Chapter 8
Root Water Transport Under Waterlogged Conditions and the Roles of Aquaporins

Helen Bramley and Steve Tyerman

Abstract Water flow through plants roots can be affected when the soil is water-logged and oxygen deficient. For species not adapted to these conditions, water flow usually decreases within minutes to days, depending on the oxygen concentration in the root and rhizosphere. During this time, the decrease in water flow is attributed to decreased root hydraulic conductance, through an inhibition of plasma-membrane aquaporins. There is increasing evidence that aquaporins may also be involved in the transport of gases, end products of anaerobic respiration, and signalling molecules; all of which are relevant to oxygen-deficient conditions. Eventually, primary roots die if continually starved of oxygen, but may be replaced with adventitious roots that can maintain the supply of water to the shoot. Here, we review the effects of waterlogging and oxygen deficiency on root hydraulic conductance and aquaporin activity.

8.1 Introduction

Some plants wilt within hours of their roots being waterlogged unless they close their stomata. This phenomenon, called physiological drought, is believed to be caused by an insufficient supply of water from the roots (Cannell and Jackson 1981). Water uptake into roots and transport to the shoots is often inhibited when the soil is waterlogged (Kramer 1949; Kramer 1983). The abundant supply of water in direct contact with roots should reduce the drop in water potential required across the root/rhizosphere interface to induce water uptake. However, although water

H. Bramley
Institute of Agriculture, The University of Western Australia, 35 Stirling Highway, Crawley, Western Australia 6009, Australia

S. Tyerman (✉)
School of Agriculture, Food and Wine, The University of Adelaide (Waite Campus), Plant Research Centre, PMB 1, Glen Osmond SA 5064, Australia
e-mail: stephen.tyerman@adelaide.edu.au

moves entirely passively in response to gradients in water potential, roots are not invariable pathways for water flow (Tyree and Zimmermann 2002), so flow rate is determined by their hydraulic conductance. Hydraulic conductance of some species varies with time of day, transpiration rate and in response to biotic or abiotic perturbation (for recent reviews see Javot and Maurel 2002; Vandeleur et al. 2005; Bramley et al. 2007; Maurel et al. 2008). Water transport through the root is a spatially and temporally complex process with many physiological mechanisms that can influence it. The activity of aquaporins (density in the membrane and gating) is seen to be a component accounting for changes in root hydraulic conductance; however anatomical factors and the distribution of water flow along the root and in different parts of the root system are also important. At the plasma-membrane and in membranes surrounding intracellular compartments aquaporins may also be involved in the flux of gases (O_2, CO_2 NO), end products of anaerobic respiration (ethanol, lactic acid and other organic acids), or of molecules important in signalling (H_2O_2, ethylene). In this chapter, we review what mechanisms may be involved during root submergence and oxygen deficiency.

8.2 Variable Root Hydraulic Conductance (L_r)

Oxygen deficiency has apparently varying effects on L_r. For example, 0.5 h of anoxia reduced L_r of *Arabidopsis thaliana* by almost half and 2–4 h of flooding *Ricinus communis* reduced L_r by 65% (Else et al. 2001; Tournaire-Roux et al. 2003). In comparison, L_r was not influenced by 3 h of anoxia in *Agave deserti*, 24 h of flooding *Lycopersicon esculentum* or 10 d of flooding conifer species (Nobel et al. 1990; Reece and Riha 1991; Jackson et al. 1996). In other studies, oxygen deficiency transiently reduced L_r of *L. esculentum*, *Helianthus annuus*, *Zea mays* and *Musa* spp. (Bradford and Hsiao 1982; Everard and Drew 1989; Gibbs et al. 1998; Aguilar et al. 2003). Some of this variability may be related to differences in methodology such as concentration of oxygen or duration of oxygen deficiency. Many studies simulated oxygen-deficient conditions by rapidly applying anoxia, which likely induces "shock" responses. In their natural environment, roots experience hypoxia before anoxia, which stimulates acclimation responses and imparts greater tolerance to subsequent anoxia (Waters et al. 1991; Xia and Roberts 1996; Dennis et al. 2000; Kato-Noguchi 2000). On the other hand, studies that attempted to simulate waterlogging by inundating root systems grown in pots often imposed the treatment for weeks at a time, so by the time L_r was measured the root systems may have deteriorated or died.

The variability in L_r may also be species dependent and few studies have addressed this variability in terms of differences in root hydraulic properties. The conductance of the tissue to water and the magnitude of the driving force determine the rate of water flow through roots. Consequently, root hydraulic properties are influenced by morphology, anatomy and activity of proteinaceous water channels

called aquaporins (Bramley et al. 2009). Changes induced by oxygen deficiency to each of these attributes can potentially influence L_r.

8.3 Changes in Root Morphology and Anatomy

Anatomy plays a major role in determining where water is absorbed along the root and the pathway water takes across the root. From the root surface to the xylem (radial pathway), water can travel extracellularly through the apoplast and/or intercellularly across membranes and through plasmodesmata. The contribution of these pathways depends on their hydraulic conductivity and can change with environmental conditions or whether water flow is induced by transpiration or accumulation of solutes (Steudle and Peterson 1998). Roots of many species exhibit phenotypic plasticity and long-term exposure to waterlogging can cause changes in morphology and anatomy, through cell death and/or modifications to cell walls.

8.3.1 Root Death and Adventitious Roots

The extent of injury induced by waterlogging depends on the tolerance of the species to oxygen deficiency (Drew 1992). Root tips tend to have high metabolic rates and are generally the most sensitive part of the root to oxygen deficiency (Drew 1997). In particularly sensitive species, such as *Lupinus*, root tips start to visually deteriorate within a few days of waterlogging (Bramley 2006). Root tips lose turgor pressure and the cortex deteriorates (Atwell 1991). Further from the root tip, turgor pressure of cortical cells decreases even with short exposure (0.5 h) to mild hypoxia (Bramley et al. 2010). For intolerant species, the longer the duration of waterlogging the greater the extent of root death, with progressive deterioration towards the basal region. Water absorption commences behind the root tip, so death of this region of the root may have little effect on L_r. It is not clear how root death under waterlogging affects L_r, as barriers to flow may increase or decrease, depending on which tissue dies and where the greatest limitation to L_r occurred prior to waterlogging. Plugging or collapse of xylem vessels as opposed to death of the cortex could have contrasting effects on L_r.

To investigate the role of roots in water absorption by transpiring plants Kramer (1933) killed root systems of seven different species by immersing them in hot water. Plants with dead roots still absorbed "considerable quantities" of water during the first few days after root death, so that leaves continued to transpire and remained hydrated. Eventually, transpiration declined, leaves wilted and shoots died because xylem vessels in stems plugged above the killed tissue. Applying suction to decapitated dead root systems induced water flux more than when root systems were alive, which was attributed to a decrease in the resistance to water movement in the radial pathway (Kramer 1933). In addition, L_r is commonly

measured by the pressure chamber technique, but pressurising flooded roots or roots in solution culture causes tissue of some species to become infiltrated with water and/or deteriorate (Bramley 2006).

The growth of adventitious roots during waterlogging is also likely to influence L_r. These roots emerge from nodes on the base of the stem and are constitutive in Gramineae (called nodal or crown roots) and many wetland species (Barlow 1994; Kovar and Kuchenbuch 1994). However, waterlogging often stimulates their growth in species that do not develop adventitious roots under ambient conditions. Adventitious roots usually develop aerenchyma (see 8.4.2), which enables them to survive and grow in waterlogged soil, albeit with limited length (Armstrong et al. 1991). The development of adventitious roots appears to be particularly important where oxygen deficiency causes extensive mortality of primary roots (Etherington 1984; Harrington 1987; Moog and Janiesch 1990; Solaiman et al. 2007) and their importance in maintaining plant growth during waterlogging was demonstrated in pruning experiments (Etherington 1984). Adventitious roots become the main source for nutrient and water uptake, but their contribution to L_r of waterlogged root systems has not been investigated.

8.3.2 Barriers to Radial Flow

Once water has been absorbed by the root, it has to cross a series of concentric tissues to reach the vasculature. Oxygen deficiency triggers physical changes to some of these tissues, which may influence L_r. The most common change is the development of large gas-filled tubes in the cortex, called aerenchyma (Fig. 8.1). Aerenchyma forms predominantly by cell lysis in adventitious roots, but can also form in new seminal roots and some above-ground organs (Colmer 2003; Vartapetian 2006). Schizogenous aerenchyma also forms constitutively in wetland species. Often coinciding with aerenchyma in the basal part of the root is an impermeable barrier to oxygen in the outer cell layers (Fig. 8.1), but the occurrence of this feature depends on the particular species (see review in Colmer 2003). Both features are adaptations to enhance internal root aeration and both constitutively occur in many species inhabiting flooded soils (Vartapetian 2006).

Aerenchyma potentially poses a large resistance to radial flow because it dramatically reduces the surface area for liquid flow. Without septa providing an apoplastic pathway across the air spaces, water would have to cross in the form of vapour. There have been few examinations of the influence of aerenchyma on L_r under waterlogging, but a decrease in L_r of phosphorus deficient *Z. mays* was associated with the formation of aerenchyma (Fan et al. 2007). Other nutrient deficiencies and abiotic stresses can induce aerenchyma formation, but these may also cause other anatomically or physiologically confounding changes. To investigate the influence of aerenchyma on L_r, it would be more appropriate to study a species that does not develop a barrier to oxygen loss, such as *T. aestivum* (Colmer 2003). *T. aestivum* develops aerenchyma in nodal roots under oxygen deficiency,
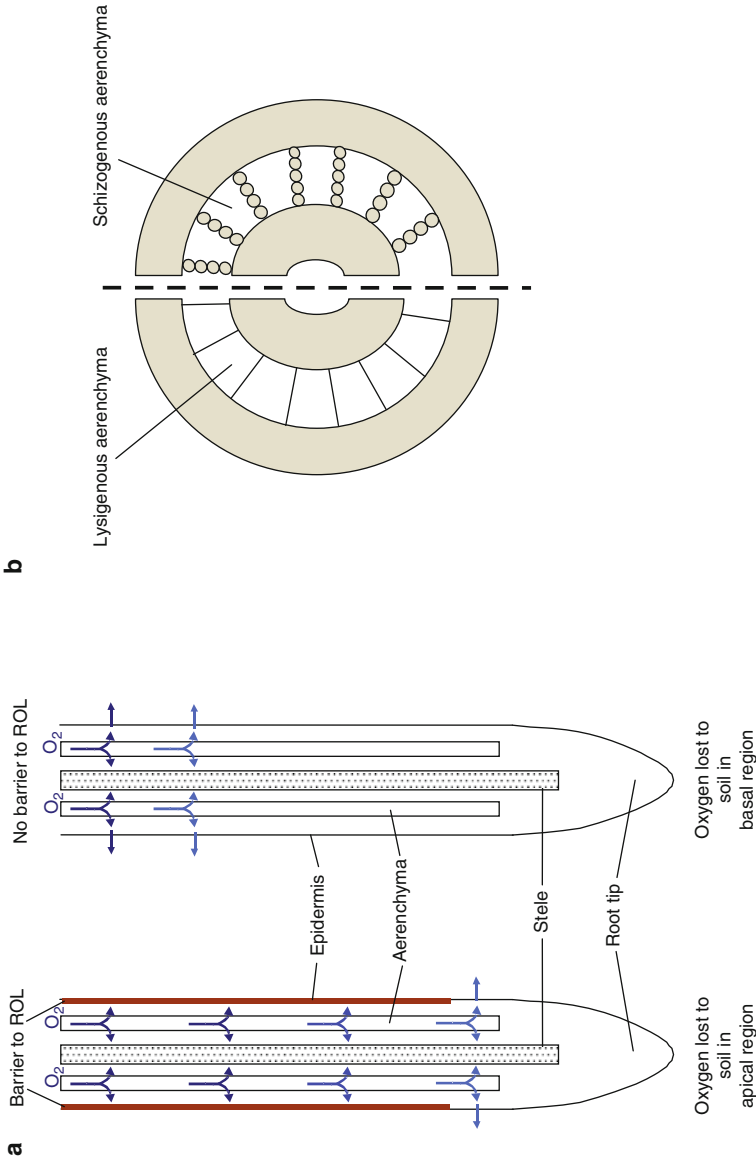
Fig. 8.1 (**a**) Diagram comparing roots with and without a barrier to radial oxygen loss (ROL). Oxygen transport occurs by diffusion from the aerial parts of the plant to the roots via aerenchyma. The barrier has a low oxygen permeability that reduces oxygen loss to the soil. A root with a barrier should have a higher concentration of oxygen in the apical part of the root, compared with an otherwise identical root without a barrier. The barrier may also block water transport via the apoplast in the outer cell layers, so water uptake may be restricted to the apical zone. The apoplast may not be blocked in a root without the barrier to ROL, but aerenchyma may still form a large resistance to radial water flow. (**b**) Diagram of a root cross-section showing lysigenous aerenchyma or schizogenous aerenchyma. Cell walls or cells form septa that could provide a pathway for water flow across aerenchyma

but has non-aerenchymatous nodal roots under ambient conditions. However, increased cellulose deposition with cell wall thickening has been observed in *T. aestivum* seedling roots exposed to 4 d hypoxia, which was speculated to provide mechanical strength where aerenchyma develops (Albrecht and Mustroph 2003).

Barriers to reduce oxygen loss may also increase the resistance to radial water flow as their low permeability to oxygen may impart similarly low permeability to water. The barrier appears to be formed by greater suberisation and/or lignification of the exodermis (Moog and Janiesch 1990; Armstrong and Armstrong 2005; Enstone and Peterson 2005; Soukup et al. 2007), whilst the endodermis often becomes less suberised (Harrington 1987; Enstone and Peterson 2005). Under stagnant conditions, adventitious roots of *O. sativa* had increased aerenchyma development and increased deposition of phenolic or lipid compounds in the hypodermis, the layer of sclerenchymatous fibre cells below and the cell layer beneath that (Insalud et al. 2006). Suberin is a waxy substance with hydrophilic properties creating an apoplastic barrier to water flow and its deposition is usually associated with the retention of water during drought (Steudle and Peterson 1998). However, whether increased suberisation of the exodermis decreases L_r is a matter of debate (Steudle and Peterson 1998).

The influence of the barrier to oxygen loss on L_r has only been studied in two species, *Hordeum marinum* and *O. sativa* (Ranathunge et al. 2003; Garthwaite et al. 2006). Adventitious roots of *H. marinum* developed a barrier to oxygen loss when grown under stagnant conditions, which did not influence L_r compared with roots grown under aerated conditions (Garthwaite et al. 2006). However, the barrier formed proximal of 60 mm from the root tip, which could be the reason it had little effect on L_r., i.e. if most water uptake occurs distall from this position. Maximal water uptake of cereal roots generally occurs in the apical region (Greacen et al. 1976), where the barrier did not form. Despite the development of a barrier in *O. sativa* roots, which reduced oxygen loss, water flow across the outer cell layers apparently occurred predominantly through the apoplast (Ranathunge et al. 2004; Ranathunge et al. 2005) and the outer part of the root was not rate limiting to water flow (Ranathunge et al. 2003). This is contrasting with Miyamoto et al. (2001) who reported that apoplastic barriers and development of the endodermis resulted in low L_r of *O. sativa* roots. *O. sativa* roots tended to have lower hydraulic conductivity per unit surface area than other herbaceous species (Miyamoto et al. 2001), which may be because water uptake occurs predominantly in the apical zone. In addition, measurements with ion-selective microelectrodes on *O. sativa* adventitious roots demonstrated that rates of NO_3^- and NH_4^+ uptake were greatest in the apical region and decreased with distance from the tip (Colmer and Bloom 1998). The decrease in ion uptake in the basal region was related to the development of sclerenchymatous fibres in the outer cortex.

Whilst the limited evidence indicates that barriers to oxygen loss and aerenchyma do not limit L_r of cereal roots under waterlogged conditions they may pose some constraint when the soil drains and there is less water available. The region of root involved in water uptake may be restricted, with little capacity for adjustment apart from growth of new roots. In addition, adventitious roots will be shallow and may be susceptible to drought and fluctuations in temperature. However, there are

some suggestions that aerenchyma may have a secondary role in water storage (van der Weele et al. 1996; Kozela and Regan 2003). Plants that inhabit environments that frequently flood tend to have dimorphic root systems, where roots that penetrate the soil are thick, with few branches and have well developed aerenchyma, but surface roots are fine and heavily branched and are responsible for most nutrient uptake (Koncalová 1990).

8.3.3 Varying the Root or Root Region Involved in Water Uptake

The combined development of adventitious roots and death of primary roots may thus explain some of the transient responses in L_r. Measurements on anoxic *H. annuus* roots also indicate other mechanisms causing transient changes in L_r. Under anaerobic treatment, L_r of *H. annuus* initially declined, but after 22 h increased to values greater than aerated roots (Everard and Drew 1989). The roots were not dead and there was no evidence of xylem blockage. Anaerobic treatment for 6 d killed roots and L_r of these roots was similar to roots killed by heat immersion. Everard and Drew (1989) argued that L_r initially decreased because water flow occurs through hydrophilic pores spanning cell membranes that require metabolic energy to sustain them; a rather insightful conclusion considering these water "pores", now called aquaporins (see 8.6.1), were not discovered in plants until a few years later. The closure or reduced expression of aquaporins also probably explains the decrease in L_r of oxygen deficient *Z. mays* root segments (Birner and Steudle 1993; Gibbs et al. 1998), although it was not tested. L_r of hypoxic root segments recovered after 4–6 h of treatment, but did not recover in anoxic roots (Birner and Steudle 1993; Gibbs et al. 1998). An interesting observation from both of these studies was that L_r measured using a hydrostatic gradient was reduced more than L_r measured with an osmotic gradient, indicating different pathways for radial water flow.

Some species can vary the root or region of root for preferential water uptake, which may be particularly beneficial in stochastic and heterogeneous environments. During waterlogging, different parts of the root system may be located in wet soil if the soil is not saturated to the surface. Even if the plant is highly sensitive to oxygen deficiency and its submerged roots die, the oxygen-sufficient roots may be able to supply adequate water to maintain shoot growth. In an experiment on *Larix laricina,* where only the lower half of the root system was flooded, water uptake in the upper non-flooded part transiently increased (Reece and Riha 1991), but decreased in the flooded part. In roots totally flooded, water uptake decreased in both parts and transpiration and stomatal conductance decreased more in full-flooded plants (Reece and Riha 1991).

T. aestivum is a particularly good example of a species that can regulate L_r, as it can alter L_r of individual roots and the hydraulic conductance of different parts of an individual root (Vysotskaya et al. 2004; Bramley et al. 2009; Bramley et al. 2010). Severing roots from plants grown in solution culture resulted in L_r of the remaining root increasing, so that water supply to the shoot was maintained (Vysotskaya et al. 2004).

In an experiment on excised root segments, 0.5 h hypoxia only reduced L_r of roots shorter than 120 mm (Bramley et al. 2010). This was explained by longer roots subjected to hypoxia being able to increase the length of root over which preferential water transport occurred, so that there was then no apparent change in root conductance. When roots were re-aerated, L_r increased in all roots irrespective of length and L_r was greater than control roots. Hypoxia dramatically decreases the hydraulic conductivity of root cortical cells through decreased aquaporin activity (Zhang and Tyerman 1991; Zhang and Tyerman 1999; Bramley et al. 2010). Modelling and measurements on cells in different cell layers indicated that variation in the water permeability of the endodermis could account for the variation in L_r (Bramley et al. 2010). In an unrelated study, L_r of *T. aestivum* grown under stagnant conditions but measured under aerated conditions, was greater than plants grown under aerated conditions (Garthwaite et al. 2006). No explanation for this observation was purported, but the response may be similar to the overshoot in L_r reported by Bramley et al. (2010).

8.4 Volatile and Toxic Compounds in Anaerobic Soils

Ethylene, CO_2 and CH_4 accumulate in flooded soils because of restricted gas exchange with the atmosphere (Vartapetian 2006). Elevated levels of these gases may have an effect on L_r through their interaction with aquaporin activity, provided water flows across membranes. Ethylene is a signalling molecule that triggers aerenchyma development. In hypoxic aspen roots, ethylene enhanced L_r, which was speculated to increase phosphorylation of aquaporins (Kamaluddin and Zwiazek 2002). In contrast, CO_2 tends to decrease L_r, but the mechanism is unknown (Newman 1976; Smit and Stachowiak 1988). Anaerobic soils also accumulate reduced ions such as nitrous oxide, ferrous ions, sulphides and organic substances such as lactic acid, ethanol, acetylaldehyde and aliphatic acids (Cannell and Jackson 1981). The effect of these substances during waterlogging is often overlooked because primary injury occurs from oxygen deficiency (Drew 1997). However, their accumulation may have an antagonistic effect with oxygen deficiency on L_r.

8.5 Water Permeability of Root Cells and Aquaporins

The water permeability of root cells during waterlogging has received little attention and yet the measurements at the cell level provide direct evidence for the temporal sensitivity to oxygen deficiency, as well as transport processes that are affected. For example, turgor pressure of cortical cells near the tip in *T. aestivum* roots decreased during 0.5 h of hypoxia, which was caused by membrane depolarisation and leakage of osmotica (Zhang and Tyerman 1991; Zhang and Tyerman 1997). In another study, cortical cells in the mature root region maintained turgor pressure during similar hypoxic treatment of *T. aestivum*, but not in two *Lupinus*

species (Bramley et al. 2010). In all of these species, hypoxia (0.04–0.05 mol O_2 m^{-3}) dramatically reduced cell hydraulic conductivity (Lp_c), which we now attribute to an inhibition of aquaporin activity (Zhang and Tyerman 1991; Zhang and Tyerman 1999; Bramley et al. 2010).

Flow across cell membranes and through cells may be part of the pathway for radial water transport and as such, may impart great influence on water flow through roots. It is therefore, important to understand the mechanisms affecting cell water permeability. Since the discovery of plant aquaporins in the early 1990's (Maurel et al. 1993; Chrispeels and Agre 1994) there has been renewed interest in plant hydraulics, because these ubiquitous proteins control water flow across membranes.

8.5.1 Plant Aquaporins

The majority of aquaporin genes are predominantly expressed in roots (Bramley et al. 2007) and therefore, their location, abundance and gating, can potentially control the rate of water flow through whole plants and the fluxes of other molecules and gasses that may need compartmentation or extrusion from the cell. In this section, we review the effects of root submergence and oxygen deficiency on cell water relations and the role of aquaporins in water transport and transport of other molecules relevant to anoxia and hypoxia.

Aquaporins are members of the major intrinsic protein (MIP) group of transmembrane channels found ubiquitously in all organisms. In the last decade MIPs have also been shown to facilitate the transport of other small neutral molecules, in addition to water. This is particularly relevant to the metabolic consequences of hypoxia and anoxia, as we elaborate further below.

MIPs form tetramers in the membrane, where each monomer appears to function as an individual functioning pore. However, interactions between tetramers that affect transport may occur and the central pore in the tetramer may function as a channel (Yu et al. 2006; Bertl and Kaldenhoff 2007; Fig. 8.2). The MIP superfamily is a large gene family in plants; 35 MIP encoding genes have been identified in *Arabidopsis thaliana* (Johanson et al. 2001), 31 in *Z. mays* (Chaumont et al. 2001), 33 in *O. sativa* (Sakurai et al. 2005), 24–28 in *Vitis vinifera* (Fouquet et al. 2008; Shelden et al. 2009) and 37 in *L. esculentum* (Sade et al. 2009). *T. aestivum* appears to have an even larger set of aquaporin genes based on the identification of 35 genes just from the PIP and TIP groups (Forrest and Bhave 2008).

Plant aquaporins have been traditionally divided into four groups based on sequence homology, and two of these groups (tonoplast intrinsic proteins, TIP; and plasma-membrane intrinsic proteins, PIP) seems to match their membrane localization; though not always for some PIPs (Kirch et al. 2000; Whiteman et al. 2008). In addition to the PIPs and TIPs, there are the NOD26-like intrinsic proteins (NIPs), and the small basic intrinsic proteins (SIPs) (Johanson et al. 2001; Johanson and Gustavsson 2002). New subgroups of MIPs have been identified from the moss *Physcomitrella patens*, which have 23 MIPs (Danielson and Johanson 2008). One of these (X Intrinsic Proteins, XIPs) has been identified in other plants
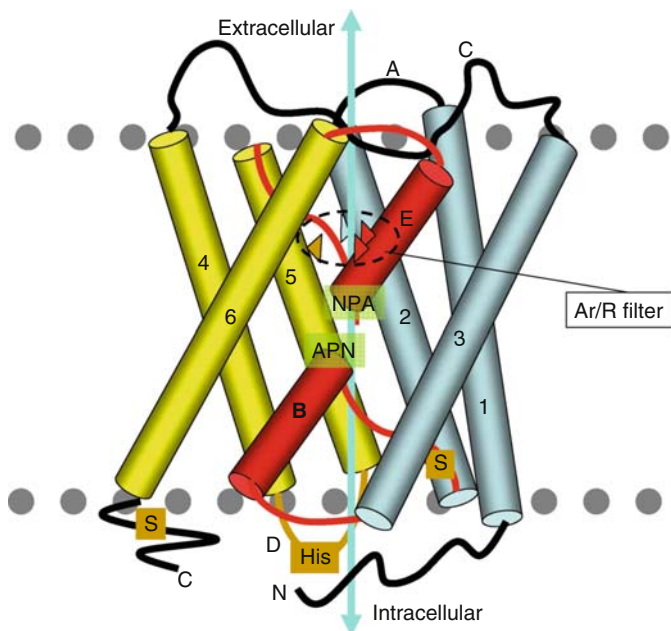
Fig. 8.2 Diagram of the structure of a PIP aquaporin monomer with six transmembrane helices (1–6) and five connecting loops (**a–e**). PIPs normally occur as a tetramer in the membrane. Shown are the highly conserved NPA motifs; histidine (H) residue, involved in cytosolic pH sensing; two phosphorylation sites [serine (S) residues] in the loop B and C-terminal tail of aquaporins of the PIP2 subgroup. The Ar/R selectivity filter is indicated. The diagram is not to scale and is only a general indicator of the structure (adapted from Luu and Maurel (2005) and Tornroth-Horsefield et al. (2006))

including *L. esculentum* (Sade et al. 2009). The NIPs have been located to both endomembranes and the plasma-membrane (Zhang and Roberts 1995; Mizutani et al. 2006; Choi and Roberts 2007). The NIPs are an important group with respect to the effects of oxygen deficiency, because of their potential to facilitate the transport of gasses and end products of anaerobic respiration.

The PIPs have been divided into two groups: the PIP1 group often show lower or no water permeability when expressed in Xenopus oocytes, in contrast with the PIP2 group that show high water permeability (Chaumont et al. 2000; Moshelion et al. 2002; Fetter et al. 2004; Vandeleur et al. 2009). The PIPs are an interesting group because: (1) They are probably the rate limiting aquaporins in transcellular water flow, because the tonoplast membrane has much higher water permeability under normal conditions (Maurel et al. 1997; Niemietz and Tyerman 1997). Though there may be exceptions given the extraordinarily high water permeability measured for *Beta vulgaris* plasma-membrane (Alleva et al. 2006); (2) They are inhibited by low pH in the physiological range (Tournaire-Roux et al. 2003; Alleva et al. 2006; Verdoucq et al. 2008); (3) They are gated by phosphorylation and cytosolic Ca^{2+} concentration (Johansson et al. 1996; Johansson et al. 1998;

Törnroth-Horsefield et al. 2006). But note that NIPs and TIPs can be regulated by phosphoryation (Maurel et al. 1995; Guenther et al. 2003; Wallace et al. 2006); (4) Some are implicated in facilitating the permeation of CO_2 (Uehlein et al. 2003; Hanba et al. 2004; Flexas et al. 2006; Uehlein et al. 2008) and there is evidence for facilitated oxygen transport by mammalian AQP1 and a link to hypoxia-inducible transcription factor (Echevarria et al. 2007).

The selectivity of aquaporins is determined by the asparagine-proline-alanine (NPA) motif at the end of the two half helices that extend into the pore (Fig. 8.2) and the aromatic-arginine (ar/R) region, which is composed of a tetrad of residues on helices 2 and 5, and two positions on loop E (Fig. 8.2). The ar/R region is on the apoplastic side of the NPA filter. The ar/R region determines both the selectivity and transport rate of MIPs. In plant MIPs, the structure of the ar/R filter has been examined for each of the four groups, revealing that PIPs have an ar/R signature typical for high water transport, TIPs have three separate conserved ar/R filters, and NIPs possess two conserved ar/R filters (Wallace and Roberts 2004). The NIPs can be subdivided into two groups that differ mainly by the substitution of an alanine (NIP subgroup II) for a tryptophan (NIP subgroup I) in the helix 2 of the filter. A subgroup I representative, NOD26, can have high water permeability and transports glycerol and formamide, while AtNIP1;6 showed no water transport but transported glycerol, formamide, and urea (Wallace and Roberts 2005). Eight *A. thaliana* NIPs (out of nine) appear to be expressed in roots, but those that have highest expression in roots, NIP1;1 and NIP5;1 represent members of subgroup I and II respectively (Wallace et al. 2006). NIP2;1, which can transport lactic acid and is upregulated, is a member of subgroup I, but does not transport water or glycerol (Choi and Roberts 2007).

8.5.2 *Responses at the Cell Level Affecting Water Permeability and Potential Mechanisms*

There is no known mechanism of direct oxygen sensing in plants, but indirect sensing may occur through changes in cytosolic pH, calcium concentration, reduction in ATP, or production of NO and H_2O_2. Various membrane transport processes are regulated directly or indirectly by cytosolic ATP, pH, free Ca^{2+} and reactive oxygen species (ROS) (Amtmann and Blatt 2009). It would not be surprising therefore, if MIPs were sensitive to these factors and hence, to hypoxia and anoxia. Indeed, we have an increased understanding now, of the mechanisms of how PIPs are regulated by pH, free Ca^{2+}, phosphorylation (Alleva et al. 2006; Hedfalk et al. 2006) and H_2O_2 (Boursiac et al. 2008). In addition, like ion channels, one might expect water channels to be sensitive to the gradients that generate flow, either osmotic gradients, pressure gradients or a combination of both.

8.5.2.1   Changes in Water Potential

Flooding may occur with solutions of different composition and salinity. Flooding with non-saline water would elevate the soil matrix potential to near zero and consequently, root cells adjacent to this medium would rapidly equilibrate (within several tens of seconds) to a high water potential. Saline water, on the other hand, brings with it both low osmotic potential and ion toxicity effects. The latter has been shown to depress hydraulic conductivity of roots (Azaizeh and Steudle 1991; Martinez-Ballesta et al. 2003), root cortical cells (Azaizeh et al. 1992) or proto-plasts and vesicles from roots (Martínez-Ballesta et al. 2008), but not always (Tyerman et al. 1989). External calcium can ameliorate this response, probably via a combination of effects on aquaporin gating and expression (Martínez-Ballesta et al. 2008). Depression of aquaporin activity (expression and density in the membrane) has been implicated in the response to salinity (Martinez-Ballesta et al. 2003; Boursiac et al. 2008) and the internalisation of PIP aquaporins from the plasma-membrane is mediated by H_2O_2. This observation is important to keep in mind when considering the mechanism of depressed water permeability under hypoxia and anoxia.

Water potential or components thereof have been implicated in the control of aquaporins, either by phosphorylation (Johansson et al. 1996; Guenther et al. 2003), or by direct interactions of osmotica (Vandeleur et al. 2005; Ye et al. 2005). Drought (low water potential) certainly has large effects on aquaporin expression and activity in roots (Parent et al. 2009; Vandeleur et al. 2009) and ABA is implicated in these responses (Parent et al. 2009).

8.5.2.2   Decreased ATP, Implications for Transport and Interactions with Aquaporins

Although it is beyond the scope of this chapter to discuss the various pathways of carbohydrate catabolism that occur during anaerobiosis, it is prudent to consider some of the end products that can accumulate under anaerobic metabolism, because these may be substrates for transport out of the cytosol (to the vacuole or apoplast) via some MIPs (Table 8.1). For roots that do not contain aerenchyma, flooding and the hypoxia/anoxia that eventuates cause a respiratory crisis where energy produc-tion is reduced by more than 65% (Gibbs and Greenway 2003). This necessitates ATP production via fermentation with the end products of lactic acid or ethanol. Much less ATP is produced per glucose molecule catabolized (2:36–38) than when the tricarboxylic acid cycle (TCA) uses O_2 as the final electron acceptor in the mitochondrial electron transport chain. Plant cells can adapt to these conditions by increasing glycolysis (Pasteur effect) and increasing the regeneration of NAD^+ for glycolysis by induction of enzymes of fermentation pathways (ethanolic fermenta-tion, lactic acid fermentation, and alanine fermentation) (Kennedy et al. 1992; Gibbs and Greenway 2003). Some anoxia tolerant plants, such as *O. sativa*, may also use a pyrophosphate (PPi) dependent glycolysis, which can increase the yield

Table 8.1 Small molecules related to anoxia, hypoxia or re-aeration after anoxia, that may or do permeate via MIPs

	Molecule	Link with MIP	pKa of acids	Reference to MIP or accumulation of molecule where relevant
Gases	O_2	HsAQP1		MIP = (Echevarria et al. 2007)
	CO_2	NtAQP1; HvPIP2;1		MIP = (Uehlein et al. 2003; Hanba et al. 2004; Flexas et al. 2006)
				Acc = (Greenway et al. 2006)
Signalling	NO	?		Acc = (Dordas 2009)
	H_2O_2	AtTIP1;1, AtTIP1;2		Acc = (Blokhina et al. 2003)
		AtPIP2;4 > AtPIP2;1 > AtNIP1;2 > AtNIP2;3		MIP = (Bienert et al. 2007; Dynowski et al. 2008)
	Ethylene	Ethylene can enhance L_r, inhibited by Hg	Neutral	Acc = (Fukao and Bailey-Serres 2008)
				MIP = (Kamaluddin and Zwiazek 2002)
Catabolic end products	Lactic acid	AtNIP2;1	3.1	MIP = (Choi and Roberts 2007)
				Acc = (Bailey-Serres and Voesenek 2008)
	Alanine	Peribacteroid membrane, Hg inhibited	Zwitterion	MIP = (Day et al. 2001)
				Acc = (Kato-Noguchi and Ohashi 2006)
	Ethanol	Chara plasma-membrane, ROS inhibited	Neutral	MIP = (Henzler et al. 2004)
	Acetaldehyde	?	Neutral	Acc = (Meguro et al. 2006)
	Acetic acid	?	4.8	Acc = (Meguro et al. 2006)
	γ-aminobutyric acid (GABBA)	?	Zwitterion	Acc = (Kato-Noguchi and Ohashi 2006)
	γ-hydroxybutyric acid	?	4.7	Acc = (Bailey-Serres and Voesenek 2008)
	Succinic acid	?	4.2, 5.6	Acc = (Bailey-Serres and Voesenek 2008)
	Malic acid	Once thought to permeate via NOD26	3.4, 5.1	MIP = (Ouyang et al. 1991)
				Acc = (Bailey-Serres and Voesenek 2008)
Lipid peroxidation	Ethane	?	Neutral	Acc = (Santosa et al. 2007)
Antioxidants	Dehydroascorbate	High affinity transporter identified	Neutral	Acc = (Blokhina et al. 2000; Garnczarska 2005)
				Tran = (Horemans et al. 2000)
	Ascorbic acid	? Transporter identified	4.1	Acc = (Blokhina et al. 2000; Garnczarska 2005)
				Tran = (Horemans et al. 2000)

For acids it is more likely the neutral form will permeate and this depends on the pKa (s). References are given for the association to MIPs (MIP=) or other transporters (Tran=), and for the accumulation of the molecules (Acc=), where relevant

of ATP per glucose molecule from 2 to 5 molecules of ATP (Huang et al. 2008). Increased PPi will maintain proton pumping across the tonoplast via the H$^+$-PPiase. Another interesting adaptation is the potential use of nitrite as an electron acceptor. Mitochondria isolated from *O. sativa* and *Hordeum vulgare*, under anaerobic conditions, can use nitrite as an electron acceptor to oxidize cytosolic NADH/NADPH and generate ATP (Stoimenova et al. 2007). Nitric oxide (NO) is an end product of this process and may be scavenged by a non-symbiotic haemoglobin and ascorbate (Igamberdiev and Hill 2009).

To conserve energy, expression and translation of many genes are suppressed with the exception of particular genes required for acclimation. Some of the genes that are upregulated in the early stages of hypoxia/anoxia include some TIPs and NIPs. Interestingly, two of the TIPs from *A. thaliana* (AtTIP1;2 and AtTIP4;1) are also implicated in urea transport across the tonoplast and are upregulated in roots under nitrogen deficiency (Liu et al. 2003), while AtNIP2;1 transports lactic acid and is located on the plasma-membrane (Choi and Roberts 2007).

The energy crisis means that electrochemical proton gradients established across the plasma-membrane and tonoplast, generated by H$^+$-ATPases, may decline. This is subject to the extent of H$^+$-ATPase deactivation and the leakage of protons down the gradients into the cytoplasm. The latter can be via direct leakage of protons, thought to be minor (but see below), or co-transport reactions which drive the much needed active influx of reduced carbon (Felle 2005). Hypoxia and anoxia depolarize the plasma-membrane voltage (Buwalda et al. 1988; Zhang and Tyerman 1997), as would be expected if the H$^+$-ATPase is inhibited (Greenway and Gibbs 2003). The voltage gradient, which is large across the plasma-membrane, can be sustained at a somewhat less hyperpolarised level for a short period without the H$^+$-pump. This occurs by slight K$^+$ leakage out of the cell via K$^+$-outward rectifier channels, which tend to maintain a membrane voltage equal to the potassium diffusion potential, the so called K$^+$-state. Proton pumping across the tonoplast may be sustained by more reliance on the H$^+$-PPiase (Greenway and Gibbs 2003). Under hypoxia, both efflux of K$^+$ (Buwalda et al. 1988) and decline in net K$^+$ flux has been observed in seminal roots of *T. aestivum* seedlings (Kuiper et al. 1994) and the mature zone of *H. vulgare* roots of a waterlogging sensitive variety, but not in a tolerant variety (Pang et al. 2006). The mature zone response has been attributed, in part, to increased K$^+$ efflux via K$^+$-outward rectifier channels (Pang et al. 2006). The activation of K$^+$-outward rectifier channels was not associated with altered cell membrane hydraulic conductivity in algae (Schutz and Tyerman 1997) but remains to be tested directly in roots. There is, however, an association of mRNA levels of PIP aquaporins and K$^+$ transporters in *O. sativa* roots (Liu et al. 2006).

The extent of activation of anion efflux transport, for which NO$_3$$^-$ and Cl$^-$ can have high intrinsic efflux rates from roots (Britto et al. 2004; Segonzac et al. 2007) may have the effect of strong membrane voltage depolarisation (going more positive). If both K$^+$ and anions are effluxed by the combined action of both K$^+$ channels and anion channels there will be a loss of osmotica that will decrease turgor pressure. At high concentrations of nutrients, where low affinity influx mechanisms dominate, there can be energy wasting recycling via efflux from root

cells (Britto and Kronzucker 2006) and this would seem to be particularly problematical under anoxia. Acid load of *A. thaliana* roots increased unidirectional NO_3^- efflux, leading to NO_3^- loss (Segonzac et al. 2007). The role of NO_3^- is particularly interesting because of its stimulatory effect on root water transport (Gloser et al. 2007), via aquaporins (Gorska et al. 2008a, b), and because of its ameliorative effects during anoxia (Libourel et al. 2006). In the study by Gorska et al. (2008a, b), anoxia treatment was used as an inhibitor of aquaporins to test the effect of nitrate on root hydraulic conductivity and to indicate a role for aquaporins in NO_3^- stimulation. Nitrate in the cytosol increased aquaporin activity, but not via increases in mRNA expression of PIP1 and PIP2. Given the ameliorative effect of NO_3^- and NO_2^- on cytosolic pH, and the proposal that stimulation of water flow by NO_3^- is to advect the ion to the roots (Gloser et al. 2007), one would predict that there may be an advantage of NO_3^- to counteract the inhibitory affect of anoxia on PIP aquaporins.

Both K^+ and Cl^- have been observed to leak from anoxia treated *T. aestivum* roots, more-so in the expansion zone (Greenway et al. 1992) where there is also concentration of water uptake (Bramley et al. 2009). It is interesting to note that when substances that block aquaporins, (which are also toxic to metabolism e.g. Hg) are applied to roots there is invariably a reduction in turgor pressure, particularly when the inhibition of water transport is severe. This reduction is not caused by a reduced reflection coefficient, which can be a consequence of large reduction in hydraulic conductivity (Schutz and Tyerman 1997), but rather a leak of K^+ and anions (Bramley and Tyerman unpublished). The link with aquaporin inhibition is interesting and it is worthwhile exploring why aquaporin inhibition, resulting in reduced cellular hydraulic conductivity, is linked to any treatment that appears to compromise the energy status of the cell.

One mechanism of post-translational regulation of aquaporin activity is reversible phosphorylation. Phosphorylation of plant MIPs can increase water permeability (Maurel et al. 1995; Johansson et al. 1998; Guenther et al. 2003). In *Spinacia oleracea* leaves, SoPIP2;1 is dephosphorylated under drought stress and therefore inactivated (Johansson et al. 1996). Dephosphorylation occurs at two highly conserved serine residues, Ser115 in cytosolic loop B and, Ser274, in the C terminus, by a Ca^{2+} dependent protein kinase (Johansson et al. 1996; Johansson et al. 1998). It is not known whether dephosphorylation of PIPs and TIPS during hypoxia and anoxia is involved in reduced water permeability of root membranes (but see 8.3. above).

8.5.2.3   Decrease in Cytosolic pH

The cytosol becomes more acidic under anoxia, depending upon species and tissue and pre-exposure to hypoxia (Vartapetian and Jackson 1997). Decreases in pH from around 7.4–7.5 to 6.5–7.3 have been recorded (Felle 2005). There has been some considerable effort to explain the origin of the excess protons. Lactate fermentation acidifies the cytoplasm and lactic acid must be effluxed from the cell, alternatively

there can be a switch to ethanolic fermentation through suppression of LDH and enhancement of PDC. Ethanol also must exit the cell. There are mixed results regarding the association between the ability to switch to ethanolic fermentation and anoxia tolerance (Vartapetian and Jackson 1997; Felle 2005). Some plants show a short initial production of lactic acid, while others show more sustained production in addition to ethanol production (Vartapetian and Jackson 1997). Nucleotide triphosphate hydrolysis has also been associated with cytoplasmic acidification (Gout et al. 2001). An alternative view of the acidification of the cytosol is that it represents a new set point for regulation (Greenway and Gibbs 2003; Felle 2005). Several organic acids besides lactic acid may accumulate as end products under anaerobic conditions and these are listed in Table 8.1. The change in cytosolic pH under anoxia links directly to the molecular basis of decreased plasma-membrane water permeability via pH gating of PIP aquaporins.

Plasma-membrane water permeability is strongly inhibited by reduced cytosolic pH and one clear example is plasma-membrane vesicles from *Beta vulgaris* storage roots, which have very high water permeabilities ($>500~\mu m~s^{-1}$). In this case, a one-hundred-fold inhibition was observed when the cytosolic face of the plasma-membrane was made acidic. The pH for half-maximum inhibition was pH 6.6 and only the cytosolic face of the membrane showed sensitivity (Alleva et al. 2006). Measurements with yeast expressing PIP aquaporin gave half-inhibition at pH 7.1 (Verdoucq et al. 2008) and plasma-membrane vesicles from *A. thaliana* roots had a half-inhibition of pH 7.2–7.5 (Gerbeau et al. 2002). Plasma-membrane vesicles from *T. aestivum* seedling roots had maximum water permeability at pH 8.5 and a minimum at pH 6.5, so that minor changes in pH near seven affected water permeability of the plasma-membrane (Vandeleur et al. 2005). The tonoplast water permeability also shows strong sensitivity to cytosolic pH (Sutka et al. 2005). These examples demonstrate that this effect is within physiological cytosolic pH and within the range of changes in cytosolic pH observed under anoxia.

Cytosolic and apoplastic pH are major factors in control of K^+ and anion/Cl^- channels at the plasma-membrane of guard cells (Amtmann and Blatt 2009) and some evidence is available also for K^+ transport and K^+-channels in root cells (Hartje et al. 2000; Babourina et al. 2001). It may not be so surprising, therefore, that PIP aquaporins are also regulated by pH. However, unlike the situation with K^+ and Cl^--channels, where there is a link between proton gradients and the energization of transport of these ions, the link between proton gradients and water transport is less obvious.

When aquaporins are expressed in Xenopus oocytes or yeast cells, cytoplasmic acidification with a weak acid can be used to examine the sensitivity of PIPs and TIPs to acidic pH (Fischer and Kaldenhoff 2008). *A. thaliana* AtTIP1;1 was insensitive while AtPIP2;1, AtPIP2;2, AtPIP2;3 were inhibited (Tournaire-Roux et al. 2003). PIP1 isoforms generally do not show water permeability in Xenopus unless co-expressed with PIP2 ioforms (Fetter et al. 2004; Vandeleur et al. 2009). Only *A. thaliana* AtPIP1;2 displayed a significant water transport activity in oocytes, which was blocked by acidifying the cytoplasm (Tournaire-Roux et al. 2003). With respect to AtPIP2;3, this isoform is located in the stele of *A. thaliana* roots (Wang and

Tyerman unpublished) and may be important in water transfer to the xylem vessels. As the stele is likely to become anoxic sooner under hypoxic conditions it would be interesting to examine this PIP's pH sensitivity in further detail.

All PIP1 and PIP2 aquaporins have conserved histidine residues and His 197 (Fig. 8.2) appears to be a major determinant of pH sensitivity for AtPIP2;2 (Tournaire-Roux et al. 2003). The decrease in cytoplasmic pH may therefore, explain the sudden reduction in hydraulic conductivity when roots were subjected to anoxic stress (Tournaire-Roux et al. 2003). A number of residues in loop D of *S. oleracea* SoPIP2;1 have been identified as being involved in gating of the channel (Törnroth-Horsefield et al. 2006). Structural studies show that loop D forms a hydrophobic gate over the pore and phosphorylation of serines in loop B and at the C terminus can open the gate (Törnroth-Horsefield et al. 2006). The situation may be more complicated than this or PIPs from different species may have different gating mechanisms, since combined effects of the serines and histidine on loop D were not supported in a study on tobacco NtPIP2;1 and NtAQP1 (Fischer and Kaldenhoff 2008). They also only observed about 50% inhibition of these PIPs under cytoplasmic acidification of living yeast cells. Complexity was also indicated in another recent study showing that serine mutation to glutamic acid (S115E and S274E) could not replicate opening the gate in SoPIP2;1 (Nyblom et al. 2009).

The question arises as to why PIPs should respond in this way under anoxic conditions, or indeed, any condition that leads to cytosolic acidification. It has been proposed that the inhibition is to divert flows from the cell-to-cell pathway to the apoplast pathway (Zhang and Tyerman 1991), which was extended to a hypothesis for diversion of flow from one part of the root system to another (Vandeleur et al. 2005), analogous to hydraulic redistribution in a root system subject to regions of dry soil. Toxic compounds accumulate in anoxic soils (Bailey-Serres and Voesenek 2008), thus if some roots were exposed to anoxic conditions it may be advantageous to divert water flow to roots in more favourable zones in the soil. One problem with this hypothesis is that all PIP aquaporins have the conserved His residues on loop D, even those that may not be present in roots and cytosolic acidification seems to inhibit water permeation even in leaves and storage organs.

One hypothesis that has not been examined, to our knowledge, is that aquaporins may disrupt the membrane pH gradient under some circumstances. Water transport must be facilitated without dissipation of proton gradients, thus protons, hydronium ions and hydroxyl ions must be excluded from the pore of aquaporins. This is accomplished by the NPA residues on loops B and E (Fig. 8.2) forming a central constriction, and the ar/R constriction in the exterior half of the pore. Mutations that remove positive charge in the ar/R constriction in AQP1 allow proton permeation (Beitz et al. 2006). Molecular dynamic simulations have shown that different exclusion mechanisms may exist for protons, hydronium ions and hydroxyl ions in the pore. Furthermore, there are differences between the periplasmic and luminal mechanisms, specifically for the hydroxyl ion (Jensen et al. 2005). There is also the possibility that the central (fifth) pore of the tetramer may be an ion conducting pathway and it is interesting that Loop D has been implicated in gating this pathway in AQP 1 (Yu et al. 2006). There is evidence for ammonia permeation through this

pathway in a TIP (Bertl and Kaldenhoff 2007). It should be noted that it is probably not energetically feasible for water flow through an aquaporin to move a proton against its normal electrochemical gradient, even when the cytosol becomes acidified. Some thousands of water molecules moving down typical gradients (0.1 MPa) would be required to move a proton in the opposite direction. A discovery that links pH gradients and aquaporin function at reduced cytosolic pH would be highly significant in plant biology.

8.5.2.4 Increase in Cytosolic Free Ca^{2+}

Cytosolic free Ca^{2+} increases rapidly after the onset of anoxia and induces increased mRNA of alcohol dehydrogenase (ADH) and sucrose synthase (Subbaiah et al. 1994a, b). Ruthenium red, an inhibitor of Ca^{2+} pumps and channels, inhibited anoxia induced Ca^{2+} influx into maize roots, but external Ca^{2+} was not required for induction of ADH activity. The increase in Ca^{2+} also occurred independently of extracellular Ca^{2+} (Subbaiah et al. 1994a, b). Aequorin expressed in *A. thaliana* seedlings revealed biphasic increases in Ca^{2+} in leaves that could not be detected in roots, but upon return to air transients were observed in roots (Sedbrook et al. 1996). Calcium-sensitive dye imaging of cultured maize cells subject to anoxia showed that the increase in Ca^{2+} can be heterogeneous within a cell (sometimes around the periphery) and between cells, but was strongly associated with mitochondria (Subbaiah et al. 1998).

Ca^{2+} effects on aquaporins appear to be quite complex. Based on structural studies, divalent binding anchors loop D to the N-terminus keeping the gate in the closed state (Nyblom et al. 2009). For plasma-membrane vesicles obtained from *B. vulgaris* storage root, Ca^{2+} showed a biphasic effect on water permeability wherein a steep effect indicating co-operativity was observed at very low concentrations (half-inhibition: 4.5 nM) and a second less intense inhibitory effect was observed at higher concentrations (half-inhibition: 200 μM) (Alleva et al. 2006). Only the high concentration effect has been observed for vesicles isolated from *A. thaliana* roots (half-inhibition: 50–100 μM) (Gerbeau et al. 2002). It was proposed that Ca^{2+} could modulate both opening and closing of the channel based on the biphasic effect observed in *B. vulgaris* (Alleva et al. 2006). Calcium may also regulate PIP aquaporins by phosphorylation via a Ca^{2+}-dependent protein kinase, as has been observed for a *Tulipa gesnerina* plasma-membrane aquaporin (Azad et al. 2004) and the extensively studied SoPIP2;1 (Johansson et al. 1998). It is likely that anoxia effects on cytosolic Ca^{2+} will also affect the function of aquaporins in addition to the effect of acidification.

8.5.2.5 Increase in ROS

ROS production may occur under hypoxia, anoxia and re-aeration (Blokhina et al. 2003). H_2O_2 accumulates in the roots of *H. vulgare* and *T. aestivum* under hypoxia (Kalashnikov et al. 1994) and re-aeration in *T. aestivum* roots (Biemelt et al. 2000).

Re-aeration after anoxia can elevate ROS because of the highly reduced state of the cytoplasm, low energy charge, depleted antioxidants and membrane damage. (Blokhina et al. 2003). The iron-catalysed Fenton reaction will produce highly reactive and damaging OH^- from H_2O_2. Lipid peroxidation products (ethane) have been detected for submerged *O. sativa* seedlings with a transient peak after de-submergence (Santosa et al. 2007). Intermittent anoxia and re-aeration in *T. aestivum* caused oxidative stress in the expanded zone of the root, but there was no evidence of terminal membrane damage (Goggin and Colmer 2005). *A. thaliana* seedlings, when exposed to hypoxia, use a Rop (RHO-like small G protein) signal transduction pathway that activates NADPH oxidase, resulting in increased H_2O_2 production. A RopGAP4 (GTPase that inactivates Rop) provides negative feedback resulting in rheostatic control of H_2O_2 and induction of ADH (Baxter-Burrell et al. 2002). Both H_2O_2 and antioxidants may need to be transported to different compartments under oxidative stress, and some high affinity transporters have been identified for ani-toxidants, ascorbate/dehydroascorbate (Horemans et al. 1998) and glutathione (Raichaudhuri et al. 2009). Compared to the ascorbate anion (pKa = 4.17), dehy-droascorbate is more membrane permeant since it is not charged. This is another candidate for permeation via aquaporins, though a high affinity DHA/ascorbate exchanger has been characterised on the plasma-membrane (Horemans et al. 2000).

ROS have been shown to be potent and reversible inhibitors of water transport across the plasma-membrane of algal cells, root cell and root segments (Henzler et al. 2004; Ye and Steudle 2006; Boursiac et al. 2008). This effect was originally suggested to be a direct blockade by hydroxyl radicals, but it has been subsequently shown that H_2O_2 induces a signalling pathway that internalises PIPs to intracellular membranes (Boursiac et al. 2008). Salicylic acid also inhibits water transport in *A. thaliana* roots and this is partially mediated by H_2O_2 signalling (Boursiac et al. 2008).

Besides affecting the density of aquaporins in the plasma-membrane, H_2O_2 also permeates through some aquaporins (Henzler and Steudle 2000; Bienert et al. 2007). Using a yeast screen for sensitivity to H_2O_2, several aquaporins were identified that could facilitate H_2O_2 transport and from plants these were TIP1;1 and TIP1; 2 (Bienert et al. 2007). Representatives of *A. thaliana* aquaporins were tested in yeast for permeation of H_2O_2 (AtPIP1;1, AtPIP2;1, AtPIP2;4, AtTIP2;3, AtNIP1;1, AtNIP1;2) (Dynowski et al. 2008). AtPIP2;4 and AtPIP2;1 showed the most permeation. AtPIP2;4 is relatively highly expressed in roots and shows relatively poor water permeations compared to other PIP2s (Wang and Tyerman unpublished). It is also interesting that AtNIP1;1 can transport glycerol, but appeared not to transport H_2O_2 with the yeast assay (Dynowski et al. 2008).

8.5.3 Other Changes Under Oxygen Deficiency that Could Affect Water Transport

Apart from the factors described above, there are other changes to membranes and in the cell that could affect water permeation under oxygen deficient conditions.

The cell becomes highly reduced under anoxia. Other plant transporters and channels are sensitive to redox potential (Scholz-Starke et al. 2005); for example, potassium channels in root hairs (Grabov and Bottger 1994), Ca^{2+} channels in epidermal cells (Demidchik et al. 2007) the slow vacuolar (SV) channel from *Daucus carota* (Scholz-Starke et al. 2004), and the sucrose transporter SUT1 from *Solanum tuberosum* (Krugel et al. 2008). Redox agents that react with SH groups affect water transport across plasma-membrane vesicles isolated from *Pisum sativum* root and shoot. Roots appeared to have more SH groups on the plasma-membrane and were more sensitive to oxidation (4-fold increase in osmotic water permeability), but the reducing agent Dithiothreitol only resulted in a 40% decrease in osmotic water permeability (Ampilogova et al. 2006). Lipid structure may also interact with aquaporin gating, since gating of voltage sensing K^+ channels is affected by the surrounding lipid (Swartz 2008). Under anoxia, the acute shortage of energy causes changes in lipid composition, with a decrease in desaturation of newly synthesised lipids and decrease in membrane fluidity (Rawyler et al. 2002).

Cell walls may also change in composition under anoxia and hypoxia that will affect extensibility and volumetric elasticity. Extensibility determines the expansion rate for growth while volumetric elasticity will affect the turgor/volume changes for changes in water potential. The volumetric elastic modulus may change under hypoxia (Bramley unpublished), and this could influence the rate of change of turgor and volume to water potential changes in the plant and soil medium. Changes in volumetric elastic modulus and hydraulic conductivity will equally affect the half-time for water potential equilibration. If both are reduced, this will considerably increase the half-time. Stress strain characteristics of *Z. mays* coleoptiles show classic viscoelastic hysteresis loops (Hohl and Schopfer 1995) that can affect the water relations in other systems (Tyerman 1982), and pretreatment of segments with anoxia and H_2O_2 modify the hysteresis loop consistent with wall stiffening (Hohl and Schopfer 1995). ROS production has been observed in the apoplast of the apical region of the elongation zone of water-stressed *Z. mays* roots and was proposed to enhance wall loosening for growth at reduced turgor (Zhu et al. 2007).

8.5.4 Transport of Other Molecules Besides Water Through MIPs Relevant to Flooding

MIPs have been shown to transport, or have been implicated in the transport, of specific neutral molecules relevant to mineral nutrition (Holm et al. 2005; Ma et al. 2006; Tanaka et al. 2008), toxic substances (Bienert et al. 2008), and gas exchange (Flexas et al. 2006). However, only one of them (AtNIP2;1) has been linked to flooding as a facilitator of lactic acid efflux from root cells (Choi and Roberts 2007). Lactic acid is an interesting example because it has a relatively low pKa (3.1). If this

acid can permeate a NIP from cells with a cytosolic pH > 6.5ish, as the neutral lactic acid, there are numerous other candidate acids that may be transported by other MIPs, and which may prevent toxic levels of the acids building up in the cytosol and assist in maintaining cytosolic pH. Table 8.1 lists some acids and amino acids that may accumulate under flooding and may be worth examining as MIP substrates. Note that some preliminary evidence exists for permeation of alanine via a MIP (Day et al. 2001). In addition, gases such as CO_2, NO and O_2 may be facilitated by MIPs, that may reduce diffusion gradients both in and out of root cells. H_2O_2 has already been shown to be transported by some MIPs, but a role in flooding has not been examined. Transport of ethanol may be enhanced by MIPs (Henzler et al. 2004). If water transporting MIPs are also involved in the transport of other molecules, such as O_2, it would seem counterintuitive for conditions of reduced O_2 to inhibit aquaporins in one way or another, as is indicated from the information above. Similarly the pH dependence of some aquaporins that may transport molecules from the cytoplasm needs to be investigated, e.g. TIP1;2 and TIP4;1.

8.6 Signalling

We stated previously that decreased root water transport is the cause of leaf wilting during waterlogging. However, the accuracy of this statement requires comment. Often there is no clear correlation between wilting, stomatal closure and changes in L_r. For example, L_r did not change in two waterlogged conifer species, but stomatal conductance and transpiration decreased (Reece and Riha 1991). Contrary to this, flooding influenced L_r in *Populus trichocarpa* and xylem water potential was more negative, but stomatal closure was not observed (Harrington 1987). *Lupinus* species wilt and close their stomata so that the shoot rehydrates (Dracup et al. 1998), but oxygen deficiency does not influence L_r, at least in the short-term (Bramley et al. 2010). In flooded *Ricinus communis* plants, gas exchange, leaf wilting and L_r also did not correlate temporally (Else et al. 2001). In waterlogged *L. esculentum,* the relationship between L_r and gas exchange depended on whether the plants were flooded starting in the evening or during the day (Bradford and Hsiao 1982). However, stomatal closure was accompanied by a decline in L_r (due to occlusion of xylem vessels) of *Pyrus* species (Andersen et al. 1984).

There appear, therefore, complex regulation of gas exchange, leaf water potential and L_r, which is species dependent. Hormonal imbalance is suspected to be involved, but as yet the signalling molecule remains elusive (Jackson 2002). Some of the molecules described above, could also be involved, and facilitated by aquaporins and/or aquaporins may be involved in the signalling pathway. Parallels may be drawn with isohydric and anisohydric behaviour of plants experiencing water deficit. Isohydric species tend to close stomata to maintain constant leaf water potential, in comparison with anisohydric species where leaf water potential becomes more negative (Tardieu and Simonneau 1998). An element of iso/anisohydry

appears to be the regulation of L_r (Vandeleur et al. 2009). The same mechanisms may thus, also occur during waterlogging. There appears to be a signal from leaves to roots that allows matching of the transpirational demand to water supplied by the roots (Vandeleur et al. 2009). Thus, if anoxia tends to close stomata, this may feedback also to further reduce L_r. Hydraulic signalling has also been proposed for coordination of hydraulic conductance in root systems in response to nitrate in different regions (Subbaiah et al. 1994a, b).

8.7   Conclusion and Future Perspectives

The pathways for water flow through roots are dynamic, so hydraulic conductance is variable. During waterlogging, oxygen deficiency has spatial and temporal effects on the pathways that are not mutually exclusive. These effects influence root hydraulic conductance controlling the rate of water flow to the shoot. The spatial effects have two components; (1) depth of root submergence, i.e. which parts of the root system experience oxygen deficiency. In some species, aquaporins may facilitate increased hydraulic conductance of oxygen-sufficient roots or root regions to compensate for decreased hydraulic conductance of oxygen deficient regions. (2) changes in morphology and anatomy of root systems. Primary roots that die may be replaced by adventitious roots, but changes in anatomy to increase internal aeration may act as barriers to radial water flow. The temporal effects depend on the concentration of oxygen in the root and rhizosphere, length of exposure to oxygen deficiency and the prevailing energy crisis.

Prior to root death or changes in anatomy, decreased root hydraulic conductance may be accounted for by inhibition of aquaporin activity. PIPs are regulated by cytoplasmic pH, free Ca^{2+}, phosphorylation and H_2O_2, all of which are pertinent to cell anoxia/hypoxia. It is likely that anoxia effects on cytosolic Ca^{2+} will also affect the function of aquaporins in addition to the effect of acidification. During re-aeration the increase in ROS may inhibit aquaporins at the plasma-membrane, so recovery from anoxia may also impose a water stress. In addition, expression of aquaporins may by reduced by the energy crisis. On the other hand, at the plasma-membrane and in membranes surrounding intracellular compartments, aquaporins may be involved in the flux of gases (O_2, CO_2 NO), end products of anaerobic respiration (ethanol, lactic acid and other organic acids), or of molecules important in signalling (H_2O_2, NO, ethylene). But the role of these aquaporins during water-logging needs further investigation.

There is now a wealth of information regarding root hydraulics and aquaporins that has greatly advanced our understanding of the potential mechanisms influencing root water flow. More integrated research is required to understand these mechanisms during waterlogging and oxygen deficiency.

References

Aguilar EA, Turner DW et al (2003) Oxygen distribution and movement, respiration and nutrient loading in banana roots (*Musa* spp. L.) subjected to aerated and oxygen-depleted environments. Plant Soil 253(1):91–102

Albrecht G, Mustroph A (2003) Localization of sucrose synthase in wheat roots: increased in situ activity of sucrose synthase correlates with cell wall thickening by cellulose deposition under hypoxia. Planta 217:252–260

Alleva K, Niemietz CM et al (2006) Plasma membrane of *Beta vulgaris* storage root shows high water channel activity regulated by cytoplasmic pH and a dual range of calcium concentrations. J Exp Bot 57(3):609–621

Ampilogova Y, Zhestkova I et al (2006) Redox modulation of osmotic water permeability in plasma membranes isolated from roots and shoots of pea seedlings. Russ J Plant Physiol 53(5):622–628

Amtmann A, Blatt MR (2009) Regulation of macronutrient transport. New Phytol 181(1):35–52

Andersen P, Lombard P et al (1984) Effect of root anaerobiosis on the water relations of several *Pyrus* species. Physiol Plant 62(2):245–252

Armstrong J, Armstrong W (2005) Rice: sulfide-induced barriers to root radial oxygen loss, Fe^{2+} and water uptake, and lateral root emergence. Ann Bot 96(4):625–638

Armstrong W, Beckett PM et al (1991) Modelling and other aspects of root aeration. In: Jackson MB, Davies DD, Lambers H (eds) Plant Life Under Oxygen Stress. SPB Academic, The Hague, The Netherlands, pp 267–282

Atwell BJ (1991) Factors which affect the growth of grain legumes on a solonized brown soil. I. Genotypic responses to soil physical factors. Aust J Agric Res 42(1):95–105

Azad AK, Sawa Y et al (2004) Phosphorylation of plasma membrane aquaporin regulates temperature-dependent opening of tulip petals. Plant Cell Physiol 45(5):608–617

Azaizeh H, Gunsé B et al (1992) Effects of NaCl and $CaCl_2$ on water transport across root cells of maize (*Zea mays* L.) seedlings. Plant Physiol 99:886–894

Azaizeh H, Steudle E (1991) Effects of salinity on water transport of excised maize (*Zea mays* L.) roots. Plant Physiol 97:1136–1145

Babourina O, Hawkins B et al (2001) K^+ transport by *Arabidopsis* root hairs at low pH. Funct Plant Biol 28(7):637–643

Bailey-Serres J, Voesenek LACJ (2008) Flooding stress: acclimations and genetic diversity. Annu Rev Plant Biol 59(1):313–339

Barlow PW (1994) The origin, diversity and biology of shoot-borne roots. In: Davis TD, Haissig BE (eds) Biology of adventitious root formation, vol 62. Plenum, New York

Baxter-Burrell A, Yang Z et al (2002) RopGAP4-Dependent Rop GTPase rheostat control of *Arabidopsis* oxygen deprivation tolerance. Science 296(5575):2026–2028

Beitz E, Wu B et al (2006) Point mutations in the aromatic/arginine region in aquaporin 1 allow passage of urea, glycerol, ammonia, and protons. Proc Natl Acad Sci USA 103(2):269–274

Bertl A, Kaldenhoff R (2007) Function of a separate NH_3-pore in Aquaporin TIP2;2 from wheat. FEBS lett 581(28):5413–5417

Biemelt S, Keetman U et al (2000) Expression and activity of isoenzymes of superoxide dismutase in wheat roots in response to hypoxia and anoxia. Plant Cell Environ 23(2):135–144

Bienert G, Thorsen M et al (2008) A subgroup of plant aquaporins facilitate the bi-directional diffusion of $As(OH)_3$ and $Sb(OH)_3$ across membranes. BMC Biol 6(1):26

Bienert GP, Moller ALB et al (2007) Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. J Biol Chem 282(2):1183–1192

Birner TP, Steudle E (1993) Effects of anaerobic conditions on water and solute relations, and on active transport in roots of maize (*Zea mays* L.). Planta 190:474–483

Blokhina O, Virolainen E et al (2003) Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot 91(2):179–194

Blokhina OB, Virolainen E et al (2000) Antioxidant status of anoxia-tolerant and -intolerant plant species under anoxia and reaeration. Physiol Plant 109(4):396–403

Boursiac Y, Boudet J et al (2008) Stimulus-induced down regulation of root water transport involves reactive oxygen species-activated cell signalling and plasma membrane intrinsic protein internalization. Plant J 56(2):207–218

Bradford KJ, Hsiao TC (1982) Stomatal behaviour and water relations of waterlogged tomato plants. Plant Physiol 70:1508–1513

Bramley H (2006) Water flow in the roots of three crop species: the influence of root structure, aquaporin activity and waterlogging. PhD thesis, Plant Biology, The University of Western Australia, Crawley, WA, Australia

Bramley H, Turner DW et al (2007) Water flow in the roots of crop species: The influence of root structure, aquaporin activity, and waterlogging. Adv Agron 96:133–196

Bramley H, Turner NC et al (2009) Roles of morphology, anatomy and aquaporins in determining contrasting hydraulic behavior of roots. Plant Physiol 150:1–17

Bramley H, Turner NC et al (2010) Contrasting influence of mild hypoxia on hydraulic properties of cells and roots of wheat and lupin. Funct Plant Biol (in press)

Britto D, Kronzucker H (2006) Futile cycling at the plasma membrane: a hallmark of low-affinity nutrient transport. Trends Plant Sci 11:529–534

Britto D, Ruth T et al (2004) Cellular and whole-plant chloride dynamics in barley: insights into chloride-nitrogen interactions and salinity responses. Planta 218:615–622

Buwalda F, Thomson CJ et al (1988) Hypoxia induces membrane depolarization and potassium loss from wheat roots but does not increase their permeability to sorbitol. J Exp Bot 39 (206):1169–1183

Cannell RQ, Jackson MB (1981) Alleviating aeration stresses. In: Arkin GF, Taylor HM (eds) Modifying the root environment to reduce crop stress. American Society of Agricultural Engineers, St. Joseph, Michigan, pp 141–192

Chaumont F, Barrieu F et al (2000) Plasma membrane intrinsic proteins from maize cluster in two sequence subgroups with differential aquaporin activity. Plant Physiol 122:1025–1034

Chaumont F, Barrieu F et al (2001) Aquaporins constitute a large and highly divergent protein family in maize. Plant Physiol 125(3):1206–1215

Choi W-G, Roberts DM (2007) *Arabidopsis* NIP2;1, a major intrinsic protein transporter of lactic acid induced by anoxic stress. J Biol Chem 282(33):24209–24218

Chrispeels MJ, Agre P (1994) Aquaporins: water channel proteins of plant and animal cells. Trends Biochem Sci 19:421–425

Colmer TD (2003) Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. Plant Cell Environ 26:17–36

Colmer TD, Bloom AJ (1998) A comparison of NH_4^+ and NO_3^- net fluxes along roots of rice and maize. Plant Cell Environ 21(2):240–246

Danielson J, Johanson U (2008) Unexpected complexity of the Aquaporin gene family in the moss *Physcomitrella patens*. BMC Plant Biol 8(1):45

Day DA, Poole PS et al (2001) Ammonia and amino acid transport across symbiotic membranes in nitrogen-fixing legume nodules. Cell Mol Life Sci 58(1):61–71

Demidchik V, Shabala SN et al (2007) Spatial variation in H_2O_2 response of *Arabidopsis thaliana* root epidermal Ca^{2+} flux and plasma membrane Ca^{2+} channels. Plant J 49(3):377–386

Dennis ES, Dolferus R et al (2000) Molecular strategies for improving waterlogging tolerance in plants. J Exp Bot 51(342):89–97

Dordas C (2009) Nonsymbiotic hemoglobins and stress tolerance in plants. Plant Sci 176 (4):433–440

Dracup M, Turner NC et al (1998) Responses to abiotic stress. In: Gladstones JS, Atkins CA, Hamblin J (eds) Lupins As Crop Plants: Biology, Production and Utilisation. CAB International, Oxon, pp 227–262

Drew MC (1992) Soil aeration and plant root metabolism. Soil Sci 154(4):259–268

Drew MC (1997) Oxygen deficiency and root metabolism: Injury and acclimation under hypoxia and anoxia. Annu Rev Plant Physiol Plant Mol Biol 48:223–250

Dynowski M, Schaaf G et al (2008) Plant plasma membrane water channels conduct the signalling molecule H_2O_2. Biochem J 414(1):53–61

Echevarria M, Munoz-Cabello AM et al (2007) Development of cytosolic hypoxia and HIF stabilization are facilitated by aquaporin 1 expression. J Biol Chem 282(41):30207–30215

Else MA, Coupland D et al (2001) Decreased root hydraulic conductivity reduces leaf water potential, initiates stomatal closure and slows leaf expansion in flooded plants of castor oil (*Ricinus communis*) despite diminished delivery of ABA from the roots to shoots in xylem sap. Physiol Plant 111:46–54

Enstone DE, Peterson CA (2005) Suberin lamella development in maize seedling roots grown in aerated and stagnant conditions. Plant Cell Environ 28:444–455

Etherington JR (1984) Comparative studies of plant growth and distribution in relation to water-logging: X. Differential formation of adventitious roots and their experimental excision in *Epilobium Hirsutum* and *Chamerion Angustifolium*. J Ecol 72(2):389–404

Everard JD, Drew MC (1989) Mechanisms controlling changes in water movement through the roots of *Helianthus annuus* L. during continuous exposure to oxygen deficiency. J Exp Bot 40(210):95–104

Fan M, Bai R et al (2007) Aerenchyma formed under phosphorus deficiency contributes to the reduced root hydraulic conductivity in maize roots. J Integr Biol 49(5):598–604

Felle HH (2005) pH regulation in anoxic plants. Ann Bot 96(4):519–532

Fetter K, Van Wilder V et al (2004) Interactions between plasma membrane aquaporins modulate their water channel activity. Plant Cell 16(1):215–228

Fischer M, Kaldenhoff R (2008) On the pH regulation of plant aquaporins. J Biol Chem 283 (49):33889–33892

Flexas J, Ribas-Carbo M et al (2006) Tobacco aquaporin NtAQP1 is involved in mesophyll conductance to CO_2 in vivo. Plant J 48(3):427–439

Forrest K, Bhave M (2008) The PIP and TIP aquaporins in wheat form a large and diverse family with unique gene structures and functionally important features. Funct Integr Genomics 8(2):115–133

Fouquet R, Léon C et al (2008) Identification of grapevine aquaporins and expression analysis in developing berries. Plant Cell Rep 27(9):1541–1550

Fukao T, Bailey-Serres J (2008) Ethylene – a key regulator of submergence responses in rice. Plant Sci 175(1–2):43–51

Garnczarska M (2005) Response of the ascorbate-glutathione cycle to re-aeration following hypoxia in lupine roots. Plant Physiol Biochem 43(6):583–590

Garthwaite AJ, Steudle E et al (2006) Water uptake by roots of *Hordeum marinum*: formation of a barrier to radial O_2 loss does not affect root hydraulic conductivity. J Exp Bot 57(3):655–664

Gerbeau P, Amodeo G et al (2002) The water permeability of *Arabidopsis* plasma membrane is regulated by divalent cations and pH. Plant J 30(1):71–81

Gibbs J, Greenway H (2003) Mechanisms of anoxia tolerance in plants. I. Growth, survival and anaerobic catabolism. Funct Plant Biol 30:1–47

Gibbs J, Turner DW et al (1998) Response to oxygen deficiency in primary maize roots. II. Development of oxygen deficiency in the stele has limited short-term impact on radial hydraulic conductivity. Aust J Plant Physiol 25:759–763

Gloser V, Zwieniecki MA et al (2007) Dynamic changes in root hydraulic properties in response to nitrate availability. J Exp Bot 58(10):2409–2415

Goggin DE, Colmer TD (2005) Intermittent anoxia induces oxidative stress in wheat seminal roots: assessment of the antioxidant defence system, lipid peroxidation and tissue solutes. Funct Plant Biol 32(6):495–506

Gorska A, Ye Q et al (2008a) Nitrate control of root hydraulic properties in plants: translating local information to whole plant response. Plant Physiol 148(2):1159–1167

Gorska A, Zwieniecka A et al (2008b) Nitrate induction of root hydraulic conductivity in maize is not correlated with aquaporin expression. Planta 228(6):989–998

Gout E, Boisson AM et al (2001) Origin of the cytoplasmic pH changes during anaerobic stress in higher plant cells. Carbon-13 and phosphorous-31 nuclear magnetic resonance studies. Plant Physiol 125(2):912–925

Grabov A, Bottger M (1994) Are redox reactions involved in regulation of K^+ channels in the plasma membrane of *Limnobium stoloniferum* root hairs? Plant Physiol 105(3):927–935

Greacen EL, Ponsana P et al (1976) Resistance to water flow in the roots of cereals. In: Lange OL, Kappen L, Schulze E-D (eds) Water and plant life, vol 19. Springer, Berlin, pp 86–100

Greenway H, Armstrong W et al (2006) Conditions leading to high CO_2 (>5 kPa) in waterlogged-flooded soils and possible effects on root growth and metabolism. Ann Bot 98(1):9–32

Greenway H, Gibbs J (2003) Mechanisms of anoxia tolerance in plants. II. Energy requirements for maintenance and energy distribution to essential processes. Funct Plant Biol 30:999

Greenway H, Waters I et al (1992) Effects of anoxia on uptake and loss of solutes in roots of wheat. Aust J Plant Physiol 19:233–247

Guenther JF, Chanmanivone N et al (2003) Phosphorylation of soybean nodulin 26 on serine 262 enhances water permeability and is regulated developmentally and by osmotic signals. Plant Cell 15(4):981–991

Hanba YT, Shibasaka M et al (2004) Overexpression of the barley aquaporin HvPIP2;1 increases internal CO_2 conductance and CO_2 assimillation in the leaves of transgenic rice plants. Plant Cell Physiol 45(5):521–529

Harrington CA (1987) Responses of red alder and black cottonwood seedlings to flooding. Physiol Plant 69(1):35–48

Hartje S, Zimmermann S et al (2000) Functional characterisation of LKT1, a K^+ uptake channel from tomato root hairs, and comparison with the closely related potato inwardly rectifying K^+ channel SKT1 after expression in *Xenopus* oocytes. Planta 210(5):723–731

Hedfalk K, Tornroth-Horsefield S et al (2006) Aquaporin gating. Curr Opin Struct Biol 16(4): 447–456

Henzler T, Steudle E (2000) Transport and metabolic degradation of hydrogen peroxide in *Chara corallina*: model calculations and measurements with the pressure probe suggest transport of H_2O_2 across water channels. J Exp Bot 51(353):2053–2066

Henzler T, Ye Q et al (2004) Oxidative gating of water channels (aquaporins) in *Chara* by hydroxyl radicals. Plant Cell Environ 27:1184–1195

Hohl M, Schopfer P (1995) Rheological analysis of viscoelastic cell wall changes in maize coleoptiles as affected by auxin and osmotic stress. Physiol Plant 94(3):499–505

Holm LM, Jahn TP et al (2005) NH_3 and NH_4^+ permeability in aquaporin-expressing Xenopus oocytes. Pflugers Archiv 450(6):415–428

Horemans N, Asard H et al (1998) Carrier mediated uptake of dehydroascorbate into higher plant plasma membrane vesicles shows trans-stimulation. FEBS lett 421(1):41–44

Horemans N, Foyer CH et al (2000) Transport and action of ascorbate at the plant plasma membrane. Trends Plant Sci 5(6):263–267

Huang S, Colmer TD et al (2008) Does anoxia tolerance involve altering the energy currency towards PPi? Trends Plant Sci 13(5):221–227

Igamberdiev AU, Hill RD (2009) Plant mitochondrial function during anaerobiosis. Ann Bot 103 (2):259–268

Insalud N, Bell RW et al (2006) Morphological and physiological responses of rice (*Oryza sativa*) to limited phosphorus supply in aerated and stagnant solution culture. Ann Bot 98(5):995–1004

Jackson MB (2002) Long-distance signalling from roots to shoots assessed: the flooding story. J Exp Bot 53(367):175–181

Jackson MB, Davies WJ et al (1996) Pressure-flow relationships, xylem solutes and root hydraulic conductance in flooded tomato plants. Ann Bot 77:17–24

Javot H, Maurel C (2002) The role of aquaporins in root water uptake. Ann Bot 90(3):301–313

Jensen MX, Rvthlisberger U et al (2005) Hydroxide and proton migration in aquaporins. Biophys J 89(3):1744–1759

Johanson U, Gustavsson S (2002) A new subfamily of major intrinsic proteins in plants. Mol Biol Evol 19(4):456–461

Johanson U, Karlsson M et al (2001) The complete set of genes encoding major intrinsic proteins in Arabidopsis provides a framework for a new nomenclature for major intrinsic proteins in plants. Plant Physiol 126(4):1358–1369

Johansson I, Karlsson M et al (1998) Water transport activity of the plasma membrane aquaporin PM28A is regulated by phosphorylation. Plant Cell 10(3):451–459

Johansson I, Larsson C et al (1996) The major integral proteins of spinach leaf plasma membranes are putative aquaporins and are phosphorylated in response to Ca^{2+} and apoplastic water potential. Plant Cell 8:1181–1191

Kalashnikov Y, Balakhnina T et al (1994) The effect of soil hypoxia on oxygen activation and the enzyme-system protecting barley roots and leaves against oxygen destruction. Russ J Plant Physiol 41:512–516

Kamaluddin M, Zwiazek JJ (2002) Ethylene enhances water transport in hypoxic aspen. Plant Physiol 128:962–969

Kato-Noguchi H (2000) Abscisic acid and hypoxic induction of anoxia tolerance in roots of lettuce seedlings. J Exp Bot 51(352):1939–1944

Kato-Noguchi H, Ohashi C (2006) Effects of anoxia on amino acid levels in rice coleoptiles. Plant Prod Sci 9(4):383–387

Kennedy RA, Rumpho ME et al (1992) Anaerobic metabolism in plants. Plant Physiol 100(1):1–6

Kirch H-H, Vera-Estrella R et al (2000) Expression of water channel proteins in Mesembryanthemum crystallinum. Plant Physiol 123(1):111–124

Koncalová H (1990) Anatomical adaptations to waterlogging in roots of wetland graminoids: limitations and drawbacks. Aquat Bot 38(1):127–134

Kovar JL, Kuchenbuch RO (1994) Commercial importance of adventitious rooting to agronomy. In: Davis TD, Haissig BE (eds) Biology of aventitious root formation, vol 62. Plenum, New York

Kozela C, Regan S (2003) How plants make tubes. Trends Plant Sci 8(4):159–164

Kramer PJ (1933) The intake of water through dead root systems and its relation to the problem of absorption by transpiring plants. Am J Bot 20(7):481–492

Kramer PJ (1949) Plant and soil water relationships. McGraw-Hill, New York

Kramer PJ (1983) Water relations of plants. Academic, New York

Krugel U, Veenhoff LM et al (2008) Transport and sorting of the Solanum tuberosum sucrose transporter SUT1 is affected by posttranslational modification. Plant Cell 20(9):2497–2513

Kuiper P, Walton C et al (1994) Effect of hypoxia on ion uptake by nodal and seminal wheat roots. Plant Physiol Biochem 32:267–276

Libourel IGL, van Bodegom PM et al (2006) Nitrite reduces cytoplasmic acidosis under anoxia. Plant Physiol 142(4):1710–1717

Liu H-Y, Sun W-N et al (2006) Co-regulation of water channels and potassium channels in rice. Physiol Plant 128(1):58–69

Liu L-H, Ludewig U et al (2003) Urea transport by nitrogen-regulated tonoplast intrinsic proteins in Arabidopsis. Plant Physiol 133(3):1220–1228

Luu DT, Maurel C (2005) Aquaporins in a challenging environment: molecular gears for adjusting plant water status. Plant Cell Environ 28(1):85–96

Ma JF, Tamai K et al (2006) A silicon transporter in rice. Nature 440(7084):688–691

Martínez-Ballesta M, Cabañero F et al (2008) Two different effects of calcium on aquaporins in salinity-stressed pepper plants. Planta 228(1):15–25

Martinez-Ballesta MC, Aparicio F et al (2003) Influence of saline stress on root hydraulic conductance and PIP expression in Arabidopsis. J Plant Physiol 160(6):689–697

Maurel C, Kado RT et al (1995) Phosphorylation regulates the water channel activity of the seed-specific aquaporin alpha-TIP. Embo J 14(13):3028–3035

Maurel C, Reizer J et al (1993) The vacuolar membrane-protein gamma-TIP creates water specific channels in *Xenopus* oocytes. Embo J 12(6):2241–2247

Maurel C, Tacnet F et al (1997) Purified vesicles of tobacco cell vacuolar and plasma membranes exhibit dramatically different water permeability and water channel activity. Proc Natl Acad Sci USA 94(13):7103–7108

Maurel C, Verdoucq L et al (2008) Plant aquaporins: membrane channels with multiple integrated functions. Annu Rev Plant Biol 59(1):595–624

Meguro N, Tsuji H et al (2006) Analysis of expression of genes for mitochondrial aldehyde dehydrogenase in maize during submergence and following re-aeration. Breed Sci 56(4): 365–370

Miyamoto N, Steudle E et al (2001) Hydraulic conductivity of rice roots. J Exp Bot 52(362):1835–1846

Mizutani M, Watanabe S et al (2006) Aquaporin NIP2;1 is mainly localized to the ER membrane and shows root-specific accumulation in *Arabidopsis thaliana*. Plant Cell Physiol 47 (10):1420–1426

Moog PR, Janiesch P (1990) Root growth and morphology of *Carex* species as influenced by oxygen deficiency. Funct Ecol 4:201–208

Moshelion M, Becker D et al (2002) Plasma membrane aquaporins in the motor cells of *Samanea saman*: Diurnal and circadian regulation. Plant Cell 14(3):727–739

Newman EI (1976) Water movement through root systems. Phil Trans R Soc B 273:463–478

Niemietz CM, Tyerman SD (1997) Characterisation of water channels in wheat root membrane vesicles. Plant Physiol 115:561–567

Nobel PS, Schulte PJ et al (1990) Water influx characteristics and hydraulic conductivity for roots of *Agave deserti* Engelm. J Exp Bot 41(225):409–415

Nyblom M, Frick A et al (2009) Structural and functional analysis of SoPIP2;1 mutants adds insight into plant aquaporin gating. J Mol Biol 387(3):653–668

Ouyang L-J, Whelan J et al (1991) Protein phosphorylation stimulates the rate of malate uptake across the peribacteroid membrane of soybean nodules. FEBS Lett 293(1–2):188–190

Pang JY, Newman I et al (2006) Microelectrode ion and O_2 fluxes measurements reveal differential sensitivity of barley root tissues to hypoxia. Plant Cell Environ 29(6):1107–1121

Parent B, Hachez C et al (2009) Drought and abscisic acid effects on aquaporin content translate into changes in hydraulic conductivity and leaf growth rate: a trans-scale approach. Plant Physiol 149(4):2000–2012

Raichaudhuri A, Peng M et al (2009) Plant vacuolar ATP-binding cassette transporters that translocate folates and antifolates *in vitro* and contribute to antifolate tolerance *in vivo*. J Biol Chem 284(13):8449–8460

Ranathunge K, Kotula L et al (2004) Water permeability and reflection coefficient of the outer part of young rice roots are differently affected by closure of water channels (aquaporins) or blockage of apoplastic pores. J Exp Bot 55(396):433–447

Ranathunge K, Steudle E et al (2003) Control of water uptake by rice (*Oryza sativa* L.): role of the outer part of the root. Planta 217:193–205

Ranathunge K, Steudle E et al (2005) A new precipitation technique provides evidence for the permeability of Casparian bands to ions in young roots of corn (*Zea mays* L.) and rice (*Oryza sativa* L.). Plant Cell Environ 28(11):1450–1462

Rawyler A, Arpagaus S et al (2002) Impact of oxygen stress and energy availability on membrane stability of plant cells. Ann Bot 90(4):499–507

Reece CF, Riha SJ (1991) Role of root systems of eastern larch and white spruce in response to flooding. Plant Cell Environ 14(2):229–234

Sade N, Vinocur BJ et al (2009) Improving plant stress tolerance and yield production: is the tonoplast aquaporin SlTIP2;2 a key to isohydric to anisohydric conversion? New Phytol 181(3):651–661

Sakurai J, Ishikawa F et al (2005) Identification of 33 rice aquaporin genes and analysis of their expression and function. Plant Cell Physiol 46(9):1568–1577

Santosa I, Ram P et al (2007) Patterns of peroxidative ethane emission from submerged rice seedlings indicate that damage from reactive oxygen species takes place during submergence and is not necessarily a post-anoxic phenomenon. Planta 226(1):193–202

Scholz-Starke J, Angeli AD et al (2004) Redox-dependent modulation of the carrot SV channel by cytosolic pH. FEBS lett 576(3):449–454

Scholz-Starke J, Gambale F et al (2005) Modulation of plant ion channels by oxidizing and reducing agents. Arch Biochem Biophys 434(1):43–50

Schutz K, Tyerman SD (1997) Water channels in *Chara corallina*. J Exp Bot 48:1511–1518

Sedbrook JC, Kronebusch PJ et al (1996) Transgenic AEQUORIN reveals organ-specific cytosolic Ca^{2+} responses to anoxia in *Arabidopsis thaliana* seedlings. Plant Physiol 111(1):243–257

Segonzac C, Boyer J et al (2007) Nitrate efflux at the root plasma membrane: Identification of an *Arabidopsis* excretion transporter. Plant Cell 19:3760–3777

Shelden MC, Howitt SM et al (2009) Identification and functional characterisation of aquaporins in the grapevine, *Vitis vinifera*. Funct Plant Biol 36:1–14

Smit B, Stachowiak M (1988) Effects of hypoxia and elevated carbon dioxide concentration on water flux through *Populus* roots. Tree Physiol 4(2):153–165

Solaiman Z, Colmer TD et al (2007) Growth responses of cool-season grain legumes to transient waterlogging. Aust J Agric Res 58(5):406–412

Soukup A, Armstrong W et al (2007) Apoplastic barriers to radial oxygen loss and solute penetration: a chemical and functional comparison of the exodermis of two wetland species, *Phragmites australis* and *Glyceria maxima*. New Phytol 173(2):264–278

Steudle E, Peterson CA (1998) How does water get through roots? J Exp Bot 49(322):775–788

Stoimenova M, Igamberdiev A et al (2007) Nitrite-driven anaerobic ATP synthesis in barley and rice root mitochondria. Planta 226(2):465–474

Subbaiah CC, Bush DS et al (1994a) Elevation of cytosolic calcium precedes anoxic gene expression in maize suspension-cultured cells. Plant Cell 6(12):1747–1762

Subbaiah CC, Bush DS et al (1998) Mitochondrial contribution to the anoxic Ca^{2+} signal in maize suspension-cultured cells. Plant Physiol 118(3):759–771

Subbaiah CC, Zhang J et al (1994b) Involvement of intracellular calcium in anaerobic gene expression and survival of maize seedlings. Plant Physiol 105(1):369–376

Sutka M, Alleva K et al (2005) Tonoplast vesicles of *Beta vulgaris* storage root show functional aquaporins regulated by protons. Biol Cell 97(11):837–846

Swartz KJ (2008) Sensing voltage across lipid membranes. Nature 456(7224):891–897

Tanaka M, Wallace IS et al (2008) NIP6;1 is a boric acid channel for preferential transport of boron to growing shoot tissues in *Arabidopsis*. Plant Cell 20(10):2860–2875

Tardieu F, Simonneau T (1998) Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. J Exp Bot 49(suppl 1):419–432

Törnroth-Horsefield S, Wang Y et al (2006) Structural mechanism of plant aquaporin gating. Nature 439(7077):688–694

Tournaire-Roux C, Sutka M et al (2003) Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. Nature 425:393–397

Tyerman SD (1982) Water relations of seagrasses – stationary volumetric elastic-modulus and osmotic-pressure of the leaf-cells of halophila-ovalis, zostera-capricorni, and posidonia-australis. Plant Physiol 69(4):957–965

Tyerman SD, Oats P et al (1989) Turgor-volume regulation and cellular water relations in *Nicotiana tabacum* roots grown in high salinities. Aust J Plant Physiol 16:517–531

Tyree MT, Zimmermann MH (2002) Xylem structure and the ascent of sap. Springer, New York

Uehlein N, Lovisolo C et al (2003) The tobacco aquaporin NtAQP1 is a membrane CO_2 pore with physiological functions. Nature 425:734–737

Uehlein N, Otto B et al (2008) Function of *Nicotiana tabacum* aquaporins as chloroplast gas pores challenges the concept of membrane CO_2 permeability. Plant Cell 20:648–657

van der Weele CM, Canny MJ et al (1996) Water in aerenchyma spaces in roots. A fast diffusion path for solutes. Plant Soil 184(1):131–141

Vandeleur R, Niemietz C et al (2005) Roles of aquaporins in root responses to irrigation. Plant Soil 274(1–2):141–161

Vandeleur RK, Mayo G et al (2009) The role of plasma membrane intrinsic protein aquaporins in water transport through roots: Diurnal and drought stress responses reveal different strategies between isohydric and anisohydric cultivars of grapevine. Plant Physiol 149(1):445–460

Vartapetian BB (2006) Plant anaerobic stress as a novel trend in ecological physiology, biochemistry, and molecular biology: 2. Further development of the problem. Russ J Plant Physiol 53(6):711–738

Vartapetian BB, Jackson MB (1997) Plant adaptations to anaerobic stress. Annals of Botany 79 (suppl 1):3–20

Verdoucq L, Grondin A et al (2008) Structure-function analysis of plant aquaporin AtPIP2;1 gating by divalent cations and protons. Biochem J 415(3):409–416

Vysotskaya LB, Arkhipova TN et al (2004) Effect of partial root excision on transpiration, root hydraulic conductance and leaf growth in wheat seedlings. Plant Physiol Biochem 42:251–255

Wallace IS, Choi WG et al (2006) The structure, function and regulation of the nodulin 26-like intrinsic protein family of plant aquaglyceroporins. Biochim Biophys Acta 1758(8):1165–1175

Wallace IS, Roberts DM (2004) Homology modeling of representative subfamilies of Arabidopsis major intrinsic proteins. Classification based on the aromatic/arginine selectivity filter. Plant Physiol 135(2):1059–1068

Wallace IS, Roberts DM (2005) Distinct transport selectivity of two structural subclasses of the nodulin-like intrinsic protein family of plant aquaglyceroporin channels. Biochemistry 44(51):16826–16834

Waters I, Morrell S et al (1991) Effects of anoxia on wheat seedlings. II. Influence of O_2 supply prior to anoxia on tolerance to anoxia, alcoholic fermentation, and sugar levels. J Exp Bot 42(244):1437–1447

Whiteman SA, Nuhse TS et al (2008) A proteomic and phosphoproteomic analysis of Oryza sativa plasma membrane and vacuolar membrane. Plant J 56(1):146–156

Xia J-H, Roberts JKM (1996) Regulation of H^+ extrusion and cytoplasmic pH in maize root tips acclimated to a low-oxygen environment. Plant Physiol 111:227–233

Ye Q, Muhr J et al (2005) A cohesion/tension model for the gating of aquaporins allows estimation of water channel pore volumes in Chara. Plant Cell Environ 28(4):525–535

Ye Q, Steudle E (2006) Oxidative gating of water channels (aquaporins) in corn roots. Plant Cell Environ 29(4):459–470

Yu J, Yool AJ et al (2006) Mechanism of gating and ion conductivity of a possible tetrameric pore in Aquaporin-1. Structure 14:1411–1423

Zhang W-H, Tyerman SD (1991) Effect of low O_2 concentration and azide on hydraulic conductivity and osmotic volume of the cortical cells of wheat roots. Aust J Plant Physiol 18:603–13

Zhang W-H, Tyerman SD (1997) Effect of low oxygen concentration on the electrical properties of cortical cells of wheat roots. J Plant Physiol 150:567–572

Zhang W-H, Tyerman SD (1999) Inhibition of water channels by $HgCl_2$ in intact wheat root cells. Plant Physiol 120:849–857

Zhang YX, Roberts DM (1995) Expression of soybean Nodulin-26 in transgenic tobacco – targeting to the vacuolar membrane and effects on floral and seed development. Mol Biol Cell 6:109–117

Zhu J, Alvarez S et al (2007) Cell wall proteome in the maize primary root elongation zone. II. Region-specific changes in water soluble and lightly ionically bound proteins under water deficit. Plant Physiol 145(4):1533–1548