CHAPTER THREE

WATER FLOW IN THE ROOTS OF CROP SPECIES: THE INFLUENCE OF ROOT STRUCTURE, AQUAPORIN ACTIVITY, AND WATERLOGGING


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The hydraulic properties of plant roots depend on the morphology and anatomy of the root system, the length of the absorbing region and the influence of aquaporins (AQPs). These features change during development and in response to environmental stimuli, and alter the hydraulic conductivity of the root system ($L_p$). AQPs are proteins that form water selective channels to facilitate water flow across membranes. A large proportion of AQP isoforms are predominantly expressed in roots and their localization indicates a putative role in the transport of water across the root. AQP activity can finely regulate the rate of water flow across the root by changes in abundance and opening/closing the water channels. Since water will flow by the pathway of least resistance, AQPs will only influence radial water flow if the hydraulic conductivity of the apoplast is relatively less than that of the cell-to-cell pathway. There is growing evidence that AQPs influence water flow through the roots of some, but not all, species.

Waterlogging is a significant environmental constraint to crop growth, but its influence on $L_p$ is poorly understood. Depending on the tolerance of the species, waterlogging through oxygen deficiency reduces root growth and tends to reduce $L_p$. Oxygen deficiency can directly or indirectly close AQPs or alter their abundance. Changes in AQP activity may be the key component which ultimately influences water transport through waterlogged roots.

1. INTRODUCTION

Aquaporins (AQPs) are proteins that form channels to facilitate the transport of water across biological membranes. By altering their abundance and/or opening and closing the channel AQPs can control the rate of water flow into and out of cells and intracellular compartments. Since water is a fundamental requirement for most life processes, there has been a prodigious amount of research into AQP-facilitated water transport since the first AQP was discovered in the early 1990s. AQPs have now been found to exist in the membranes of almost all organisms, with the largest number of AQP genes expressed in plants.

The sedentary lifestyle of plants presents a fundamental challenge in the uptake and transport of water, to meet the demands of transpiration and growing tissues. AQPs have the potential to mediate not only the rate of flow across membranes, but also through tissues and organs, and to provide regulation that may minimize adverse effects during abiotic perturbations. It is now widely accepted that water flow through the roots of plants is...
regulated by AQP activity (Javot and Maurel, 2002), but the majority of
the current research tends to overlook the rudimentary features that make
plant roots successful as absorbing organs. The hydraulic properties of roots
are dependent not only on AQP activity, but also on the anatomy and
morphology of the root system, as well as the length of the absorbing
regions. These features vary between species, depend on the physical
characteristics of the soil, and can change in response to changes in the
environment. The nature of these changes in root structure can alter the
hydraulic conductivity of the pathways for water flow through roots, either
to conserve water during adverse conditions or function in some other way,
for example, to increase the transport of gases during waterlogging. AQP
activity also varies between species and can be regulated by development,
time of day, and in response to abiotic perturbation. Water flow through
roots is therefore a multifaceted process where a number of variables control
the mechanisms.

There has been very little research into the effects of waterlogging and/or
O₂ deficiency on water transport through roots, despite waterlogging being a
significant constraint to crop growth. The influence of waterlogging on water
flow through roots appears to be an enigma, as unlike drought and salinity
there is an abundance of freely available water and yet root water transport
tends to be reduced. Despite the effects of waterlogging on root water
transport being known for more than a century, there has been no adequate
explanation for this phenomenon. A significant discovery linked the closure of
AQPs with cytoplasmic acidosis, which occurs when cells respire anaerobically
during anoxic conditions (Tournaire-Roux et al., 2003). This mechanism of
reducing membrane permeability may be the mechanism that reduces the rate
of water flow through roots, when roots are submerged during waterlogging
and the O₂ concentration in the rhizosphere declines.

This chapter begins with an introduction into the processes driving
water flow and the definitions used to describe water fluxes through
roots. The review then details some of the physical characteristics that
influence water transport through roots and discusses how AQPs may be
involved in regulating water flow through the radial pathway. Finally, the
effects of waterlogging on root growth and water flow through roots are
reviewed. The response of AQPs to O₂ deficiency is reviewed and related
to their potential control of water flow through waterlogged roots.

2. Water Movement Through the Plant

2.1. Driving forces

Water movement in a plant is driven by gradients in water potential (Boyer,
1985; Passioura, 1982). Water potential (Ψ) is a measure of the free energy
associated with water and is expressed in units of pressure, usually
megapascals (MPa). The net water potential of a plant is composed of two principal components, hydrostatic \( P \) and osmotic \( \pi \) pressure:

\[
\Psi = P - \pi
\]

\( P \) is the result of internal or external pressures, such as the tension generated by transpiration. In cells, \( P \) includes turgor, which is an internally generated positive pressure, due to the rigidity of plant cell walls (Tyree and Jarvis, 1982). \( \pi \) depends on the concentration of solutes in solution. A gravitational component is sometimes included in Eq. (1), but only for tall trees (Boyer, 1985; Passioura, 1982).

Water passively moves down a gradient in total \( \Psi \), from high to low energy, until reaching equilibrium. Hence, gradients in \( \Psi \) can drive water transport through the soil-plant-atmosphere continuum (SPAC), and by manipulating its \( \Psi \) through transpiration or the accumulation of salts, a plant can control the process, within limits.

Water movement through the SPAC occurs in two phases, in the liquid phase by bulk flow through the soil and plant, and in the gas phase through the stomatal region (Baker, 1989). The traditional theory describing the ascent of water through the SPAC is the cohesion-tension (CT) theory, although the theory is still vigorously challenged and debated (Steudle, 2001; Zimmermann et al., 1993, 2004). Transpiration during the day generates hydrostatic pressure gradients to draw water into the roots and through the xylem of the plant. This hydrostatic pressure gradient is created by the surface tension that develops at the air–water interface in leaves and is transmitted as a negative pressure throughout the water column, where it lowers \( \Psi \) of the roots below the soil \( \Psi \) (Tyree, 1997). The negative pressure is equivalent to a tension or pulling force, drawing water upward (Tyree, 1997). The tension within the xylem increases as the soil water decreases and/or as the transpiration rate increases (Tyree and Sperry, 1988). By controlling the size of the stomatal apertures, the plant can regulate transpiration, and therefore \( P \), to avoid an excessive amount of cavitation. However, when stomata are closed, photosynthesis is also inhibited, creating a compromise between CO₂ uptake and water loss.

The accumulation of solutes across semipermeable membranes also establishes a gradient in \( \Psi \) that induces water uptake (Oertli, 1991). An osmotic gradient generates root pressure, causing xylem sap to exude from a cut shoot or detopped root system (reviewed by Zhoklevich, 1991). The endodermis probably forms a semipermeable barrier, preventing the net efflux of solutes from the stele. Solutes secreted into the xylem lower \( \Psi \) and induce water uptake across the root. An upward flow of solution results as more water is drawn into the xylem.

Osmotic gradients are only important in driving water transport when transpiration is low (Kramer, 1983). When transpiration increases, the
increasing mass flow of water dilutes the solutes in the xylem sap until the osmotic component is negligible and the uptake of water is predominantly controlled by the hydrostatic pressure within the xylem (Section 2.3). Transpiration can produce a much steeper gradient in $\Psi$, from soil to roots, than an osmotic mechanism, as reviewed by Kramer (1983). Even so, water uptake may be limited when a plant experiences water deficit through drying soil or saline conditions because the plant cannot lower its $\Psi$ sufficiently to create a gradient between the soil and roots.

2.2. Hydraulic conductance

The flux of water (rate of water flow) through a plant depends not only on the size of the driving forces, but also on the conductance (reciprocal of resistance) of the pathways, through which water flows (Boyer, 1985):

$$Q = L(\Delta P - \sigma \Delta \pi)$$

(2)

where $Q$ is the water flux or volume flow rate (m$^3$ s$^{-1}$), $L$ the hydraulic conductance (m$^3$ s$^{-1}$ MPa$^{-1}$), $\sigma$ the reflection coefficient, and $\Delta P$ and $\Delta \pi$ the hydrostatic and osmotic pressure differences (MPa), respectively. $\sigma$ is a unitless parameter, relating the interaction between water and solute crossing a membrane. The value of $\sigma$ depends on the particular solute, with a value of 1 for a perfect osmometer and 0 when the membrane does not reflect the solute relative to water. If $\sigma = 1$, then Eq. (2) reduces to (Boyer, 1985):

$$Q = L(\Delta \Psi) = \frac{(\Delta \Psi)}{R}$$

(3)

where $R$ is the apparent hydraulic resistance (MPa s m$^{-3}$) and hence, Eq. (3) is a simple analogy to Ohm’s law (van den Honert, 1948).

A common error in plant water relations is the interchangeable use of hydraulic conductance and conductivity ($L_p$). Equation (4) shows the relationship between the two parameters. $L$ is a measure of the ability of an entity to conduct water, independent of the entity’s dimensions, whereas $L_p$ is a property of an entity with specified dimensions, usually surface area ($A$).

$$L = L_p \cdot A$$

(4)

The SPAC has been described as a system of hydraulic resistors arranged in series (van den Honert, 1948). Plants can vary the resistance (and conductance) of the pathways to maintain the water balance of the shoot (Steudle, 2000). When water exists in the vapor phase, the greatest resistance is the stomatal aperture. However, in the liquid phase, the root system constitutes
a highly significant and important resistance to overall flow of water in the plant (Steudle, 2000).

2.3. Hydraulic conductivity of roots ($Lp_r$)

The surface area limits water uptake by the root (Steudle, 2001; Tyree, 2003). Therefore, water transport parameters are usually normalized per unit surface area and revision of Eq. (2) incorporates this:

$$J_v = Lp_r(\Delta P - \sigma \Delta \pi)$$  \hspace{1cm} (5)

where $J_v$ is the volume flow, per unit area ($m^3$ m$^{-2}$ s$^{-1}$ or m s$^{-1}$), $Lp_r$ the hydraulic conductivity of the root (m s$^{-1}$ MPa$^{-1}$), and $\sigma$, the apparent root reflection coefficient (Passioura, 1988). The relative influences of $\Delta P$ and $\Delta \pi$ depend on the rate of salt secretion into the xylem and the rate of water flow (Weatherley, 1982). When $\Delta P$ is zero, water flow is driven by the osmotic gradient, but the xylem sap becomes diluted when water flow increases through transpiration, and $\Delta \pi$ becomes negligible (Kramer, 1983).

The majority of studies have used detopped root systems to measure $J_v$, where the exudation of sap from the cut stump is collected under root pressure, or water flow is induced either by applying suction to the cut stump or externally pressurizing the root system (Fiscus, 1975; House and Findlay, 1966; Nobel et al., 1990). In the root pressure exudation technique, the driving force is osmotic and $\Delta \pi$ is determined by measuring the osmotic pressure of the exudate and the medium bathing the roots.

In the external pressure technique, the root system is sealed in a pressure chamber with the cut stem protruding through the lid of the chamber. The root system is pressurized and $J_v$ determined at different pressure increments. Plotting $J_v$ against $\Delta P$ typically produces a curvilinear relationship (Passioura, 1984, 1988), due to the osmotic component dominating flow at low flow rates (Dalton et al., 1975; Fiscus, 1975). At high flow rates, the solutes are so diluted that $J_v(\Delta P)$ is inherently linear and the slope gives $Lp_r$ [Eq. (5)]. If the linear part of the $J_v(\Delta P)$ relationship is extrapolated to the $x$-axis, the intercept should equal the osmotic pressure of the external medium ($\pi_o$), but invariably it exceeds $\pi_o$ (Passioura, 1984). This anomaly has not been satisfactorily explained (Passioura, 1984), but the offset depends on the species and can vary diurnally or with abiotic perturbations (Boursiac et al., 2005; Emery and Salon, 2002; Munns and Passioura, 1984; Murphy, 2003; Passioura and Munns, 1984; Rieger and Litvin, 1999).

The root and cell pressure probes have been developed to measure water flow through individual roots and across cell membranes (Steudle, 1993; Tomos and Leigh, 1999). In summary, the root pressure probe (RPP) measures root pressure and water flow is induced either by applying
hydrostatic pressure (with the aid of the probe) to the root, or by changing
the osmotic pressure of the bathing medium. The rate of water flow across
the root, and subsequently, \( L_{pr} \), is calculated from the rate of transient
relaxations in root pressure (Steudle, 1993).

The cell pressure probe (CPP) measures turgor pressure via the tip of a
microcapillary introduced into an intact cell. Analogous to the RPP, water
flow is induced by applying hydrostatic pressure, with the aid of the probe
(Steudle, 1993). If the dimensions of the cell are known, the CPP can be
used to determine the volumetric elastic modulus \( \varepsilon \) and hydraulic con-
ductivity \( (L_{pc}) \) of the cell. Osmotic flows can also be induced, but due to the
uncertainty of the effects of unstirred layers for cells located within tissue,
the values of \( L_{pc} \) may be erroneous.

The CPP and RPP are appealing because they provide real-time measure-
ments that are useful for estimating the location of the principal resistances to
water transport through roots. Measurements of cell turgor have revealed
gradients in \( \Psi \) across a radial profile in the roots of some species (Pritchard
et al., 1989; Zimmermann et al., 1992). Combining results from CPP and
RPP measurements on roots of the same species can potentially identify the
main radial pathway for water transport across the root (Section 3.2.2). For
example, \( L_{pc} \) of epidermal and cortical cells was much greater than \( L_{pr} \) of
Hordeum distichon and Phaseoluscocineus roots, indicating that water flows via
the cell-to-cell pathway (Steudle and Brinckmann, 1989; Steudle and Jeschke,
1983). In comparison, analogous measurements on maize (Zea mays) roots
revealed a predominantly apoplastic flow (Steudle et al., 1987). Comparing the
measured values of \( L_{pc} \) and \( L_{pr} \) for each cortical cell layer indicated that radial
water flow through wheat (Triticum aestivum) roots occurs by a combination of
the parallel pathways, but radial water flow in the roots of narrow-leafed lupin
(Lupinus angustifolius) and yellow lupin (L. luteus) appears to be predominantly
apoplastic (Bramley, 2006).

2.3.1. Transport models
The root has been modeled as a system containing either two or three
compartments, a series of membranes or as a perfect osmometer, to explain
the processes of water flow across plant roots (Dalton et al., 1975; Fiscus,
1975; Pickard, 2003; Tyree et al., 1994). However, there has been consid-
erable debate about whether \( L_{pc} \) of roots was dependent on \( J_w \) (reviewed by
Kramer, 1983; Passioura, 1982). Before the models of Fiscus (1975) and
Dalton et al. (1975), the nonlinear relationship between flow and driving
force was interpreted as \( L_{pc} \) increasing with flow rate, so water flux was
considered to be independent of \( \Delta \Psi \) across the root (reviewed by
Weatherley, 1982). The landmark papers of Passioura and Munns (Munns
and Passioura, 1984; Passioura and Munns, 1984) demonstrated a linear
relationship between driving force and flow. The slope of the relationship
varied with time and treatment, indicating that the apparent hydraulic conductance was truly variable.

Steudle (1994) extended the two-compartment models and developed the Composite Transport model. The model incorporates the composite nature of water flow across different tissues, the parallel radial pathways, the hydrostatic and osmotic driving forces, variable $L_p$, and also accounts for $\sigma_r < 1$ (Steudle and Peterson, 1998). The model explains the variability in $L_p$ in relation to the nature and intensity of the driving forces (Steudle and Peterson, 1998). Osmotic forces will primarily drive water flow through the cell-to-cell pathway, as $\sigma \approx 1$ for cell membranes. Since there are no membranes in the apoplastic pathway, the apoplastic $\sigma \approx 0$. Thus, osmotic gradients will drive very little water transport in the apoplast. When the driving force is primarily hydrostatic (e.g., generated by transpiration), water can flow via a combination of apoplastic and cell-to-cell pathways.

3. Root Characteristics and Water Flow

3.1. Factors that influence root growth and water uptake

External factors can influence water uptake by the roots, such as interactions between the roots and the soil, and the distribution of roots within the soil profile (Passioura, 1988). Close contact with the soil is imperative for roots to maintain hydraulic continuity at the soil–root interface and minimize the interfacial resistance to flow. Poor hydraulic contact necessitates a large drop in water potential across the interface to induce water uptake (Passioura, 1988). Roots growing through large soil pores may have poor hydraulic contact with the soil, but there is also evidence that some roots shrink when plant $\Psi$ is low, so the root–soil contact may be reduced (Huck et al., 1970; Palta et al., 1987). The growth of root hairs or the exudation of compounds that adhere to soil particles may assist roots in maintaining an intimate connection with the soil.

The distribution of roots in the soil profile depends on soil texture and structure, and the type of inherited root system (Kramer, 1983). Roots tend to grow through preexisting soil pores, toward regions with nutrients and water, and avoid unfavorable regions, so the distribution is generally not uniform. Roots of many crop species can penetrate to depths of several meters in well-aerated, deep, soft soils (Kramer, 1983). In addition, tolerance to the range of abiotic limitations encountered by roots in a growing season may determine root growth behavior.

There are two main types of root system. Monocots develop a fibrous root system with initial roots (3–5 axes for wheat) emerging from the seed (seminal roots) and subsequent roots emerging from the basal nodes of the stem (nodal roots, also called adventitious roots) (Greacen et al., 1976). In comparison, the radicle of eudicots (the first root to emerge from the
seed) can develop into the dominant root of a taproot system that can become extensively branched. Although monocot and eudicot root systems can extend to similar depths in the field, the total root length density (root length/soil volume) of monocots is often much greater. For example, the root length density of wheat is two- to tenfold larger than narrow-leaved lupin in Western Australian soils (Dracup et al., 1993; Gallardo et al., 1996; Gregory and Eastham, 1996; Hamblin and Tennant, 1987). Despite these differences in root length, the roots of eudicotyledon species tend to have a higher specific rate of water uptake than cereals (Bremner et al., 1986; Hamblin and Tennant, 1987; Mason et al., 1983). Moreover, these greater rates of water uptake appear to be due to a greater hydraulic conductivity. For example, $L_p$ of lupin root systems is at least twofold greater than $L_p$ of wheat root systems (Bramley, 2006; Gallardo et al., 1996).

### 3.2. Root anatomy

Kramer (1983) stated that the “effectiveness of roots as absorbing organs” depends on anatomical and morphological features. The dynamics of root water permeability in relation to these features were succinctly summarized by Moreshet and Huck (1991). The root apex typically has high axial and radial resistances to water flow compared with the remainder of the root with its developed xylem (Steudle, 2001). Water and nutrient absorption generally commences >10 mm behind the tip, which coincides with the root hair zone. The length of the absorbing region depends on the species, but may change with transpirational demand and during adverse conditions (reviewed by Kramer, 1983). $L_p$, of wheat roots decreases with distance from the root tip, indicating that water absorption occurs preferentially in the apical region (Bramley, 2006; Jones et al., 1988). In addition, water absorption by several or all of the individual roots may contribute to $L_p$ of the whole root system (Bramley, 2006). Cereal roots apparently have maximum water absorption within a region <100 mm from the root tip (Greacen et al., 1976; Sanderson, 1983). There is also evidence that individual roots are able to vary their hydraulic conductivity. Vysotskaya et al. (2004a,b) excised four of the seminal roots of durum wheat (*Triticum durum*), and this increased $L_p$ of the remaining root, so that the water supply to the shoot was maintained. In comparison, lupin roots absorb water more evenly along the length of the taproot as $L_p$ is constant with length (Bramley, 2006). Using a novel CAT-scanning and microelectrode technique, Hamza and Aylmore (1992a) observed uniform water absorption along the length of roots of 17-day-old narrow-leaved lupin plants, in comparison with radish (*Raphanus sativus*) plants that extracted more water closer to the soil surface. Lupins had lower potential difference between leaves and root surface (i.e., smaller $\Delta \Psi$, which equates to a smaller driving force) and higher water flow rates than radish, indicating that lupins had lower plant resistances (Hamza and Aylmore, 1992b).
This disparity in root water uptake and $L_p$, were initially thought to be due to differences in axial and radial anatomy (Gallardo et al., 1996; Hamblin and Tennant, 1987; Hamza and Aylmore, 1992a), but this was proposed before AQPs were discovered. Nevertheless, differences in anatomy can influence the hydraulic conductivity of the pathways for water flow.

The root has been described as a porous pipe or leaky cable, where the ratio of the radial (across the pipe wall) and axial (through the length of the pipe) resistances determines the resistance of the whole root and the distribution of water uptake (Landsberg and Fowkes, 1978; Zwieniecki et al., 2003). In the radial pathway, water taken up by the root has to traverse living tissue before reaching the lumen of the xylem vessels. In the axial plane, water flow occurs through the xylem vessels and tracheids.

3.2.1. Axial pathway

Mature xylem vessels are dead cells that form a continuum of tube-like structures, separated by thin perforated walls, so that longitudinal flow through the vessels is analogous to flow through a conduit or pipe (Zwieniecki et al., 2003). The driving force for longitudinal flow through the vessels will be hydrostatic, unless membranes (e.g., living cells) interrupt the pathway. If the perforated walls provide negligible resistance, the radius of the vessels (to the fourth power) determines the rate of water flow through the xylem continuum, according to the Poiseuille-Hagen equation (Steudle and Peterson, 1998). The development of the vasculature is different between monocots and eudicots. For example, the stele of wheat contains one central pitted metaxylem vessel, which increases in diameter away from the root tip (40- to 100-$\mu$m diameter), and seven or eight xylem strands containing one small metaxylem vessel (10-$\mu$m diameter). Consequently axial conductance does not appear to change much along the length of the root (Bramley, 2006). Within the stele of lupins, the vasculature develops in a diarch pattern, with bundles of metaxylem vessels up to 100 $\mu$m in diameter (Hamblin and Tennant, 1987). The abundance and diameter of vessels increase with distance from the root tip, which result in axial conductance also increasing in a similar pattern (Bramley, 2006).

The axial conductance may be associated with $L_p$, probably reflecting the capacity of the axial pathway in relation to all water absorption distal to any point. The axial conductance may be several orders of magnitude greater than the total hydraulic conductance of a root and hence the radial pathway creates the greatest constraint on water flow through the root (reviewed by Steudle and Peterson, 1998).

3.2.2. Radial pathway

Water taken up by the root has to cross a series of concentric cell layers: the epidermis, cortex, endodermis, and stele, before reaching the lumen of the xylem vessels (Fig. 1).
The epidermis consists of a single layer of elongated, tightly packed cells. Epidermal cell walls are generally thin, but may contain deposits of suberin or cutin (Kramer, 1983; Moreshet and Huck, 1991). Epidermal walls of the majority of 181 species surveyed appeared autoflourescent when viewed under ultraviolet light, indicating the presence of suberin or lignin (Perumalla et al., 1990). Root hairs arise from epidermal cells, and their abundance and longevity depend on the plant species and environment. A structurally different subepidermal layer may exist, called the hypodermis (Perumalla et al., 1990). In some species, the hypodermal walls contain a radial strip of suberin (Casparian bands) or suberized secondary walls and the layer is called an exodermis (Enstone and Peterson, 1997, 1998; Perumalla and Peterson, 1986; Perumalla et al., 1990). Suberin is composed of hydrophobic, fatty compounds, believed to act like a waterproofing agent (Nawrath, 2003; Zeier and Schreiber, 1998; Zeier et al., 1999). The presence of a suberized exodermis can change with root maturity and environmental conditions. For example, in maize roots, aeroponic culture (mist culture) induces the development of an exodermis (Hose et al., 2000; Zimmermann et al., 2000). In comparison, the roots of lupin species do not form a hypodermis or exodermis, at least up to 300 mm from the tip when grown in hydroponic or sand culture (Bramley, 2006; Hartung et al., 2002; Perumalla et al., 1990) and cannot be stimulated by aeroponics (Hartung et al., 2002). A suberized exodermis does not form in members of the Tritaceae family (Greacen et al., 1976; Perumalla et al., 1990) possibly because the cortex eventually deteriorates.

The cortex consists of cell layers, the number of which varies depending on species, root development, and environment. The cortex of cereal roots generally consists of 6–8 cell layers, but this shrivels with maturity (Greacen et al., 1976; Hamblin and Tennant, 1987). The cortex also shrinks when the water potential of the root decreases, which implies it is not buffered against...
large changes in $\Psi$ in the xylem. Hence, the greatest resistance to radial water flow may occur in the epidermis (Passioura, 1988). Cortical cell walls are thin and usually contain no suberin. The cubic or hexagonal arrangement of cells determines the shape of the intercellular spaces between cells, which influences the air-filled root porosity and the size of the apoplast.

The endodermis is a single cell layer that forms a sheath around the stele (Moreshet and Huck, 1991). The cell walls of the endodermis may contain suberin and thicken to form a similar structure to the exodermis (Perumalla and Peterson, 1986; Zeier and Schreiber, 1998; Zeier et al., 1999). During root growth, the endodermis develops in three stages: (1) appearance of Casparian strip; (2) continuous layer of suberin (suberin lamellae) covering the protoplast, between plasmalemma and cell wall; and (3) inner tangential walls thicken, and a layer of cellulose is deposited over the suberin lamellae (Ma and Peterson, 2003). The location of the different endodermal stages along the root axis depends on species, but appears to mature earlier in cereals compared with eudicots (Bramley, 2006; Greacen et al., 1976; Hartung et al., 2002; Perumalla et al., 1990; Sanderson, 1983). Water deficit may also promote earlier development of the endodermis (Enstone et al., 2003). The endodermis may also contain unsuberized passage cells, opposite xylem poles (Esau, 1977).

The pericycle, which is the layer from which lateral roots arise, bounds the stele. The stele contains small, tightly packed parenchyma cells and the vascular tissue (xylem and phloem). Primary xylem forms a core in the root that develops in an exarch pattern, that is, centripetally (Esau, 1977). Protoxylem elongates and matures (deposition of secondary walls) to become a functioning conduit, before eventually differentiating into metaxylem.

3.2.2.1. Parallel pathways for radial water flow There are three parallel pathways for radial water flow: symplastic, transmembrane, and apoplastic (Fig. 2).

The symplastic pathway is from cell to cell, through the cytoplasmic continuum, via plasmodesmata (Fig. 2). Water must cross at least two membranes, once to enter and once to exit the symplast.

The transmembrane pathway involves water flow from cell to cell (vacuole to vacuole), and the most direct pathway is by crossing cell walls, plasma membrane, cytoplasm, and tonoplast (Fig. 2). Water crossing each cell layer in series, via the transmembrane pathway, would hence cross four membranes per cell (Fig. 2).

The apoplastic pathway is around protoplasts, via the cell walls and intercellular spaces (Fig. 2). Analogous to flow through the xylem, hydrostatic pressure gradients drive water flow in the apoplastic pathway because it contains no membranes. However, the apoplastic path has small cross-sectional surface area of the radial pathway and may be interrupted at the
endodermis and exodermis (if present) by suberin, forcing water to cross at least the plasma membrane to reach the apoplast of the stele. Suberization of the endodermis and exodermis is thought to block the apoplast and prevent the transport of water and ions (Enstone et al., 2003), but Steudle et al. (1993) demonstrated that the endodermis of young maize roots did not influence $L_p$.

Water flow across the root can occur by any combination of apoplastic and cell-to-cell pathways, or one pathway may dominate. The pathways may differ between species, change in different regions of the root, and alter with different environmental conditions. Identifying the contribution of the different pathways is important for understanding the nature of the resistances to root water transport. Some researchers believe the cell-to-cell pathway is the main pathway for water flow, and that the epidermis poses the greatest resistance (reviewed by Passioura, 1988). Transport through the cell-to-cell pathway, crossing membranes or via plasmodesmata, provides the opportunity for regulatory control and hence the potential to influence the size of the resistance, without necessitating anatomical changes. Moreover,
the discovery of AQP s is changing the traditional view that water transport across membranes has a high invariant resistance to water flow (Tyerman et al., 1999).

The apoplast is often considered to be the dominant pathway, despite the fact that it only constitutes ~5% of total root volume and an even smaller fraction of the cross-sectional surface area. If the apoplast were the main pathway, the plant would have little control of water flow through the root, unless the endodermis forms a tight barrier and therefore, plays a central regulatory role.

If the radial conductance is indeed the rate-limiting factor in water transport through roots, then theoretically, thicker roots should have a smaller $L_p$, than thin roots because the path length for flow is longer. However, anatomy and AQP activity (Section 6.3) influence the resistivity of the radial pathways to flow, which can counteract the significance of the path length. Rieger and Litvin (1999) found that root diameter was negatively correlated with $L_p$, in five species with contrasting root anatomy. In addition, Rieger and Litvin (1999) found that drought stimulated suberization and other anatomical changes that reduced $L_p$. The development of an exodermis in maize roots decreases the radial hydraulic conductivity (Hose et al., 2000; Zimmermann et al., 2000). Conversely, Barrowclough et al. (2000) discovered that in developing onion (Allium cepa) roots, the greatest values of radial hydraulic conductivity were correlated with the presence of an exodermis. Huang and Eissenstat (2000) also found that structural differences in the radial pathway were the primary factor determining $L_p$, of roots of citrus rootstocks.

4. Changes in $L_{pr}$

$L_p$, for individual roots and whole root systems typically ranges between $10^{-8}$ and $10^{-7}$ m s$^{-1}$ MPa$^{-1}$, for herbaceous species, reflecting differences in root structure and experimental techniques (Table 6 of Steudle, 1989; Table 2 of Rieger and Litvin, 1999). For any given species, $L_p$, can alter with development, environment, and other regulatory controls.

During the day, water flux through the plant varies with time, usually following the fluctuation in transpiration. A number of studies have found $L_p$, to vary diurnally in excised roots of wheat, maize, Lotus japonicus, castor oil (Ricinus communis), white lupin (L. albus), and sunflower (Helianthus annuus) (Carvajal et al., 1996; Clarkson et al., 2000; Else et al., 2001; Everard and Drew, 1987, 1989; Henzler et al., 1999; Passioura and Munns, 1984).

Exogenous addition of phytohormones to the root-bathing medium tends to influence root water transport, although the responses to abscisic acid (ABA) treatment are contradictory. The hydraulic conductivity of maize, sunflower, and common bean (Phaseolus vulgaris) roots increased in
response to exogenous ABA (Aroca et al., 2006; Freundl et al., 2000; Hose et al., 2000; Quintero et al., 1999). ABA also increased $L_p$ of maize root cortical cells, but the effect was only transient with $L_p$, returning to control levels after 2 h (Hose et al., 2000). Conversely, ABA did not influence water flux from detopped *Populus tremuloides* roots, but reduced $L_p$ of *P. coccineus* roots (Fiscus, 1981; Wan and Zwiazek, 1999).

Abiotic factors also affect hydraulic conductivity. Gradual soil drying or addition of external osmoticants (e.g., NaCl, polyethylene glycol) decreased root water transport by 27–100% in long- and short-term experiments (Aroca et al., 2006; Azaizeh and Steudle, 1991; Carvajal et al., 1999, 2000; Joly, 1989; Liu et al., 2006; Lu and Neumann, 1999; Martre et al., 2001; Munns and Passioura, 1984; Nobel et al., 1990; Rieger and Litvin, 1999). Other factors that can reduce $L_p$ include $O_2$ deficiency (Section 7.3), nutrient deficiencies, chilling, and high concentrations of aluminium or other toxicants (Aroca et al., 2005; Carvajal et al., 1996; Clarkson et al., 2000; Gunsé et al., 1997; Kamaluddin and Zwiazek, 2002a, 2003; Lee et al., 2004). However, the results are not consistent, and for example, starving the roots of potassium more than doubled $L_p$ of rice (*Oryza sativa*) roots, which was correlated with an increase in expression of AQP genes (Liu et al., 2006). The response of $J_v$ and $L_p$ is frequently rapid, occurring within minutes and when the treatment is removed the recovery in $L_p$ is often just as rapid (Carvajal et al., 1996; Gaspar et al., 2001; Kamaluddin and Zwiazek, 2002a, 2003). The magnitude of the effect on $L_p$ appears to depend on the nature of the driving force with salinity and chilling inhibiting osmotic $L_p$, more than hydrostatic $L_p$ (Azaizeh and Steudle, 1991; Lee et al., 2004; Martinez-Ballesta et al., 2000).

Physical changes in root structure and anatomy occur in some species (Section 3) and these may decrease the conductance to bulk water flow, for example deposition of suberin in hypodermal cell walls (Steudle, 2000). However, anatomical changes are slow and dependent on growth, and may act as a survival strategy to reduce $L_p$, in the long term, when environmental changes in the field are slow. There is no evidence where anatomical changes provide the means to regulate $L_p$, diurnally, nor do they account for the rapid and reversible changes in $L_p$, measured under laboratory conditions. If a significant proportion of radial water flow occurs through the cell-to-cell pathway by crossing cell membranes, $L_p$ may be controlled by AQP activity.

### 5. Plant Aquaporins (AQPs)

Plant AQPs are ubiquitous and expressed at such high levels that they can constitute up to 15% of total membrane protein (Johansson et al., 1996; Rivers et al., 1997). Plant AQPs are also highly diverse. The *Arabidopsis*
thaliana genome contains 35 sequences that are AQP homologues and at least 31 AQPs have been identified in maize and 33 in rice (Chaumont et al., 2001; Quigley et al., 2001; Sakurai et al., 2005). The high number of AQPs alludes to their importance in plant membrane transport and possibly plant hydraulics (The Arabidopsis Genome Initiative, 2000). Based on the sequence alignment of their amino acids, the plant AQP family is classified into four subfamilies: plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodulin-like intrinsic proteins (NIPs), and small basic intrinsic proteins (SIPs). The PIPs are further subdivided into two groups, PIP1 and PIP2. The PIP and TIP nomenclature implies that these proteins are localized to the respective plasma and vacuolar membranes, but they may also be localized to other membrane compartments (Barkla et al., 1999; Frangne et al., 2001; Kirch et al., 2000; Liu et al., 2003).

The diversity of plant AQPs implies different functional roles, although the nature of these roles is still generally unclear. Phylogenetic analysis shows close similarities between monocots and eudicots and the genomes of A. thaliana, rice and maize contain similar numbers of AQP homologues within each subfamily/group (Table 1). Rice has two less PIP members than A. thaliana and maize, and maize has fewer NIPs compared with the other two species. Although there is a high degree of homology between AQPs within each subfamily, each species also has unique AQP members (Chaumont et al., 2001; Johanson et al., 2001; Quigley et al., 2001; Sakurai et al., 2005).

5.1. AQP structure

In AQP proteins there are six transmembrane helices connected by five loops of varying lengths and the amino (NH₂) and carboxy (COOH) termini are located on the cytoplasmic side of the membrane (Fig. 3). Amino acid motifs in loops C and E are highly conserved in a wide range of PIPs, but are not present in AQPs localized in the tonoplast, and so these motifs are thought to be necessary for signal recognition and targeting to the respective membrane (Barone et al., 1997).

The two longest loops, B and E, contain the signature AQP motifs asparagine-proline-alanine (NPA) that fold into the membrane as a component of the single aqueous pore (Fig. 3). The novel folding of the polypeptide creates a pore shape that is analogous to an hourglass, with a narrow constriction in the center of the membrane and widens at the membrane surfaces (Jung et al., 1994; Murata et al., 2000). The obverse symmetry of the pore provides AQPs with bi-directionality without rectification (Meinild et al., 1998; Murata et al., 2000). Loop D in PIPs is four to seven amino acid residues longer than other AQPs, which folds under the protein on the cytosolic side of the membrane and forms a cap occluding the cytosolic side of the pore (Törnroth-Horsefield et al., 2006, Section 5.3.3.1.1).
A cysteine residue on the third transmembrane domain (Fig. 3) has been found to impart mercury sensitivity to several plant AQPs and this residue is highly conserved in PIPs and TIPs (Daniels et al., 1996; Quigley et al., 2001). Barone et al. (1997, 1998) also demonstrated that Hg\(^{2+}\) induces a conformational change to the plant plasma membrane AQPs instead of direct occlusion of the water channel. Several PIPs and at least one TIP appear to be mercury insensitive, despite the presence of several cysteine residues on the protein (Biela et al., 1999; Daniels et al., 1994; Krajinski et al., 2000). Despite the caveats of using this compound (Section 5.3.3.1.3), the application of mercury is one of the main tools used to estimate the activity of AQPs by determining its effect on reducing membrane permeability (Maurel, 1997).

Other features that are highly conserved in plant AQPs include a pH-sensitive histidine residue on Loop D (Fig. 3), present in all PIPs (Tournaire-Roux et al., 2003). Serine residues, which are putative phosphorylation sites, are located in the first cytosolic loop of most PIPs and

<table>
<thead>
<tr>
<th>AQP subfamily/group</th>
<th>Arabidopsis thaliana</th>
<th>Oryza sativa</th>
<th>Zea mays</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIP1</td>
<td>5</td>
<td>3</td>
<td>6</td>
<td>Chaumont et al., 2001; Johanson et al., 2001; Quigley et al., 2001; Sakurai et al., 2005</td>
</tr>
<tr>
<td>PIP2</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>Chaumont et al., 2001; Johanson et al., 2001; Quigley et al., 2001; Sakurai et al., 2005</td>
</tr>
<tr>
<td>TIP</td>
<td>10</td>
<td>10</td>
<td>11</td>
<td>Chaumont et al., 2001; Johanson et al., 2001; Quigley et al., 2001; Sakurai et al., 2005</td>
</tr>
<tr>
<td>NIP</td>
<td>9</td>
<td>10</td>
<td>3</td>
<td>Chaumont et al., 2001; Johanson et al., 2001; Quigley et al., 2001; Sakurai et al., 2005</td>
</tr>
<tr>
<td>SIP</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>Chaumont et al., 2001; Johanson et al., 2001; Quigley et al., 2001; Sakurai et al., 2005</td>
</tr>
</tbody>
</table>
TIPs, and the C-terminal of all PIP2s and NIPs (Fig. 3) (Guenther et al., 2003; Johansson et al., 1998; Quigley et al., 2001).

In the membrane, AQPs form tetrameric structures with each monomer acting as an independent water channel (Murata et al., 2000; Walz et al., 1997). There is increasing evidence that at least some plant AQPs form heterotetramers through interaction with different PIP isoforms and this interaction activates water channel function (Fetter et al., 2004; Temmei et al., 2005). However, for functional importance to be implied, studies need to demonstrate that the relevant isoforms are coexpressed in the same tissue and cells.

5.2. AQP selectivity

AQP activity is predominantly assayed by heterologous expression of individual isoforms in *Xenopus laevis* oocytes. The oocytes have an intrinsically low permeability to water and are invariably unperturbed when exposed to a hypotonic bathing medium. After expression of exogenous AQPs the oocytes swell rapidly and burst within a few minutes. The swelling rate is used to calculate the osmotic water permeability of the membrane ($P_f$). Although this method does not reveal the true function of AQPs in native membranes, the procedure does demonstrate that many AQP homologues increase the rate of water flow across membranes. The activity of individual

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**Figure 3** Overall topology of a plant PIP2 showing the highly conserved and important residues (circled). AQPs contain six membrane-spanning domains connected by loops A to E. Loop D of PIPs contains four to seven more amino acid residues than other AQPs and this loop folds under the protein, on the cytosolic side, to block the pore. The NPA motifs form the aqueous pore. Serine (S) and histidine (H) residues are involved in phosphorylation and protonation of the protein, respectively. The cysteine (C) residue on the third transmembrane domain confers mercury sensitivity in some AQPs.
AQP varies considerably, with some isoforms having very low water permeability, while others are also permeable to small nonelectrolytes, such as CO₂, glycerol (aquaglyceroporins), boric acid, and urea (Biela et al., 1999; Dordas et al., 2000; Liu et al., 2003; Uehlein et al., 2003). PIP1s appear to have little or no water permeability, although when expressed individually they may not be activated in oocytes (Chaumont et al., 2000; Fetter et al., 2004; Li et al., 2000; Suga and Maeshima, 2004). Conversely, PIP2s have high water permeability and can increase \( P_f \) by at least tenfold (Chaumont et al., 2000; Suga and Maeshima, 2004). NOD26 from soybean (Glycine max) and its ortholog LIMP2 from L. japonicus have moderate water permeability and only increase oocyte swelling rates by threefold, but are also permeable to glycerol (Guenther and Roberts, 2000; Guenther et al., 2003). NtAQP1 from tobacco (Nicotiana tabacum), a PIP1 ortholog, is moderately permeable to water, glycerol, and CO₂ (Biela et al., 1999; Siefritz et al., 2001; Uehlein et al., 2003). TIPs appear to have constitutively high permeability to water and several A. thaliana TIPs also transport urea (Daniels et al., 1996; Liu et al., 2003). No plant AQPs that have been functionally characterized are permeable to ions, unlike the mammalian AQP6 that is permeable to anions, particularly nitrate, when exposed to HgCl₂ or when the cytosol is acidified (Hazama et al., 2002; Ikeda et al., 2002; Yasui et al., 1999). HgCl₂ can cause turgor pressure of plant cells to decrease due to a leakage of ions (Zhang and Tyerman, 1999), albeit the loss of ions is probably through ion channels.

The shape of the pore and the size of the permeating molecule confer the selectivity of the individual AQP for water or other small neutral solutes (Murata et al., 2000). However, a simple steric model does not account for the diversity of solutes that can permeate some AQPs and not others. As Meinild et al. (1998) demonstrated, there is no connection between the permeation of mammalian AQPs and the solute size. Selectivity of the protein is more likely determined by the properties of the amino acid residues lining the pore. Identification of the important residues for selectivity is progressing for prokaryotic and mammalian AQPs (Sui et al., 2001; Tajkhorshid et al., 2002), but no specific signature motifs have been identified in solute-transporting plant AQPs thus far (reviewed by Santoni et al., 2000; Tyerman et al., 2002). There is also some conjecture whether the fourfold axis in the center of the AQP tetramer could function as a channel, and possibly as an ion channel, which is activated when the state of the water channels is altered (Chrispeels et al., 1999; Tyerman et al., 2002).

Considerable advances have been made in determining the permeability of different AQPs to various compounds, but knowledge of the specifics of permeability alone does not infer a physiological role. With new technology and molecular techniques, research is progressing in understanding of the role of AQPs in situ.
5.3. Control of water permeability

The existence of AQPs is profoundly significant for living cells, because they endow the organism with the potential to control water flow (Tyerman et al., 2002). AQPs can control flow across the membrane (and tissue/organs) in two ways: (1) by changing their abundance in the membrane and/or (2) by changing the rate of flow through the water channel. Changing the rate of flow through the AQP may be achieved by the opening and closing of the channel (gating), or by changing the gradient of the driving force (Tyerman et al., 2002).

5.3.1. AQP abundance and patterns of expression in roots

The expression pattern of AQPs tends to be greater in tissues and cells with high water permeability. In plants, the patterns of expression are complex, varying between organs and tissues and are dependent on the species. Very few AQP isoforms appear to be cell specific, but rather, are expressed at varying levels in a range of cell types under developmental control or in response to environmental stimuli.

When focusing on roots, the literature reveals several principal features. First, a high proportion of AQP isoforms is preferentially expressed in roots (Table 2). PIP2;1 and/or PIP2;2 homologues are abundantly expressed in the roots of most species studied (Table 2). The tissue-specific expression of these isoforms also reveals a putative role in the radial transport of water across the root with the high abundance of PIP2 homologues particularly in the endodermis (Hachez et al., 2006; Javot et al., 2003; Suga et al., 2003). If suberization of the endodermis blocks the apoplastic pathway, then water would have to cross membranes in this tissue to reach the apoplast of the stele (Section 3.2.2.1). Geometrically, the endodermis also creates a high resistance to water flow, because of the decreasing surface area toward the center of the root. A high expression of PIP2s with high water permeability (Section 5.2) in the endodermis could increase the radial hydraulic conductance and confer an efficient mechanism of regulating Lp_r.

Additional support for PIP2s facilitating radial water transport across membranes where the apoplast is blocked comes from aeroponically grown maize roots. Roots grown under these conditions develop a suberized exodermis and endodermis, in comparison with roots grown hydroponically (Hose et al., 2000; Zimmermann et al., 2000). Labeling of ZmPIP2;1 and ZmPIP2;5 by immunocytochemical localization was strongest in the epidermis, exodermis, and cortex of primary root tissue, correlating with the regions that were highly suberized (Hachez et al., 2006). Moreover, localization of ZmPIP2;5 in the epidermis exhibited polarity, with a stronger signal on the plasma membrane facing the external medium. The development of an exodermis in aeroponically grown maize roots tends to reduce Lp, compared with the less suberized hydroponic roots,
### Table 2  AQP transcript (shown in italics) and protein isoforms highly and/or preferentially expressed in roots

<table>
<thead>
<tr>
<th>Species</th>
<th>Isoform</th>
<th>Cell/tissue-specific localization</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Arabidopsis thaliana</em></td>
<td>PIP2;2</td>
<td>Cortex, endodermis (highly expressed), outer layers of stele.</td>
<td>Javot et al., 2003</td>
</tr>
<tr>
<td></td>
<td>“PIP1;1, PIP1;2, PIP1;5 protein, “PIP2;1, “PIP2;2, “PIP2;7, TIP1;1, TIP1;2, TIP2;1”</td>
<td>Expression levels varied among the different studies, reflecting different growth conditions (soil versus hydroponic) and growth stage.</td>
<td>Alexandersson et al., 2005; Boursiac et al., 2005; Daniels et al., 1996; Jang et al., 2004; Santoni et al., 2003</td>
</tr>
<tr>
<td></td>
<td>SIP2;1</td>
<td>Primarily expressed in roots, but in low amounts.</td>
<td>Johanson and Gustavsson, 2002</td>
</tr>
<tr>
<td><em>Craterostigma plantagineum</em></td>
<td><em>CpPIPa</em> (PIP1), <em>CpPIPb</em> (PIP1)</td>
<td></td>
<td>Mariaux et al., 1998</td>
</tr>
<tr>
<td><em>Hordeum vulgare</em></td>
<td><em>HvPIP2</em>;1 protein</td>
<td>All cells near the tip, but epidermis and vascular bundles in maturing region.</td>
<td>Katsuhara et al., 2002</td>
</tr>
<tr>
<td><em>Juglans regia</em></td>
<td>JrPIP2;2</td>
<td></td>
<td>Sakr et al., 2003</td>
</tr>
<tr>
<td></td>
<td>LIMP1 (TIP protein)</td>
<td>Roots and symbiosome membrane.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PIP1-like protein</td>
<td></td>
<td>Henzler et al., 1999</td>
</tr>
<tr>
<td><em>Lyospermos exsulatum</em></td>
<td><em>LaAQP2</em></td>
<td></td>
<td>Werner et al., 2001</td>
</tr>
<tr>
<td></td>
<td>MIP-B (PIP protein)</td>
<td>Endodermis.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MIP-C (PIP protein)</td>
<td>All cells of elongation zone.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MIP-F (TIP protein)</td>
<td>All root cells.</td>
<td></td>
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</table>

(continued)
<table>
<thead>
<tr>
<th>Species</th>
<th>Isoform</th>
<th>Cell/tissue-specific localization</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Nicotiana glauca</em></td>
<td><em>NgMIP1</em> (TIP),</td>
<td>Transcript highly abundant in roots. Root tip and meristem. Associated with xylem. Protein detected in exodermis, endodermis, cortex, near vascular bundles, xylem parenchyma, and apical region.</td>
<td>Smart et al., 2001</td>
</tr>
<tr>
<td><em>Nicotiana tabacum</em></td>
<td><em>NtAQP1</em> (PIP1b)</td>
<td></td>
<td>Biela et al., 1999; Otto and Kaldenhoff, 2000; Siefritz et al., 2001</td>
</tr>
<tr>
<td><em>Olea europaea</em></td>
<td><em>OePIP1;1, OePIP2;1</em></td>
<td>Highest in roots.</td>
<td>Secchi et al., 2007</td>
</tr>
<tr>
<td><em>Oryza sativa</em></td>
<td><em>nc1</em> (PIP1)</td>
<td>Predominantly expressed in roots.</td>
<td>Li et al., 2000</td>
</tr>
<tr>
<td></td>
<td><em>OsPIP2;3, OsPIP2;4,</em>&lt;br&gt; and <em>OsPIP2;5</em></td>
<td>Silicon transporter primarily expressed in roots, localized at the plasma membrane of the distal side of the exo- and endodermis.</td>
<td>Sakurai et al., 2005</td>
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<tr>
<td></td>
<td><em>Lsi1</em> (NIP)</td>
<td></td>
<td>Ma et al., 2006</td>
</tr>
<tr>
<td><em>Pisum sativum</em></td>
<td><em>PsPIP1;1, PsPIP2;1, PsTIP1;1</em></td>
<td>Highest in roots.</td>
<td>Schuurmans et al., 2003</td>
</tr>
<tr>
<td><em>Populus tremula x</em></td>
<td><em>PtPIP1;1, PtPIP2;2, PtPIP2;3,</em>&lt;br&gt; and <em>PtPIP2;5</em></td>
<td>Preferentially expressed in roots.</td>
<td>Marjanovic et al., 2005</td>
</tr>
<tr>
<td><em>tremuloides</em></td>
<td><em>α</em>-VM23 (TIP)</td>
<td>Growing roots and taproot.</td>
<td>Suga et al., 2001</td>
</tr>
<tr>
<td><em>Raphanus sativus</em></td>
<td><em>αPAQ1s (PIP1s)</em>&lt;br&gt; and <em>αPAQ2s (PIP2s)</em>&lt;br&gt; <em>RsPIP1</em> and <em>RsPIP2</em> proteins</td>
<td>Highest in young roots, but also high in mature taproots.</td>
<td>Suga et al., 2003</td>
</tr>
<tr>
<td></td>
<td><em>RsTIP</em></td>
<td>Vasculature of taproots, but endodermis of seedling.</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>RsPIP2;1</em></td>
<td>All tissue of taproots, epidermis of seedling roots.</td>
<td></td>
</tr>
<tr>
<td><em>Spinacia oleracea</em></td>
<td><em>pm28a</em> (PIP2;1)&lt;br&gt; <em>αSoPIP1;2</em></td>
<td>Highest in roots.</td>
<td>Suga et al., 2002; Fraysse et al., 2005; Johansson et al., 1996</td>
</tr>
<tr>
<td>Species</td>
<td>Genes</td>
<td>Expression</td>
<td>References</td>
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<tr>
<td>Vitis hybrid Richter 110</td>
<td><em>PIP1;1</em> and <em>TIP1</em></td>
<td>Highest in root tip and lateral roots.</td>
<td>Baiges <em>et al.</em>, 2001</td>
</tr>
<tr>
<td></td>
<td><em>PIP2;1</em> and <em>PIP2;2</em></td>
<td>Highest in roots.</td>
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<tr>
<td><em>Zea mays</em></td>
<td><em>ZmPIP1;2</em>, <em>ZmPIP2;4</em></td>
<td>Highest in mature compared to elongating tissue.</td>
<td>Hukin <em>et al.</em>, 2002</td>
</tr>
<tr>
<td></td>
<td>^<em>ZmPIP1;5</em></td>
<td>Preferentially expressed in roots. Stele and cortex.</td>
<td>Gaspar <em>et al.</em>, 2003</td>
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<td></td>
<td><em>ZmPIP1a</em>, <em>ZmPIP2a</em></td>
<td><em>ZmPIP2a</em>—root specific.</td>
<td>Chaumont <em>et al.</em>, 2000</td>
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<td><em>ZmPIP1;2</em>,</td>
<td>Xylem parenchyma.</td>
<td>Fetter <em>et al.</em>, 2004</td>
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<td><em>ZmPIP2;5</em></td>
<td>Cortex and xylem parenchyma.</td>
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<td></td>
<td>^<em>ZmPIP1;1</em>,</td>
<td>Developmentally regulated in aeroponically grown roots, greatest expression in mature primary root tissue. <em>ZmPIP2;1</em> and <em>ZmPIP2;5</em> labeling in mature zones greatest in epidermis, exodermis, and cortex. Also in opericlinal plasma membrane of epidermis exposed to external medium.</td>
<td>Hachez <em>et al.</em>, 2006</td>
</tr>
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<td></td>
<td>^<em>ZmPIP2;1</em>, ^<em>ZmPIP2;5</em>,</td>
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<td></td>
<td>^<em>ZmPIP1;5</em></td>
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<td><em>ZmPIP1;5</em></td>
<td>Primarily expressed in roots.</td>
<td>Chaumont <em>et al.</em>, 2001</td>
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<td><em>ZmPIP2;4</em>, <em>ZmTIP2;1–2;3</em></td>
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<td><em>ZmTIP2;3</em></td>
<td>Root specific.</td>
<td>Lopez <em>et al.</em>, 2004</td>
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</table>

^ Indicates expression of both transcript and protein.
but does not appear to influence \( L_p \) (Hose et al., 2000; Zimmermann et al., 2000). Therefore, it would have been interesting if Hachez et al. (2006) had examined whether the expression of these PIP2s only occurred in roots with an exodermis.

Certain PIP1 homologues are also highly expressed in roots, but there is less correlation with tissue-specific expression (Table 2). PIP1;1 homologue has highest expression associated with the apical meristem in \( Vitis \) R-110 roots, but is associated with vascular tissue in mature radish roots and a variety of cell types in tobacco roots (Baiges et al., 2001; Otto and Kaldenhoff, 2000; Suga et al., 2003). These differences may reflect different functional roles for PIP1s that vary with developmental stage, but because most PIP1s have low water permeability when expressed in \( Xenopus \) oocytes (Section 5.2), they may serve some regulatory role through interaction with other PIPs (Fetter et al., 2004), or as osmotic or turgor sensors (Hill et al., 2004).

Some TIP homologues are specifically expressed in roots and no other organs (e.g., ZmTIP2;3, Lopez et al., 2004). Conversely, other TIPs are ubiquitous, being expressed in all root cells (e.g., MIP-F, Kirch et al., 2000), suggesting their importance in “housekeeping” processes such as the osmo-regulation of the cytosol. Some studies have shown that the tonoplast has a much higher \( P_t \) than the plasma membrane, which would allow the vacuole to protect against deleterious cytoplasmic volume changes (reviewed by Chrispeels et al., 2001; Maurel, 1997; Tyerman et al., 1999, 2002). This role in osmotic equilibration is further supported by the observation that several TIPs are predominantly expressed in tissue, such as the epidermis, that potentially experience the greatest fluctuations in apoplastic water potential (e.g., ZmTIP1, Barrieu et al., 1998, Table 2). However, according to Hill et al. (2004) the greater AQP activity in the tonoplast serves some function other than buffering against rapid changes in cytoplasmic volume because pressure changes induced through transpiration or osmotic perturbations in the soil are not instantaneously transmitted through the apoplast. Several TIP1 homologues are also primarily expressed in root tips of grapevines (\( V. \ vinifera \)), radish, and maize (Table 2), implying a putative role in turgor maintenance that is required to drive the cell expansion process (Chaumont et al., 1998; Dolan and Davies, 2004).

### 5.3.2. Changes in AQP abundance

The stages of protein synthesis provide mechanisms for controlling the amount of functional AQPs present in a membrane. The majority of studies investigating changes in AQP abundance have focused on expression levels of AQPs mRNA transcripts, but this does not always correlate with abundance of the respective proteins. AQP gene expression or protein abundance in roots changes both temporally and in response to environmental stimuli, and the expression patterns are multifaceted, varying between...
genes and treatment (Table 3). Some AQPs are upregulated by abiotic factors, whereas others are either downregulated or not affected.

One of the most comprehensive studies undertaken was conducted by Jang et al. (2004), who analyzed the transcript levels of all PIPs expressed in roots and aerial parts of A. thaliana, in response to drought, salinity, cold, or ABA treatments. No consistent expression patterns existed in roots. Drought and low temperature downregulated a large number of PIPs in roots, including most PIP2 transcripts, whereas salinity had a stimulatory effect. In comparison, Boursiac et al. (2005) found that salinity generally depressed AQP gene expression, although the effect depended on the particular transcript and the time of exposure to the salinity treatment. An increase in AQPs may facilitate water uptake under harsh conditions, without necessitating large osmotic or hydraulic gradients between soil, xylem, and shoot. Alternatively, if water uptake is low, the presence of AQPs may increase the backflow of water into the dry soil and cause tissues to dehydrate. Therefore, it is critical where AQPs or their activities are localized.

In comparison with the downregulation of a large number of AQP transcripts in response to chilling in A. thaliana and rice roots (Table 3), PIP1 and PIP2 transcripts, as well as PIP2 protein abundance appear to increase with cold acclimation of wheat crowns, which is associated with improved freezing tolerance (Herman et al., 2006). An increase in PIP2 abundance possibly enhances water export to the apoplast to reduce intracellular injury from the development of ice crystals, although no physiological measurements were undertaken.

The phytohormones ABA and gibberelic acid (GA) appear to be involved in facilitating transcription of PIPs and TIPs from different plant species (Table 3). ABA is involved not only in signaling and stomatal closure during drought, but ABA also potentially enhances Lpr (Section 4).

Most PIP genes appear to be diurnally regulated (Table 3). PIP transcript expression gradually increases, peaking 2–8 h into the photoperiod (depending on the plant species) and then declines to a basal level at the start of or during the dark period (Gaspar et al., 2003; Henzler et al., 1999; Lopez et al., 2003; Sakurai et al., 2005). However, peak transcript and protein expression occur at midnight in barley (Hordeum vulgare) roots (Katsuhara et al., 2003a). Lopez et al. (2003) observed that the diurnal pattern continues during continuous darkness suggesting circadian regulation, although the amplitude of expression diminishes with time. The changes in AQP expression during the day/night cycle could contribute to diurnally fluctuating Lp, (Section 4). Henzler et al. (1999) found that the diurnal fluctuation of AQP transcripts of L. japonicus roots coincided with fluctuations in Lp. The oscillation in transcript abundance and Lp was slightly offset, with transcript expression increasing in anticipation of the light period, before changes in Lp. Paradoxically, the permeability of root cell membranes did not vary diurnally. However, regulation
<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Plant species</th>
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<th>Downregulated</th>
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<tr>
<td>Drought</td>
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<td>Jang et al., 2004</td>
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<td></td>
<td><em>Craterostigma plantagineum</em></td>
<td>CpPIPα2, CpPIPα6, CpPIPα7, CpPIPc</td>
<td>GmPIP1, GmPIP2 (effect greater for mycorrhizal plants)</td>
<td>Mariaux et al., 1998</td>
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<td></td>
<td><em>Glycine max</em></td>
<td>SunTIP7, SunTIP20 (transient)</td>
<td>SunTIP18</td>
<td>Porcel et al., 2006</td>
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<td></td>
<td><em>Helianthus annuus</em></td>
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<td></td>
<td><em>Lactuca sativa</em></td>
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<td>LsPIP1, LsPIP2</td>
<td>Porcel et al., 2006</td>
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<td></td>
<td><em>Mesembryanthemum crystallinum</em></td>
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<td>Vera-Estrella et al., 2004</td>
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<td><em>Nicotiana glauca</em></td>
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<td>NgMIP2, NgMIP3 (TIPs), NgMIP4 (PIP)</td>
<td>Smart et al., 2001</td>
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<td>OsPIP1;1, OsPIP1;2 (24 h PEG treatment)</td>
<td>OsPIP1;3, all OsPIP2s (24 h PEG treatment)</td>
<td>Li et al., 2000</td>
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<td>Aroca et al., 2006</td>
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<td>RsPIP2;1 protein</td>
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<td>PIP1;5</td>
<td>Jang et al., 2004</td>
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<td>PIP1;1 (24 h 60–100 mM NaCl)</td>
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<td>Li et al., 2000; Sakurai et al., 2006</td>
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<td>Tritium aestivum</td>
<td>PIP2b, PIP2 protein (cold acclimation)</td>
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<td>Otto and Kaldenhoff 2000; Siefritz et al., 2001</td>
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<td>Suga et al., 2001</td>
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<td>Katsuhara et al., 2003a</td>
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<td><em>Lotus japonicus</em></td>
<td>PIP1a</td>
<td>Henzler et al., 1999</td>
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<td><em>Oryza sativa</em></td>
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<td>Sakurai et al., 2006</td>
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<td><em>Raphanus sativus</em></td>
<td>PAQ1 and PAQ2 proteins</td>
<td>Suga et al., 2001</td>
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<td>Gaspar et al., 2003; Lopez et al., 2003</td>
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<td>Light</td>
<td><em>Arabidopsis thaliana</em> PIP1b (white or blue light activated)</td>
<td>Kaldenhoff et al., 1996; Sato-Nara et al., 2004</td>
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<td>Dark</td>
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<td>Sato-Nara et al., 2004</td>
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<td>Circadian</td>
<td><em>Zea mays</em> ZmPIP1 protein</td>
<td>Lopez et al., 2003</td>
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<td></td>
<td>ZmPIP2 protein</td>
<td>Lopez et al., 2003</td>
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in \( L_p \), could be controlled at the endodermis or stele, and the cell pressure probe measurements were only conducted on cortical cells.

Lopez et al. (2003) observed a positive correlation between the amount of ZmPIP2 proteins (but not PIP1 proteins) and the rate of water flow through de-topped maize seedlings. Under an osmotic gradient, stimulated water flux from the root system, but continuous darkness stopped sap flow and caused the relative amount of PIP2 proteins to decline. Other regulatory mechanisms also appeared to be involved, as the abundance of PIP2 proteins began to increase before the start of the light period and before the start of sap-flow. Katsuhara et al. (2003a) also correlated AQP protein abundance with \( L_p \) of barley roots. Contrary to ZmPIP2 proteins, HvPIP2;1 protein accumulated during late evening and peaked at night, following the same diurnal oscillation as \( L_p \). HvPIP2;1 was detected in most cells 2 mm from the tip, but was localized to the epidermis, outer cortical layer and surrounding the vascular cylinder, 50 mm from tip. Katsuhara et al. (2003a) speculated that posttranslational modifications of HvPIP2;1 could also be modulating \( L_p \), because the amplitude of the oscillation in \( L_p \), was greater than that of HvPIP2;1 protein abundance. The studies of Katsuhara et al. (2003a) and Lopez et al. (2003) are also important because they demonstrate that the turnover rate of AQPs is rapid. For example, the abundance of ZmPIP1 proteins in maize roots can increase more than 20-fold within 4 h (Lopez et al., 2003).

Gaspar et al. (2003) also found that ZmPIP1;5 transcript varied diurnally, with high expression in the stele and cortex during the day and lower expression during the night. However, analogous to HvPIP2;1, the expression of ZmPIP1;5 during the night was limited to the epidermis. The cell walls of the epidermis, or (if present) the exodermis, of some plant species are suberized so expression of AQPs in the epidermis may facilitate water uptake in the symplast when the apoplast is blocked. However, this does not explain why the localized expression of ZmPIP1;5 changes diurnally. If epidermal expression during the evening is a common feature of certain PIP isoforms, it raises the question about possible involvement in the redistribution of soil water by plant roots. The roots of several tree species “redistribute” soil water during the night by absorbing water from the wetter parts of the soil profile and releasing it to the dryer regions (Burgess et al., 1998). The water is then taken up during the day, when transpiration resumes. The expression of AQPs in the epidermis of roots, during the night, could mediate this hydraulic redistribution by either enhancing the backflow of water into the dry soil and/or enhancing the uptake of water from wet soil.

5.3.3. Posttranslational regulation
When extrapolating information on transcription levels, care is needed as protein abundance or activity is not necessarily altered (reviewed by Chrispeels et al., 2001; Tyerman et al., 2002). Translation of the mRNA
transcript into protein may be delayed or inhibited until a signal is received, or the RNA may require molecular changes after transcription. The amount of protein translated often does not correlate with the amount of transcript expressed (Alexandersson et al., 2005; Aroca et al., 2005; Kirch et al., 2000; Lopez et al., 2003; Suga et al., 2001). Once the message in mRNA is translated, a polypeptide chain forms, but the protein may still not function as a water channel. The majority of AQPs have putative glycosylation and phosphorylation sites where attachment of carbohydrate and phosphate groups, respectively, may be required to enable correct folding of the protein and/or insertion into the membrane. Little is known about endocytosis processes in plants, but hormones and environmental conditions can regulate the internalization and recycling of plasma membrane proteins, including some AQPs (Murphy et al., 2005). Santoni et al. (2000), Chrispeels et al. (2001), and Tyerman et al. (2002) have reviewed trafficking of AQPs to and from the membrane, as a regulatory mechanism of activity. Since then, Vera-Estrella et al. (2004) demonstrated in *Mesembryanthemum crystallinum* that McTIP1;2 becomes glycosylated, in response to osmotic perturbation, and is trafficked to other compartments, where it then becomes de-glycosylated. Activation of the cAMP-signaling pathway and phosphorylation of the protein by protein kinase are also involved in redistribution of this AQP. An innovative proteomic study by Santoni et al. (2003) demonstrated that individual PIP isoforms can be present in different forms and at least one of these forms was due to posttranslational modification by phosphorylation.

Methylation is also a potential posttranslational regulatory mechanism of AQP function (Santoni et al., 2006). Several residues of the N-terminal tail of PIPs can be methylated, which may influence protein stability and subcellular localization. However, expression of PIP2;1 with altered methylation sites in *A. thaliana* suspension cells did not significantly change their osmotic water permeability (Santoni et al., 2006).

### 5.3.3.1 AQP gating

Once the AQP is located in the membrane, opening and closing of the channel can then control water flow through the pore. Phosphorylation, cytoplasmic pH and heavy metals directly control AQP gating, either through conformational changes in the shape of the pore or direct blockage of the pore. Ca$^{2+}$ also potentially acts by direct or indirect obstruction of the pore (Alleva et al., 2006; Gerbeau et al., 2002). Other mechanisms that affect water permeability, speculated to be via interaction with the AQP pore, include mechanical stimuli and osmotic pressure (Wan et al., 2004; Ye et al., 2005).

#### 5.3.3.1.1 Phosphorylation

By site-directed mutagenesis, Johansson et al. (1996, 1998) demonstrated that the phosphorylation/dephosphorylation of two serine residues regulates PM28A (now called SoPIP2;1) of spinach.
(Spinacia oleracea) leaf, which increases/decreases $P_f$, respectively. In vivo, phosphorylation of Ser-274 depends on the water potential of the leaf apoplast and based on their observations, Johansson et al. (1998) developed a model for the regulation of flow through PM28A. During ambient conditions, the leaf apoplast has a high water potential and cell turgor is high. Submicromolar $Ca^{2+}$ activates a membrane-associated protein kinase that phosphorylates PM28A, on Ser-274, and the water channel opens. When the leaf experiences water deficiency, cell turgor begins to fall, the protein kinase inactivates (possibly by closure of a stretch-activated $Ca^{2+}$ channel) and dephosphorylation of PM28A closes the pore. It is likely that water-potential-induced phosphorylation regulates water channel activity in roots as well as leaves, as PM28A is predominantly expressed in roots and Ser-274 is conserved in all PIP2s.

Using X-ray crystallography Törnroth-Horsefield et al. (2006) made a remarkable breakthrough by identifying the phosphorylation-induced structural mechanism that gates SoPIP2;1 in spinach. Plant PIPs typically have a longer Loop D (four to seven amino acid residues) that folds under the protein and occludes the pore. Phosphorylation of Ser-115 and Ser-274 causes the “cap” of Loop D to be displaced so that the pore is open. Through molecular modeling, Törnroth-Horsefield et al. (2006) also demonstrated how $Ca^{2+}$ and protonation of His 193 may regulate the gating of PIPs (Section 5.3.3.1.2).

The increasing evidence implies that phosphorylation of AQPs is an important regulator of water channel activity. The water channel of radish RsPIP2;2 and soybean NOD26 is activated by phosphorylation (Guenther et al., 2003; Suga and Maeshima, 2004). Phosphorylation of RsPIP2;2 could be a fundamental factor influencing $L_p$, particularly during changes in external osmotic pressure, since this AQP is highly expressed in roots (Table 2). Phosphorylation of NOD26 is dependent on the developmental state of the nodule, with maximum activity in mature nodules (Guenther et al., 2003). Contrary to their effects on PM28A, drought (and salinity) enhances the phosphorylation of NOD26 in planta, although a concurrent increase in water permeability was not assessed in planta, phosphorylation induced by okadaic acid increased $P_f$ in Xenopus oocytes (Guenther et al., 2003). However, decreasing the external osmotic potential reduced $P_f$ of membranes vesicles isolated from the peribacteriod membranes (PBM) of soybean root nodules, in which NOD26 is highly abundant (Vandeleur et al., 2005). The response of NOD26 in PBM vesicles is consistent with Johansson’s model (Johansson et al., 1998), but conflicts with the findings of Guenther et al. (2003) and highlights the importance of measurements on native membranes.

Temmei et al. (2005) demonstrated that phosphorylation of PIP1 proteins was involved in the cooperative regulation of water channel activity with PIP2 proteins. While phosphorylation did not affect the formation of
heterotetramers, phosphorylation of Ser-131 of MpPIP1;1 (in *Mimosa pudica*) activated the water channel activity of MpPIP2;1.

Presently, no studies have investigated phosphorylation-regulated Lp_r in response to abiotic perturbations in root systems. However, Kamaluddin and Zwiazek (2002b) applied exogenous ethylene to hypoxic or aerated *P. tremuloides* seedlings, which increased Lp_r. They interpreted this response as an increase in water channel activity by phosphorylation, as ethylene induces protein phosphorylation with involvement of Ca^{2+}-dependent protein kinases. In plants that are waterlogged, hypoxia strongly stimulates the biosynthesis of ethylene, but at the same time tends to reduce Lp_r (Drew, 1997; and Section 7.3), which conflicts with Kamaluddin and Zwiazek’s ideas. Experiments inhibiting ethylene biosynthesis or action, or phosphorylation during hypoxia, or stimulating phosphorylation in the absence of ethylene during hypoxia, may resolve this issue.

5.3.3.1.2. Cytoplasmic pH

Studies on the vacuoles and plasma membranes of *Beta vulgaris*, and the plasma membranes of *A. thaliana*, have shown that cytoplasmic pH affects the gating of AQPs and that acidification of the cytosol reduces the membrane permeability to water (Alleva *et al.*., 2006; Amodeo *et al.*, 2002; Gerbeau *et al.*, 2002; Sutka *et al.*, 2005). This is important as respiratory inhibitors, anoxia, and some other abiotic perturbations induce acidification of the cytosol (Dat *et al.*, 2004; Felle, 1987, 2005).

The connection between anoxia-induced cytoplasmic acidification and AQP gating by internal pH is particularly relevant in understanding the waterlogging-induced reductions in Lp_r (Section 7.3). A pioneering study by Tournaire-Roux *et al.* (2003) identified a histidine residue conserved in all PIPs, which is a major site for cytosolic pH sensing. Tournaire-Roux *et al.* (2003) also found that a group of charged amino acid residues on Loop D of the protein manipulate AQP gating by controlling either the conformation and/or the conductance of the pore, in response to cytosolic protons. When a cell experiences anoxia, the cytoplasm acidifies due to an accumulation of by-products from anaerobic respiration. The histidine of PIPs becomes protonated, which closes the water channel and so reduces the permeability of the membrane to water (Tournaire-Roux *et al.*, 2003).

It should also be noted that during O_2 deficiency root cells leak ions, which is assumed to be due to depolarization of the plasma membrane that stimulates the opening of ion channels (e.g., wheat; Greenway *et al.*, 1992; Zhang and Tyerman, 1997). Given the acid-induced gating of the mammalian AQP6 (Hazama *et al.*, 2002), anoxia could stimulate similar ion permeability in analogous AQPs in plants.

5.3.3.1.3. Heavy Metals

Mercuric chloride has been widely used to demonstrate the function of AQPs in isolated native membranes and *in planta*. Hg^{2+} binds to cysteine residues on the protein and acts either by direct obstruction of the pore or by conformational change (Section 5.1, Barone
et al., 1997, 1998). Blocking water channels reduces the membrane permeability to water and this has been demonstrated in *Xenopus* oocyte swelling assays, membrane vesicles, isolated protoplasts, and intact cells (Daniels et al., 1996; Kaldenhoff et al., 1998; Niemietz and Tyerman, 1997; Zhang and Tyerman, 1999).

AQP inhibitors are valuable because they provide an estimate of AQP activity in real time, without destruction of the tissue and can be used to examine AQP activity in relation to changing environmental conditions. Mercury is nonspecific (cannot target individual isoforms or may affect other channel proteins), may be phytotoxic and may reduce cellular metabolism and other biochemical processes (reviewed by Santoni et al., 2000; Tyerman et al., 1999), but despite all these caveats a reduction in cell water permeability indicates a decrease in AQP activity.

Niemietz and Tyerman (2002) demonstrated that gold and, in particular, silver ions inhibit AQPs more effectively than mercury. Silver ions reduced the water permeability of membrane vesicles from different organisms, to almost the predicted inherent water permeability of the lipid bilayer. The effect of silver was not reversible and the mechanism of inhibition is unknown, but Niemietz and Tyerman (2002) reasoned that Ag⁺ and Au³⁺ react with sulfhydryl groups of cysteine residues, analogous to mercury. Silver is considered less toxic than mercury and provides a promising alternative for AQP inhibition studies (Niemietz and Tyerman, 2002). Confirming Niemietz and Tyerman’s observations, gold and silver inhibited the activity of AQP1 in erythrocytes and epithelial cells from mice, but to a similar extent as mercury (Yang et al., 2006).

Despite Tazawa and Iwasaki (1996) demonstrating that Zn²⁺ reduced *Lp* of *Chara corallina* cells, Zn²⁺ and other transition elements did not significantly reduce the water permeability of membrane vesicles (Niemietz and Tyerman, 2002). However, Tazawa and Iwasaki (1996) used a high concentration of Zn²⁺ (5 mM) and the speed of the inhibition on *Lp* depended on the pH of the external medium, suggesting that AQP activity may have been indirectly affected.

### 5.3.3.1.4. Other AQP gating mechanisms

Meinild et al. (1998) found that AQP pore dimensions are not fixed and that lowering the temperature caused the pore to narrow, which increased the reflection coefficient for various solutes. Wan et al. (2004) speculated that mechanical stimulus closes AQPs, either by creating tension that collapses the pore or causes a conformation change in the pore shape. However, Ca²⁺ entering through stretch-activated ion channels may also indirectly close AQPs during pressure pulses. Large positive or negative pressure pulses (>0.1 MPa) induced by the cell pressure probe reduced the water permeability of cortical cells in maize roots (Wan et al., 2004). The reduction was transient with 0.1–0.2 MPa pressure pulses, but pulses >0.2 MPa were only reversible by the addition of high concentrations of ABA. Wan et al. (2004) verified that the
reduction in water permeability was by a reduction in AQP activity, as those cells whose water permeability had been reduced by large pressure pulses were unaffected by mercury. This finding by Wan et al. (2004) is important as the cell pressure probe is widely used for measuring the water permeability of cells in intact tissue. Future measurements with the cell pressure probe should be restrained to applying small pressure changes (pressure relaxations) to prevent confounding effects on cell water permeability.

Osmotic pressure has also been suggested to gate AQPs through dehydration of the pore (Ye et al., 2004, 2005). The observation that the permeability of the membrane to water decreases with increasing external solute concentration has been reviewed by Tyerman et al. (2002) and Vandeleur et al. (2005). They speculate that high osmotic pressure could not only restrict the pore through dehydration of the protein but also could create tension in the central vestibule of the tetramer causing conformation changes of the monomers. The inhibition of AQP activity by high external solute concentration has relevance to water flow through root systems, as salinity tends to reduce $L_p$ (Section 4). Salinity appears to stimulate the expression of AQPs in A. thaliana roots (Jang et al., 2004), but this will probably be ineffectual at influencing $L_p$ if high solute concentrations close these AQPs.

6. The Role of AQPs in Root Water Transport

6.1. Inhibition studies

The inhibition of water flow by mercuric chloride has illustrated that AQPs are an important component in regulating water transport across the roots of some species (Barrowclough et al., 2000; Maggio and Joly, 1995; Martinez-Ballesta et al., 2000; Martre et al., 2001; Quintero et al., 1999; Wan and Zwiazek, 1999). The use of mercuric chloride has revealed that AQPs can account for up to 90% of total root water flow either through occlusion of the water channel or indirect inhibition by lowered metabolism (Martre et al., 2001 and references therein).

The employment of mercury, in combination with an abiotic perturbation, has confirmed the inhibition of AQP activity. For example, salinity reduced $L_p$ (based on flux per gram root weight) of Cucumis melo and Capsicum annuum roots by 80% and 100%, respectively (Carvajal et al., 1999, 2000). Mercuric chloride reduced $L_p$ of control plants, to a similar extent as the effect of salinity, but barely influenced $L_p$ of salinity-treated roots (Carvajal et al., 1999, 2000). Similarly, nutrient deficiency reduced $L_p$ of wheat roots by 70–80%, which was not further inhibited by mercury, but mercury reduced $L_p$ of nutrient-sufficient roots by 63% (Carvajal et al., 1996).

Measurements of the radial hydraulic conductivity after removal of tissue layers and application of mercury demonstrated the varying activity of AQPs in different regions of roots of cacti and desert succulents (Martre et al., 2001;
North et al., 2004). $L_p$, decreased with soil drying in the distal and mid-root regions of *Opuntia acanthocarpa*, with no additional reduction by mercury (Martre et al., 2001). In wet soil, mercury reduced $L_p$ of the distal region, but not the mid-root region. However, mercury may not have fully permeated the mid-root region. After removing the cortex and periderm, mercury reduced the radial hydraulic conductivity of the stele, which accounted for almost 75% of $L_p$ (Martre et al., 2001). In an analogous study on *Agave deserti* roots, AQP s were found to be active external to the stele, being important in the epidermis/exodermis of the basal region, but more important in the endodermis of the distal region (North et al., 2004).

Wan and Zwiazek (1999) and Siemens and Zwiazek (2003) used an apoplastic permeant dye to demonstrate that mercury inhibits the water flux from *P. tremuloides* roots, but the relative contribution of the apoplastic pathway increased. The relative contribution of the apoplastic pathway also increased when AQP activity was inhibited by mercury treatment in *A. thaliana* roots (Martinez-Ballesta et al., 2003). The contribution of the different pathways to $L_p$ could also be estimated using the cell pressure probe to measure the effects of mercury on the water permeability of root cells and comparing them with measurements of root hydraulic conductivity. However, such comparisons in the literature are rare. Barrowclough et al. (2000) combined cell pressure probe and root potometer measurements, to identify the dominance of apoplastic transport in the young region of onion roots, but AQP s facilitated water flow in the mature root regions containing a suberized exodermis. Although Zhang and Tyerman (1999) did not measure $L_p$, the inhibition of $L_p$ by mercury was similar to that observed by Carvajal et al. (1996), in whole roots, suggesting a significant contribution of the cell-to-cell pathway to $L_p$ of wheat roots. The combination of CPP, RPP, and mercury treatment demonstrated the contribution of the radial pathways to flow in wheat and lupin roots (Bramley, 2006). Mercury treatment reduced $L_p$ to 15–20% of untreated cells, indicating a significant influence of AQP s in membrane water permeability. This inhibition at the cellular level was associated with a similar inhibition in $L_p$ (40–50% of control roots) of wheat roots, indicating that a significant proportion of radial water flow occurs through the cell-to-cell pathway and AQP s may be involved in regulating that flow. However, despite the presence of mercury sensitive AQP s in lupin roots, mercury did not inhibit $L_p$ (Bramley, 2006) suggesting that radial water flow may be predominantly apoplastic. Moreover, the study by Bramley (2006) implies that AQP s may have other, as yet undiscovered, functional roles in roots.

AQP activity in roots may be an important component in the refilling of embolized vessels in shoots of grapevine. The inhibition of root water flow by mercuric chloride impaired the recovery of shoot hydraulic conductance after a water deficit (Lovisolo and Schubert, 2006). Despite using a high concentration of mercury, Lovisolo and Schubert (2006) were able to
reverse the inhibition of root hydraulic conductance and consequently the shoots rehydrated. The mechanism of repair after drought is unknown, but embolized vessels in grapevines refill in spring under root pressure (Scholander et al., 1955; Sperry et al., 1987). Inhibition of AQP activity in roots may therefore have some regulatory effect on root pressure, although due to the nonspecificity of mercury nutrient transport would also have decreased, which would affect root pressure.

6.2. Expression and transformation studies

Molecular transformation techniques creating AQP-deficient or overexpressing mutants have corroborated the mercury inhibitory experiments in assessing the function of AQPs in plants. Knocking out *NtAQP1* from tobacco reduced *Lp* by 58% compared with control plants, without altering plant morphology (Siefritz et al., 2002). Conversely, downregulation of PIPs in antisense *A. thaliana* plants increased the root mass, but had no effect on root water uptake (Kaldenhoff et al., 1998; Martre et al., 2002). Kaldenhoff et al. (1998) speculated that antisense plants compensated for the lack of PIPs by increasing the absorption area of the root system. In support of this, root systems of transgenic tobacco plants overexpressing PIP1b were able to support a larger shoot system (Aharon et al., 2003).

Knocking out *NtAQP1* from tobacco or PIP1s from *A. thaliana* reduced the tolerance of antisense plants to a water deficit (Kaldenhoff et al., 1998; Siefritz et al., 2002), which is surprising given that PIP1 genes seem to be downregulated under adverse conditions (Table 3). However, transgenic tobacco and rice plants over expressing PIP1s and HvPIP2;1, respectively, were also less tolerant of drought or salinity (Aharon et al., 2003; Katsuhara et al., 2003b). There appears to be a fine balance between the abundance of AQPs, root morphology, and water transport, so that some plants compromise performance under favorable conditions to minimize deleterious effects in the event of adverse conditions.

Javot et al. (2003) knocked out PIP2;2 that is predominantly expressed in *A. thaliana* roots. *Lp* of cortical cells was almost one-third less for mutants than wild-type plants, but this reduction did not translate into an equivalent reduction in *Lp*. The *Lp* of detopped roots was only reduced by 14% under an osmotic driving force (natural sap exudation) compared with zero reduction under a hydrostatic driving force (pressurized root system). Javot et al. (2003) interpreted their observations according to the composite transport model, that is under a hydrostatic driving force, water flow was predominantly via the apoplast (Section 2.3.1). However, expression of PIP2;2 is predominantly localized in the endodermis (Table 2), implying an important function of this AQP in this tissue. Manipulating AQP activity in the endodermis would be a geometrically efficient method of controlling *Lp*, given the decreasing surface area for water flow toward the root.
axis. If the endodermis is suberized, then the apoplastic pathway may be blocked and water would have to cross the plasma membrane at least twice (i.e., through AQP)s to reach the apoplast of the stele. Therefore, even under a hydrostatic driving force there should be some transcellular flow. It would have been useful, if Javot et al. (2003) had examined the anatomy of the roots, not only to confirm the above speculations, but more importantly, to ensure that A. thaliana mutants did not compensate for an absence of PIP2:2 by some other mechanism.

The analysis of AQP expression in conjunction with measurements of the water permeability of cells or protoplasts has been used to identify the contribution of AQPs to membrane permeability. Hukin et al. (2002) observed a higher $L_p$, of cells in mature compared with elongating tissue of maize roots, which was correlated with a greater expression of PIP genes. They suggested that AQPs are required in mature tissue to mediate radial water flow because there was also less symplastic connection between cells via plasmodesmata in mature tissue. Suga et al. (2003) measured $P_f$ of cells with different AQP protein content. The osmotic water permeability of cortical or endodermal protoplasts, isolated from radish roots, was the same despite the endodermis having much greater expression levels of PIP1, PIP2, and a TIP. High $P_f$ of protoplasts isolated from young rape (Brassica napus) and flax (Linum usitatissimum) roots were considered to be related to high amounts of PIP1 and PIP2 proteins, but this was not true for two wheat genotypes that had low $P_f$, despite the presence of PIPs (Morillon and Lassalles, 2001). Interestingly, wheat species with low $P_f$ values were more tolerant of severe water deficit and were still able to germinate, suggesting stronger regulation of AQP activity may mediate the effects of water deficit, at least in germinating seeds. It should also be noted that the $P_f$ values for wheat protoplasts and plasma membranes vesicles are much lower than in situ values obtained with the CPP (Bramley, 2006; Niemietz and Tyerman, 1997; Zhang and Tyerman, 1999), indicating that the isolation procedures may affect AQP activity. Gerbeau et al. (2002) demonstrated that plasma membrane vesicles isolated in the presence of chelating agents had higher $P_f$ than those isolated in the presence of divalent cations.

6.3. The contribution of AQPs to radial water flow

Given the ubiquitous nature, diversity, and high expression levels of many AQPs, it is tempting to speculate that they have an important role in water flow through the plant. However, AQPs will only have an influence on $L_p$, if a significant proportion of water flow occurs by the cell-to-cell pathway (Steudle, 1997). In some species, water appears to flow predominantly via the apoplast, for example maize and lupins, despite an abundance of AQPs expressed in the roots (Bramley, 2006; Chaumont et al., 2001; Steudle et al., 1987).
Therefore, the function of AQPs in these plants is an enigma, although the contribution of the radial pathways to flow may vary under different conditions, as Gibbs et al. (1998b) thought that the contribution of the apoplast in maize had been overestimated.

There is the potential for finer control of $L_{p_r}$, mediated by AQP activity, when water flows via the cell-to-cell pathway. The apoplast only constitutes a small fraction of the root cross-sectional surface area, so the cell-to-cell pathway could potentially transport larger volumes of water. Additionally, AQPs create parallel pathways for water flow across a membrane and therefore their respective conductances are additive, so their abundance can have a dramatic effect on $L_{p_r}$. Increasing $L_{p_r}$ requires a lower gradient to drive the same amount of flow. Under conditions of high transpiration, increasing the abundance of AQPs could considerably increase $L_{p_r}$ and hence avert large tensions in the xylem. Unless negative pressure (tension), generated by transpiration and transmitted throughout the apoplast, causes a conformational change in the protein and closes the pore, water flow through the cell-to-cell pathway would be advantageous. Measurements demonstrating that abiotic perturbation, respiratory inhibitors, and mercury reduce hydrostatically induced water flux, point toward a significant proportion of root water flow occurring via the cell-to-cell pathway, in many species (Section 4).

7. Waterlogging

Waterlogging occurs when the infiltration of water from rainfall or flooding is greater than the rate of subsurface drainage and evapotranspiration, leading to saturation of the soil. The frequency and duration of waterlogging, and the depth of soil saturation, depend not only on the soil properties, but also on location in the landscape and climate. For example, during winter in Western Australia, when rainfall exceeds pan evaporation, the soil profiles susceptible to waterlogging often saturate close to the soil surface, for days to several weeks at a time (Cox and McFarlane, 1995; Tennant et al., 1992; Zhang et al., 2004). Waterlogging is a significant environmental constraint for crop growth and can ultimately reduce yields by as much as 80% (Dracup et al., 1992; McFarlane and Williamson, 2002).

7.1. Effect on $O_2$ in the rhizosphere

Oxygen diffuses 10,000 times slower in water than in air (Grable, 1966). Consequently, when the soil is saturated the $O_2$ concentration in the rhizosphere declines due to respiring roots and microorganisms (Armstrong et al., 1991; Cannell and Jackson, 1981). Greater respiration rates caused by high temperatures lead to a greater consumption of $O_2$ and a more rapid decline in $O_2$ concentration in waterlogged soils over time (Belford, 1981
and Drew, 1992). The lack of soil aeration leads to the formation of many biochemically reduced compounds that can accumulate to concentrations that are phytotoxic, but a low O_2 supply is the primary factor detrimental to plant growth (Drew, 1992).

Even in winter, when the average daily temperatures are low, the rhizosphere can become hypoxic (low O_2) within a few days of waterlogging. Barrett-Lennard et al. (1987) used specially designed plots to control waterlogging in the field, at a nonplanted site in Western Australia. Waterlogging gradually reduced the O_2 concentration in the soil profile to ~4% O_2 (0.05 mol m^{-3}) within 4–8 days of waterlogging, depending on the depth in the profile. The decline in O_2 would be more rapid in cropped fields, depending on root density and root depth (Drew, 1992).

### 7.2. Effect on root growth

The lack of O_2 causes respiration to become anaerobic, which affects metabolism and leads to a cascade of biochemical and physiological changes that may ultimately cause cell injury or death (Drew, 1992). Aerobic respiration switches over to glycolysis, once the O_2 supply drops below the critical oxygen pressure (COP). The COP varies depending on the species but at 25 °C is generally less than 10% O_2 (Drew, 1997).

Glycolysis only produces one-eighteenth of the energy of aerobic respiration and therefore growth ultimately becomes inhibited (reviewed by Drew, 1992). In general, roots are more adversely affected than shoots (Davies et al., 2000a; Trought and Drew, 1980). Because of their high rates of O_2 consumption the root apical meristems are the most sensitive parts of the root to O_2 deficiency (Drew, 1992). In maize roots the COP for the root tip is equal to, or slightly greater than the concentration of O_2 in equilibrium with air (Saglio et al., 1984). The COP of wheat roots is 0.16 mol m^{-3} O_2 in the apical region (0–2 mm from the tip) compared with a COP of 0.05 mol m^{-3} O_2 for mature tissue (10–12 mm from the tip) (Thomson et al., 1989). Peak water uptake in cereal roots occurs behind the root tip (Section 3.2), so growth should be inhibited before water uptake is affected during O_2-deficient conditions.

The effects of waterlogging and/or O_2 deficiency depends on the species and the duration of submergence, with the complete cessation of root growth or even root death in the more sensitive species (Cannell and Jackson, 1981; Grable, 1966). Waterlogging or hypoxia reduces the growth of wheat roots and the extent of the inhibition depends on the genotype (Huang and Johnson, 1995; Huang et al., 1994; Thomson et al., 1992). Seminal roots of wheat appear to be more adversely affected by waterlogging than nodal roots, which often increase in abundance and continue to elongate (Huang et al., 1994; Thomson et al., 1992; Trought and Drew, 1980; Wiengweera et al., 1997).
Based on their growth response, yellow lupin was more tolerant to waterlogging than other lupin species (Broué et al., 1976; Davies et al., 2000a, b). Narrow-leafed lupin is very sensitive to excess water and mild O$_2$ deficiency. Watering to 130% field capacity only reduced the air-filled porosity from 28% to 21%, but depressed root growth (White and Robson, 1989). The air-filled porosity is the proportion of the bulk soil volume filled with air and the critical value considered to affect plant growth is <10% (Grable, 1966). By reciprocal grafts of roots and shoots of narrow-leafed lupin with yellow lupin, Davies et al. (2000c) identified that it was the root system of lupins that conferred the genotypic tolerance to waterlogging.

Wheat is considered to be sensitive to waterlogging, but anecdotal evidence in the field indicated that wheat was more tolerant to waterlogging than lupin, which was confirmed in a glasshouse study (Bramley, 2006; Dracup et al., 1992). Lupins have thick taproots (1- to 2-mm diameter), which would require a higher rate of O$_2$ supply from the rhizosphere for normal functioning, compared with thin wheat roots (Dracup et al., 1992). Wheat and lupin roots also differ in their waterlogging tolerance due to anatomical changes that occur during waterlogging. The tolerances of different wheat genotypes to hypoxia were associated with changes in root system anatomy that increased the internal aeration of roots (Boru et al., 2003; Erdmann and Wiedenroth, 1986; Huang et al., 1994; Thomson et al., 1992).

An increase in internal aeration or root porosity, in response to hypoxia, is a common feature of many wetland species including rice and some dryland species including the cereal crops *Hordeum marinum*, maize and wheat, and the legume species soybean, cowpea (*Vigna unguiculata*) and *Lotus* species (Colmer, 2003; Evans, 2003; Thomas et al., 2005 and references therein). Hypoxia stimulates the development of gas spaces, called lysigenous aerenchyma, through ethylene-induced cell death (reviewed by Evans, 2003). In roots, aerenchyma usually forms in the cortex and these air spaces are connected longitudinally, creating a pathway for the diffusion of gases, from the aerial parts of the plant to the roots (Colmer, 2003; Evans, 2003). The effectiveness of internal aeration depends on root porosity (i.e., the amount of aerenchyma), respiration, and radial O$_2$ loss (ROL) to the soil (Armstrong et al., 1991). Many wetland species, such as rice, develop a barrier in the epidermis or outer hypodermal layers that reduces the loss of O$_2$ from the root to the soil (Colmer, 2003). The nature of the barrier is unresolved but may involve tightly packed cells and wall modifications such as thickening and lignification (Armstrong et al., 2000).

Waterlogging and/or low O$_2$ supply stimulates the development of aerenchyma in wheat roots, predominantly in nodal roots, although the seminal roots of some genotypes also increase their root porosity (Erdmann et al., 1986; Huang and Johnson, 1995; Huang et al., 1994; Thomson et al., 1992;
Wheat roots do not appear to develop a tight barrier to ROL (Colmer, 2003), so even with aerenchyma the internal aeration may be inefficient. Without a barrier to ROL, O\textsubscript{2} can diffuse across the cortex in the basal region of the root and be lost to the soil environment, so that the root tips have insufficient O\textsubscript{2} to maintain viable meristems. Root tips have tightly packed cells with high metabolic rates and few air spaces. It is therefore not surprising that the root tips are the most sensitive part of the root when the O\textsubscript{2} supply becomes limited. Modeling the diffusion of O\textsubscript{2} within graminaceous roots Armstrong et al. (1991) demonstrated that even with the development of aerenchyma, root growth would only extend into anaerobic media to a depth of ~30 cm. In addition, they predicted that the stele would become anoxic before the cortex, which was later experimentally confirmed in maize roots (Darwent et al., 2003; Gibbs et al., 1998a).

The majority of research has focused on the effects of waterlogging during the waterlogging event. Far less is known about recovery, when the wet soils have drained. Dracup et al. (1992) suggested that wheat roots might resume growth more rapidly than lupins, based on observations in different field studies. Yellow lupin roots are better at recovering after waterlogging than narrow-leaved lupin, but only when waterlogged at an early stage of growth (Davies et al., 2000a). In a glasshouse study, Bramley (2006) found that wheat roots survived a waterlogging event better than lupins and recovery in root growth was more rapid, with a significant amount of growth resuming from the apical region. In comparison, narrow-leaved lupin roots died with no subsequent recovery. Although the majority of the root system of yellow lupin roots died, root growth resumed from the basal region, and this was associated with the weak development of aerenchyma (Bramley, 2006).

### 7.3. Effect on water use

Synonymous with the majority of abiotic perturbations, waterlogging tends to alter nutrient and plant water use. Lowered metabolism and subsequently less available energy to drive nutrient transport across plasma membranes may reduce nutrient absorption by roots (Cannell and Jackson, 1981; Drew and Stolzy, 1991). Water uptake can also decrease (Kramer, 1983 and references therein). Reductions in leaf gas exchange indicate the adverse effect of waterlogging on plant water use (Davies et al., 2000b; Huang et al., 1994). Despite closure of the stomata, wilting and a decrease in leaf \( \Psi \) imply that the water transport from the root is insufficient to maintain the water status of the shoot (Davies et al., 2000b).

Waterlogging and/or O\textsubscript{2} deficiency tends to reduce \( L_p \). Kramer (1949, 1983) reviewed the earlier literature and in the more recent literature the extent of the inhibition on \( L_p \) appears to be species and treatment
dependent (Table 4). Apart from root death, $L_p$, may be influenced by waterlogging in the longer term (days to weeks) through changes in root structure and anatomy (Section 3.2). For example, Erdmann et al. (1986) found that low O$_2$ increased cell wall lignification and Huang et al. (1994) found that the xylem vessels of nodal roots decreased in diameter, although no measurements of root water transport were undertaken. Aerenchyma and barriers to ROL could also influence radial water flow, but this would depend on the length of the absorbing region. The formation of aerenchyma and barriers to ROL do not appear to affect $L_p$, of rice and H. marinum roots (Garthwaite et al., 2006; Miyamoto et al., 2001; Ranathunge et al., 2003), but these measurements were undertaken in O$_2$-sufficient conditions and water uptake in cereals occurs preferentially in the apical region (Section 3) where these anatomical features do not occur.

A metabolically controlled mechanism could also influence $L_p$, under O$_2$ deficiency, providing that a significant proportion of water flow across roots occurs via the cell-to-cell pathway, since respiration rates decline when the O$_2$ supply is low. In further support of this, respiratory inhibitors have been shown to reduce water flow through roots (Kamaluddin and Zwiazek, 2001; Tyerman et al., 1992).

### 7.4. Anoxia and AQP activity

To date, no studies have directly investigated AQP transcript or protein abundance (altered production or breakdown of in situ proteins) in plant roots in response to anoxia or hypoxia. However, several microarray analyses monitored the expression of hundreds of A. thaliana genes, when the O$_2$ concentration surrounding the roots or seedlings was reduced (Klok et al., 2002; Loreti et al., 2005; Liu et al., 2005). Cross-referencing those lists of genes with GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html; Benson et al., 2000) reveals that O$_2$ deficiency modulates the expression of some AQP genes (Table 5).

Klok et al. (2002) exposed cultured A. thaliana roots to hypoxia (5% O$_2$ and 95% N$_2$), which highly upregulated TIP1;2 in the first 0.5 h of treatment and TIP4;1 during 2–20 h of hypoxia. Submergence of the roots will dilute the apoplast leading to uptake of water by cells and so TIPs may be induced as a defence against adverse cytoplasmic volume changes (Section 5.3.1).

Several studies identified NIP genes that were rapidly and strongly upregulated by anoxia or hypoxia (Table 5). Since these NIPs are aquaglyceroporins, they may be induced to transport glycerol that accumulates during hypoxia (Weig et al., 2004). Additionally, NIPs could also be upregulated to facilitate the transport of gases.
Table 4  Summary of the effects of waterlogging or O₂ deficiency on water transport in roots

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Waterlogging treatment</th>
<th>Reduction in Lp, (or Lp,) (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agave deserti</td>
<td>Anoxia (3 h)</td>
<td>0</td>
<td>Nobel et al., 1990</td>
</tr>
<tr>
<td>Arabidopsis thaliana</td>
<td>Anoxia (30 min)</td>
<td>42</td>
<td>Tournaire-Roux et al., 2003</td>
</tr>
<tr>
<td>Helianthus annuus</td>
<td>Anoxia (16 h – 6 d)</td>
<td>46 (transient)</td>
<td>Everard and Drew, 1989</td>
</tr>
<tr>
<td>Lupinus species</td>
<td>Hypoxia (30 min)</td>
<td>10</td>
<td>Bramley, 2006</td>
</tr>
<tr>
<td>Lupinus species (cortical cells in expanded region)</td>
<td>Hypoxia (30 min)</td>
<td>70</td>
<td>Bramley, 2006</td>
</tr>
<tr>
<td>Lycopersicon esculentum</td>
<td>Flooding (8 h)</td>
<td>50 (transient)</td>
<td>Bradford and Hsiao, 1982</td>
</tr>
<tr>
<td>Lycopersicon esculentum</td>
<td>Flooding (24 h)</td>
<td>0</td>
<td>Jackson et al., 1996</td>
</tr>
<tr>
<td>Populus tremuloides</td>
<td>Hypoxia (1–10 d)</td>
<td>26–75</td>
<td>Kamaluddin and Zwikszek, 2002a</td>
</tr>
<tr>
<td>Ricinus communis</td>
<td>Flooding (2–24 h)</td>
<td>&gt;50</td>
<td>Else et al., 2001</td>
</tr>
<tr>
<td>Triticum aestivum (root cells near tip)</td>
<td>Hypoxia (30 min)</td>
<td>85</td>
<td>Zhang and Tyerman, 1991</td>
</tr>
<tr>
<td>Triticum aestivum (cortical cells in expanded region)</td>
<td>Hypoxia (30 min)</td>
<td>46</td>
<td>Bramley, 2006</td>
</tr>
<tr>
<td>Triticum aestivum</td>
<td>Hypoxia (30 min)</td>
<td>20</td>
<td>Bramley, 2006</td>
</tr>
<tr>
<td>Zea mays</td>
<td>Anoxia (12 h)</td>
<td>28</td>
<td>Birner and Steudle, 1993</td>
</tr>
<tr>
<td>Zea mays</td>
<td>Anoxia (&gt;20 h)</td>
<td>&lt;70</td>
<td>Everard and Drew, 1987</td>
</tr>
<tr>
<td>Zea mays</td>
<td>Hypoxia (2 h)</td>
<td>27 (transient)</td>
<td>Gibbs et al., 1998b</td>
</tr>
<tr>
<td>Zea mays</td>
<td>Hypoxia (30 min)</td>
<td>18</td>
<td>Tyerman et al., 1992</td>
</tr>
<tr>
<td>Zea mays (root cells)</td>
<td>Hypoxia (30 min)</td>
<td>60</td>
<td>Tyerman et al., 1992</td>
</tr>
</tbody>
</table>

Downregulation of AQP genes occurs later than upregulation, but overall, hypoxia appears to repress water channel activity, especially after 12 to 24 h of treatment (Liu et al., 2005; Table 5). Several of the PIPs downregulated are predominantly expressed in roots (Table 2). Therefore,
<table>
<thead>
<tr>
<th>Expression</th>
<th>Locus</th>
<th>Transcript</th>
<th>Details</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upregulated</td>
<td>At3g26520</td>
<td>TIP1;2</td>
<td>Highly upregulated within 0.5 h of hypoxia treatment</td>
<td>Klok et al., 2002</td>
</tr>
<tr>
<td></td>
<td>At2g25810</td>
<td>TIP4;1</td>
<td>Very highly upregulated 2–20 h of hypoxia treatment</td>
<td>Klok et al., 2002</td>
</tr>
<tr>
<td></td>
<td>At2g34390</td>
<td>NIP2;1</td>
<td>Rapidly (within 1 h) and highly upregulated in response to anoxia or hypoxia</td>
<td>Liu et al., 2005; Loreti et al., 2005</td>
</tr>
<tr>
<td></td>
<td>At2g29870</td>
<td>NIP</td>
<td>Highly upregulated in the central cylinder of roots</td>
<td>Liu et al., 2005</td>
</tr>
<tr>
<td></td>
<td>AJ 781013</td>
<td>NIP</td>
<td>Highly upregulated in flooding tolerant rice roots</td>
<td>Weig et al., 2004; Agarwal and Grover, 2005</td>
</tr>
<tr>
<td></td>
<td>At4g01470</td>
<td>TIP1;3</td>
<td>Transiently upregulated during 2–6 h of hypoxia</td>
<td>Liu et al., 2005</td>
</tr>
<tr>
<td></td>
<td>At1g31885</td>
<td>NIP3;1</td>
<td>Transiently upregulated during 6–12 h hypoxia</td>
<td>Liu et al., 2005</td>
</tr>
<tr>
<td>Downregulated</td>
<td>At3g61430</td>
<td>PIP1;1</td>
<td>Rapid (2 h hypoxia) and sustained downregulation, but expression not downregulated when exogenous sucrose applied</td>
<td>Liu et al., 2005; Loreti et al., 2005</td>
</tr>
<tr>
<td></td>
<td>At2g45960</td>
<td>PIP1;2</td>
<td>Same as PIP1;1</td>
<td>Liu et al., 2005; Loreti et al., 2005</td>
</tr>
<tr>
<td></td>
<td>At4g17340</td>
<td>TIP2;2</td>
<td>Same as PIP1;1</td>
<td>Liu et al., 2005; Loreti et al., 2005</td>
</tr>
</tbody>
</table>

(continued)
<table>
<thead>
<tr>
<th>Expression</th>
<th>Locus</th>
<th>Transcript</th>
<th>Details</th>
<th>References</th>
</tr>
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<tr>
<td>At5g47450</td>
<td>TIP2;3</td>
<td></td>
<td>Same as PIP1;1, but effects of sucrose not known</td>
<td>Liu et al., 2005</td>
</tr>
<tr>
<td>At2g39010</td>
<td>PIP2;6</td>
<td></td>
<td>Downregulated with addition of exogenous sucrose</td>
<td>Loreti et al., 2005</td>
</tr>
<tr>
<td>At3g56950</td>
<td>SIP2;1</td>
<td></td>
<td>Same as PIP2;6</td>
<td>Loreti et al., 2005</td>
</tr>
<tr>
<td>At4g19030</td>
<td>NIP1;1</td>
<td></td>
<td>Sustained downregulation after 12 h hypoxia</td>
<td>Liu et al., 2005</td>
</tr>
<tr>
<td>At2g45960</td>
<td>PIP1;2</td>
<td></td>
<td>Same as NIP1;1</td>
<td>Liu et al., 2005</td>
</tr>
<tr>
<td>At1g01620</td>
<td>PIP1;3</td>
<td></td>
<td>Same as NIP1;1</td>
<td>Liu et al., 2005</td>
</tr>
<tr>
<td>At3g53420</td>
<td>PIP2;1</td>
<td></td>
<td>Same as NIP1;1</td>
<td>Liu et al., 2005</td>
</tr>
<tr>
<td>At2g37170</td>
<td>PIP2;2</td>
<td></td>
<td>Same as NIP1;1</td>
<td>Liu et al., 2005</td>
</tr>
<tr>
<td>At5g60660</td>
<td>PIP2;4</td>
<td></td>
<td>Same as NIP1;1</td>
<td>Liu et al., 2005</td>
</tr>
<tr>
<td>At4g35100</td>
<td>PIP2;7</td>
<td></td>
<td>Same as NIP1;1</td>
<td>Liu et al., 2005</td>
</tr>
<tr>
<td>At3g26520</td>
<td>TIP1;2</td>
<td></td>
<td>Same as NIP1;1</td>
<td>Liu et al., 2005</td>
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<tr>
<td>At3g16240</td>
<td>TIP2;1</td>
<td></td>
<td>Same as NIP1;1</td>
<td>Liu et al., 2005</td>
</tr>
<tr>
<td>At4g23400</td>
<td>PIP1;5</td>
<td></td>
<td>Not downregulated until 24 h of hypoxia</td>
<td>Liu et al., 2005</td>
</tr>
<tr>
<td>At3g54820</td>
<td>PIP2;5</td>
<td></td>
<td>Same as PIP1;5</td>
<td>Liu et al., 2005</td>
</tr>
<tr>
<td>At2g16850</td>
<td>PIP2;8</td>
<td></td>
<td>Same as PIP1;5</td>
<td>Liu et al., 2005</td>
</tr>
<tr>
<td>At2g36830</td>
<td>TIP1;1</td>
<td></td>
<td>Same as PIP1;5</td>
<td>Liu et al., 2005</td>
</tr>
</tbody>
</table>

The loci, identified by microarray analyses of genes significantly altered by low O2, were given in the supplemental tables attached to the references listed above and were used to search the genetic database GenBank (Benson et al. 2000) for the description of the respective AQP transcripts.
such a dramatic reduction in AQP gene transcripts could negatively impact on root water transport and potentially explain the increased root resistance to water flow during waterlogging. Downregulation of TIPs and PIPs in the apical meristem could also be a component that reduces root growth during waterlogging.

Interestingly, Loreti et al. (2005) reported that the addition of exogenous sucrose altered which particular genes were downregulated by anoxia. Under anoxic conditions PIP1;1, PIP1;2 and TIP2;2 were downregulated, but not when sucrose was added to the bathing medium. Conversely, PIP2;6 and SIP2;1 were not downregulated by anoxia unless sucrose was present. Both of these latter genes are expressed in low amounts in roots and the proteins have unknown functions (Jang et al., 2004; Quigley et al., 2001). The addition of sugars to the bathing medium often increases the tolerance of roots to anoxia, but by unknown mechanisms. Exogenous sucrose could indirectly mitigate the reduction in $L_{pr}$ induced by $O_2$ deficiency.

Under field conditions, waterlogging is often transient, but no studies have analyzed the expression of AQPs in roots after $O_2$ deficiency followed by re-aeration. A problem with these studies could be separating the effects of tissue death from adaptation under $O_2$ deficiency. Few studies have examined AQP expression during a recovery period after other forms of abiotic perturbations. However, Martre et al. (2002) found that PIPs are important in the recovery of *A. thaliana* after a water deficit. A key component in the recovery of a species after waterlogging could be the installation of new AQPs in root membranes, particularly if $O_2$ deficiency leads to the breakdown of AQPs or root death. Moreover, given that the turnover rate of some AQPs can occur within a few hours, the resumption of water uptake and growth could also be rapid. After a short-term (0.5 h) mild hypoxia treatment followed by 1 h re-aeration, $L_p$, of wheat roots was $\sim$1.5 times greater than initial values of $L_p$, which was associated with an increase in AQP activity (Bramley, 2006). Moreover, in a long-term glasshouse experiment waterlogged wheat roots rapidly resumed growth, when the pots were drained and the growth rate was greater than before the plants were waterlogged (Bramley, 2006). An increase in AQP activity could not only facilitate the rapid transport of water to the shoots, but may also mediate changes in growth rate during the recovery period.

Rapid responses in $L_p$, to $O_2$ deficiency may also be mediated by the gating of AQPs. As detailed in Section 5.3.3.1.2, protonation of PIPs during cytosolic acidification closes the AQP pore and reduces the membrane permeability to water (Tournaire-Roux et al., 2003). Anoxia causes the cytoplasm to become acidified (Menegus et al., 1991), which therefore may close AQPs. The significance of this reaction on $L_p$ depends on the contribution of AQPs to radial water flow, and also on the degree of cytoplasmic acidification, which increases over time during anoxic conditions. However, some species such as rice are more tolerant of anoxia and
regulate their cytoplasmic pH to avoid acidosis (Menegus et al., 1991), but it is not known what effect this has on $L_p$, and hence on $P_f$. Furthermore, in nature plants experience a gradual decline in $O_2$ rather than a sudden exposure to anaerobic conditions (i.e., hypoxia prior to anoxia), which triggers acclimatic responses such as avoidance of proton accumulation and expression of proteins required for anaerobic respiration. A pretreatment with hypoxic conditions increases the tolerance of many species to subsequent anoxia, and one of the processes related to this enhanced tolerance is better regulation of cytoplasmic pH (Germain et al., 1997; Kato-Noguchi, 2000; Waters et al., 1991; Xia and Saglio, 1992). When exposed to hypoxic or anoxic conditions, the internal pH is generally not as low as that used to demonstrate the inhibitory effects of pH on $L_p$, or $P_f$ (Alleva et al., 2006; Menegus et al., 1991; Tournaire-Roux et al., 2003; Xia and Roberts, 1994, 1996). However, the permeability of wheat and lupin root cell membranes can decrease significantly, when exposed to only 0.5 h of 3–4% $O_2$ (Bramley, 2006; Zhang and Tyerman, 1991), which is considered a relatively mild hypoxic treatment. It is therefore, not clear whether a reduction in internal pH is the initial factor that reduces $L_p$ through a closure of AQP s. The effect may also be related to other mechanisms of AQP gating such as changes in apoplastic water potential and osmoregulation that arises from the effect of $O_2$ deficiency on ion leakage from a depolarization of the membrane potential (Zhang and Tyerman, 1991, 1997). Calcium fluxes also change in response to $O_2$ deficiency, which can affect AQP gating (Dat et al., 2004). Moreover, it has never been tested whether $O_2$ itself affects AQP gating as could be demonstrated with stopflow spectrophotometry.

Overall, AQP activity does appear to decrease in response to $O_2$ deficiency. This could have significant consequences on the rate of water flow through roots, provided that a large proportion of water flow occurs through the cell-to-cell pathway (Section 3.2.2.1). In addition, given that some AQP s such as PIP1s are permeable to other small neutral molecules, a reduction in AQP activity may influence other cellular processes under $O_2$ deficiency. For example, $CO_2$ and ethanol build up under anaerobic conditions (Gibbs and Greenway, 2003), which may be because these molecules are permeable to some AQP s (Steudle and Henzler, 1995; Uehlein et al., 2003).

8. Conclusion

Water flow through roots can be controlled by a variety of mechanisms. The structure of the root and the anatomy of the radial pathways for flow dominate the process because they determine the physical hydraulic conductance of the root system to water flow. Changes to this hydraulic conductance will be slow and growth dependent, as the root lays down new
tissue, extends and/or becomes suberized. AQPs can influence the rate of water flow through the radial pathway of roots, but only if the hydraulic conductance of the apoplast is less than that of the cell-to-cell pathway. AQPs may increase the hydraulic conductivity of the cell-to-cell pathway by increasing their abundance and/or opening their pores. The distinct advantage of a significant proportion of radial water flow through AQPs is that it provides the ability for the plant to rapidly and reversibly regulate flow in response to a fluctuating environment. AQP transcripts and proteins have been found to vary significantly and rapidly in response to abiotic perturbations. Moreover, AQP gating provides an even finer control. Despite the abundance of AQP homologs in roots, indicating that they play a part in regulating root water flow, there are also many other important functional roles that they may perform. The majority of research to date has focused on the molecular aspects of AQP activity, but more detailed physiology is required to understand AQP functions.

Of those species where radial water flow occurs through the cell-to-cell pathway, AQPs may influence water transport through roots when plants are waterlogged, since AQP genes tend to be downregulated under O₂-deficient conditions. However, long-term exposure to O₂ deficiency causes root systems to die, particularly in less tolerant species, which hence would reduce the absorbing surface area for water uptake. Root systems that do not die avoid anoxia by forming aerenchyma and barriers to ROL. These anatomical features may obstruct water flow through the radial pathway and measurements of their effect on Lp have not been undertaken during O₂ deficiency. AQPs in the apical region are likely to be downregulated and/or close as the cytoplasm becomes acidified, since this region is the most sensitive to O₂ deficiency. Conversely, AQP activity in O₂-sufficient cells (e.g., adjacent aerenchyma) could increase and hence compensate for the reduced hydraulic conductance caused by anatomical changes. Although little has been investigated, AQPs are likely to be beneficial during recovery when the root system is no longer submerged and the O₂ levels in the rhizosphere return to normal. The speed in the resumption of growth and transport of water and nutrients are key features that influence the tolerance of a species to waterlogging and all these attributes can be influenced by AQP activity. For us to understand the physiological mechanisms that enhance a species’ tolerance to waterlogging, it is important that more research focuses on AQP-mediated processes that are affected by O₂ deficiency.

ACKNOWLEDGMENTS

A scholarship for HB and funding for research were supported by the Grains Research Development Corporation (GRDC) of Australia.
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intrinsic proteins in Arabidopsi provides a framework for a new nomenclature for major intrinsic proteins in plants. **Plant Physiol.** **126**, 1358–1369.


