Plant Aquaporins and Cell Elongation

Wieland Fricke and Thorsten Knipfer

Abstract There exists an increasing number of reports which show that the gene transcript, and in some cases also protein level, of particular aquaporin (AQP) isoforms is higher in growing than in nongrowing plant tissues. This suggests that AQPs play a role in the process of cell expansion. The most likely role of AQPs is that of facilitating water inflow into cells as they expand to a multiple of their original volume. The question is whether this is the major role which AQPs play in expanding cells and whether expanding cells actually need AQPs given the rate at which they expand and the hydraulic conductivity (Lp) of their membranes. These questions are addressed in this chapter by using a combination of molecular (AQP), biophysical (Lp, driving forces and water potential difference), anatomical (apoplastic barriers) and physiological (cell dimensions and relative growth rates) data for growing plant tissues and cells. The focus of analyses is on growing root and leaf tissues and on plasma membrane intrinsic (PIPs) and tonoplast intrinsic proteins (TIPs). It is concluded that a high expression of AQPs and a high Lp in growing plant cells are required more for facilitating water transport at significant (and high) rates *through* cells and tissues rather than for facilitating water transport *into* cells to sustain the (comparatively smaller) water uptake rates required for the volume expansion of these cells.

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F. Chaumont, S.D. Tyerman (eds.), *Plant Aquaporins*, Signaling and Communication in Plants, DOI 10.1007/978-3-319-49395-4_5

Abbreviations

AQP	Aquaporin
Lp	Hydraulic conductivity
NIP	Nodule-26 like intrinsic protein
PIP	Plasma membrane intrinsic protein
SIP	Small basic intrinsic protein.
TIP	Tonoplast intrinsic protein
$\Delta\Psi$	Water potential difference
Ψ	Water potential

1 Introduction

1.1 Plants and Animals: The Little Difference

Terrestrial higher plants are sessile organisms. In contrast to most animals, which can move physically and often keep the next generation in close proximity, plants reflect the opposite evolutionary strategy – if there exists anything like an evolutionary 'strategy' – in that they literally form roots and try to spread through the next generation (seeds). Being able (animals) or not being able (plants) to move as vegetative organism has many implications for the design of such an organism. An almost endless list of such implications and differences in design between sessile and mobile multicellular organisms could be listed here, yet possibly the 'single'-most basic differences which impact organ growth and development are the absence (animals) and presence (plants) of a cell wall and a large central vacuole in cells.

Not being able to move as a vegetative organism from A to B means that self-sufficiency in nutrition and temporal and spatial variation in nutrient supply has to be optimised; this also applies to the access of water. It also implies that some cells and tissues of the organism must be exposed directly, and in a highly conductive manner, to an environment in which the water availability and osmotic strength can change quickly. Furthermore, dealing with waste becomes a major issue as waste cannot simply be discharged next to the organism as this would lead in the longer-term to the build-up of toxic concentration of waste products. The solution to these challenges in plants is that all mature living cells are surrounded by a wall and contain a large central vacuole. The wall prevents cells from bursting when cells, such as root surface cells, are exposed to a hypo-osmotic environment. The large central vacuole enables plants to optimise various processes: (i) excess nutrients can be stored transiently; (ii) waste products or toxic compounds can be stored indefinite (for as long as the cell is alive); (iii) water is stored; and (iv), maybe of most relevance to the process of cell expansion, the bulk of protoplast volume is occupied by a low-protein, resource-efficient aqueous compartment – the large central vacuole

or, at very early stages of cell development, the sum of many smaller vacuoles ('vacuon'). The presence of a significant vacuolar compartment renders cell expansion 'cheap' in terms of protein and nitrogen use, and as nitrogen often limits plant production in a natural environment, it potentially provides an evolutionary advantage. To complete this comparison of evolutionary strategies between plants and animals, the latter substitute the lack of a cell wall through a tight control of the osmolarity of interstitial and extracellular fluid, and take in food and discharge waste products in either liquid or solid form through specialised body openings. Cells in animals play a minor role in waste product storage, with few exceptions such as liver tissue.

1.2 Meristems

Having cells with a mechanically tough wall has some potential disadvantages. Because plants must be able to respond in their growth and tissue/organ repair/replacement to changes in their environment, this 'plasticity' requires the growth of new tissue which in turn requires the production of new cells. The presence of a cell wall in plants precludes the option that cells can be produced at one part of the body, such as through stem cells in the human body, and migrate to the site of tissue damage and growth. Rather, the ability to produce new cells must persist throughout the lifetime of plant and throughout the plant body. Secondary meristems such as the root lateral meristem, the vascular cambium in stem and the leaf axillary meristem fulfil such a function.

In contrast to secondary meristems, primary meristems facilitate the growth of the main axis of the two primary organs of plant, root and shoot, in the form of apical meristems. These meristems form already early during embryo development. Most studies that are concerned with the function of AQPs in cell growth have focused on primary meristems. Once cells have been produced in the apical meristem, or 'cell division zone', they start to expand to a multiple of their original volume in the so-called cell expansion/elongation zone before they differentiate in the 'differentiation zone' to reach their final form and function. All three zones together will be referred to as 'growth zone' in the following. The spatial and sequential arrangement of meristem, cell expansion and cell differentiation zone makes it easy to study the development-dependent expression of genes, particularly in roots and in grass leaves. One aspect of cell and tissue differentiation which becomes particularly important when trying to understand how growing tissues take up water is the circumstance that neither the water conduction transport paths (xylem, phloem) along the main axis of organ (from root to shoot or from shoot to root) are fully developed along the entire growth zone, nor are possible apoplastic barriers within the radial transport paths (roots, between soil and root xylem; leaf, between leaf xylem and epidermis) (e.g. Hukin et al. 2002; Fricke 2002; Enstone et al. 2003; Hachez et al. 2006; Knipfer and Fricke 2011). This means that accepted

views of water flows and hydraulic barriers in mature tissues cannot necessarily be adopted to explain flows and barriers in growing tissue. In fact, as will be shown, our knowledge about the hydraulic architecture of the meristematic and proximal cell expansion zone in roots and leaves is far from complete.

In the following, we will first have a closer look at the hydraulics of growing plant tissues, their architecture and water potential gradients, and what they tell us about the possible limitation of growth through cell and tissue hydraulic properties – and therefore also AQPs. We will then move on to data on the expression of AQPs in growth zones and on the hydraulic conductivity of the plasma membrane of growing leaf cells. Finally, we will use this information together with data on the rate of cell volume expansion to ask the question whether growing plant cells actually need AQPs to take in water at sufficiently high rates to support their volume expansion rates.

2 Water Potential Differences in Growing Plant Tissues

2.1 Quantitative Relationships

From the biophysical point of view, the process of cell expansion and factors limiting cell expansion are best described by the Lockhart equation (Lockhart 1965; e.g. Touati et al. 2015). This equation relates the relative growth rate of a cell to (i) the mechanical and hydraulic forces which drive volume expansion and (ii) the mechanical and hydraulic conducting properties which affect the gain in cell volume per unit driving force. The mechanical properties are those of the wall of growing cells, and the hydraulic properties are those of the plasma membrane and, for tissues, of the water conducting path. We are not so much interested here into addressing the question whether growth is limited more through hydraulic or wall properties of cells and tissues (e.g. Boyer et al. 1985; Cosgrove 1993; Boyer 2001; Boyer and Silk 2004; Touati et al. 2015), but rather in the specific hydraulic properties of growing tissues and the contribution that AQPs make to these properties.

The volume flux density of water (J; volume [m^3] of water per unit surface (m^2) and time (s^{-1}); unit: $m^3\ m^{-2}\ s^{-1}$, or $m\ s^{-1}$) from location A to B equals the product of hydraulic conductivity (Lp; unit: $m\ s^{-1}\ MPa^{-1}$) and the driving force (water potential difference between A and B, $\Delta\Psi$; unit: MPa); so $J = Lp \times \Delta\Psi$. Locations A and B could be the apoplast (A) and cytosol (B), respectively, of a cell; they could also be the root environment (A) and xylem (B), respectively, of a root system. Note that $\Delta\Psi$ includes two components, an osmotic component and a hydrostatic pressure component; the osmotic component can only drive water flow between A and B in the presence of a semipermeable structure (containing AQPs; see below) that separates both compartments and largely inhibits the free diffusion of osmotically active solutes (i.e. allows the build-up of an osmotic gradient between A and B). The flux of water which is of relevance when studying cell expansion is that flux which is required to let the growing tissue expand at a certain relative rate. Maximum relative

growth rates of roots are typically higher than those of leaf tissues, and values range from about 20 to 40 % h^{-1} (for a review, see Pritchard 1994). It can be seen from the relation 'J = Lp x ΔΨ' that the smaller Lp is for a given J, the larger ΔΨ needs to be. The smaller the Lp is, the less hydraulically conductive is the path between A and B; in other words, a small Lp and a significant ΔΨ are indicative of some hydraulic limitation of growth. If so, one could predict that an increase in Lp, such as through increased expression and activity of AQPs – provided that water has to cross at least one membrane – should lead to an increase in J and cell expansion rate, provided ΔΨ can be maintained at its original level, for example, by maintaining an osmotic gradient across a semipermeable membrane barrier through active solute transport (Fricke and Flowers 1998). It follows from the above that an analysis of ΔΨ, for a given J, provides an indication whether growth is potentially limited through Lp and hydraulic properties of cells or not.

2.2 Water Potential Difference ΔΨ

There exists evidence in support and not in support of a significant (ca 0.05 MPa and larger) ΔΨ, between the plant-internal water source (e.g. leaf xylem) and expanding cell (e.g. leaf epidermis) in growing plant tissues (for reviews, see Cosgrove 1993; Fricke 2002; Boyer and Silk 2004). A significant ΔΨ has been reported in particular for the elongation zone of grass leaves and growing hypocotyls and epicotyls (stem sections) (e.g. Boyer et al. 1985; Fricke et al. 1997; Fricke and Flowers 1998; Martre et al. 1999; Tang and Boyer 2002; Touati et al. 2015). The apparent discrepancy between studies may be related to differences in the species analysed and in the growth and experimental conditions used. Significant differences in Ψ have also been reported between the root medium and growing regions of roots (e.g. Miyamoto et al. 2002; Hukin et al. 2002).

If we accept that there can exist a significant ΔΨ in some growing plant tissues, does this necessarily mean that a change in plasma membrane or tonoplast AQP activity alters the growth rate of cells and tissues? No, it does not. Figure 1 shows two possible scenarios for a setup where six elongating cells are located, in sequence, along a radial water path. Water is provided just outside cell 1 and moves radially along cells 2–5 to cell 6. In both cases, water moves from cell to cell by crossing membranes, and if water only crosses the plasma membrane (and not also tonoplast), it has to cross 11 plasma membranes, in a polar manner (entering one side and exiting the opposite side of a cell) until it has entered cell 6. The hydraulic resistance of the path calculates as the additive resistance of the 11 plasma membranes and the six sections of wall that have to be crossed, by analogy to Ohm's law for an electric circuit (Van den Honert 1948). In scenario 'a', the hydraulic resistance of the wall sections is negligible compared with that of the plasma membranes, and the overall Lp of flow path is dominated by the Lp of plasma membranes. In scenario 'b', water moves the same path from cell 1 to cell 6, but before water enters cell 1, it has to pass through an apoplast which has a very high hydraulic

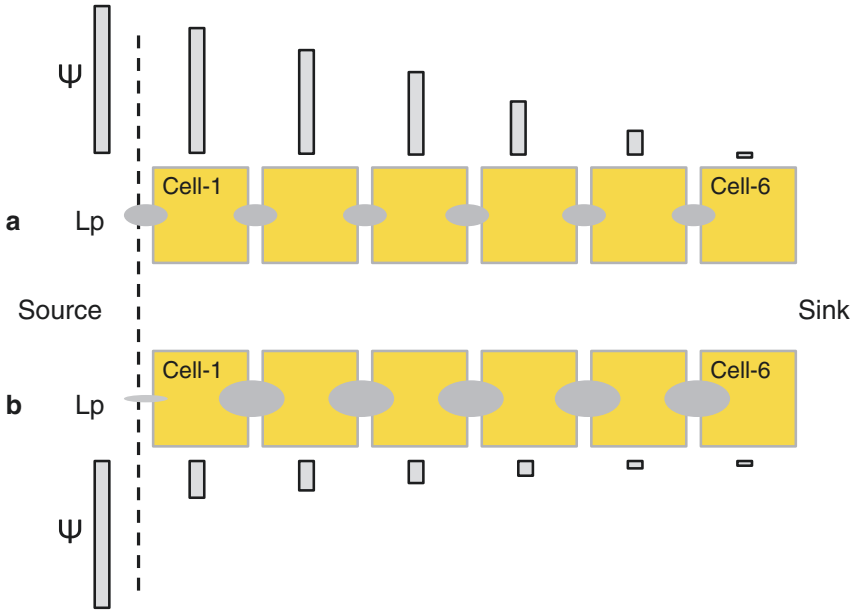


Fig. 1 The significance of a water potential difference across tissues for a hydraulic limitation of growth through cell hydraulic properties. The scheme shows a cross-sectional view of six cells that are arranged next to each other along the radial flow path of water from source (e.g. root medium) to sink (e.g. cell 6 or xylem). Each cell has a hydraulic conductivity (*Lp*) at its membrane, and the size of Lp symbol reflects the size of Lp; the same applies to the symbols used for water potential (*Ψ*), aquaporin (AQP) activity and expression and for plasmodesmatal (PD, symplastic) connection between cells. In (**a**), the Lp of all six cells contributes equally to the difference in Ψ (ΔΨ) across the tissue, and any change in Lp will affect ΔΨ and a hydraulic limitation of growth. In (**b**), the by far smallest Lp (highest resistance to water movement) is found at the entry point of water into cell 1; this could be due to, e.g. suberinisation of the apoplast. The Lp of cells contributes little to ΔΨ, and changes in cell Lp will have little effect on ΔΨ. The value of ΔΨ is similar in (**a**, **b**) and points to a hydraulic limitation of growth, yet only in (**a**) could such a limitation be significantly affected by changes in cell Lp, for example, through changes in AQP activity

resistance (low Lp, as represented by the small size Lp symbols in Fig. 1), e.g. due to intense suberisation (hydrophobic hydraulic barrier). The resistance of the suberised wall section dominates the overall resistance of the flow path, and any alteration of the Lp of plasma membranes will have little effect on the overall Lp. Both scenarios lead to a significant ΔΨ. In 'a', the significant ΔΨ is due to an inherently low Lp of the plasma membrane of cells, and upregulation of PIPs may increase Lp and growth. In contrast, in 'b', upregulation of PIP activity will have little effect on the overall Lp and growth rate. Thus, the existence of a significant ΔΨ in growing tissues is a pre-requisite but not proof per se that any alteration in plasma membrane AQP activity leads to an altered growth rate of cells. The possible existence of local and significant apoplast barriers to water movement needs to be considered too.

2.3 Root Growth Zones Are Special

There exist one fundamental difference between the hydraulic arrangement of growing tissues in roots on the one hand and stem and leaf on the other. Growth zones of stems, such as hypocotyls, and of leaves of monocot crop plants (e.g. wheat, barley and maize), which are exposed to the atmosphere (as opposed to, e.g. leaves of seagrass that are submerged under water), are located along the axial transport route of water from roots to shoot (Fricke 2002; for water flow in submerged plants such as seagrass, *Posidonia australis*, see Tyerman et al. 1984). In contrast, growth zones of roots are located at the very starting point, or even prior to that starting point, where water is transported axially from root to shoot (Fig. 2). Which implications does this have for a hydraulic limitation of growth and a role that AQPs could play?

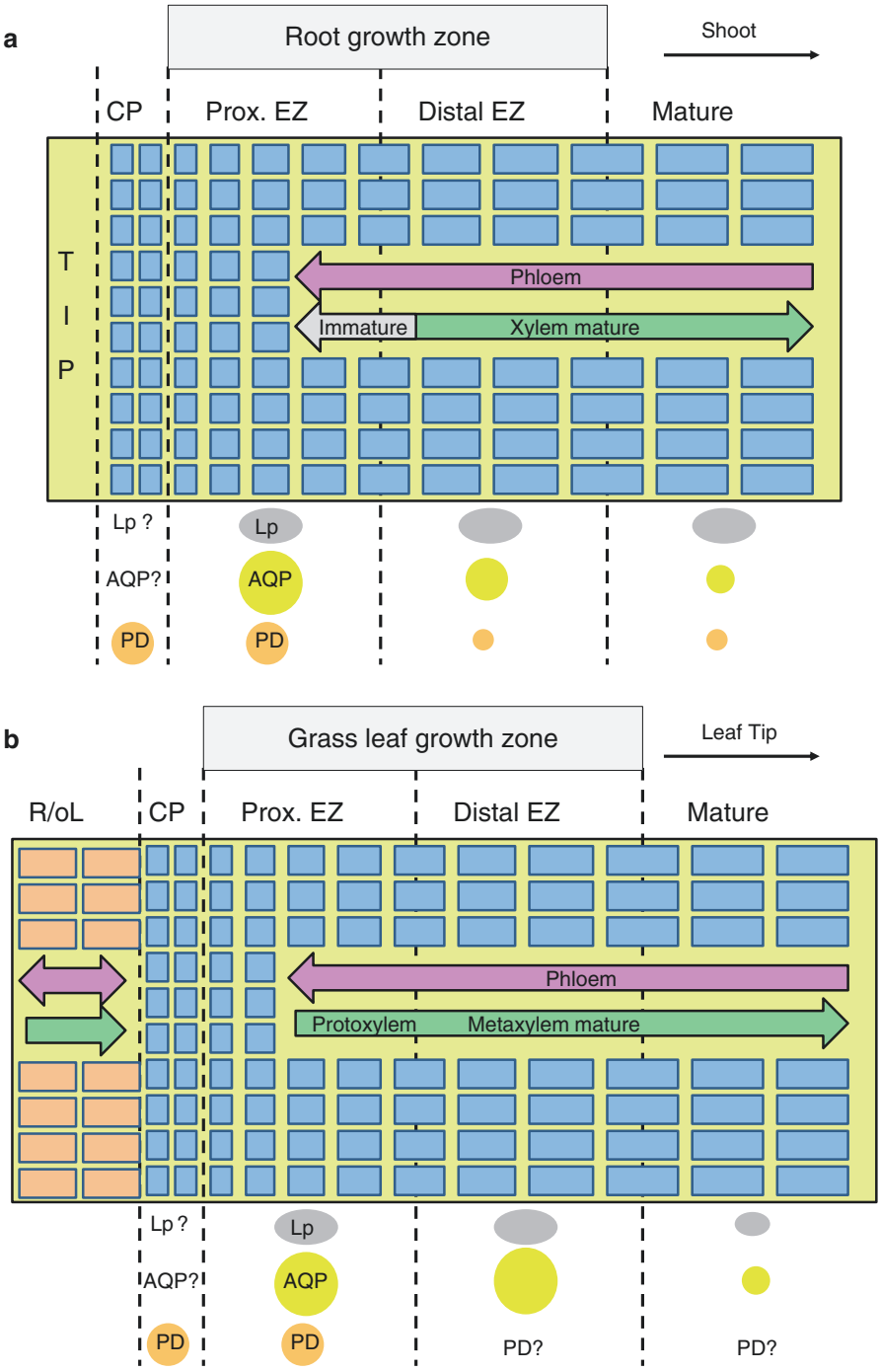
In roots, transpiration water in xylem moves away from the growth zone and may not reach any of the proximal (closest to the tip) regions. In the latter regions, phloem-delivered water may constitute the main source of water to expanding cells, in addition to water entering from the soil/root interface. In addition, water may move along a symplastic path, through plasmodesmata (Hukin et al. 2002). This is supported by a study on maize roots, which showed that AQP inhibitors have little or no effect on the half-time of water exchange (and by implication plasma membrane Lp) in the growing tip region (Hukin et al. 2002). By linking the expansion of cells in roots to the supply of water, and resources such as carbon and energy through the phloem from shoots, shoot productivity and transpirational surface can be fine-tuned with growth of water and mineral nutrient absorbing root surface. In contrast, the distal portion of root growth zones is more directly linked to xylem water flow. Radial movement of water can occur along the transmembrane path, from cell-to-cell crossing membranes, and AQP activity and AQP inhibitors impact on this water movement (e.g. Hukin et al. 2002; Frensch and Steudle 1989; Knipfer et al. 2011).

3 AQP Expression in Growing Root and Leaf Tissues

A comprehensive literature review of data on AQP expression in growing plant tissues, including root, leaf, stem, petals and hairs, is provided by Obroucheva and Sin'kevich (2010). We will focus here on roots and leaves, with some information also on fibre elongation.

3.1 Roots

There exist a few studies in which the expression of the majority of AQP isoforms of a particular plant species has been compared between root and shoot tissue. These studies show two trends: (i) when there are AQP isoforms within a species


a
Root growth zone
Shoot
CP
Prox. EZ
Distal EZ
Mature
T
I
P
Phloem
Immature
Xylem mature
Lp ?
Lp
AQP?
AQP
PD
PD
b
Grass leaf growth zone
Leaf Tip
R/oL
CP
Prox. EZ
Distal EZ
Mature
Phloem
Protoxylem
Metaxylem mature
Lp ?
Lp
AQP?
AQP
PD
PD
PD?
PD?

that are almost exclusively expressed in one vegetative plant organ, then the exclusive expression occurs mostly in roots and not in shoots, and (ii) roots show a higher total expression of AQPs compared with shoots. For example, relatively (compared with shoot tissue) high or exclusive expression of AQPs in roots was observed for *Oryza sativa* (OsPIP2;3, OsPIP2;5, OsTIP2;1, Sakurai et al. 2005), *Zea mays* (ZmPIP2;1, ZmPIP2;5, ZmPIP1;5, Hachez et al. 2006; Heinen et al. 2009; Lopes et al. 2003), *Arabidopsis thaliana* (AtPIP2;2, AtPIP1;1, AtPIP1;2, AtPIP2;1, AtTIP1;1, AtTIP1;2, Javot et al. 2003; Alexanderson et al. 2005), *Vitis vinifera* (VvPIP1;1, VvPIP2;2, Vandeleur et al. 2009), *Hordeum vulgare* (HvPIP1;2, HvPIP2;1, HvPIP2;2, HvPIP2;5, Katsuhara et al. 2002; Katsuhara 2007; Besse et al. 2011; Knipfer et al. 2011) and *Pisum sativum* (PsPIP2;1, Beaudette et al. 2007). As some of these AQPs were also expressed in growing root tissue, the data show that there is not a single set of AQPs in a particular plant species that facilitates growth in growing root *and* leaf tissue, but that this role can be organ-specific and involve isoform-specific AQPs.

Using *in situ* hybridisation and also immunocytochemical approaches, AQP isoforms have been localised in certain root tissue types (e.g. cortex, epidermis) and in dependence on root developmental stage (Hachez et al. 2006; Sakurai et al. 2005, 2008; Vandeleur et al. 2009; Knipfer et al. 2011). The most complete studies so far exist for rice (Sakurai et al. 2005, 2008), vine (*Vitis vinifera*, Vandeleur et al. 2009; Gambetta et al. 2013), maize (Hachez et al. 2006, 2012), *Arabidopsis* (Brady et al. 2007) and barley (Knipfer et al. 2011). For example, it was shown for maize that ZmPIP2;1/2;2 proteins are most abundant in the stelar cells at the root tip, encompassing the growth zone, whereas in more mature root regions, these two AQP isoforms were preferentially localised to the cortex and epidermis; ZmPIP2;5 protein was in particular found in cortex tissue at the root tip and in the endodermis in mature root tissue (Hachez et al. 2006). In rice, Sakurai et al. (2008) showed that OsPIP1s and OsPIP2;1, OsPIP2;3 and OsPIP2;5 proteins were localised preferentially in the endodermis at the root tip. In more mature root regions, OsPIP2s proteins were distributed rather evenly between tissues, whereas OsTIP2;1 and OsTIP2;2 proteins were localised preferentially in stelar cells. In grapevine, Vandeleur et al. (2009) observed that VvPIP1s and VvPIP2s gene expression and protein level were localised evenly in cortex tissue and vascular tissue at the root tip, but showed lower signals in the cortex of mature root regions. Gambetta et al. (2013) compared the root zone-dependent gene expression of isoforms in grapevine with

Fig. 2 Hydraulic architecture of the growth zone of (**a**) roots and (**b**) grass leaves. The scheme gives a longitudinal view of the arrangement of expanding cells and the relative location of tissues (xylem, phloem) through which water can be supplied internally to growing tissues. The size of Lp symbol reflects the size of Lp; the same applies to the symbols used for aquaporin (*AQP*) activity and expression and for plasmodesmatal (*PD*, symplastic) connection between cells. A '*?*' indicates that we lack detailed information here, and *arrows* for phloem and xylem point to possible main directions of flow; for details, see text. *CP* cell production zone (meristem), *prox. EZ* proximal elongation zone, *distal EZ* distal elongation zone, *mature* mature root or leaf region where cells have attained their full size, *TIP* root tip region including the root cap

their homologues in *Arabidopsis* (using data from Brady et al. 2007) and concluded that most AQP isoforms distributed similar in the two species. There was a much higher gene expression of PIPs in the root tip compared with more mature regions along the main root axis. In barley, tissue localisation of the gene expression of six AQP isoforms was tested (*HvPIP2;2*; *HvPIP2;5*, *HvPIP2;7*, *HvPIP1;2*, *HvTIP1;1*, *HvTIP2;3*) (Knipfer et al. 2011). There was generally high expression of AQP genes in the epidermis and protoxylem. Expression in cortex tissue was evident in all root developmental zones and in both seminal and adventitious roots. Expression in the endodermis and stele was observed particularly in less mature adventitious roots, highlighting a potential role in regulating radial water transport. Of all barley AQPs tested, *HvTIP1;1* was the most ubiquitously expressed gene, while *HvPIP2;5* was expressed particularly in cortex tissue.

In barley, adventitious roots had a threefold higher cortex cell hydraulic conductivity and total expression of PIP2s and TIPs compared with seminal roots (Knipfer et al. 2011). This difference in AQP expression was due to higher expression of three aquaporins, *HvPIP2;*2, *HvPIP2;5* and *HvTIP1;1*, all of which display water channel activity (Besse et al. 2011). These aquaporins were expressed in the epidermis, cortex, endodermis and stele of the transition zone of adventitious roots, where cells have completed some of their elongation yet are not fully mature as judged from endodermis development. *HvPIP2;5* and *HvTIP1;1* were the highest-expressed AQPs tested.

In seminal roots of barley, *HvTIP1;1* was expressed lowest in the mature zone, and this coincided with the lowest cortex cell hydraulic conductivity in this root region compared with the immature and transition zone, which, together encompassed the entire growth zone (Knipfer et al. 2011). The expression of three HvPIPs (HvPIP1;2, HvPIP2;2, HvPIP2;5) was compared between growing (immature + transition zone) and nongrowing (mature zone) root regions in seminal roots. None of the three genes tested was expressed higher in growing tissue, despite cell hydraulic conductivity being almost four times higher in growing compared with nongrowing tissue. Other PIPs, which were not tested in the study by Knipfer et al. (2011), may be responsible for the higher cell Lp in growing barley root tissue. What the study shows, though, is that AQPs such as HvPIP2;5 may facilitate water flow in growing root tissue in one type (adventitious roots) but not another type (seminal roots) of roots, even within one species.

Sequence comparison between barley and maize PIPs shows that ZmPIP2;1 and ZmPIP2;2 share highest sequence identity with HvPIP2;5. ZmPIP2;1 is among the highest-expressed PIPs in maize roots, The tissue localisation of ZmPIP2;1 protein changes during maize root development from a predominant location in the stele and endodermis to a location in the cortex and epidermis (Hachez et al. 2006). Such a change in tissue localisation was not observed for *HvPIP2;5* in barley (Knipfer et al. 2011), where gene expression was analysed. *HvPIP2;5* was expressed in cortex tissue in both transition and mature zones. Sakurai et al. (2008), using immunocytochemistry, observed for rice roots that candidate AQPs occurred predominantly in the endodermis and stele, with some protein in the rhizodermis and very little in the cortex. The difference in results between the study on barley (Knipfer et al.

2011) and the studies on maize (Hachez et al. 2006) and rice (Sakurai et al. 2008) may reflect differences between species or the circumstance that AQP gene and protein abundance do not correlate in time and space.

TIP1;1 isoforms are generally the most abundantly expressed members of the TIP family of AQPs (e.g. Alexandersson et al. 2005; Sakurai et al. 2005) and share a high sequence identity among the plant species tested. The ubiquitous and abundant expression of *HvTIP1;1* in barley roots (Knipfer et al. 2011) suggests that this AQP is a 'housekeeping' type of AQP, which provides a 'baseline' level tonoplast hydraulic conductance to guarantee rapid osmotic equilibration between vacuole and cytosol (Maurel et al. 1993). This does not preclude a role of HvTIP1;1 in growth-facilitated water uptake. A complete loss of (water channel) function of HvTIP1;1 is not expected to cause a phenotype in barley (see also Schüssler et al. (2008) for *Arabidopsis*), as another TIP (HvTIP2;3), which shows water channel activity (Besse et al. 2011), is expressed in roots, though with a different tissue (e.g. the cortex, stele, epidermis) pattern (Knipfer et al. 2011). It remains to be shown why multiple TIP isoforms which show water channel activity are co-expressed abundantly in root cells and whether any of these TIP isoforms carries a growth-specific function.

3.2 Leaves

Besse et al. (2011) conducted a detailed study on the development-dependent expression of AQPs in growing barley leaves. At the time of study, the entire set of almost 40 barley AQPs (Hove et al. 2015) was not known, and 23 AQPs were studied. Five AQP genes, including one PIP1 (*HvPIP1;2*), were expressed at such low levels in leaf regions that it was difficult to conclude on their pattern of expression. A sixth gene (*HvSIP2;1*) was expressed so uniformly between leaf developmental zones that it turned out to be a suitable reference gene of expression. The 17 remaining genes analysed, which included most known barley PIPs, were expressed particularly in either growing (seven genes) or in emerged, mature leaf tissue (ten genes). It can be concluded from these data that differential expression during leaf development is the rule rather than exception for barley MIPs and that all MIPs that facilitate diffusion of water across the plasma membrane are under developmental or environmental control. There is no obvious reason why control of water channel activity of one particular AQP through post-translational regulation and trafficking (Johansson et al. 1998; Tournaire-Roux et al. 2003; Maurel 2007; Zelazny et al. 2007; Boursiac et al. 2008; Hachez et al. 2014) should not provide sufficient means to meet requirements specific to growing and mature leaf tissue. Therefore, the observation that many different barley AQP isoforms show development-specific expression points to these AQPs fulfilling tissue-specific functions. Individual AQPs with localisation to specific tissues may play important roles during tissue and cell expansive growth in barley, but their relative abundance in whole tissue extracts is lower because of their specific localisation.

None of the AQPs tested in the study by Besse et al. (2011) were expressed highest or lowest in the non-elongation zone – that zone during grass leaf cell development, where cells have ceased to elongate, yet still show some residual expansion in width before being displaced through growth of more basal regions from subtending sheaths into the ambient atmosphere. Instead, the start (elongation zone) and end point (emerged, mature blade) of a cell's ontogeny were accompanied by maximum or minimum expression of a particular AQP isoform (Besse et al. 2011). This contrasts with the only other comparable study, on maize (Hachez et al. 2008), where expression of AQPs was analysed in detail and at high spatial resolution in different developmental zones of a leaf. In the study on maize, expression of PIPs in developing leaves was highest in the zone near the emergence point from the sheath of older leaves, with subsequent decrease in expression in the mature part of blade. A continuous increase in expression during leaf development was only observed for *ZmPIP1;5*. As the studies on maize and barley are the only ones of their kind, it cannot be said which study presents the rule and which the exception with respect to AQP isoform expression during leaf development. The most notable difference between barley and maize is that barley is a C_3 and maize a C_4 plant. How, and whether, this could explain differences in the expression pattern of AQP in leaves of the two species remains to be shown.

It is not known what regulates the differential expression of barley (or maize or any other grass) MIPs during leaf development. The most pronounced difference in microenvironment between the elongation zone and the more mature leaf regions is the intensity and quality of light that reaches cells; also relative humidity in the air next to the elongation zone enclosed by subtending sheaths of older leaves should be considerably higher than ambient. The elongation zone of leaf three is enclosed by sheaths of leaf one and two, whereas the non-elongation zone is only enclosed by the sheath of leaf two. The sheaths are green and photosynthetic. As a result, the light that reaches the non-elongation and, particularly, elongation zone will have a higher ratio of far-red to red light than that striking the emerged and mature blade. This could enable regulation of AQP expression through the phytochrome system in a development- and therefore also growth-dependent manner.

3.3 Roles of Particular AQP Isoforms During Leaf Growth

In barley, *HvPIP2;5*, *HvTIP1;1* and *HvTIP2;3* were expressed abundantly and highest in growing tissue of roots and leaves (Besse et al. 2011). The same was observed for *HvPIP1;1* (identical to the barley AQP annotated as *HvPIP1;6*) in a previous study on barley (Wei et al. 2007). These four MIPs, all of which show water channel activity (Wei et al. 2007; Besse et al. 2011), seem to have a role that is specific to cell growth in barley, irrespective of the organ. In contrast, the water channel HvPIP2;2 was expressed particularly in growing tissue of leaves (Besse et al. 2011) and seems to have a growth-related function that is more leaf-specific.

High expression of TIP1;1 isoforms in meristematic and elongating shoot tissue has been reported, e.g. maize (Chaumont et al. 1998), tulip (*Tulipa gesneriana*, Balk and de Boer 1999), cauliflower (*Brassica oleracea*, Barrieu et al. 1998) and oilseed

rape (*Brassica napus*, Frangne et al. 2001), and appears to be a common characteristic associated with growth. *Arabidopsis* plants lacking AtTIP1;1 (and AtTIP1;2) protein do not show any phenotype or change in growth rate under normal growth conditions (Schüssler et al. 2008). This does not preclude a role of AtTIP1;1 or the barley homologue HvTIP1;1 in facilitating water uptake and vacuole enlargement during leaf cell expansion, nor in playing a role in any cell expansion-related event such as lateral root formation (see recent work on role of TIPs in lateral root growth using triple TIP mutants of *Arabidopsis*; Reinhardt et al. 2016). For example, the high expression of the water channel HvTIP2;3 in the growth zone of barley leaves points to some redundancy in function among TIPs (Besse et al. 2011).

In the leaf elongation zone of barley, *HvPIP1;1* and *HvPIP2;5* accounted for 90 % or more of the expression of PIP1s and PIP2s, respectively (Besse et al. 2011). Their closest maize homologues (based on sequence identity), *ZmPIP1;1* and *ZmPIP2;1* together with *ZmPIP2;2*, also accounted for the bulk of expression of PIP1s and PIP2s in the elongation zone of maize (Hachez et al. 2008). It appears from these two studies on grasses that dominant PIP isoforms are conserved in elongating leaf tissue. As these isoforms include members of the PIP1 and PIP2 subfamily, they may regulate cell Lp and growth through formation of PIP1/PIP2 heteromers (for a review, see Chaumont and Tyerman 2014) (see chapter "Heteromerization of Plant Aquaporins").

In barley, the water channel *HvPIP2;5* was expressed abundantly in the leaf elongation zone, and this included the mesophyll in this leaf region (Besse et al. 2011). Mesophyll constitutes most of tissue volume of leaves, and this could explain why *HvPIP2;5* accounted for more than 90 % of PIP2 expression in the elongation zone (Besse et al. 2011). This renders HvPIP2;5, a prime candidate to mediate plasma membrane water flow in growing mesophyll cells. It would also explain the higher cell hydraulic conductivity in growing compared with nongrowing mesophyll tissue in barley, as concluded from swelling assays of the osmotic water permeability of mesophyll protoplasts (Volkov et al. 2007). In growing leaf epidermal cells of barley, the function of HvPIP2;5 appears to be carried out by HvPIP1;1 (HvPIP1;6) and HvPIP2;2, both of which are expressed highest in the epidermis (Wei et al. 2007; Besse et al. 2011). Trans-tonoplast movement of water in growing leaf tissues seems to be facilitated by the abundantly expressed HvTIP1;1 and HvTIP2;3 (Besse et al. 2011).

3.4 The Role of Vascular Bundles in the Hydraulics of Leaf Growth

It is not known whether water reaches epidermal cells in the elongation zone of grass leaves directly through mesophyll or through bundle sheath extensions, from where it diffuses radial within the epidermis. In the latter case, many membranes and hydraulic resistances have to be overcome. A potential hydraulic limitation of cell expansion growth could be avoided by high expression of AQPs such as *HvPIP1;1/1;6* in the epidermis, leading to a higher cell hydraulic conductivity in the epidermis of elongation compared to mature leaf tissue (Volkov et al. 2007). The comparatively low water transport activity of HvPIP1;1/1;6 (Wei et al. 2007) may

be partially compensated for by high expression levels of, and heteromerisation (Fetter et al. 2004; Zelazny et al. 2007) (see chapter "Heteromerization of Plant Aquaporins") with, the concurrently expressed *HvPIP2;2* and *HvPIP2;5*.

The mestome sheath of grass leaves can be suberised (O'Brien and Carr 1970; O'Brien and Kuo 1975; for a review, see Lersten 1997; Fricke 2002). The study by Besse et al. (2011) on barley showed that Casparian-band like structures increased during leaf development. The mestome sheath may fulfil a role in leaves that is comparable to that of the endodermis in roots (Fricke 2002; Enstone et al. 2003; Wu et al. 2005; Heinen et al. 2009). This view receives increasing experimental support through studies which emphasise the role of the bundle sheath and bundle sheath AQPs as potential hydraulic bottlenecks, through which the radial movement of water from xylem to substomatal cavity is controlled in transpiring leaves of monocot and dicot plant species (e.g. Shatil-Cohen et al. 2011; Sade et al. 2014).

Increase in expression of HvPIP2;7 during barley leaf development, with smaller expression in elongating and higher expression in mature leaf tissue, in vascular bundles (Besse et al. 2011) could compensate for the formation of any apoplastic barriers by facilitating radial movement of transpiration water through membranes along a cell-to-cell pathway. Such a pathway has been supported by a study on *Tradescantia* (Ye et al. 2008). In rice, *OsPIP2;7* is expressed in leaves predominantly in mesophyll, and not in vascular bundles as in barley. Overexpression of *OsPIP2;7* in rice results in increased transpirational water loss (Li et al. 2008). The data on PIP2;7 expression in barley and rice support a role of this PIP in facilitating radial movement of transpiration water in both species, yet the tissue site where this facilitation occurs differs between barley (vascular bundle) and rice (mesophyll). The considerable expression of *HvPIP2;7* in the non-transpiring non-elongation zone in barley (Besse et al. 2011) might be in preparation of the displacement of cells into the open atmosphere (past the point of emergence from the sheath of leaf two). This displacement can occur in as little as 10 h (Richardson et al. 2005).

Between 98 and 99 of every 100 water molecules that enter the leaf elongation zone of barley along the xylem are lost through the emerged blade; only one to two molecules are used to support cell expansive growth (Fricke 2002). Therefore, it is surprising that water channels such as HvPIP2;5 and HvTIP1;1 are expressed at so much lower levels in mature, transpiring compared with growing, non-transpiring leaf tissue (Besse et al. 2011). Could it be that their water channel activity is an experimental disguise of their true function *in planta* (e.g. Hill et al. 2004) or that the need to rapidly osmotically equilibrate water across membranes is much higher in growing than in mature plant tissue? We do not know.

3.5 Examples of Other Experimental Systems

Fibres, which grow on/around seeds, such as cotton (*Gossypium hirsutum*) seed fibres offer a great experimental system to study the molecular processes accompanying cell elongation. The fibres and fibre cells are large; they are arranged in

series, are easy accessible, are easy to observe and are comparatively easy to analyse; and in addition, they are also commercially very important. In *Gossypium hirsutum*, four PIP2s (GhPIP2;3, GhPIP2;4, GhPIP2;5 and GhPIP2;6; Li et al. 2013) and one PIP1 and TIP (GhPIP1;2, GhγTIP; Yang and Cui 2009) have been shown to be expressed particularly in fibres and to peak in expression during a period when fibres and fibre cells elongate at their highest rate. Knockdown of expression of GhPIP2 genes in cotton significantly decreased the rate of fibre elongation (Li et al. 2013). Furthermore, the authors also observed that most of the PIP2s that were expressed particularly in cotton fibre cells were able to form heterotetramers and, through this, increased water channel activity further.

In the milkweed *Calotropis procera*, which produces long seed trichomes, CpPIP2 AQPs were also implicated in the process of fibre cell elongation (Aslam et al. 2013). This conclusion was based on the observation that the expression of CpPIP2s in fibre cells was highest during the period of highest rates of fibre cell elongation. In addition, transgenic tobacco plants that expressed CpPIP2s had a larger number of trichomes on leaves and stem.

In rose (*Rosa hybrid* 'Samantha'), the rose PIP2 RhPIP2;1 was shown to be involved in the ethylene-regulated expansion of petals (Ma et al. 2008). RhPIP2;1 was localised primarily in the abaxial subepidermal cells of petals, and its expression during petal development was highly correlated with petal expansion rate. Furthermore, treatments that reduced the rate of petal expansion, such as ethylene and silencing RhPIP2;1 in transgenic plants, also reduced the gene expression level of RhPIP2;1.

In deepwater rice (*Oryza sativa* L. ssp. indica), shoot internodes must elongate quickly in response to flooding to minimise anoxia stress to leaf tissues. Muto et al. (2011) observed that the gene transcript levels of several OsAQPs (OsTIP1;1, OsTIP2;2, OsPIP1;1, OsPIP2;1, OsPIP2;2) increased significantly in stems in response to submersion. This occurred in parallel to the stimulation of other processes that facilitate elongation growth of cells, such as expression of vacuolar proton pumps (OsVHP1;3). The authors also observed that AQPs (OsNIP2;2 and OsNIP3;1), which are not primarily involved in water transport, but in the transport of substances (silicic acid and boric acid) that can interfere with wall expansion, were reduced in expression in response to flooding. The latter provides a good example for a role of AQPs in cell elongation not related to their water transport function.

4 Membrane Hydraulic Conductivity in Growing Plant Cells

The above section was concerned with identifying AQP isoforms in a range of species, which facilitate water uptake into growing leaf or root tissue. Now we have a closer look at the hydraulic conductivity (Lp) of growing cells. First, we want to know whether growing cells have a higher hydraulic conductivity, at their plasma

membrane, compared with mature cells. Then we ask whether such differences in plasma membrane Lp are indicative of growing cells taking in more water per unit time and driving force to support volume expansion.

4.1 Roots

Most of the data on the Lp of higher plant cells and tissues exist for roots. This is because roots provide an easier experimental system compared with leaves: (i) roots can be grown hydroponically and enable an easier application of experimental treatments; (ii) roots are cylinders in shape, which facilitates modelling of water flows; and (iii) the discovery of AQPs in plants has opened up the possibility that plant water flow can be regulated also in the short term through roots rather than through stomata in shoots. There exist surprisingly few data on the plasma membrane Lp of growing root cells mainly due to the experimental challenges involved, in particular for cells that are located very close to the root tip in the proximal half of root elongation zone. The majority of cell Lp data on roots has been obtained on mature tissues, often with the aim to link cell Lp to root tissue Lp and to address the role which AQPs and particular root developmental regions play in root water uptake (e.g. Hachez et al. 2006; Bramley et al. 2009; Ehlert et al. 2009; Knipfer et al. 2011; Gambetta et al. 2013). This has made it possible to conclude on the main path of radial water movement (apoplast versus cell to cell) across the root cylinder and to test the composite model of water transport across roots (Frensch and Steudle 1989; Steudle 2000; Steudle and Peterson 1998). The implicit assumption of these analyses is that a higher expression of AQPs in conjunction with a higher Lp supports the idea that AQPs contribute to root water uptake in a particular root region and that a significant portion of water moves along the transmembrane component of cell-to-cell path. This assumption may apply to mature root regions. However, one has to be careful when applying this assumption to growing and meristematic root regions. This is demonstrated nicely by the studies of Hukin et al. (2002) on maize, Gambetta et al. (2013) on grapevine and Miyamoto et al. (2002) on pea roots.

Hukin et al. (2002) studied the expression of two AQPs, and cell Lp in the presence and absence of the AQP inhibitor Hg and the symplastic connectivity in the tip region of maize roots. The authors analysed locations in the proximal half of the growth zone, half-way along the growth zone (where relative elemental growth rates were highest) and at the distal end of the growth zone and the mature zone just beyond that. The main observations were that (i) Lp of cells averaged about $1.5–2 \times 10^{-7}$ m s^{-1} MPa^{-1} throughout the growth zone and increased two- to threefold in mature tissue; (ii) Hg reduced cell Lp half-way along and in the distal portion of the growth zone and in the mature zone but not closer to the root tip; and (iii) symplastic continuity was observed only closer to the root tip, in the proximal portion of growth zone. The conclusions that can be drawn from these observations are that maize root AQPs contribute to cell Lp and radial root water flow across the root cylinder in the distal half of the growth zone and mature root regions. However,

cells closer to the root tip, where xylem is not fully developed and functional, have a high symplastic connectivity to enable the import of water through the phloem (compared with xylem earlier developed) and transport of water through tissue. The two AQPs studied were expressed at much lower levels in the proximal compared with distal half of the growth zone, yet cell Lp was similar in the two root regions. It is possible that other AQP isoforms that were not studied showed a different pattern of expression, with expression being higher in the proximal portion of the growth zone. The disparity between AQP expression and cell Lp could also be due to the technique used to determine cell Lp: the cell pressure probe. This instrument can be used to induce water flow across the plasma membrane of a cell and follow the subsequent turgor pressure relaxation, which in turn is used to determine cell Lp. What this technique measures is water flow across the plasma membrane (and through plasmodesamata, as water flow through these two structures cannot be measured separated with currently available techniques) of a cell, yet it cannot distinguish between the path of water flow, which could be through simple diffusion through the lipid bilayer, facilitated diffusion through AQPs or through movement of water through plasmodesmata. Thus, the above data could also be interpreted in such a way that the Lp in cells close to the root tip reflects plasmodesmatal water transport, whereas the Lp of cells in more distal regions of the growth zone and in the mature region reflects water transport through AQPs. If this is the case, we cannot simply ask the question whether AQPs contribute to water uptake into expanding root cells, but we must distinguish between earlier and later stages of cell expansion. Also, at earlier stages of cell expansion, water supply appears to be through a root internal source (phloem), whereas at later stages of cell expansion, water supply occurs through an external source (root medium). This has implications for the direction of water movement (early, axial, tip-wards, radial from inner to outer tissues; late, axial, towards shoot; radial from outer to inner tissues) during cell elongation in roots and the driving forces that facilitate water movement through tissues.

Miyamoto et al. (2002) studied the gravitropic bending response of pea roots. Using the cell pressure probe, the authors observed that cell Lp in the part of growth zone where xylem was not fully developed averaged about 1.7×10^{-6} m s^{-1} MPa^{-1}. This value was higher than cell Lp in more mature root regions and about 100 times higher than tissue Lp in the growth zone. The authors explained the latter observation such that the growing cells with a high Lp obtained their water not from the root medium but through xylem vessels that were up to 50 cells (and 100 plasma membranes) located away from those growing cells. This supports some of the above data by Hukin et al. (2002) on maize.

Gambetta et al. (2013) carried out a detailed analysis of the expression of AQPs and tissue Lp in different developmental regions of the woody plant grapevine. The authors also distinguished between the very proximal portion of growth zone, which was rather meristematic, and the more distal portion of growth zone, where most cell elongation occurred. The authors observed much higher expression of most AQPs in the proximal portion of growth zone compared with the other root regions. This coincided with a higher tissue Lp in the proximal part, and Lp could be

inhibited through Hg. The authors concluded that even though mature root regions have lower AQP gene expression and tissue Lp compared with the other root regions, they contribute significantly to root water uptake of plants. The authors questioned the significance of the very tip region of roots for root water uptake, despite having the highest tissue Lp and AQP expression level, and raised the possibility that such high values may have little to do with root water uptake but with the volume expansion of cells. The above cited studies of Hukin et al. (2002) and Miyamoto et al. (2002) would support such an interpretation of data. Gambetta et al. (2013) observed highest expression of many AQP isoforms in the proximal and meristematic portion of growth zone, whereas Hukin et al. (2002) observed a much lower expression of the two AQP isoforms studied in proximal compared with distal regions. This difference may be due to the number of AQP isoforms and species studied. Our own data on the expression of AQPs in the proximal portion of growth zone of barley roots (Knipfer, Besse and Fricke, unpublished results) supports the data by Gambetta et al. (2013).

4.2 Relevance of Cell Lp for Root Growth

The above analyses highlight four major aspects, which must be considered when studying the role of AQPs in root cell expansion. Firstly, a high cell Lp as determined with the cell pressure probe does not necessarily reflect water flow through AQPs across the plasma membrane; it may actually reflect water flow through plasmodesmata (see Zhang and Tyerman 1991). Secondly, proximal and distal portions of the growth zone must be distinguished during analyses. Thirdly, the direction of water flow and the source of water supply to expanding root cells may differ between proximal and distal portions of the growth zone. Fourthly, and in relation to the previous aspect, the Lp of a growing root cell should be seen more in context of facilitating water flow through a tissue rather than facilitating water uptake at a sufficiently high rate into an individual cell to support that cell's expansion growth. This can be demonstrated through calculations where data on cell Lp, growth rates and cell dimensions are used.

Published values of the Lp of growing root cells are in the region 10^{-6} to 10^{-7} m s^{-1} MPa^{-1} (Miyamoto et al. 2002; Hukin et al. 2002) and generally not that different from values for mature root cells (e.g. Ehlert et al. 2009; Azaizeh et al. 1992; Lee et al. 2012), though differences in Lp between root regions have been reported (e.g. barley, Knipfer et al. 2011). Maximum relative growth rates of root cells are generally higher than those of leaf cells, with peak values half-way along the elongation zone of about 20–40 % h^{-1} (e.g. Miyamoto et al. 2002; Hukin et al. 2002). The volume of a typical root cortex cell half-way along the growth zone is in the range of 30–50 pl ($30–50 \times 10^{-15}$ m^3). The surface area of such a cortex cell, if we assume that it is shaped like a cube with 35 µm of each side, is about 40×10^{-9} m^2 (compare also, e.g. Miyamoto et al. 2002; Hukin et al. 2002). If we take here a relative growth rate of 30 % h^{-1}, a cell volume of 40×10^{-15} m^3 (40 pl) and a surface area of

40×10^{-9} m^2, we obtain a net rate of water uptake into growing cells of ($0.3\ h^{-1} \times 40 \times 10^{-15}\ m^3$) $12 \times 10^{-15}\ m^3\ h^{-1}$, or $3.3 \times 10^{-18}\ m^3\ s^{-1}$; the water flow rate per unit cell surface area calculates to ($3.3 \times 10^{-18}\ m^3\ s^{-1}$ divided by $40 \times 10^{-9}\ m^2$) $8.3 \times 10^{-11}\ m\ s^{-1}$. If we take an Lp of about $5 \times 10^{-7}\ m\ s^{-1}\ MPa^{-1}$, we see that a water potential difference of ($8.3 \times 10^{-11}\ m\ s^{-1}$ divided by $5 \times 10^{-17}\ m\ s^{-1}\ MPa^{-1}$) 1.7×10^{-4} MPa across the plasma membrane of the growing cortex cell is required to sustain such a net water uptake rate for growth. Thus, we calculate here theoretical water potential differences as small as 0.1–0.2 kPa. We do not know how large the actual water potential difference is across the plasma membrane of a growing root cortex cell. On the one hand, cells are thought to be in local water potential equilibrium with their immediately surrounding apoplast, and the water potential difference may be as small as, e.g. 1.7×10^{-4} MPa (0.17 kPa); on the other hand, water potential differences across the root cylinder of larger than 0.1 MPa (100 kPa) have been measured in growing root tissues (e.g. Miyamoto et al. 2002; Hukin et al. 2002). Even if 20 cells were located along the radial path, and provided that the hydraulic resistances of cells are comparable, the resulting water potential step across each cell would be by factor 100 to 1,000 larger than that required to sustain the volume expansion of these cells.

The most likely conclusion from these data is that cell Lp and plasma membrane localised AQPs do not limit the growth of individual cells through restricting their capacity to take in water. Rather, cell Lp and AQPs can limit the growth of root cells through limiting the rate of water supply to these cells through tissues. This conclusion is supported through the observation that the expression of AQPs in growing regions of roots is generally higher in stelar compared with more peripherally located tissues (e.g. Gambettta et al. 2013; Knipfer et al. 2011). The cells in the root stele are mostly much smaller than the cells in the cortex. If it was only for their own water demand during cell expansion, the smaller stelar cells would not need a higher expression of AQPs (and by implication plasma membrane water flow). However, the cells in the stele encounter much high radial water flow densities per unit cell surface compared with, e.g. cortex cells (Bramley et al. 2009), and this is independent of the direction of flow (from the epidermis towards the xylem or from the xylem towards the epidermis). The higher water flow densities are the most likely reason for the higher expression of AQPs in stelar tissue. In other words: a high expression of AQPs in growing stelar tissue relates more to the position of cells than to particular volume expansion rates.

4.3 Relevance of Cell Lp for Leaf Growth

There exist some detailed studies on the hydraulics of cell expansion in leaves, in particular leaves of grasses. Most studies have been carried out on maize, tall fescue and barley (e.g. Ehlert et al. 2009; Martre et al. 1999; Fricke et al. 1997; Fricke and Peters 2002; Bouchabké et al. 2006; Parent et al. 2009; Touati et al. 2015). These studies showed that there exist growth-induced water potential differences between

growing tissue and leaf internal water source (xylem). The implication is that growth is co-limited by hydraulic properties in addition to a mechanical limitation through wall-yielding properties. For example, the Lp of growing leaf epidermal cells in barley ranged from 0.4 (Volkov et al. 2007) to 2×10^{-6} m s^{-1} MPa^{-1} (Touati et al. 2015) and was slightly, though significantly, larger than the Lp of mature epidermal cells (Volkov et al. 2007). The same applies to the osmotic water permeability of mesophyll protoplasts when comparing growing with mature leaf regions (Volkov et al. 2007). One can, similar to the above calculations for roots, estimate the water potential difference across the plasma membrane of a growing leaf cell required to support water uptake for growth, given the dimension, growth rate and Lp of the cell. The conclusion is the same as for roots (Touati et al. 2015), in that the measured water potential difference across a tissue exceeds the one required to sustain growth by a factor of 1,000 or more. These data suggest that cell Lp and any growth-dependent expression of AQPs in leaves match more the need for trans-tissue transport of water than for sustaining volume expansion rates of an individual cell. The Lp values in the leaf epidermis are slightly at odds with this conclusion: as the epidermis is at the end of the water transport path from leaf xylem to leaf periphery, any high cell Lp in the epidermis contributes little to speeding up the overall transport of water from xylem to epidermis. So, why is cell Lp in growing epidermal cells high and also higher than in the mature leaf region, and why does this coincide with the tissue-specific expression of AQPs, as shown for barley (HvPIP1;1/1;6)? One possible explanation is that the cell Lp reflects extensive symplastic movement of water between epidermal cells, though previous studies on barley make this explanation unlikely (Fricke 2000). Another explanation is that water reaches the epidermis along bundle sheath extensions and then has to pass through 10, 20 or more lateral-located epidermal cells in succession to reach the end of its transport route (Fricke 2000); similarly, water may move over significant distances axially along a file of epidermal cells, from cell to cell, rather than being supplied to epidermal cells throughout along a radial transport route from xylem, via mesophyll/bundle sheath extension to epidermis. A third explanation could be that close to 100 % of the difference in water potential difference between epidermis and leaf xylem (ca 0.2–0.3 MPa) is generated upstream of the transport route of water, for example, at the parenchymatous and mestome sheath of vascular bundles (Fricke 2002; Heinen et al. 2009). In this case, the difference in water potential between two adjacent cells in the epidermis, or between adjacent mesophyll and epidermal cells, may actually be so small that it requires the high cell Lp observed for growing cells.

As for roots, we could ask for leaves whether the high cell Lp in the growth zone has also the potential function to speed up water transport in the proximal portion of growth zone, which is close to the meristem. This question is of particular interest when studying grasses, as these have an intercalary ‘apical’ meristem located at the base of leaves during the early vegetative growth stage of plants. This means that the cell production zone and portion of growth zone containing undifferentiated cells including xylem are located between the water supply route from the root (xylem) and more distally located growing and mature leaf region (Fig. 2). There exist some anatomical and biophysical studies on the hydraulics of this proximal meristematic

region, yet we do not know for sure how water gets through the very base of growing grass leaves (for a review, see Fricke 2002); at least the author is not aware of any study that answers this question conclusively. Therefore, it is possible that similar to roots, growing cells close to the leaf meristem require a high cell Lp to facilitate the movement of water through tissues. Detailed analyses of the Lp of cells in this leaf region are required.

5 AQPs: Facilitators of Water Uptake into Growing Plant Cells or…?

The above considerations allow several conclusions as to any involvement of AQPs in cell expansion in plants.

(i) There exist AQP isoforms that are expressed particularly in growing compared with nongrowing tissues, in roots and leaves and in all species examined so far.
(ii) Higher expression of AQPs in growing compared with nongrowing tissues is often associated with a higher cell Lp, and sometimes also tissue Lp, in growing tissue.
(iii) The portion of growth zone closest to the meristem – the 'proximal' growth zone – seems to be hydraulically isolated from the major internal source (xylem) of water supply, particularly in roots. This has implications for the interpretation of data on AQPs and cell Lp and renders the (very) proximal growth zone different from the more distal regions of the growth zone, which are located further away from the meristem.
(iv) Growing cells have a cell Lp that should easily satisfy their need for water uptake associated with volume expansion. Rather, the high cell Lp is required to facilitate water transport at a sufficient rate through the tissue. Exceptions from this rule could be cells that are located at the very periphery (epidermis) of growing organs.
(v) It is concluded from the above that any specific expression of AQPs and high values of cell Lp in growing tissues reflect less any growth-specific requirements of individual cells but are a consequence of the overall developmental state and water flow pattern in these tissues. The primary role of AQPs in growing plant tissues is not so much the net transport of water *into* cells but *through* cells.
(vi) To test the above conclusion requires the isolation of growing cells from their usual tissue environment and a comparison of the AQP expression pattern and cell Lp between such cells and cells that are retained within tissues. Also, detailed data are needed on the hydraulic architecture of the very proximal portion of growth zone in roots and leaves. In addition, analyses of cell/tissue growth in multiple AQP knockout/knockdown lines would be interesting to address this question (e.g. compare Reinhardt et al. 2016), especially if this can be combined with a downregulation of AQP expression in a tissue-/cell-specific manner.

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