The Role of Aquaporins in Root Water Uptake

HÉLÈNE JAVOT and CHRISTOPHE MAUREL*

Biochimie et Physiologie Moléculaire des Plantes, Agro-M/INRA/ CNRS/UM2 UMR5004, 2 place Viala, F-34060 Montpellier Cedex, France

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The capacity of roots to take up water is determined in part by the resistance of living tissues to radial water flow. Both the apoplastic and cell-to-cell paths mediate water transport in these tissues but the contribution of cell membranes to the latter path has long been difficult to estimate. Aquaporins are water channel proteins that are expressed in various membrane compartments of plant cells, including the plasma and vacuolar membranes. Plant aquaporins are encoded by a large multigene family, with 35 members in Arabidopsis thaliana, and many of these aquaporins show a cell-specific expression pattern in the root. Mercury acts as an efficient blocker of most aquaporins and has been used to demonstrate the significant contribution of water channels to overall root water transport. Aquaporin-rich membranes may be needed to facilitate intense water flow across root tissues and may represent critical points where an efficient and spatially restricted control of water uptake can be exerted. Roots, in particular, show a remarkable capacity to alter their water permeability over the short term (i.e. in a few hours to less than 2–3 d) in response to many stimuli, such as day/night cycles, nutrient deficiency or stress. Recent data suggest that these rapid changes can be mostly accounted for by changes in cell membrane permeability and are mediated by aquaporins. Although the processes that allow perception of environmental changes by root cells and subsequent aquaporin regulation are nearly unknown, the study of root aquaporins provides an interesting model to understand the regulation of water transport in plants and sheds light on the basic mechanisms of water uptake by roots.

Key words: Hydraulic conductivity, membrane, MIP, stress, water relation, water channel.

INTRODUCTION

Roots usually provide the input of water in higher plants and, hence, play an essential role in their water balance. While most of the resistance to water flow through plants is due to the stomatal aperture, the root system can still represent a significant barrier (Steudle, 1994; Steudle and Peterson, 1998) and can contribute up to approx. 50 % of the overall hydraulic resistance of the plant (Marré et al., 2001a, and references therein). Thus, roots establish a critical link in the soil–plant–air continuum and this link has to be maintained in the most adverse environmental or physiological conditions (Jackson et al., 2000; Steudle, 2001). For instance, under a high transpiration rate some terrestrial plants can lose to the atmosphere the equivalent of their water content in a few hours and under such conditions water uptake should not be limiting. Also, root morphology has to adapt to specific physical and nutritional constraints encountered in different types of soil (Jackson et al., 2000). In all these conditions, the water transport properties of the root have to be adjusted to the physiological demand of the whole plant.

In recent years, there has been a renewed interest in the processes that underlie the regulation of root water transport in response to environmental factors. Upon long-term exposure to stresses (>3 d), roots can respond with marked anatomical and growth alterations which induce profound changes in their transport capacity. Before these changes occur, rapid and significant (>30 %) increases or decreases in root water permeability (hydraulic conductivity, Lp) can be observed. Water channel proteins named aquaporins, which are found in the membranes of root cells, are thought to mediate these adjustments. Their study provides an interesting model to understand the regulation of water transport in plants and sheds light on the basic mechanisms of water uptake by roots.

BASIC PRINCIPLES OF ROOT WATER UPTAKE

Anatomic and hydraulic properties of roots

To pass from the soil solution into root vascular tissues, water has to flow radially across a series of concentric cell layers (Fig. 1). These layers include the epidermis, an exodermis in roots where it is differentiated, several layers of cortex cells, the endodermis, pericycle, xylem parenchyma cells and finally the vessels (Steudle and Peterson, 1998). As in other organs, three pathways co-exist for water transport across living root tissues: through the cell walls (apoplastic path) or from cell to cell, along the symplasm through plasmodesmata (symplastic path) or across membranes (transcellular path) (Fig. 2). The cell walls of exodermal and endodermal cells possess a particular structure, the Casparian band, which consists of a deposit of suberin and/ or lignin. It has been shown that in the exodermis, this structure represents an effective impediment to water flow (Zimmermann and Steudle, 1998). In the endodermis, it is...
generally accepted that the Casparian band creates a tight apoplastic barrier to solutes and prevents their backflow from the stele (Tester and Leigh, 2001), but its significance for water transport has been more disputed (Steudle and Peterson, 1998). The endodermal Casparian band is most commonly viewed as a hermetic barrier to hydraulic flow. Suberization of the endodermis is more pronounced in trees than in herbaceous species, and accordingly roots of the former are less permeable to water and solutes (Steudle and Heydt, 1997). The barrier formed by the Casparian band suggests that to reach the stele, water has to enter the symplasm and cross the membranes of endodermal cells or other cell types that sit external to the endodermis. However, this mechanism may not always operate. For instance, injury of the endodermis of young maize (Zea mays) roots by insertion of a glass tube did not result in any increase in root $L_{wp}$, suggesting that the endodermis was not a major impediment to water flow in these roots (Steudle and Peterson, 1998). Also, at the tip of growing roots, neither the endodermis nor the exodermis are differentiated and upon the emergence of secondary roots the continuity of these cells layers is disrupted (Steudle, 1994). Thus, cell division and differentiation during root growth can account for differences in the hydraulic resistance of the radial path along the root growth axis. A typical example of this can be found in a study by French et al. (1996) who dissected the osmotic and hydrostatic water transport properties of developing maize roots, considering 50-mm segments that were taken along the root axis. When the mean distance of the segment from the root tip was varied from 25 to 100 mm, osmotic $L_{wp}$ and solute permeability decreased by a factor of five and ten, respectively, indicating that during development the root tissues become less permeable for cell-to-cell water and solute movement.

Once it has reached the vascular tissues, water moves axially towards the aerial parts. Xylem is made up of long cells (tracheids or vessels) that have lost their protoplasts. Mature vessels and tracheids have thick lignified walls but there are differentiated un lignified areas called pits that facilitate water loading from the surrounding cells. The hydraulic resistance of xylem vessels can be estimated using Poiseuille’s law. This law indicates that when compared with other structures, differentiated xylem vessels oppose a
negligible resistance to water flow. For instance, a cylindrical vessel with a diameter of 23 μm would require a length of 24 km to exhibit a resistance comparable with that of a single cell membrane (Steudle and Peterson, 1998). This shows how the removal of vascular cell membranes can dramatically decrease root axial hydraulic resistance and allows water to move easily under bulk flow. Because xylem resistance drops with the 4th power of vessel radius, differences in xylem morphology along the root growth axis can also account for striking differences in resistance: in the root tip, xylem has not differentiated, resulting in an area with a high axial resistance (Steudle, 1993), whereas in older parts of the root, the successive differentiation of xylem into protoxylem and early and late metaxylem, with increasing diameters, dramatically reduces the resistance to water flow (Steudle, 1994; Steudle and Peterson, 1998).

In summary, water transfer across living tissues, i.e. essentially along the radial path, offers the greatest resistance for water uptake by the root (Peterson and Steudle, 1993). More specifically, it was estimated that 70–90% of the radial resistance to water flow is due to cell types external to the vessels, whereas the remaining resistance is due to water transfer across the vessel walls (pits) (Steudle and Peterson, 1998). Following the differentiation of cells and tissues along the root growth axis, profound changes in the hydraulic properties of both the axial and the radial path can be observed.

Driving forces

Biophysical analyses have established that in plants, as in any other organism, water moves passively in response to water potential gradients (Steudle, 1994; Schultz, 2001). When a plant transpires, water evaporation in the stomatal chamber results in highly negative pressures (i.e. tensions up to −1 MPa) in the xylem vessels, which draw water from the roots up into the aerial parts (Steudle, 2001). In the absence of transpiration, i.e. when stomata are closed during the night or in water-stress conditions, residual water movement is driven by the active pumping of solutes in the root. This creates an osmotic driving force that results in a positive, hydrostatic root pressure which, in turn, pushes the sap in the xylem.

The existence of parallel apoplastic and cell-to-cell water movements is the basis of the composite water transport model proposed by Steudle and his co-workers [reviewed in Steudle and Peterson (1998) and Steudle (2001)]. Because of its lack of selectivity with respect to water and solute transport, the apoplast cannot maintain osmotic driving forces (Steudle, 1994) and water transport in this compartment is essentially driven by hydrostatic forces (i.e. pressures or tensions). In contrast, cell membranes allow the establishment and maintenance of osmotic gradients along the cell-to-cell path, and these gradients in addition to hydrostatic forces will drive water flow (Steudle, 1994; Steudle and Peterson, 1998). Because plant cells have relatively short hydraulic equilibration times (in the range of a few to some ten seconds) (Steudle, 1992), it is considered that cell and apoplastic water potentials will be locally equilibrated nearly all the time. Nevertheless, depending on the predominant forces and the respective resistance of the apoplastic or the cell-to-cell paths, the respective contribution of the two pathways to water transport might change. For instance, Kamaluddin and Zwiazek (2001) studied apoplastic water transport in roots of red-osier dogwood (Cornus stolonifera) using 3-hydroxy-5,8,10-pyrenetrisulfonate (PTS) as an apoplastic tracer. Roots that were metabolically poisoned by addition of sodium azide (NaN₃), a potent blocker of cell respiration, showed a subsequent decrease in $L_p$. It was also found that the concentration of PTS in the exuded sap was increased 11-fold. This was interpreted to mean that the contribution of the apoplastic path to water transport had increased by the same factor, possibly because the cell-to-cell path had been significantly blocked after NaN₃ treatment (Kamaluddin and Zwiazek, 2001). The predominance of one path over another may also depend on the species. In maize and onion (Allium cepa) roots, water transport was mediated mostly by the apoplastic or by the cell-to-cell pathways depending on the nature of the driving force (i.e. hydrostatic or osmotic), whereas in barley (Hordeum vulgare), bean and rice the cell-to-cell path was favoured in all of the conditions examined (Steudle, 1994; Miyamoto et al., 2001).

Changes in root hydraulic properties

It appears that either a change in the nature or intensity of driving forces or changes in the intrinsic $L_p$ of root cells and tissues can be responsible for variations in the water transport properties of roots. This allows plants to adjust their water uptake capacity to ever-changing environmental conditions or during development.

A first level of adjustment follows directly from the composite model of root water transport. In plants with a high rate of transpiration, hydrostatic forces are predominant; water transport through the apoplast can be enhanced if cell walls have a low resistance and roots can then exhibit a high $L_p$. In contrast, when stomata are closed, osmotic forces are predominant, water movement occurs via the cell-to-cell pathway, and the apparent root $L_p$ might be lower (Steudle and Peterson, 1998). Thus, depending on the environmental conditions and transpiration demand, plants can modify the respective contributions of the apoplastic and cell-to-cell pathways and the overall root $L_p$ may be adjusted accordingly.

Roots also adjust their transport properties in response to a changing environment by undergoing profound metabolic and anatomical alterations. These processes are relevant mostly to long-term adaptation to stresses such as drought or soil salinity. In general, water stress induces the differentiation of a Casparian band in the exodermis, which limits the apoplastic by-pass and significantly reduces root $L_p$ (Zimmermann and Steudle, 1998).

Plant roots also have a remarkable capacity to alter their $L_p$ over the short term (i.e. <3 d) in response to many stimuli, such as day/night cycles, nutrient deficiency or stress, and before any change in root anatomy and morphology can be observed (Clarkson and Touraine, 1994; Steudle and Peterson, 1998; Clarkson et al., 2000; Steudle, 2000). The variety of stimuli and mechanisms
involved will be discussed in greater detail later in the text. Recent data suggest that these changes can be mostly accounted for by changes in cell membrane permeability. The study of aquaporins (Maurel et al., 2002) offers a new paradigm to understand these processes, and more generally, the basic mechanisms of root water uptake (Steudle and Peterson, 1998).

**EXPRESSION AND ACTIVITY OF AQUaporIN WATER CHANNELS IN ROOT CELL MEMBRANES**

*Subcellular localization of aquaporins*

Aquaporins are membrane proteins that belong to the major intrinsic protein (MIP) family, with members found in nearly all living organisms (Agre et al., 1998). Plants appear to have a particularly large number of MIP homologues. The complete genome of Arabidopsis thaliana has 35 full-length MIP genes (Johanson et al., 2001; Maurel et al., 2002). The count of MIP genes in other plant species is still incomplete but a similarly high number is expected (Kirch et al., 2000; Chaumont et al., 2001). In part because of this very high diversity, our knowledge about plant aquaporin expression and subcellular localization is far from complete. However, data collected over recent years clearly indicate that plant aquaporins fulfill expression and regulation properties that are expected from proteins specialized in root water transport.

Based on sequence homology, plant MIPs cluster in four subgroups which to some extent reflect different subcellular localizations. Members of the two major subgroups, the plasma membrane intrinsic proteins (PIPs) and the tonoplast intrinsic proteins (TIPs), have been initially localized in the plasma membrane and in the tonoplast, respectively, hence their names (Johnson et al., 1989, 1990; Daniels et al., 1994; Kammerloher et al., 1994). Soybean Nodulin26 (NOD26) is specifically expressed in the peribacteroid membrane of *N₂*-fixing symbiotic root nodules (Fortin et al., 1987) and defines another type of MIP. Homologues of NOD26, i.e. Nodulin26-like intrinsic proteins (NIPs), have been identified in plant species that do not develop any *N₂*-fixing symbiosis and their subcellular localization is still unclear (Weig et al., 1997). The small basic intrinsic proteins (SIPs) form the fourth MIP subgroup. They were recently identified in *siliquo* during the arabidopsis genomic sequencing project and their subcellular localization remains unknown (Johanson et al., 2001).

*Water transport properties of root subcellular membranes*

Following the identification of aquaporins in intracellular and plasma membranes of plant cells, there have been several attempts to measure the osmotic water permeability (*Pₜ*) of subcellular membrane compartments. [The osmotic water permeability coefficient (*Pₜ*) is equivalent to the hydraulic conductivity (*Lₜ*) and can be derived from the latter according to the following equation: *Pₜ* = *Lₜ*RT/Vₙ, where *R* is the universal gas constant, *T* is absolute temperature, and *Vₙ* is the partial volume of water.] The contribution of water channels to water transport in these membranes was probed using mercury inhibition. The effects of mercury, commonly applied as mercuric chloride (HgCl₂), were first assessed on plant aquaporins that had been functionally expressed in *Xenopus* oocytes. Mercurials were found to block most plant aquaporins even though a few turned out to be resistant to these compounds (Daniels et al., 1994; Biela et al., 1999). Mercurials act by binding to the sulfhydryl group of Cys residues located in the vicinity of the aqueous pore, thereby blocking water permeation (Agre et al., 1998).

Plasma membrane and tonoplast-enriched vesicles were purified from wheat (*Triticum aestivum*) roots using aqueous two-phase partitioning and their *Pₜ* was investigated by stopped-flow spectrophotometry (Niemietz and Tyerman, 1997). Intracellular membrane vesicles exhibited a seven-fold higher *Pₜ* than plasma membrane vesicles. The increased water permeability of the former vesicles, together with sensitivity to mercury inhibition and reduced sensitivity to temperature (activation energy), suggested the presence of active water channels. Similar observations (tonoplast vesicles with active water channels and a *Pₜ* higher than that in plasma membrane vesicles) have been reported in radish taproots (Ohshima et al., 2001), tobacco suspension cells (Maurel et al., 1997) and leaves of the CAM plant *Graptopetalum paraguayense* (Ohshima et al., 2001). The activity of water channels in the membranes of root cells has also been investigated by cell pressure probe measurements in the cortex of wheat roots (Zhang and Tyerman, 1999). Such activity was revealed by a > 75 % inhibition of cell *Lₜ* by mercurials. However, data from isolated vesicles (Niemietz and Tyerman, 1997) and intact cells (Zhang and Tyerman, 1999) of wheat roots could only be reconciled provided that (1) mercury-sensitive water channels were present in the native plasma membrane and (2) metabolic inhibition of these channels had occurred during the isolation of plasma membrane vesicles, which resulted in a low *Pₜ* and insensitivity to mercury inhibition.

The comparison of *Pₜ* values in isolated protoplasts and vacuoles provides another means by which to estimate the relative water permeability of the vacuolar and plasma membranes. This approach has recently become more reliable after *Pₜ* measurements in isolated protoplasts and vacuoles were optimized using a special transference chamber which allowed an accurate monitoring of fast osmotically induced volume changes (Morillon and Lassalles, 1999; Ramahaleo et al., 1999). While these studies indicated a constitutively high *Pₜ* in the tonoplast of all plants investigated, measurements in isolated protoplasts pointed to a lower *Pₜ* in the plasma membrane, which was lower than, or in a few cases equal to, tonoplast *Pₜ* (reviewed in Maurel et al., 2002). These results are consistent with data from isolated vesicles. Altogether they suggested that in most living plant cells, the plasma membrane must be the limiting barrier for water uptake and that water channels in this membrane must be tightly regulated.
Cell-specific expression of root aquaporins

Aquaporins have been the object of a wealth of expression studies (for a review, see Maurel et al., 2002). Numerous aquaporin isoforms have been detected in plant roots and there are intricate expression patterns for MIP homologues in plants. Whereas most aquaporins expressed in roots are also found in the aerial parts, a few aquaporin isoforms, such as the soybean NOD26 (Fortin et al., 1987), are specific for roots, suggesting that independent regulation of root cell and tissue $L_p$ might be needed under certain conditions (Fortin et al., 1987; Yamamoto et al., 1991).

Also, a high number of MIP isoforms, including close members of the same subfamily, can be expressed simultaneously in the root system. For example, the highly homologous PIP1:1 (PIP1α), PIP1:2 (PIP1β), and PIP1:3 (PIP1γ) aquaporins, together with PIP2:1 (PIP2α) and PIP2:2 (PIP2β) are all expressed in the roots of A. thaliana (Kammerloher et al., 1994).

Due to the high diversity of aquaporin isoforms in plants, no exhaustive study on cell-specific expression patterns has yet been performed for all MIP homologues in any plant root system. However, from data obtained in different plant species, it appears that expression of aquaporins has been detected in virtually all root cell types examined. Thus, aquaporin homologues have been detected in the root apical meristem and lateral primordia (Opperman et al., 1994; Jones and Mullet, 1995; Chaumont et al., 1998; Otto and Kaldenhoff, 2000), in the elongation and differentiation zones (Miao and Verma, 1993; Jones and Mullet, 1995; Kaldenhoff et al., 1995; Chaumont et al., 1998; Kirch et al., 2000; Otto and Kaldenhoff, 2000), in the epidermis (Yamada et al., 1995; Chaumont et al., 1998; Kirch et al., 2000), in root hairs (Kirch et al., 2000), in the cortex (Jones and Mullet, 1995; Sarda et al., 1999; Kirch et al., 2000), and in a variety of cell types of the vascular bundle: tracheary elements, cells around the xylem, phloem, secondary phloem, phloem-associated cells and cambium (Opperman et al., 1994; Jones and Mullet, 1995; Kaldenhoff et al., 1995; Chaumont et al., 1998; Sarda et al., 1999; Kirch et al., 2000; Otto and Kaldenhoff, 2000). It appears that some aquaporin isoforms have a very large expression spectrum; for instance, the TIP homologue of ice plant (Mesembryanthemum crystallinum), MIP F, was detected in almost all root cells (Kirch et al., 2000). In contrast, its close homologue, TIP1:1 (γ-TIP), from A. thaliana showed a preferential expression in the elongation zone (Ludevid et al., 1992), whereas expression of another TIP homologue of spinach, So8TIP, was associated with mature, highly vacuolated cells (Karlsson et al., 2000). Close aquaporin homologues can have overlapping expression patterns; for instance, the co-expression of two PIP1 isoforms (PIP A and MIP B) was detected by immunocytochemistry in the cortex cells of M. crystallinum (Kirch et al., 2000).

Functions of PIPs and TIPs in the plasma membrane and in the tonoplast

Because of their distinct subcellular localization and regulation profiles, PIP and TIP aquaporins appear as good candidates to account for the differential regulation of water transport in the tonoplast and plasma membrane. It is assumed that a relatively high resistance to water flow in the plasma membrane would allow an efficient control of cell-to-cell water transport (Chrispeels and Maurel, 1994). Thus, regulation of PIPs in the plasma membrane of root cells may play a key role in controlling radial water uptake, whereas the role of tonoplast water channels in controlling transcellular water transport may well be marginal. By setting the tonoplast $P_f$ to values higher than those in the plasma membrane, TIPs may rather determine a general role for the vacuole in buffering osmotic fluctuations in the cytoplasm (for discussion of this topic, see Maurel et al., 2002). However, expression analyses show that like PIPs, TIPs exhibit complex expression patterns under the control of developmental and environmental cues. Thus, a constitutively high water permeability in the tonoplast may not always be needed, and the regulation of water transport across this membrane in response to as yet unknown stimuli may be uncovered.

It has also been noted that many aquaporins are preferentially expressed in elongating cells; the reason for this is still under debate. It is now well established that the major determinant of cell growth is the expansion capacity of cell walls, and the early idea that a low plasma membrane $P_f$ could be limiting for water uptake in growing cells and could, in turn, restrict their expansion has been discarded by most authors (Cosgrove, 1993). However, efficient cell-to-cell water equilibration in expanding tissues may be needed to facilitate water supply from vascular tissues and to avoid growth-induced water potential gradients. It is also interesting to observe that cell types that correspond to putative control points for water uptake or to convergence points for radial water flow appear to be particularly rich in water channels (Schaffner, 1998). In particular, this remark applies to epidermal and endodermal cells and to cells surrounding the xylem vessels. Aquaporin-rich membranes may be needed to facilitate intense water flow across these cells. They may thus represent critical points where an efficient and spatially restricted control of water uptake can be exerted.

THE CONTRIBUTION OF WATER CHANNELS TO OVERALL ROOT WATER TRANSPORT AS REVEALED BY MERCURY INHIBITION

Effects of mercurials on root $L_p$

Because of its efficiency in blocking most aquaporins, HgCl$_2$ has been used extensively to evaluate the contribution of water channels to overall root water transport. Various concentrations (10$^{-6}$ to 10$^{-3}$ M) and exposure times (10 min to >1 h) were used in a wide range of plant species, and there is now a consistent set of reports showing that treatment by HgCl$_2$ can significantly inhibit the $L_p$ of plant roots (Fig. 3). For instance, mercurials reduced $L_p$ by 32 % in Opuntia acanthocarpa (Cactaceae; Martre et al., 2001b), 47 % in aspen (Populus tremuloides; Wan and Zwiazek, 1999), 57 % in tomato (Lycopersicon esculentum; Maggio and Joly, 1995) (Fig. 3), 57–84 % in onion (Barrowclough
roots with H2S, resulting in the formation of insoluble HgS, tissues may have not been optimal in the above-mentioned L external cell layers in root water uptake. In the same study, up to 85 %. This suggested a major role for the two most contrast to the former study in onion, was predominant in cortex and periderm removed. Water channel-mediated water channels. In another study (Martre et al, 2001b) accounted for by different activities of mercury-sensitive O. acanthocarpa mercury inhibition was compared in intact roots of plants, and in roots with the epidermis, which can be seen as a black deposit in cross-sections. Such porins to mercury. Also, the penetration of HgCl2 in root directly metabolic effects (Zhang and Tyerman, 1999). Vital water channels since closure might be induced through that mercurials acted on water transport without profoundly on sap osmotic potential induced in wheat roots could not be irreversibly damaged the root and, for instance, the changes in sap osmotic potential induced in wheat roots could not be reversed (Carvajal et al., 1999).

However, reversion of HgCl2 effects on root Lp is not sufficient to establish that this compound directly blocked water channels since closure might be induced through indirect metabolic effects (Zhang and Tyerman, 1999). Vital dyes were used to check cell viability upon exposure of onion roots to mercury (Barrowclough et al., 2000). Wan and Zwiazek (1999) have shown that in aspen, the rate of root respiration was kept fairly constant over the first hour following HgCl2 application. It can also be useful to prove that mercurials acted on water transport without profoundly disrupting other types of transport. In particular, a conserved osmotic potential or a conserved potassium concentration in the sap exuded by excised roots can confirm that the overall solute pumping activity of the root was not altered by mercurials (Maggio and Joly, 1995; Carvajal et al., 1996, 1999, 2000; Wan and Zwiazek, 1999; Barrowclough et al., 2000; Martre et al., 2001b), or only partially (Morillon and Lassalles, 1999; Zhang and Tyerman, 1999).

In conclusion, a consistent set of data collected by an increasing number of laboratories has established that provided control experiments are properly designed, HgCl2 treatment can provide a rough estimate of water channel contribution to root water transport. Most importantly, this approach has been widely used to reveal the occurrence of water channel regulation in plant roots in response to environmental stimuli, such as light, salt stress or nutrient deprivation (see below). As long as plant materials with genetically altered aquaporin expression are not commonly available, mercurials will remain a useful tool. However, the concentrations used and the duration of exposure should be as low as possible (Barrowclough et al., 2000).
ALTERATION OF ROOT HYDRAULIC CONDUCTIVITY ($L_p$) IN RESPONSE TO ENVIRONMENTAL STIMULI SUGGESTS A CRITICAL ROLE FOR WATER CHANNELS

Stimulus-induced changes in $L_p$

More than 25 years ago, Parsons and Kramer (1974) observed that cotton (Gossypium hirsutum) roots exhibit a diurnal cycling of their resistance to water uptake that was maintained for 2–3 d following root excision. Root $L_p$ was minimal at night, and was increased two- to three-fold at midday. Similar diurnal fluctuations have been described in many other plant species including tomato (Dell’Amico et al., 2001), wheat (Carvajal et al., 1996), paprika pepper (Carvajal et al., 1999) and L. japonicus (Henzler et al., 1999) (Fig. 4A). These fluctuations may allow coupling of root $L_p$ to stomatal functions, and thus reduce xylem tension and prevent cavitation of root vessels at high transpiration rates (Jackson et al., 2000).

The nutrient status of plants also seems to be a major determinant of root hydraulics (Clarkson et al., 2000). Roots excised from young maize and barley plants grown under nitrate or sulfate deprivation, respectively, exhibited an exudation rate and an $L_p$ reduced to 20% of values from control plants grown in replete conditions (Hoarau et al., 1986; Karmoker et al., 1991). In maize, these effects could be completely reversed in 15 h following nitrate supply. Potassium deficiency for >7 d reduced the $L_p$ of sunflower (Helianthus annuus) roots by a factor of two (Graham and Ulrich, 1972), but the short-term effects of K⁺ ions have not been investigated.

Salt exposure can also reduce root $L_p$ in a wide range of species, although some plants such as tobacco do not show such a response (Tyerman et al., 1989). In tomato roots, inhibition of $L_p$ was observed as early as 40 min after the addition of 100 mM NaCl to the nutrient solution (Peyrano et al., 1997). In excised melon roots, $L_p$ was maximally inhibited by 80% after 3 d of an NaCl treatment (Carvajal et al., 2000). These effects are specific for NaCl: in paprika pepper, an equivalent increase inionic strength, as imposed by a concentrated nutritive solution, did not alter root $L_p$ (Carvajal et al., 1999). In melon, both Na⁺ and Cl⁻ independently contributed to root $L_p$ inhibition (Martinez-Ballesta et al., 2000). In many species, Ca²⁺ can counteract $L_p$ inhibition by NaCl, provided that it is available before or at the same time as salt (Azaizeh and Steudle, 1991; Carvajal et al., 2000; Martinez-Ballesta et al., 2000). Competition between Na⁺ and Ca²⁺ for the same membrane binding sites has been proposed (Martinez-Ballesta et al., 2000), but a role of Ca²⁺ in cell signalling in response to stress should also be considered.

While exposure of roots to salt results in a combined water and ionic stress, a pure water stress, such as soil drying, can also alter the water transport capacity of roots. These changes have been mostly investigated over a long-term period and are clearly associated with profound anatomical adaptations including the suberization and lignification of cell walls. Martre et al. (2001b) recently performed a thorough analysis of water transport in the root of O. acanthocarpa and showed that drought-induced reduction in $L_p$ could be partially reversed in a few days following soil re-wetting, before root anatomy had significantly changed. Root $L_p$ and the overall rate of transpiration also seem to be connected. Henzler et al. (1999) showed that, when Lotus japonicus plants were grown under saturating humidity to reduce transpiration, their root $L_p$ was slightly enhanced. In contrast, the $L_p$ of wheat roots decreased by a factor of two under reduced transpiration (Carvajal et al., 1996). Though contradictory, these observations surely illustrate the complex links that exist between water uptake by roots and overall plant water status. Abscisic acid (ABA) is a well-recognized mediator of water stress responses. In many species including sunflower, barley, sorghum and maize (Clarkson and Touraine, 1994; Quinto et al., 1999; Hose et al., 2000), exogenous ABA enhanced root $L_p$.

Soil flooding also results in profound physiological disturbances including an increase in CO₂ content in the soil solution and a decrease in O₂ supply (anaerobiosis) (Else et al., 1995; Dell’Amico et al., 2001). Bubbling N₂ gas into the nutrient solution can experimentally induce anaerobiosis. In less than 2 h, such a treatment induced a 50% decrease in the $L_p$ of sunflower roots (Everard and Drew, 1989). Respiration inhibitors also prevent root cell oxygenation: treatment of red-osier dogwood roots with...
0.5 mM Na₃ resulted in a 35% decrease in both O₂ uptake and root Lₚ after 2 h (Kamaluddin and Zwiazek, 2001). In many species, anaerobiosis also blocked the diurnal fluctuations in root Lₚ (Parsons and Kramer, 1974; Else et al., 1995).

The examples above illustrate the capacity of plants to regulate their root Lₚ in response to an amazing variety of stimuli. Toxic concentrations of aluminium or highly acidic pH in the soil solution are yet other treatments that interfere with root Lₚ (Günsé et al., 1997), and the effects of some other stresses possibly remain to be discovered. Thus, it appears that the down-regulation of Lₚ is a common response to adverse environmental conditions. Although the reason for this is not firmly established, this process may parallel a reduced growth and water demand of aerial parts in stress conditions. The response of roots to water stress seems to be more complex than that to other stresses and may involve a transient up-regulation of water transport, possibly mediated by ABA. This increase in Lₚ may facilitate osmotic water transport and the capture of remaining soil water under conditions of reduced transpiration, with plant water uptake relying mostly on the solute pumping capacity of the root (Steudle, 2000). Over a longer term, a decrease in root Lₚ may prevent a backflow of water in the soil, in particular at night when transpiration is reduced to a minimum.

**Functional evidence for water channel regulation**

As discussed previously, cell-to-cell water transport predominates in root exudation conditions, whereas apoplastic water transport is somehow favoured in conditions of pressure-induced water flow. Inhibition of melon root Lₚ by salinity was more pronounced using the former (~91%) than the latter type of measurements (~68%) (Martinez-Ballesta et al., 2000), supporting the idea that the short-term effects of environmental stimuli on root water uptake are mostly exerted on cell membranes. Also, it was observed in some systems that the relative effects of mercurials were reduced in conditions where root Lₚ was down-regulated. In wheat roots for instance, mercury inhibition of Lₚ was greater in replete than in nutrient-deprived conditions and yielded similar residual Lₚ values (Carvajal et al., 1996). These observations were taken as a basis to suggest that water channel regulation could account for the observed nutrient-dependent fluctuations in root Lₚ (Carvajal et al., 1996) and a similar conclusion was drawn for the response of root Lₚ to salinity (Carvajal et al., 1999). In rice (Oryza sativa) seedlings, the application of mercurials had an effect on whole plant conductance only when plants were water stressed by the presence of PEG (polyethylene glycol), but not in control growth conditions (Lu and Neumann, 1999). This intriguing result was interpreted to mean that apoplastic water transport predominated in normal conditions, and that water channels had been up-regulated in water-stress conditions. This result is at variance with observations made in roots of O. acanthocarpa where the Lₚ of the root stele decreased during the course of soil drying (Martre et al., 2001b) and where down-regulation of the mercury-sensitive component (i.e. aquaporins) could account for >70% of the observed reduction in the Lₚ of this tissue.

Studies at the cell level provide more direct evidence for cell membranes and aquaporins being involved in stimulus-induced regulation of root Lₚ. In most systems investigated, root cells displayed alterations in water permeability consistent with changes seen at the root level, with an even more pronounced amplitude. For instance, cell pressure-probe analyses showed that exposure of maize roots to salinity reduced the Lₚ of their cortical cells by 65–85%, whereas root Lₚ was reduced by 30–60% only (Azaizeh and Steudle, 1991; Azaizeh et al., 1992). Treatment of wheat roots by NaN₃ or oxygen deficiency for 30 min decreased the Lₚ of cortex cells by a factor ≥3 (Zhang and Tyerman, 1991; Zhang and Tyerman, 1999). Interestingly, a similar decrease in cell Lₚ was induced by HgCl₂ in control wheat roots, whereas no effect was observed in cells of roots pretreated in low-O₂ conditions. Once again, this was interpreted to mean that water channels had already been down-regulated in the latter conditions (Zhang and Tyerman, 1999). ABA is a stimulus which, in contrast to stresses, is known to enhance the Lₚ of root cells. In maize, ABA induced a spectacular, time- and dose-dependent stimulation of cell water permeability (Hose et al., 2000). The Lₚ of root cortex cells was maximally stimulated ≥30-fold after a 1 h exposure to 1 μM ABA, but these effects disappeared over the next hour. In the same studies it was also noted that in contrast to ABA, auxin (IAA) and cytokinin (kinetin) reduced the cell Lₚ by three- to four-fold (Hose et al., 2000). In contrast to other stimuli acting on root water transport, the cellular bases for diurnal fluctuation in root Lₚ are not yet established. In L. japonicus, the Lₚ of cells in the outer root cortex was stable over a day/night cycle suggesting that diurnal control of membrane permeability might be exerted in inner cell layers of the root, i.e. in the endodermis and stellar tissue (Henzler et al., 1999).

**Stimulus-induced changes in aquaporin expression**

Light-dependent expression of aquaporin genes and, more specifically, diurnal fluctuations of specific aquaporin transcripts have been reported in the roots of several plant species, including radish (Higuchi et al., 1998), arabidopsis (Harmer et al., 2000) and Nicotiana excelsior (Yamada et al., 1997). The most convincing parallel between root Lₚ fluctuations and variations in aquaporin expression has clearly been established in L. japonicus (Henzler et al., 1999) (Fig. 4). Northern blot analyses of root mRNA using an arabidopsis PIP1:2 cDNA probe, which probably cross-hybridized with several PIP1 homologues in L. japonicus, revealed that the overall level of PIP1 transcript was maximal 4–8 h after dawn and was minimal just after the beginning of the dark period (Fig. 4B).

Salt exposure is another treatment that induces complex changes in overall gene expression. A thorough analysis of this phenomenon has recently been achieved using microarrays in the roots of two rice cultivars differing in their sensitivity to salt (Kawasaki et al., 2001). Two aquaporin-encoding transcripts were found to be down-regulated during the first 15 and 60 min of a salt treatment,
Molecular and cellular mechanisms of root aquaporin regulation

There is evidence that environmental stimuli can be sensed by the root itself and in turn alter root \( L_p \). For instance, the \( P_t \) of protoplasts isolated from melon roots was reduced upon direct exposure to salt, consistent with effects observed on whole roots (Martinez-Ballesta et al., 2000). Split-root experiments suggested that the perception by roots of the incoming flow of nutrient rather than the nutrient status of the overall plant determines the adjustment of root \( L_p \) (Clarkson et al., 2000).

The molecular and cellular mechanisms that link hormonal, nutrient, or stress stimuli to the activity of aquaporins in root cell membranes remain poorly understood. Kinetic studies indicate that salt stress- or nutrient-induced changes in \( L_p \) can occur in less than 1 h (Carvajal et al., 1996, 1999). Transcriptional regulation of aquaporin genes can operate within this or an even shorter time scale (Kawasaki et al., 2001) but may be combined to other regulatory mechanisms. Phosphorylation of aquaporins, for instance, has been described in several types of plant membranes including bean or lentil seed storage vacuoles, spinach leaf plasma membranes or soybean root peribacteroid membranes (reviewed in Johansson et al., 2000). This process provides a mechanism for gating plant aquaporin water channels. For instance, okadaic acid, a potent inhibitor of phosphoprotein phosphatase 1 and 2A, promoted the water transport activity of spinach PM28a in Xenopus oocytes (Johansson et al., 1998). Interestingly, the same molecule counteracted the inhibition of \( L_p \) by salt stress in melon protoplasts (Martinez-Ballesta et al., 2000). In arabidopsis, salt stress signalling is mediated through a phosphorylation cascade involving the SOS2/SOS3 calcium-dependent protein kinase (Zhu, 2000) but it is not yet established whether plasma membrane aquaporins are directly regulated by phosphorylation in salinity conditions.

Calcium signalling is a common path in the response of plants to stresses or hormones and cell-specific fluctuations in cytosolic Ca\(^{2+}\) occur in the epidermis, endodermis and pericycle of arabidopsis roots in response to drought and salt (Kiegle et al., 2000). When exogenously supplied to roots, Ca\(^{2+}\) enhanced the stimulation of \( L_p \) by ABA (Quintero et al., 1999), whereas it counteracted the inhibitory effects of a saline treatment (Azaizeh et al., 1992; Carvajal et al., 2000). However, a link between these observations and cell signalling and/or calcium-dependent water channel gating remains to be established. Recent work in our laboratory has revealed that water channels in the plasma membrane of arabidopsis suspension cells are regulated by divalent cations, including Ca\(^{2+}\), and pH (Gerbeau et al., 2002). Water channel activity in purified membrane vesicles was reduced upon exposure to calcium (half inhibition at 75 \( \mu \)M free Ca\(^{2+}\)) or acidic pH (half inhibition at pH 7.3). Cytosolic acidification occurs in response to salt stress and anaerobiosis (Katsuhara et al., 1989; Kurkdjian and Guern, 1989) and blockade of root aquaporins by protons could provide a common mechanism for down-regulating \( L_p \) in these conditions.
four-fold increase in root mass was observed, the significance of which is not yet clear (Kaldenhoff et al., 1998). It has been proposed that the phenotypic trait of antisense arabidopsis plants reflects a compensation of reduced root $L_p$ by an increase in root surface area (Kaldenhoff et al., 1998). Differential growth of roots and shoots is a typical response of plants to water stress and an increased root/shoot ratio in antisense plants might reflect an alteration of the overall water status in these plants.

From these observations, it can be anticipated that plants with single aquaporin knockout mutations should allow an even more accurate dissection of aquaporin function in the root. However, the high diversity of aquaporins clearly poses problems for this approach. Because all genes cannot be considered at once, reverse genetic studies will have to focus on those aquaporins that are supposed to play the most important role in the root. Also, the possible functional redundancy of close aquaporin homologues may limit the detection of a phenotype and accurate biophysical measurements will certainly be required to reveal the function of a single aquaporin gene. Recent results obtained in our laboratory on an arabidopsis PIP2 knockout mutant (H. Javot et al., unpubl. res.) suggest that these problems may not be insurmountable; a reduced cell $L_p$ was observed in the root cortex of mutant plants. The water transport properties of roots excised from these plants are currently under investigation.

A variety of functions for aquaporins in plant roots

While there is now circumstantial evidence that aquaporins play an important role in regulating water uptake by the root, these proteins surely fulfill other functions in this organ. An overall role for aquaporins in cell osmoregulation has been proposed (reviewed in Maurel et al., 2002) and the interaction of root cells with living symbiotes or parasites creates specific constraints in these respects. In the root nodules of legumes for instance, the symbiosome membrane surrounds intracellular $N_2$-fixing bacteroids. The aquaglyceroporin NOD26 sits in this membrane and may participate in the osmoregulation of the peribacteroid space (Dean et al., 1999). Expression of TobRB7, a tonoplast aquaporin of N. tabacum roots is induced in giant cells that differentiate as a result of infection by root knot nematodes (Opperman et al., 1994). These cells are used as a feeding site by the parasite and TobRB7 may allow the cells to maintain their water status. Enhanced expression of aquaporins homologous to TobRB7 has also been detected in parsley and Medicago truncatula roots harbouring arbuscular mycorrhizeae (Roussel et al., 1997; Krajinski et al., 2000). Arbuscule cells show highly fragmented vacuoles and specific osmoregulatory functions might be needed (Krajinski et al., 2000).

More than simple channels, aquaporins are multifunctional channels that can exhibit a wide range of transport selectivity profiles (reviewed in Tyerman and Niemietz, 2002). In roots, they may play particular roles in the transfer of mineral nutrients and metabolites. For instance, a role for NOD26 in NH$_3$ transport across the symbiosome membrane has been proposed on the basis of measurements in isolated membrane vesicles (Niemietz and Tyerman, 2000). A similar approach showed that the permeation of boric acid across squash (Cucurbita pepo) root membranes is mediated by mercury-sensitive channels. A role for aquaporins was further suggested by oocyte expression experiments using a PIP1 homologue of maize (Dordas et al., 2000). The bacterial aquaglyceroporin GlpF is permeable to antimonite (Sanders et al., 1997) and aquaporins may well contribute to metalloid transport in plant roots.

In conclusion, roots play a crucial role in the water relations of plants and, as a consequence, show very sophisticated hydraulic properties. The predominant function for aquaporins in plant roots seems to be in water uptake, and the study of aquaporins now provides a solid molecular basis to physiological and biophysical investigations on root water transport. In particular, the discovery of aquaporins and the subsequent use of mercurials have led to the discovery that cell membranes significantly contribute to overall root water permeability although differences among plant species may exist. More profound insights are expected from plants with genetically altered aquaporin expression. It is now assumed that aquaporin-rich membranes are necessary to facilitate intense water flow across roots, and the cell-specific expression patterns of root aquaporins suggest the presence of critical points where an efficient and spatially restricted control of water uptake can be exerted. However, due to the high diversity of aquaporin isoforms in plants, a comprehensive knowledge of cell-specific expression, localization and function of root aquaporins is still lacking.

Most importantly, a link has been established between rapid changes in root $L_p$ and the expression and function of aquaporins. Thus, it appears that the regulation of root aquaporins can provide a very tight coupling between root water uptake and whole plant physiology. While facilitated water uptake is necessary in optimal growth conditions, it may also be critical for the plant to have a tighter root system in stress conditions, to reduce water uptake or losses towards the soil. The molecular and cellular processes that allow perception of environmental changes by roots and subsequent aquaporin regulation are nearly unknown and are a clear objective for future research. Nevertheless, root water uptake has already emerged as one of the most relevant models to address the function of aquaporins in plant water relations.

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LITERATURE CITED


Kammerloher W, Fischer U, Piechotta GP, Schäffer AR. 1994. Water channels in the plant plasma membrane cloned by...


Steudle E. 2001. The cohesion-tension mechanism and the acquisition of


