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REVIEW PAPER

The divining root: moisture-driven responses of roots at the micro- and macro-scale

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Abstract

Water is fundamental to plant life, but the mechanisms by which plant roots sense and respond to variations in water availability in the soil are poorly understood. Many studies of responses to water deficit have focused on large-scale effects of this stress, but have overlooked responses at the sub-organ or cellular level that give rise to emergent whole-plant phenotypes. We have recently discovered hydropatterning, an adaptive environmental response in which roots position new lateral branches according to the spatial distribution of available water across the circumferential axis. This discovery illustrates that roots are capable of sensing and responding to water availability at spatial scales far lower than those normally studied for such processes. This review will explore how roots respond to water availability with an emphasis on what is currently known at different spatial scales. Beginning at the micro-scale, there is a discussion of water physiology at the cellular level and proposed sensory mechanisms cells use to detect osmotic status. The implications of these principles are then explored in the context of cell and organ growth under non-stress and water-deficit conditions. Following this, several adaptive responses employed by roots to tailor their functionality to the local moisture environment are discussed, including patterning of lateral root development and generation of hydraulic barriers to limit water loss. We speculate that these micro-scale responses are necessary for optimal functionality of the root system in a heterogeneous moisture environment, allowing for efficient water uptake with minimal water loss during periods of drought.

Key words: Hydropatterning, osmosensing, plant–water relations, root development, root system architecture, water stress.

Introduction

Water performs a diverse array of functions within plants, including acting as a carrier for solutes and nutrients, providing structural support through turgor pressure, and as a reactant in photosynthesis. Despite the central importance of water in the life of the plant, knowledge of the mechanisms by which plants perceive and respond to water availability in the environment is still limited. How do plants find water, and how do they respond when water availability is limited? Addressing these questions is interesting from a basic research standpoint, but is also crucial in improving water-use efficiency in agriculture.

A major challenge of the 21st century will be to meet global food demands as the world population approaches nine billion people (Godfray *et al.*, 2010). Agricultural output must increase, requiring more efficient use of finite resources if these needs are to be balanced with the preservation of natural ecosystems. Optimizing use of water by plants will be critical in this endeavour, as water is one of the most important limiting factors for crop production worldwide (Boyer, 1982; Premanandh, 2011). Worse, access to fresh water will probably become more unpredictable in the face of global climate change and extreme weather patterns (Godfray *et al.*, 2010).

The multi-year drought ongoing in the state of California carries with it a projected economic cost of $2.2 billion statewide, with losses in the agricultural sector totalling $1.5 billion (Howitt *et al.*, 2014). These economic and social considerations place greater emphasis upon the need to develop a detailed understanding of plant–water relations in the near future.

Plants are sessile organisms, and their physiology and development are closely intertwined with local environmental conditions. The water within a plant is first taken up from the soil by the roots, and the efficiency of uptake in a heterogeneous soil setting is largely dependent upon the architecture of the root system. An understanding of how roots sense and respond to local water availability is thus a key area in plant biology that requires particular attention. Many studies of plant–water relations have focused on processes that occur at the whole-plant or multi-organ level. However, this overlooks what happens at the micro-scale, where sensing and responses to water availability are likely to take place through the activity of specific protein complexes expressed in individual cells. The collective response of cells within tissues and organs ultimately gives rise to the larger-scale emergent phenotypes observed. This review highlights recent literature exploring responses to water at these various spatial scales within the root. There will be a focus on responses generated in both drought and non-drought conditions, and there will be additional discussion of potential mechanisms the root may use to sense local moisture.

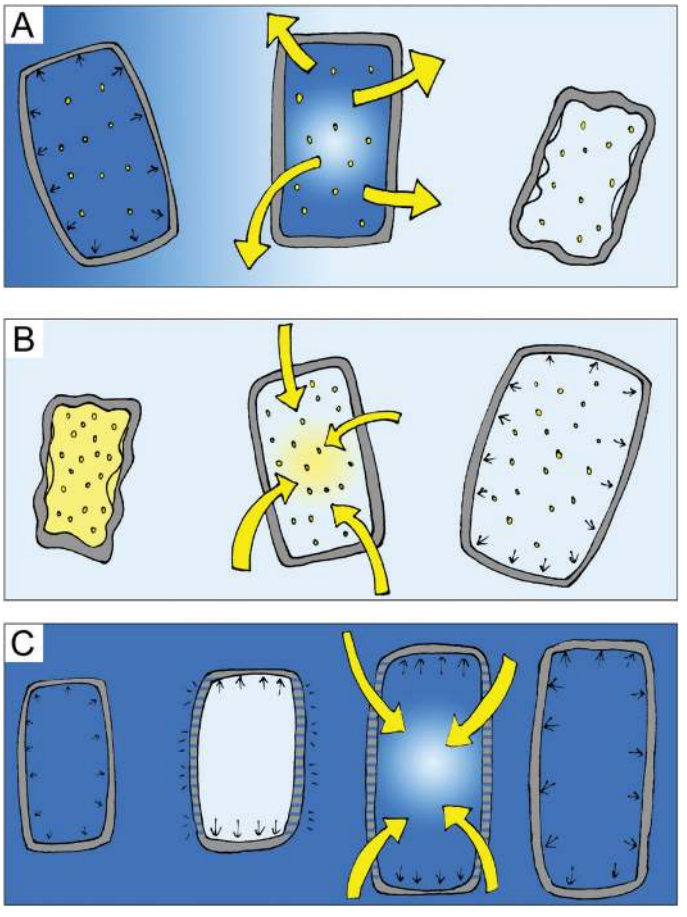

Fig. 1. Water potential is the driving force for water movement during growth and maintenance of turgor under stress. (A) A cell in non-stress conditions is depicted at full turgor due to the presence of intracellular solutes, which decreases its solute potential below the external water potential (left). When exposed to water stress, water loss from the cell leads to a loss of turgor pressure (centre) along with changes in cell volume and possible detachment of the plasma membrane from the cell wall (plasmolysis) (right). (B) Solute accumulation in the cytoplasm decreases the cell's water potential (left), driving water uptake (centre) to allow for restoration of turgor under water stress (right). (C) A cell at full turgor (far left) initiates growth by loosening of the cell wall, involving disruption of covalent and non-covalent bonds between the wall polymers in regions where the wall will be extended (centre-left). This decreases turgor pressure and concomitantly water potential, allowing for water uptake and extension of the cell wall (centre-right). Deposition of new wall material secures the cell wall at its new dimensions (far right).

Moisture signalling at the cellular scale

The plant cell is composed of an aqueous cytoplasm surrounded by a plasma membrane and a cell wall. The membrane is permeable to the movement of small, uncharged molecules such as water, but is relatively impermeable to the movement of many dissolved solutes (Kramer and Boyer, 1995). As a result, an increase in solute concentration on one side of the membrane dilutes the concentration of water molecules on that side, and water moves by osmosis from the opposite side until the two compartments come to equilibrium (Fig. 1A, B). The tendency of water to flow into a particular compartment is quantified by water potential (Kramer and Boyer, 1995). The water potential gradient between two compartments acts as the driver for water movement: water flows from areas of high potential to low potential. Water will continue to move between the cell and its environment until the two come to water potential equilibrium. Swelling and shrinking of the cell owing to water flow is largely prevented by the presence of a rigid cell wall, which resists deformation and allows turgor pressure to build within the cell (Kramer and Boyer, 1995). Thus, the total water potential of a cell is the sum of both osmotic potential, dictated by the concentration of solutes within the cell, and pressure potential, caused by pressure against the cell wall. Although several methods exist for quantifying the water status of plant tissues and growth media (Jones, 2007), water potential is based on a physically defined reference state and is thus directly comparable between laboratories and experimental conditions (Kramer and Boyer, 1995).

In a drying soil, the cells of the root lose water to the outside environment owing to a decrease in both soil osmotic potential and matric potential (caused by the adsorption of water molecules on surfaces of soil particles) (Kramer and Boyer, 1995). This water loss causes a loss of turgor pressure that may be accompanied by a decrease in cell volume depending on the hardness of the cell wall (Verslues *et al.*, 2006). The cells of the root must activate processes to limit water loss and mitigate its harmful effects.

Before acclimatory responses can be triggered, the cell must first perceive changes in the availability of water in the external environment. The mechanism by which perception

of such stimuli occurs has until recently been poorly understood. An early response to hyperosmotic stress is an increase in the concentration of intracellular calcium, which is thought to act as a signalling intermediate to activate downstream stress-response pathways (Knight *et al.*, 1997). As this calcium increase occurs quickly after exposure (within seconds), it is assumed that it must take place soon after the initial perception event in the sensory pathway. A recent advance in this effort was published by Yuan *et al.* (2014) detailing the identification of *REDUCED HYPEROSMOLALITY-INDUCED* $[Ca^{2+}]_i$ *INCREASE1* (*OSCA1*), a gene of *Arabidopsis thaliana* (*Arabidopsis*) that encodes a calcium channel protein involved in the osmotic stress response. *OSCA1* was identified from a mutant screen of *Arabidopsis* seedlings for defects in hyperosmotic-induced calcium increases. Mutant *osca1* plants exposed to osmotic stress exhibit reduced root growth and reduced stomatal closure compared with wild-type controls. The authors also observed an apparent decrease in leaf area in *osca1* mutants exposed to hyperosmotic stress, but given the time scale of the experiment (30 min), this change is probably attributable to increased leaf curling in the mutant. Changes in root growth and stomatal aperture induced by the water stress-associated hormone abscisic acid (ABA) occurred similarly to wild type, implying that OSCA1 functions upstream of ABA in the response to water stress. Heterologous expression of OSCA1 in cultured human embryonic kidney cells and electrophysiological experiments demonstrated that the protein could induce changes in intracellular concentrations of calcium and other cation species in response to hyperosmolality. These data support a role for OSCA1 in the initial perception and response of cells to water stress, providing an exciting tool for follow-up studies of the osmosensory pathway.

Is the book finally closed on identification of the elusive osmosensor in plants? The mutant screen identifying *OSCA1* had the additional criterion that mutant plants should be phenotypically normal throughout development under non-stress conditions (Yuan *et al.*, 2014). Although *OSCA1* is clearly involved in the response to hyperosmotic stress, its phenotype is normal outside of these conditions. Its role in the plant's response to gradual soil drying, which may differ from rapid hyperosmotic treatment, also remains to be explored. It is possible that other osmosensory proteins may exist to activate different responses depending on the nature of the water stress experienced by the plant.

Indeed, other proteins have been implicated as putative osmosensors in plants. One prominent example is *ARABIDOPSIS HISTIDINE KINASE1* (*AHK1*), which was discovered through a screen for *Arabidopsis* genes that could complement a yeast osmosensory mutant (Urao *et al.*, 1999). Although *ahk1* mutants have significantly higher rates of water loss during soil drying, this is probably due to a higher density of stomata on the leaf surface. No defects were observed in osmotic adjustment, as proline accumulation in response to water stress occurred normally in the mutant (Kumar *et al.*, 2013). Although these experiments do not rule out a role for *AHK1* in osmosensing, they suggest that it may play a limited role in adaptive responses to water stress. The *MECHANOSENSITIVE CHANNEL OF SMALL CONDUCTANCE-LIKE* (*MSL*) genes of *Arabidopsis* are also hypothesized to act as osmosensors based on functional homology to bacterial mechanosensitive ion channels involved in hypoosmotic stress tolerance (Kloda and Martinac, 2002; Haswell *et al.*, 2008). Two of the ten *MSL* genes, *MSL2* and *3*, encode proteins which localize to the plastid envelope; knockout of these two genes leads to swelling of the plastids, indicating that the organelles are under hypoosmotic stress (Wilson *et al.*, 2014). The double-mutant also exhibits increased solute accumulation under both standard and hyperosmotic stress conditions, which has led to the hypothesis that osmosensing could occur at organellar membranes to drive changes in the water status of the rest of the cell (Wilson *et al.*, 2014).

Proteins with roles in mechanoperception may also be important in moisture sensing, as changes in the water status of the cell can have mechanical effects on the membrane and cell wall that could be detected through such mechanisms. The *FERONIA* (*FER*) gene is one such example, which encodes a receptor-like kinase putatively involved in mechanosensing (Shih *et al.*, 2014). Mutants of *FER* show altered expression of touch-responsive genes during hypoosmotic stress and also have defects in root growth kinetics under standard conditions (Shih *et al.*, 2014). The appearance of a mutant phenotype even in the absence of an external environmental stress implies that osmosensory pathways may be important during the normal growth and development of the plant. We speculate that a strong osmosensory mutant would have severe developmental defects or may even cause lethality owing to the involvement of osmosensing and controlled water uptake in basic cellular processes such as elongative growth.

Water and growth at the cellular scale

The dynamics of water movement at the individual-cell level give rise to growth of the whole organ at a higher level of spatial organization. Growth of a root occurs through cell expansion in the elongation zone and is sustained by cell divisions in the meristem. The events that occur during growth at the cellular level are depicted in Fig. 1C. Preceding the onset of growth, the cell maintains positive turgor pressure against the cell wall through accumulation of intracellular solutes, such that its internal solute potential is below the external water potential. During growth, the cell wall relaxes through the activity of expansins and other wall-modifying enzymes, which leads to a decrease in turgor pressure (Cosgrove, 2005; Ober and Sharp, 2007; Schopfer, 2006). This drop in turgor drives water uptake and causes extension of the cell wall. Deposition of new wall material then allows for reestablishment of turgor and prevents regression of the cell wall back to its previous volume (Schopfer, 2006).

Uptake of water during growth would be expected to increase cellular solute potential over time owing to dilution of intracellular solutes. As the rate of water uptake is proportional to the magnitude of the water potential difference, cells must actively maintain a relatively constant solute potential to sustain a constant rate of growth (Schopfer, 2006).

Likewise in the cell wall, a balance must be struck between extensibility and deposition of new material—a wall that is too rigid will limit growth, whereas one that is too extensible will be susceptible to rupture.

Shachar-Hill *et al.* (2013) proposed that cell wall thickening is regulated in conjunction with solute accumulation during growth through an osmosensory mechanism. They tested this hypothesis through an examination of *in vitro* pollen tube growth. Pollen tubes elongate to many times their original length to fertilize female gametes, travelling a distance that can reach upwards of 20 cm in species such as maize (Dresselhaus *et al.*, 2011). Tube growth requires cell wall loosening and uptake of water to drive cell expansion, with the important distinction from most plants cells that loosening occurs primarily at the tip (Guerriero *et al.*, 2014). Treatment of *in vitro*-cultured elongating pollen tubes with mercury ions (Hg^+) led to tip bursting concomitant with a decrease in plasma membrane water permeability (Shachar-Hill *et al.*, 2013). The kinetics of these two processes across changing Hg^+ concentrations suggested that inhibition of the same protein was responsible for both phenotypes. The authors proposed that aquaporins, membrane-bound water channels which can be inhibited by Hg^+ (Li *et al.*, 2014), acted as osmosensing proteins in this context. A protein that acts as both a transporter and a sensor for its substrate, termed a 'transceptor', is not unheard of in the literature (Krapp *et al.*, 2014; Ho *et al.*, 2009). However, an alternative explanation we propose is that aquaporins might function to relieve excessive turgor pressure in the fragile tube tip by allowing water to move out of the pollen tube during growth. To substantiate the proposed role of aquaporins as water transceptors, additional experiments are necessary. If aquaporins are truly responsible for tip bursting, a plasma membrane-localized aquaporin should be expressed in pollen tube cells, but such an aquaporin has yet to be identified (Shachar-Hill *et al.*, 2013). Bursting should also be elicited by other aquaporin inhibitors aside from Hg^+ (Li *et al.*, 2014). Regardless of the identity of the sensor, this work demonstrates a role for fine-scale osmotic control within normal growth and development of the plant.

Regulating growth of the root under water stress

Coordinated changes in cell wall properties and turgor pressure drive water uptake and allow cells to grow. When external water becomes less available, the cell may not be able to maintain sufficient turgor to increase volume after wall yielding has occurred. To circumvent this, a decrease in cellular solute potential beyond normal levels will increase turgor pressure and enable growth in drier environments (Verslues and Bray, 2006; Verslues *et al.*, 2006).

Regulation of cell wall extensibility is another strategy cells use to maintain growth under water stress (Ober and Sharp, 2007). A recent set of publications has explored the role of hydrogen peroxide (H_2O_2) in increasing the extensibility of the cell wall under drought. It was previously shown that the length of the elongation zone in maize primary roots decreases in response to water deprivation (Sharp *et al.*, 1988; Sharp *et al.*, 2004). Comparative proteomic and gene expression profiling was done on drought-stressed and well-watered maize roots to gain further insight into this phenomenon (Spollen *et al.*, 2008; Zhu *et al.*, 2007). These experiments revealed an increased abundance of proteins involved in metabolism of reactive oxygen species (ROS), implicating a potential role for H_2O_2 in maintaining growth during water stress (Voothuluru and Sharp, 2013). H_2O_2 can act as a source for production of hydroxyl radicals, which can increase wall extensibility through non-specific cleavage of polysaccharides (Fry, 1998; Müller *et al.*, 2009; Schopfer, 2006). Accordingly, water-stressed maize roots were shown to accumulate higher levels of apoplastic H_2O_2 in the growth zone, suggesting a role for wall modification in maintaining growth under drought (Voothuluru and Sharp, 2013).

Osmotic adjustment and changes to cell wall properties can help facilitate water uptake into cells to maintain growth under water deficit. But like many aspects of the soil environment, the distribution of water is not uniform. If external water is locally limiting, water from moist regions of the soil column can be transported by roots to drier regions. This phenomenon, known as hydraulic redistribution or hydraulic lift, has been observed in many species and may be a mechanism to increase water and nutrient availability in dry topsoil, amongst other roles (Neumann and Cardon, 2012).

The importance of hydraulic redistribution in maintaining root growth was examined by Boyer *et al.* (2010). Growth of wheat roots was monitored following removal of external and/or internal sources of water to assess the relative contributions of each source to supporting elongation. Root tips suspended in air, supplied only by water transported internally, elongated at a rate that was 45% of soil-grown roots. The role of externally supplied water alone was examined by excising the tips of soil-grown roots 12–22 mm distal to the root tip; these tips continued their growth 30 min after excision at a rate that was 74% of intact roots. Removal of both internal and external water supplies, done by placing excised root tips in air, resulted in no growth. These results illustrate that both external and internal supplies of water play a role in elongation at the root tip.

Although much of the long-distance water transport that occurs within plants happens through the xylem, phloem tissue differentiates and develops transport capabilities closer to the root tip where internal water would have to be delivered for growth (Boyer *et al.*, 2010; Wiegers *et al.*, 2009). Boyer and colleagues reasoned that internally supplied water used during growth is delivered through the phloem based on calculations of changes in dry weight of the tissue during growth (a measurement of delivery of fixed carbon) and the concentration of photosynthate in phloem sap (Boyer *et al.*, 2010).

The effect of phloem-delivered water on the water potential of cells at the maize root tip has been modelled computationally (Wiegers *et al.*, 2009). A series of analyses was performed to assess how changes in various parameters, such as the size of the root and hydraulic conductivity of the tissues, would affect phloem water delivery and water potential of the tissues.

Although water flows in the direction of the water potential gradient, hydraulic conductivity quantifies the rate at which water will move along a particular path owing to the resistance of that path to water movement. Interestingly, the authors observed in their *in silico* model that development of active phloem closer to the tip led to higher tissue water potentials than if the phloem developed further away (Wiegers *et al.*, 2009). Vascular development is known to occur closer to the root tip at higher temperatures (Beauchamp and Lathwell, 1966); modulation of this process under drought could serve as an additional mechanism for acclimation to water stress by enhancing delivery of phloem water.

Although plants are often discussed as sedentary organisms victim to the whims of a changing climate, tropisms allow plants to direct growth towards more favourable conditions and potentially avoid stresses such as water deficit. One such process is hydrotropism, the directed growth of the root tip toward regions of higher water availability (Moriwaki *et al.*, 2012). Increasingly sophisticated experimental setups have been devised for detailed characterization of the hydrotropic response both on artificial growth media (Kobayashi *et al.*, 2007) and in a more natural soil context (Iwata *et al.*, 2013). These have allowed for the identification of the first molecular components involved in hydrotropism that may play a role in supporting the growth of the plant under drought (Kobayashi *et al.*, 2007; Miyazawa *et al.*, 2009). However, much concerning this adaptive response remains to be discovered, including the full cohort of genes involved in the process and the mechanism of perception of the environmental signal; a more extensive summary of the current understanding of hydrotropism can be found in a recent review (Moriwaki *et al.*, 2012).

Patterning lateral root development: from organs to an organ system

The cells of the root tip are capable of integrating information about the local moisture environment into their physiology to allow for continued growth when water becomes less available. Recent work has shown that the distribution of water also influences the form and function of tissues that are left behind following growth. These tissues tune their development to take advantage of spatial heterogeneity of moisture in the environment, tailoring the root system for optimal water uptake and transport (Fig. 2; Bao *et al.*, 2014).

Lateral branching is an important target of moisture-regulated developmental patterning. Lateral roots make up the largest portion of the absorptive surface area of the root system, and their development is strongly linked to environmental inputs including water availability (Gruber *et al.*, 2013; Malamy, 2005). Babé *et al.* (2012) showed that the response of lateral root development to water stress occurred within specific developmental windows. Roots of *Hordeum vulgare* (barley) and *Zea mays* (maize) grown in aeroponics were subjected to transient water stress by withholding water for a period of 4–8 h. This led to inhibition of lateral root development in a specific region of the root. The investigators

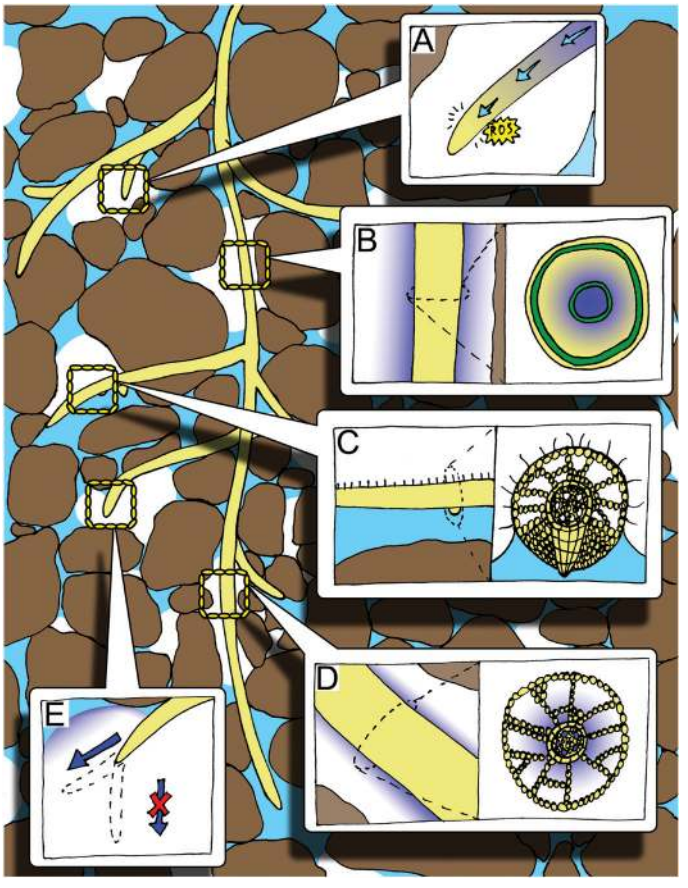

Fig. 2. Root adaptations for growth, water uptake, and transport in an environment of heterogeneous moisture. The root experiences small-scale variations in contact with soil particles (brown), water (light blue), and air (white) that can elicit various physiological and developmental responses. (A) Phloem delivery of water (arrows) and enhanced wall loosening mediated by reactive oxygen species (ROS) support the growth of a root tip in a region of low water availability. (B) Accumulation of hydrophobic barriers in the endodermis (inner ring) and exodermis (outer ring) limit water loss of the root to dry soil during long-distance transport. Areas of higher water potential near the centre of the root that could result in such an environment are depicted using a blue gradient. (C) A root growing along an air–liquid interface exhibits hydropatterning, developing new lateral branches toward available water and aerenchyma and root hairs on the side exposed to air. (D) A segment of the root exposed to an air pocket can be subject to water loss, but is buffered externally owing to the low hydraulic conductivity of air. Development of cortical aerenchyma reduces the metabolic cost of maintaining this root and may further limit radial loss of water from the vasculature. (E) Hydrotropism directs growth of a root toward regions of higher water availability.

determined a 'target window' for responsiveness to the stress—regions that had already developed above the window produced lateral roots regardless of exposure to water deficit, and regions that newly formed below the window produced lateral roots in response to rewatering (Babé *et al.*, 2012).

Research performed by our group refined our understanding of the spatial scale at which moisture regulates root branching, and revealed that the root is sensitive to the spatial distribution of moisture across the circumferential axis (Bao *et al.*, 2014). It was observed that roots growing along the surface of a wet medium produce more lateral roots on the side directly contacting the surface, with relatively few emerging toward air. In contrast, the air side has a higher abundance

of root hairs, single-cell extensions from the epidermis. While root hairs are typically thought to increase the absorptive surface area of the root, their high abundance on the air side has led to our hypothesis that they may also serve to bridge low-conductivity air gaps in the soil and connect the root with liquid water adsorbed to more distant soil particles.

Although it was clear that contact of the root with a wet surface induced spatial bias in the development of tissues, additional investigation was required to determine what aspect of the environment could be responsible for driving such responses. It was demonstrated that the bias in lateral root development was not an artefact of the *in vitro* growth conditions, as the phenotype was consistently observed across many types of growth media including natural soil. Thus, a gradient of an environmental signal that was common across growth conditions was probably at work. The distribution of available water, mechanical contact, and gradients of gases such as oxygen were all potentially informative for developmental patterning, amongst other possibilities.

Whether water availability affected this process was determined by locally decreasing the water potential of the media. This was done by placing the root between two blocks of growth media with different water potentials (Bao *et al.*, 2014). Fewer lateral roots emerged on the side of the root exposed to low-water-potential media without a significant decrease in the number on the side exposed to high-water-potential media. It was also observed that placing various non-water-conducting materials between the root and one of the agar blocks (e.g. a thin sheet of plastic or rubber) eliminated the inductive effect of that surface on lateral root development. These results support the hypothesis that local water availability serves as a cue to position lateral roots, and also rule out a role for mechanical contact alone in activating the process. Hence, we have coined the term 'hydropatterning' to describe this novel developmental phenomenon.

These results also provide an explanation for the key difference between air and wet media that is detected by the root during hydropatterning. The culture plates for our *in vitro* growth conditions are sealed from the external environment to maintain sterility and limit loss of moisture. Thus, the air in the plates is expected to be at water potential equilibrium with the growth media. This makes it unlikely that water potential alone is the signal used by the root to distinguish between the two environments, since there may be little to no difference in this parameter. Rather, hydraulic conductivity may be the key difference; air has a remarkably low hydraulic conductivity compared with soil and other growth media, which greatly limits the rate at which water moves through it (Nobel and Cui, 1992). We hypothesize that the root locally measures its ability to take up water from the external environment, which depends upon both the water potential gradient and hydraulic conductivity, as the cue for this process.

How might the root detect water flux to pattern development? To address this question, the region of the root that is receptive to the hydropatterning signal must be considered first. Contacting roots of *Arabidopsis* with a wet surface does not induce new lateral roots from previously air-exposed tissues, suggesting that developmental plasticity becomes limited with developmental maturation (Bao *et al.*, 2014). Results of further experiments performed on maize primary roots has suggested that the root loses its ability to respond to the hydropatterning signal beyond the elongation zone (NER, unpublished results). Once this transition is made, the developmental programme is determined and generally cannot be altered by new changes in the moisture environment. Based on these observations, we hypothesize that the root detects water availability during cell growth to drive hydropatterning.

Cell elongation requires water uptake, and the rate of flux depends upon the level of water availability in the environment. Growth may therefore be a way for the cell to 'test the waters', measuring water availability based on how hard it has to pull on the surrounding water source to attain a certain rate of growth. Cells that cannot take up water from the external environment might then pull in water through neighboring cells that are directly contacting a source of available water, which would require a water potential gradient across the tissue. This gradient could serve as a signal for establishing the developmental differences across the radial axis that are observed in hydropatterning. However, if hydraulic conductivity, and thus the rate of water movement through the tissues, is high, then the magnitude of such a gradient may not be sufficiently large to be detectable by the cells. Bulk movement of water between tissue layers could also carry dissolved solutes, creating chemical gradients across the diameter of the root that may also be informative for environmental sensing and developmental patterning.

Biosynthesis and transport of auxin, a plant hormone involved in lateral root production, are locally activated in response to contact with water, providing a link between the external environmental signal and activation of lateral root development deeper within the root (Bao *et al.* 2014). Hydropatterning acts very early in the process of lateral root development, and probably controls the positioning of the founder cells that will later undergo divisions to generate new lateral root primordia (Bao *et al.*, 2014). This is further corroborated by the observation that roots lose their developmental plasticity for hydropatterning as they mature. Local suppression of lateral root development by transient water deficit also seems to be permanent based on rewatering experiments (Babé *et al.*, 2012); however more quantitative analysis of these effects are needed. Together, these results imply that short-term water deficit near the root tip, or lack of contact of the root tip with available water in the case of hydropatterning, can have long-term impacts on root system architecture. Roots may still be able to explore previously dry patches of soil upon rewatering if lateral roots that have already emerged elsewhere grow into such regions, possibly guided by hydrotropism, and generate new lateral branches.

Patterning of hydraulic barriers to water movement

Along with the distribution of lateral roots, other root anatomical traits can be shaped by the local distribution of moisture in the environment. The root seems to utilize several

types of hydraulic barriers to locally control water movement between itself and the environment. The positioning of these low-conductivity blockades may be an important strategy to maximize water uptake and minimize water loss in a heterogeneous soil environment.

Owing to its low hydraulic conductivity, air may serve as an important buffering agent to limit water movement. The root can generate such a barrier internally through the development of aerenchyma, a type of tissue which contains large air pockets that can appear in cortical layers of larger roots. Local development of cortical aerenchyma may be an important adaptation to water-stress conditions. Plants with higher levels of cortical aerenchyma seem to have greater tolerance for drought stress (Lynch, 2013; Zhu *et al.*, 2010), which is thought to be due to the reduced metabolic cost of maintaining a highly aerenchymatous root in a dry environment. Aerenchyma development may play the additional role of allowing for local control of water flow between the root and the environment. We speculate that, in tissues exposed to water-stress conditions, internal air spaces could act as buffers to water loss from the central vasculature out to the external environment owing to the low hydraulic conductivity of air. A similar phenomenon was proposed by Nobel and Cui (1992) concerning the role of air gaps that form between the root and soil as the soil dries. Roots of *Opuntia ficus-indica* were observed to shrink in response to low external water potential, which was thought to occur owing to decreased cell volume as a result of water loss to the soil. As this progressed, low-conductance air gaps formed owing to separation of the root from the surrounding soil, restricting further water loss from the root. Rewatering would then lead to an increase in cell volume and root diameter and restore root–soil contact for water uptake. This strategy was proposed to provide the root with 'rectifier-like' qualities, permitting water uptake in moist soils while limiting its loss in dry soils (Nobel and Cui, 1992; Nobel and Sanderson, 1984).

Aerenchyma development occurs more prominently in air-exposed tissues of maize and *Oryza sativa* (rice) roots when grown along a wet surface (Bao *et al.*, 2014). Although aerenchyma is primarily thought to act as a conduit for gas movement during flooding stress (Colmer, 2003; Evans, 2003), it can also form in response to other environmental signals such as water deficit. Karahara *et al.* (2012) provided the first demonstration that the radial distribution of aerenchyma can be patterned by local differences in osmotic potential of the surrounding media. The local induction of aerenchyma in response to air that is observed in hydropatterned roots suggests that differences in external hydraulic conductivity can also influence the patterning of aerenchyma, highlighting the rate of water uptake as being an important signal in this process similar to our observations regarding lateral root patterning. Roots can also modify other tissue layers to establish barriers to water loss under drought. A well-known barrier layer in roots is the endodermis, in which a lignin-based Casparian strip forms between cells to limit radial movement of solutes in and out of the stele through the apoplast (Geldner, 2013; Naseer *et al.*, 2012; Robbins *et al.*, 2014). Solutes must enter the symplast and travel intercellularly through plasmodesmal connections to traverse the Casparian strip. Accumulation of suberin lamellae on the surfaces of endodermal cells later in development further requires that entry into the symplast occur in more outer tissue layers before reaching the endodermis (Geldner, 2013).

The exodermis, a specialized tissue that develops one layer below the outermost epidermal cell layer in some species, can serve as an additional barrier to solute movement (Hose *et al.*, 2001; Enstone *et al.*, 2003). Lignin and suberin are both composed of hydrophobic polymers, suggesting that their deposition might influence water flow through the root. However, the literature gives contradictory accounts of their roles in radial hydraulic conductivity, with studies indicating significant (Hose *et al.*, 2001; Meyer *et al.*, 2011; Pfister *et al.*, 2014) or insignificant (Garthwaite *et al.*, 2006; Ranathunge *et al.*, 2011) effects. Variations in results could be due to differences in environmental conditions and methods of quantifying hydraulic conductivity. The conductivity of non-barrier tissues could be another confounding variable, as these would permit entry and movement of water by the symplastic route that would be less subject to restriction by the endodermis and exodermis (Hachez *et al.*, 2012). Nonetheless, development of these two barriers can be affected by external water availability (Hachez *et al.*, 2012; Henry *et al.*, 2012; Meyer *et al.*, 2011), including in the context of hydropatterning (Fig. 3; NER, unpublished results), leaving the possibility open that they play a role in limiting water loss under some conditions.

Expression, localization, and gating of aquaporins provide another route for locally modulating tissue hydraulics. The role of aquaporins in water stress has been recently reviewed (Aroca *et al.*, 2012); although tissue conductivity tends to be decreased during drought, aquaporin activity does not follow a consistent trend between isoforms, species, and experimental conditions. Such inconsistencies could be indicative of the myriad ways in which aquaporin activity can be regulated; besides transcriptional regulation, other regulatory mechanisms could also play important roles under drought, such as protein localization and post-translational modifications (Li *et al.*, 2014). Additionally, isoform-specific or cell type-specific regulation of aquaporins may be important to regulate water flow between different tissue layers of the root and between the root and the soil environment. For example, water flux between cell layers is tightly regulated during lateral root development, where both knockout and overexpression of a single aquaporin gene can lead to defects in emergence of developing primordia (Péret *et al.*, 2012). Greater focus on individual aquaporin isoforms will be necessary to further our understanding of the functionality of these channels, although the level of genetic redundancy inherent to the family may hinder these efforts. This will be assisted by various tools for analysing and altering gene expression and protein activity at the cell type-specific level, such as those used in a study of aquaporin expression in roots of grapevine (*Vitis berlandieri* × *Vitis rupestris*) (Gambetta *et al.*, 2013) and in past studies by our group (Duan *et al.*, 2013; Geng *et al.*, 2013).

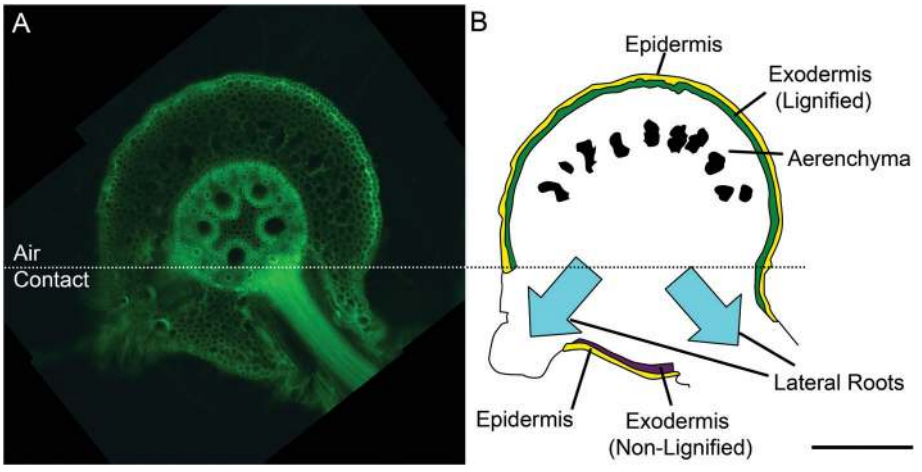

Fig. 3. Accumulation of lignin in the exodermis is patterned by the distribution of moisture in maize roots. (A) Maize kernels were sterilized and roots were grown along agar media to observe hydropatterning as previously described (Bao *et al.*, 2014). 5 days post-imbibition, primary roots were embedded in 2% agar and 200 μm-thick radial cross-sections were taken about 5 cm behind the root tip using a vibratome. Sections were cleared using the protocol from Malamy and Benfey (1997) and imaged on an inverted compound microscope using a GFP bandpass filter to observe lignin autofluorescence (Alassimone *et al.*, 2010). This representative image shows higher fluorescence intensity on the air side in the exodermis, the tissue layer below the outermost epidermal layer. The air and contact sides were distinguished by the positioning of root hairs and lateral roots. (B) Diagram highlighting various features of the fluorescence image. Scale bar=0.5 mm.

Conclusion and perspectives

Water has an impact on all levels of root development, from the activity and growth of single cells and organs to the architecture and functionality of the entire root system. Low water availability in the soil can act as a stress, but water can also act as a positional cue to pattern development outside of drought-stress context. How does the plant perceive and transduce this signal to generate a response? Activation of calcium signalling through the hyperosmotic stress-responsive calcium channel OSCA1 provides one mechanism, but others are likely to exist.

What proteins might serve as sensors of the moisture environment? A candidate approach can often prove fruitful in identifying genes involved in this process. Based on recent literature (Shachar-Hill *et al.*, 2013), the aquaporin family is one starting point for reverse-genetic analyses. Additional candidates can be identified through searches for plant homologues to components of known osmosensory pathways in other organisms. Such a route led to identification of the aforementioned AHK1 and MSL proteins, which have homology to proteins in yeast and bacteria, respectively. Forward genetics can also be a powerful tool in this endeavour, as has been demonstrated for hydrotropism (Kobayashi *et al.*, 2007) and the hyperosmotic stress response (Yuan *et al.*, 2014). However, care must be taken in experimental design to avoid isolating mutants in downstream moisture-associated pathways such as ABA. Strict screening criteria to focus on one or a few processes may also be beneficial—even processes that occur outside of severe water stress may proceed through different signalling networks with different levels of sensitivity. A water potential gradient *per se* is sufficient but not necessary to generate differential developmental outcomes between the air and contact sides of the root during hydropatterning, whereas such water potential gradients seem to be necessary for hydrotropism (Moriwaki *et al.*, 2012).

One can easily conceive a set of criteria for narrowing down a list of candidate proteins in order to identify a true osmosensor. For instance, the protein will likely be necessary for generating one or several moisture-associated responses. Such a protein might also act far upstream in its signalling pathway, which will assist in distinguishing sensors from related proteins involved in signal transduction downstream of the initial sensing event. In addition, the protein must be in the right place at the right time: that is, expressed in the cell or tissue type in which sensing is hypothesized to occur, and present at times preceding onset of the moisture stimulus. However, the key defining feature of an osmosensor is its ability to directly link the moisture environment to a molecular-genetic output. Demonstrating this link experimentally will likely be the most challenging step in identification of a novel osmosensor, as the most appropriate experimental design for validation may be unclear. In such cases, perhaps the best approach lies in first generating a hypothesis on how moisture-sensing is likely to occur on a molecular level for a particular process, and then testing whether a candidate osmosensor acts through this mechanism.

Thus, the elucidation of novel moisture-sensing pathways will no doubt be assisted by a bit of creativity. If a strong candidate for an osmosensor is identified with no clear mechanism of action, what moisture-sensing mechanisms can we hypothesize? Perhaps the water status of the cell is measured based on sensing of an internal signalling molecule whose concentration changes based on the total amount of water in the cell. Such a mechanism has been demonstrated for gibberellins, a class of hormones that can activate cell growth. The resulting water uptake during growth leads to dilution of the gibberellin and termination of the signal in a negative feedback loop (Band *et al.*, 2012). Alternatively, increases in water content of the cell could change the conformation of a regulatory protein whose folding depends upon its hydration status, such as a LEA protein (Shih *et al.*, 2008). Developing theoretical frameworks such

as these for how moisture sensing might occur can be informative for candidate approaches, forward genetics, and other approaches in order to identify the molecular actors involved, and may lead to elucidation of novel osmosensory pathways.

Significant strides have been made in furthering our understanding of root responses to water availability in recent years. However, water may have effects on the physiology and development of the plant that still remain to be discovered and characterized. Root hydropatterning is a good example of this, and future analyses of this previously unknown phenomenon may pave the way toward identification of new sensory pathways. Further, there are many well-known responses to water availability with unknown mechanisms for their activation. Putting these various responses into a molecular-genetic context—connecting physiology and development to sensing and signal transduction from the cellular to organismal scale—will be the challenge for future research in the field of plant-water relations. Bridging this gap in understanding will further our knowledge of how plants detect and respond to this fundamental molecule, and will provide the foundation for improving efficiency of water use for crop production to sustainably feed the growing population.

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