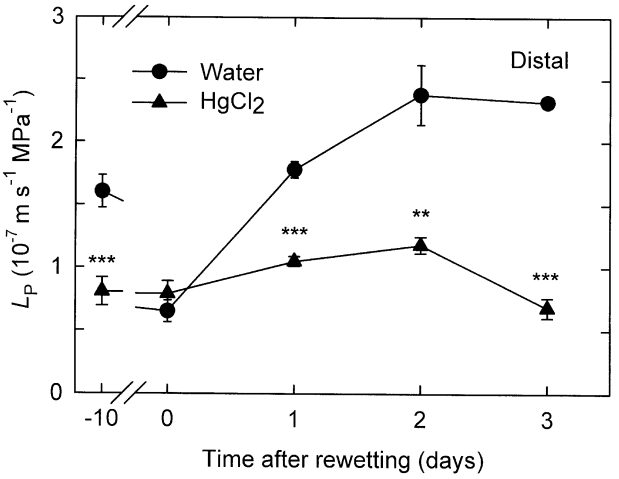

Figure 3. L_P for intact root segments from the distal region of roots of *A. deserti* measured in water and in 25 μM $HgCl_2$ under wet conditions, at 10 d of soil drying (day 0) and at 1, 2 and 3 d after rewetting. Data are means ±1 SE ($n = 4$ plants). Asterisks indicate significant differences due to the $HgCl_2$ treatment (see Fig. 2).

reduction due to $HgCl_2$ was greater, 71% ($P < 0.001$), and new apical growth averaged 9.8 ± 0.9 mm ($n = 4$).

Root hydraulic conductance and $HgCl_2$ sensitivity of concentric root tissues

For distal root segments with the epidermis and exodermis removed, L_P in water was 37% higher than L_P for intact distal root segments (Fig. 4a versus Fig. 2a). For both the mid-root and basal regions without these layers, L_P was about twice as high as for the intact segments (Fig. 4a versus Fig. 2b & c). For distal segments with the epidermis and exodermis removed, L_P in $HgCl_2$ was 23% lower than in water (Fig. 4a; $P < 0.01$). For mid-root segments with these layers removed, L_P in $HgCl_2$ was slightly but not significantly higher than in water. For basal segments with the epidermis and exodermis removed, L_P was 45% lower in $HgCl_2$ than in water ($P < 0.05$).

For distal, mid-root and basal root regions, L_P in water for the stele (the epidermis, exodermis, cortex and endodermis having been removed) was 2.1, 6.6 and 2.7 times higher, respectively, than for the intact segments (Fig. 4b versus Fig. 2). For the stele of the three root regions, $HgCl_2$ had no significant effect on L_P.

Axial hydraulic conductance (K_h) was $1.2 \pm 0.5 \times 10^{-11}$ $m^4\ s^{-1}\ MPa^{-1}$ for the distal root region, $9.8 \pm 2.6 \times 10^{-11}$ $m^4\ s^{-1}\ MPa^{-1}$ for the mid-root region and $5.4 \pm 2.3 \times 10^{-11}$ $m^4\ s^{-1}\ MPa^{-1}$ for the basal region ($n = 5$ plants). Root radial hydraulic conductance (L_R) calculated from L_P and K_h was 2.1×10^{-7} $m\ s^{-1}\ MPa^{-1}$ for intact segments from the distal region, which was 50% higher than for the mid-root region and similar to L_R for the basal region (Fig. 5). For L_R of intact segments, the effect of $HgCl_2$ was similar to its effect on L_P, with a reduction of 57% for the distal region, no reduction for the mid-root region and a reduction of 38% for the basal region.

The radial hydraulic conductance of the epidermis and exodermis ($L_{R,EpEx}$) for the distal region was about three times greater than $L_{R,EpEx}$ for the mid-root region and slightly but not significantly higher than $L_{R,EpEx}$ for the basal region (Fig. 5). $HgCl_2$ reduced $L_{R,EpEx}$ for the distal region by 77%, had no effect on $L_{R,EpEx}$ for the mid-root region and reduced $L_{R,EpEx}$ for the basal region by 24% (Fig. 5). The radial hydraulic conductance of the cortex and endodermis ($L_{R,CoEn}$) for the distal region was slightly but not significantly higher than for the mid-root region and about 60% lower than for the basal region. $HgCl_2$ reduced $L_{R,CoEn}$ by 72% for the distal region, had no effect on $L_{R,CoEn}$ for the mid-root region and reduced $L_{R,CoEn}$ by 67% for the basal region. For the stele, the radial hydraulic conductance ($L_{R,S}$) was about 40% lower for the distal region than for the mid-root and basal regions. $HgCl_2$ had no significant effect on $L_{R,S}$ for the three root regions (Fig. 5).

Root anatomy

Distal, mid-root and basal regions of roots of *A. deserti* differed in the extent of suberization and lignification of various cell layers (Fig. 6). For the distal region under wet conditions, 1.4 ± 0.2 cell layers directly inside the epidermis had suberin lamellae and were thus considered exodermis. After 10 d of soil drying, epidermal cells in the

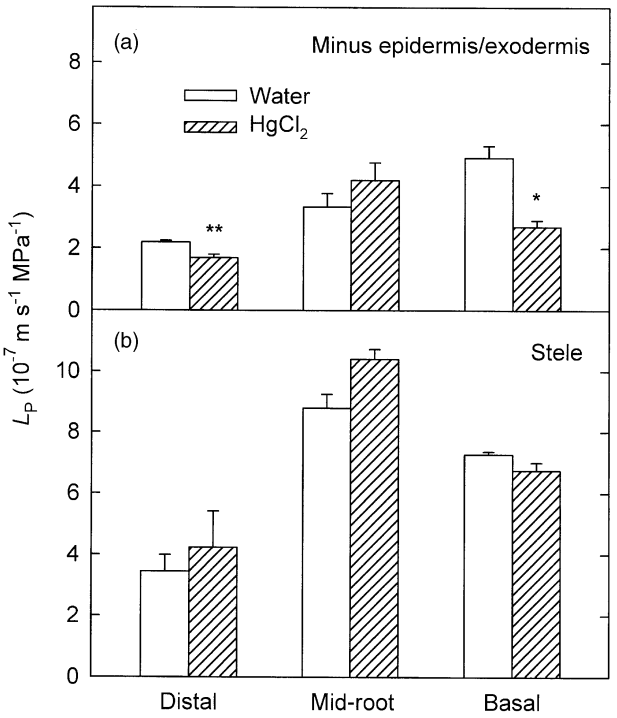

Figure 4. L_P for distal, mid-root and basal root segments of *A. deserti* (a) from which the epidermis and exodermis had been removed and (b) from which the epidermis, exodermis, cortex and endodermis had been removed, leaving the vascular cylinder (= stele), measured in water (open bars) and 25 μM $HgCl_2$ (hatched bars) under wet conditions. Data are means +1 SE (n = 4–6 plants). Asterisks indicate significant differences due to the $HgCl_2$ treatment.

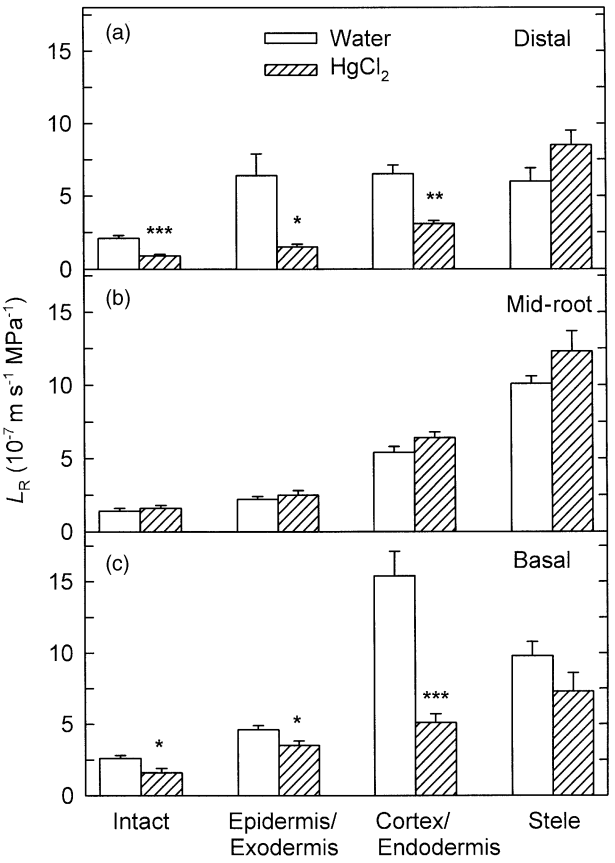

Figure 5. L_R for distal, mid-root and basal regions of roots of *A. deserti* under wet conditions. L_R for intact segments, the epidermis plus exodermis ($L_{R,EpEx}$), the cortex plus endodermis ($L_{R,CoEn}$) and the stele ($L_{R,S}$) was calculated from L_P measured in water (open bars) and in 25 μM $HgCl_2$ (hatched bars), using mean values of K_h (text data, assumed not to change in $HgCl_2$) for each root region; Eqn 2 was used for intact segments and Eqns 3 and 4 for sequential cell layers. Data are means ±1 SE (n = 5–6 plants). Asterisks indicate significant differences due to the $HgCl_2$ treatment (Student's t-test).

distal region had lost turgor and the exodermis was 2.0 ± 0.3 layers (Fig. 6a), an increase that was not significant (Mann–Whitney rank sum test, P = 0.056; n = 5 roots from different plants). Nuclei were present in exodermal layers in the distal region under both wet and drying conditions. In the inner cortex adjacent to the endodermis, 3.5 ± 0.8 cortical cell layers were lignified, although the walls remained relatively thin (Fig. 6b). The endodermis of the distal region lacked suberin lamellae (in sections taken 30–40 mm from the root tip) under both wet (Fig. 6b) and drying conditions, and lignified Casparian bands were apparent.

For the mid-root region, the exodermis consisted of 3.6 ± 0.2 suberized cell layers under both wet (Fig. 6c) and drying conditions. Nuclei were not present in the outermost layer of the exodermis, but occasionally were apparent in the inner exodermal layers. Under both wet and drying conditions, the outermost layers of the cortex at mid-root consisted of irregularly shaped, collapsed cells that were apparently dead. Outside the endodermis, 3.8 ± 0.4 cortical cell layers were lignified and had thickened cell walls under both wet (Fig. 6d) and drying conditions. The endodermis consisted primarily of cells with suberin lamellae and thickened inner tangential walls, although about 10% of endodermal cells were thin-walled and lacked suberin lamellae (i.e. were passage cells; Fig. 6d).

The basal region was anatomically more heterogeneous than the other two root regions. At 60–70 mm from the shoot base, the anatomy was similar to that of the mid-root region. At 15–40 mm from the shoot base, the root diameter increased, and cell layers tended to be less lignified and suberized than in the region further from the shoot. Specifically, the exodermis was 2.6 ± 0.2 layers at 30 mm from the base under both wet (Fig. 6e) and drying conditions, and nuclei were apparent in the outer exodermal layers. Although the outer cortical cells were collapsed, as at mid-root, most of the cortical cells just outside the endodermis were unlignified, and nuclei were often apparent under both wet and drying conditions (Fig. 6f). The percentage of endodermal cells lacking suberin lamellae (passage cells) ranged from 22 to 100% (Fig. 6f).

DISCUSSION

Except under dry conditions, treatment with 25 μM $HgCl_2$ inhibited water transport for the distal (youngest) and the basal (oldest) root regions of *Agave deserti*, suggesting the involvement of aquaporins (Chaumont *et al*. 2000; Javot & Maurel 2002). The reversibility of inhibition by 2-mercaptoethanol further suggests that the primary effect of $HgCl_2$ was to close water channels. In addition, root segments left in water for 12 h after treatment with $HgCl_2$ and 2-mercaptoethanol exhibited apical growth and gravitropism, indicating a lack of residual toxicity. Using $HgCl_2$ allowed water channel activity to be assessed on a tissue-by-tissue basis, complementing research that demonstrates gene expression and aquaporin presence in individual root cell layers (Eckert *et al*. 1999; Baiges *et al*. 2002; Javot & Maurel 2002; Tyerman *et al*. 2002).

Location of water channel activity under wet soil conditions

Under wet soil conditions, $HgCl_2$ reduced hydraulic conductance (L_P) for the distal root region of *A. deserti* by about 50%, comparable to previous results for this species (North & Nobel 2000) and within the range reported for roots of comparable age from several other species, including *Opuntia acanthocarpa* (Martre *et al*. 2001). As was the case for *O. acanthocarpa*, treatment with $HgCl_2$ did not affect water transport in the older mid-root region. Aquaporins in both the tonoplast (TIPs) and in the plasma membrane (PIPs) are more abundant in younger than in older root tissues of *Mesembryanthemum crystallinum* (Yamada *et al*. 1995), *Picea abies* (Oliviusson, Salaj & Hakman 2001) and hybrid *Vitis* (Baiges *et al*. 2002). The reduction of L_P by $HgCl_2$ for the basal root region of *A.*

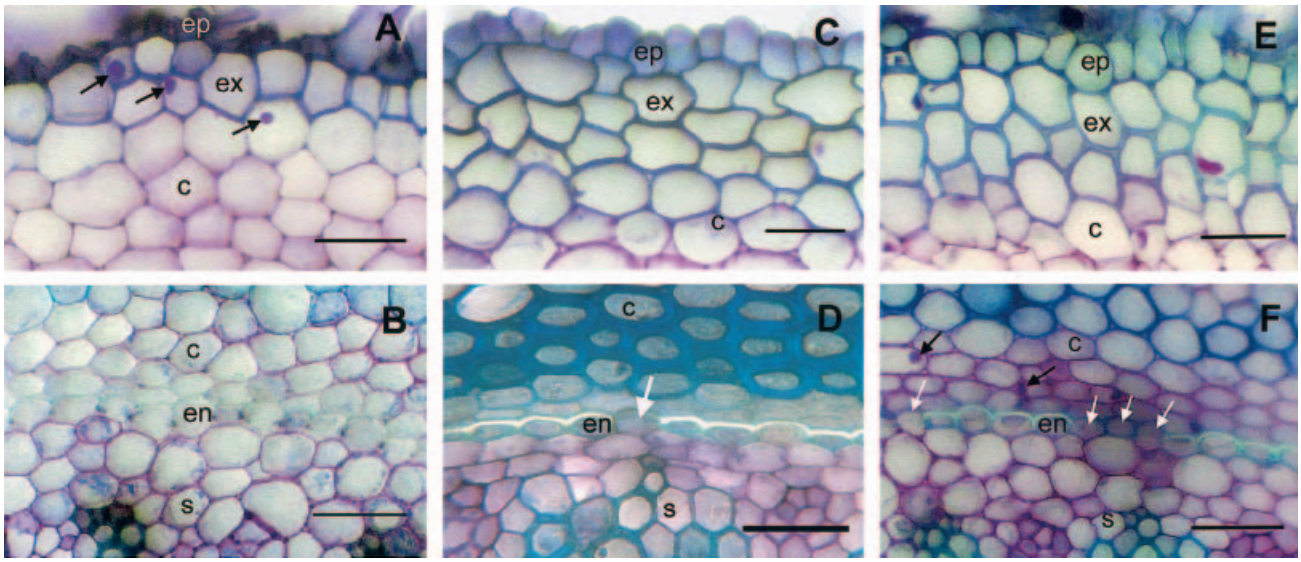

Figure 6. Photomicrographs of cross-sections of roots of *A. deserti*. Sections were made at 35 mm back from the root tip, showing the outer (a) and the inner (b) tissues of the distal region; at 170 mm from the root tip, showing the outer (c) and the inner (d) tissues of the mid-root region; and at 30 mm from the shoot base, showing the outer (e) and the inner (f) tissues of the basal region. Cross-sections in (a) and (f) were made at 10 d of soil drying and all others were made under wet conditions. All cross-sections were stained with toluidine blue O; unlignified parenchyma is purple, lignified cell walls are blue-green or blue and suberin lamellae in endodermis are unstained. Black arrows indicate nuclei; white arrows indicate passage cells in the endodermis; en, endodermis; ep, epidermis; ex, exodermis; c, cortex; s, stele. Scale bars = 50 μm.

deserti, which was similar to that for the distal region, at first appears anomalous, yet this region near the shoot base is anatomically more similar to the distal than to the mid-root region. In addition, both the distal and the basal regions had a higher proportion of living cells in all tissues than did the mid-root region. Radial hydraulic conductance (L_R) was similar for the distal and the basal regions and 50–85% higher than at mid-root. Thus, greater abundance and/or greater opening of water channels may help explain the greater hydraulic conductance for the distal and basal regions than for the mid-root region. Such a pattern may be adaptive for *A. deserti* in the desert, where new apical growth in the distal root region occurs in wet soil and where the basal root region is positioned to intercept light rainfall that may wet only the top few centimetres of soil and is channelled by the leaves toward the plant base.

For the distal region of roots of *A. deserti*, $L_{R,EpEx}$ (radial hydraulic conductance for the epidermis and exodermis), $L_{R,CoEn}$ (for the cortex and endodermis) and $L_{R,S}$ (for the stele) were all similar. In terms of resistance, each of these sequential layers thus represented about one-third of the resistance of the intact root segment. Similarly, the hydraulic resistance for young roots of *Zea mays* is relatively evenly distributed across the root (Peterson, Murrmann & Steudle 1993). For distal root segments treated with $HgCl_2$, the resistance of the epidermis and exodermis increased four-fold and became 60% of the total radial resistance, suggesting that closure of water channels in these outer cell layers had a disproportionate effect on transport across the root. Treatment of onion roots with $HgCl_2$ also indicated that closure of water channels in the epidermis and exodermis greatly increases radial hydraulic resistance (Barrowclough *et al.* 2000). In the distal region of roots of *A. deserti*, the exodermis generally consisted of one layer of cells with suberin lamellae, although this layer is actually dimorphic, with unsuberized, densely cytoplasmic cells alternating irregularly with suberized cells (North & Nobel 1991). As suberized cell walls can block the passage of $HgCl_2$ (Barrowclough *et al.* 2000), a likely site for the inhibitory effect of $HgCl_2$ on water transport is the plasma membrane of these unsuberized cells.

For the distal root region, treatment with $HgCl_2$ decreased the radial conductance of the cortex and endodermis ($L_{R,CoEn}$) by about 50% but did not change the relative contribution of these layers to the radial resistance, due in part to the decreased proportional resistance of the stele. Thus, although water transport through the cortex and endodermis appeared to involve aquaporins, their role in these layers in the distal region was not as important as in the epidermis and exodermis. Some water flow may have occurred in the apoplastic pathway through these tissues, as has been shown previously for the distal 20 mm of roots of *A. deserti* under wet conditions (North & Nobel 1995). As the endodermis lacked suberin lamellae in most of the distal region, apoplastic flow was more likely than in the mid-root and the basal regions.

For the mid-root region, treatment with $HgCl_2$ had no effect on L_R for any of the three tissue components considered. The suberized cell layers of the exodermis and endodermis as well as the lignified cortical cell layers outside the

endodermis could pose substantial barriers to the penetration of $HgCl_2$ to the plasma membrane of cells in these layers, possibly rendering the effect of $HgCl_2$ on aquaporins inconclusive (Barrowclough *et al*. 2000). However, removal of the epidermis and fully suberized exodermis did not increase sensitivity to $HgCl_2$ for the mid-root region. Passage cells lacking suberin lamellae were few in the endodermis, indicating a more limited apoplastic pathway than in the distal region. Thus, water flow in the mid-root region would be expected to occur primarily through the cell-to-cell pathway (Steudle & Peterson 1998), yet insensitivity to $HgCl_2$ indicates that aquaporin involvement was minimal. With the exception of $L_{R,S}$, radial conductance for the tissues of the mid-root region was lower than for tissues of the distal region, consistent with lower aquaporin activity and/or lower apoplastic flow.

For the basal region, $L_{R,EpEx}$ was one-third of $L_{R,CoEn}$ and one-half of $L_{R,S}$, perhaps reflecting the relatively extensive suberization of the exodermis. The epidermis and exodermis represented 56% of the total radial resistance for the intact segment in water and 46% of the total resistance in $HgCl_2$. In contrast to the case for the distal region, treatment with $HgCl_2$ increased the relative contribution of the endodermis and cortex to the total resistance, indicating greater aquaporin involvement in flow through these tissues than through the epidermis and exodermis in the basal region. Similar to the case at mid-root, a possible explanation is the relatively extensive suberization of the exodermis in comparison with the endodermis in this region. At 15–40 mm from the shoot base, many passage cells were present in the endodermis, suggesting that the 67% loss of conductance due to treatment with $HgCl_2$ could reflect closure of aquaporins in these cells. Analogously, mRNA distribution indicates abundant plasma membrane aquaporins in the endodermis of roots of *M. crystallinum* (Yamada *et al*. 1995), as does GUS expression in the endodermis and stele of roots of *Arabidopsis thaliana* (Javot *et al*. 2003). Aquaporins in cortical cells of *A. deserti* may also have been affected, although the outer cells of the cortex in the basal region were collapsed and apparently dead under both wet and drying conditions.

Treatment with $HgCl_2$ had no significant effect on water transport through the stele in any of the three root regions, in contrast to results for roots of *O. acanthocarpa* (Martre *et al*. 2001). Thus, aquaporins do not appear to have been important in radial conductance through the stele of *A. deserti*. With no suberized cell layers present (the endodermis having been removed), and under the conditions of vacuum-induced water uptake in the current study, apoplastic flow may have predominated in the radial path to the xylem. Aquaporins in stelar parenchyma, as indicated for a number of species including *M. crystallinum* (Yamada *et al*. 1995) and *Zea mays* (Barrieu, Chaumont & Chrispeels 1998), could be more important for osmotically induced water uptake than for hydrostatically induced flow, according to the composite model of root hydraulic conductivity (Steudle & Peterson 1998).

Changes in root hydraulic conductance and water channel activity during soil drying and rewetting

Soil drying reduced L_P by 50–60% for the distal and the mid-root regions and by about 30% for the basal region of roots of *A. deserti*, and treatment with $HgCl_2$ had no additional inhibitory effect on L_P. Interestingly, for both the distal and the basal root regions, L_P after 10 d of soil drying did not differ from L_P after treatment with $HgCl_2$. In most other plant species, stress similarly reduces the effect of mercurial compounds on water transport. For example, N- and P-deprived roots of *Lotus japonicus* lose their sensitivity to Hg^{2+} (Clarkson *et al*. 2000), and salinity stress reduces not only L_P for roots of *Cucumis melo* (Martinez-Ballesta *et al*. 2000), *Triticum aestivum* (Carvajal *et al*. 1996) and *Capsicum annuum* (Martinez-Ballesta, Martinez & Carvajal 2003) but also their sensitivity to further inhibition by $HgCl_2$. In such cases, the stress may down-regulate aquaporin expression, close or narrow the opening of existing water channels, or render aquaporins insensitive to $HgCl_2$ in a manner unrelated to water transport. For the roots of a number of relatively stress-tolerant species, including *M. crystallinum* (Kirch *et al*. 2000), *Nicotiana glauca* (Smart *et al*. 2001) and *Hordeum vulgare* (Katsuhara *et al*. 2002), the expression of PIPs is moderately to strongly down-regulated during water or salinity stress.

A possible mechanism for the regulation of water channel activity in roots of *A. deserti* as soil moisture varied is by the phosphorylation and dephosphorylation of aquaporins. According to a model based on work with leaves of *Spinacia oleracea*, high apoplastic water potential leads to full cell turgor and the opening of stretch-activated Ca^{2+} channels in the plasma membrane; high levels of Ca^{2+} stimulate aquaporin phosphorylation, and water channels open (Johansson *et al*. 1998). When cell turgor decreases during drying, Ca^{2+} channels close, aquaporins are dephosphorylated, and water channels close. Young roots of *A. deserti* shrink during drying and quickly regain turgor upon rewetting (Nobel & Cui 1992), changes that were paralleled in the current study by the loss and re-acquisition of sensitivity to $HgCl_2$. In particular, the complete recovery of hydraulic conductance for distal root segments of *A. deserti* after 1 d of rewetting occurred in the absence of new apical growth and was accompanied by a 41% reduction of L_P due to $HgCl_2$, similar to the 49% reduction caused by $HgCl_2$ under wet conditions. The reopening of water channels was apparently central to the recovery of root water uptake upon rewetting, corroborating results for *Arabidopsis thaliana* showing that plant recovery after soil drying and rewetting was faster and more complete for wild-type plants than for PIP1/PIP2 antisense plants (Martre *et al*. 2002).

CONCLUSIONS

The variable contribution of aquaporins to water transport has been demonstrated by differential responses to $HgCl_2$

along the length and across the tissue layers of roots of *A. deserti* under wet, dry and rewetted soil conditions. $HgCl_2$ reduced hydraulic conductance for the distal and the basal root regions but not at mid-root, suggesting a positive association between water channel opening and the frequency of living, metabolically active cells. Water channel activity was thus greatest in the root regions most likely to encounter wet or rewetted soil in the desert. In the distal root region, the epidermis and exodermis were the site of the major contribution of aquaporins to root hydraulic conductance, whereas water channel activity in the basal region was greatest in the cortex and endodermis. Soil drying led to lower root hydraulic conductance, which was not reduced further by $HgCl_2$, yet rewetting restored both hydraulic conductance and its sensitivity to $HgCl_2$ for the distal and the basal regions. The percentages of L_P inhibition due to treatment with $HgCl_2$ indicate that most of the quantitative variations in L_P for the distal and the basal root regions were due to changes in water channel activity in response to variations in soil moisture. The apparent lack of anatomical changes and the rapid recovery of water uptake after rewetting both point to a central role for aquaporins in regulating root responses to changes in water availability. Thus, aquaporins may represent an efficient way for *A. deserti* to modify root hydraulic conductance in a habitat where sporadic light rainfall must be utilized quickly.

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