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Developmental Responses to Water and Salinity in Root Systems

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Abstract

Roots provide the primary mechanism that plants use to absorb water and nutrients from their environment. These functions are dependent on developmental mechanisms that direct root growth and branching into regions of soil where these resources are relatively abundant. Water is the most limiting factor for plant growth, and its availability is determined by the weather, soil structure, and salinity. In this review, we define the developmental pathways that regulate the direction of growth and branching pattern of the root system, which together determine the expanse of soil from which a plant can access water. The ability of plants to regulate development in response to the spatial distribution of water is a focus of many recent studies and provides a model for understanding how biological systems utilize positional cues to affect signaling and morphogenesis. A better understanding of these processes will inform approaches to improve crop water use efficiency to more sustainably feed a growing population.

Contents

INTRODUCTION

Plants don't behave; they develop. Perhaps one of the most fascinating aspects of the biology of plants lies in their constant utilization of environmental context as a source of information that guides the shape and function of their bodies (Dinneny 2015). Plants lack structures that we intuitively associate with sensing of the environment (e.g., eyes, ears, a nervous system) and yet are able to detect the complexities of their environments in analogous ways (Fankhauser & Christie 2015, Hamant & Haswell 2017, Monshausen & Gilroy 2009, Robbins & Dinneny 2015).

A grand challenge for biology is to understand how sensing of environmental stimuli regulates the physiology of plants. Such discoveries have broad impact, from improving the efficiency and sustainability of agriculture, to informing our understanding of the function of ecological systems, to improving computational models that help predict the effects of climate change. Roots, in particular, are an excellent system on which to focus attention, as they develop through relatively simple mechanisms but exhibit striking sensitivity to a broad range of environmental parameters (Morris et al. 2017, Rellán-Álvarez et al. 2016, Van Norman et al. 2013, Wachsman et al. 2015). The establishment of root system architecture can be viewed as being primarily the result of three developmental processes: tissue growth, branching, and orienting growth with respect to gravity. The growth rate of roots is partly determined by anisotropic cell expansion that elongates the organ and forces the tip through the soil matrix (Bengough et al. 2011, Sharp et al. 1988). Branching increases the volume of soil that can be accessed and the number of root tips, which are key sites for resource acquisition (York et al. 2016). Finally, the angle of tip growth, with respect to the gravity vector, determines the depth and radial expanse of soil that is explored (Morris et al. 2017).

ORGAN-SCALE RESPONSES TO THE SPATIAL DISTRIBUTION OF WATER AND SALINITY

Since the 1800s, roots have been known to sense the direction of stimuli such as touch and water (Darwin & Darwin 1881, Dietrich 2018). Research in this area has recently seen a renaissance

through the use of molecular, genetic, and physiological approaches that have begun to identify the specific cell types and signaling pathways that allow roots to translate spatial information on water availability into developmental decisions. Here, I do not cover important topics such as the water-associated regulation of primary root growth or the cell type–specific differentiation of tissues such as the exodermis and endodermis, as these are rich areas of research that were recently reviewed (Augstein & Carlsbecker 2018, Doblas et al. 2017, Feng et al. 2016). Instead, I largely focus on how the sensing of water and salinity leads to local changes in growth that impact the architecture of the root system and its ability to capture resources.

Orienting Root Growth Toward Water

Hydrotropism represents one of the earliest discovered developmental responses that plants have for water. Classic experiments by the Darwins, Von Sachs, and others revealed that roots are able to grow preferentially toward environments with a higher relative water potential (Cassab et al. 2013, Dietrich 2018, Moriwaki et al. 2013). These early experiments demonstrated that plants sense both the presence and direction of moisture in the environment. Importantly, such gradients in water potential can occur in air or on hydrated media, suggesting that conductance of water into the tissues may not be a critical factor in the sensing mechanism, since air has limited conductance (Antoni et al. 2016, Eapen et al. 2015, Kobayashi et al. 2003). Species vary in their hydrotropic responsiveness, which can be masked by the dominant effects of gravitropism. For example, in cucumber, roots grown in humidified air chambers with a water potential gradient show no hydrotropic curvature, while seedlings grown on a clinostat (a rotating device that cancels out the gravitropic cue) or in which the root tip has been excised show a significant response (Fujii et al. 2018).

Gravitropism does not dominate the hydrotropic response of *Arabidopsis*, and mutant screens in this species have led to the identification of genes essential for the response (Eapen et al. 2003, Kobayashi et al. 2003). The *MIZU KUSSEI1* (*MIZ1*) gene encodes a protein of unknown molecular function residing on the cytoplasmic surface of the endoplasmic reticulum (ER) (Kobayashi et al. 2007, Yamazaki et al. 2012). *MIZ1* expression is enriched within the cortex tissue layer of the root, which is also where *MIZ1* likely functions, as tissue-specific rescue experiments have suggested (Dietrich et al. 2017, Moriwaki et al. 2013). The importance of the cortex cell layer in hydrotropism was further supported by studies of the SnRK2 subfamily. These protein kinases act in osmotic stress signaling, including in the perception of abscisic acid (ABA) (Fujii et al. 2009). The *snrk2.2/2.3* double mutant exhibits a substantially reduced hydrotropic response, and this response can be rescued through the expression of SNRK2.2 in the cortex cell layer, but not in other tissues of the root (Dietrich et al. 2017). These results are particularly exciting, as they suggest that ABA signaling in the cortex may function together with *MIZ1* to mediate a moisture response that affects differential cell elongation and root curvature (**Figure 1**). Indeed, past work has demonstrated that ABA biosynthesis is necessary for hydrotropism; however, it may not act as a spatial cue (Moriwaki et al. 2012). Perhaps ABA levels in the root act as a potentiating signal that enhances the response of roots to moisture cues without determining the orientation of growth. This response would have the predicted effect of enhancing the strength of the hydrotropism pathway when water is limiting.

Mysteries remain regarding how and where root cells sense differences in water potential and how these signals are integrated to coordinate differential cell elongation across the root axis (Dietrich 2018). Laser ablation experiments show that loss of the columella disrupts gravitropism, but not hydrotropism (Dietrich et al. 2017). Indeed, ablation of the entire meristem has little effect on the development of hydrotropic curvature, which predominantly occurs in the middle of
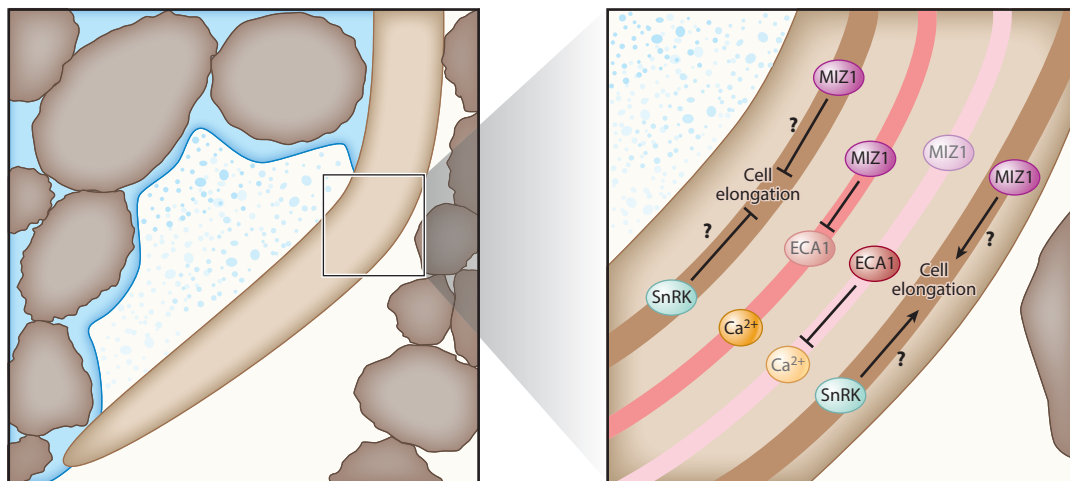
Figure 1

Water potential gradients induce hydrotropic growth. (*Left*) A root tip experiences a gradient in moisture through humid air. Curvature is induced in the elongation zone. The black box is enlarged in the right panel. (*Right*) Signaling pathways control differential cell elongation during hydrotropism.

the elongation zone, while gravitropism primarily affects cell elongation in the root tip–proximal part of this zone. The observation that sensing and response may occur in the elongation zone is intriguing, as another moisture-sensing pathway, hydropatterning, also occurs in this region of the root (Robbins & Dinneny 2018). As discussed below, Robbins & Dinneny (2018) hypothesize that tissue growth may be necessary for organs to sense spatial differences in water availability across the root axis.

Determining the molecular function of MIZ1 and the proteins that it regulates will likely provide important clues as to how moisture is sensed in the root. Recent work by Shkolnik et al. (2018) has identified a potential clue to this mystery by examining the spatiotemporal changes in Ca^{2+} accumulation during hydrotropic stimulation (**Figure 1**). Using the YC3.6 chameleon FRET sensor (Krebs et al. 2012, Nagai et al. 2004), the authors revealed that 50–80 min of exposure to a moisture gradient causes a difference in cytoplasmic $[Ca^{2+}]$ in the phloem of roots, with greater accumulation occurring in the phloem pole oriented toward the environment with the highest water potential (Shkolnik et al. 2018). This change in cytoplasmic $[Ca^{2+}]$ occurs within the root elongation zone and precedes formation of curvature in the root. *MIZ1* is necessary for the change in $[Ca^{2+}]$ but also appears important for the generally elevated levels of Ca^{2+} in the phloem even under control conditions. Interestingly, Ca^{2+} levels in the ER drop during hydrotropic stimulation, suggesting that this subcellular compartment may be the source of the Ca^{2+} that is released. Indeed, Shkolnik et al. (2018) identified a Ca^{2+}-ATPase named ECA1, which loads Ca^{2+} into the ER. Loss of function in *ECA1* results in enhanced hydrotropic curvature and increased cytoplasmic $[Ca^{2+}]$, suggesting that *ECA1* plays a negative role in mediating the response. MIZ1 directly interacts with ECA1, and in yeast, MIZ1 inhibits ECA1 Ca^{2+} transport activity. Shkolnik et al. propose that MIZ1 may mediate the response to water-potential cues by inhibiting the activity of ECA1 in the phloem and that such inhibition then causes an increase in cytoplasmic $[Ca^{2+}]$ that is transduced to neighboring tissue layers in the elongation zone and affects cell growth. Several aspects of this model remain to be further tested. An important discrepancy with the studies by Dietrich et al. (2017) is the site for MIZ1 action. The ability of ground tissue–specific promoters to fully

rescue MIZ1 function suggests that the regulation of ECA1 in the phloem may be less important overall (Dietrich et al. 2017). Indeed, Shkolnik et al. show that increases in cytoplasmic $[Ca^{2+}]$ occur in the tissues surrounding the phloem pole as well. These authors propose that the Ca^{2+} signal spreads from the phloem to the cortex through the plasmodesmata, although no direct evidence is presented to support this and cytoplasmic $[Ca^{2+}]$ may independently increase in each tissue. It will be important to perform similar tissue-specific rescue experiments with *ECA1* to determine whether its function in hydrotropism is through the phloem or other tissues such as the cortex.

Orienting Root Growth Away from Saline Soil

While most tropisms described allow organs to grow toward resources that plants need, halotropism allows roots to avoid salinity, which is pervasive in many natural and agricultural contexts and limits plant growth and water uptake from soil (Julkowska & Testerink 2015, Pierik & Testerink 2014). A relatively new addition to the canon of plant tropisms, halotropism, has been observed in *Arabidopsis*, tomato, and sorghum and allows roots to grow away from environments with the highest salinity (Galvan-Ampudia et al. 2013, Han et al. 2017). Halotropism can be observed using a split-plate system in which a gradient of NaCl is generated near the root tip of the seedling. Confirmation of the physiological relevance of this response was performed in thin sheets of soil where root growth was observed to avoid a local patch of saline soil. While the addition of NaCl to soil or media reduces its water potential, it is argued that halotropism may be distinct from hydrotropism. First, halotropism leads to a rapid change in the apparent distribution of auxin at the root tip at a timescale that is much more rapid than for hydrotropism (Galvan-Ampudia et al. 2013, Shkolnik & Fromm 2016). Second, halotropism acts at osmolarities that are ineffective at causing a robust hydrotropic response. The accumulation of sodium inside the cell is apparently the critical environmental cue, as KCl does not cause a similar response while mutants that accumulate more sodium in their cytosol show halotropic responses at lower thresholds of NaCl in the media.

The formation of an internal auxin gradient, which is presumably necessary to induce root curvature, is correlated with a change in the subcellular distribution of the PIN2 auxin efflux carrier (Galvan-Ampudia et al. 2013). PIN2 is expressed in the epidermis and cortex tissue layers and is thought to transport auxin away from the root tip (Abas et al. 2006, Grieneisen et al. 2007). On the saline side of the root, PIN2 is endocytosed through a clathrin-dependent mechanism (Galvan-Ampudia et al. 2013). The function of the removal of the PIN2 protein was recently characterized using computational models of auxin transport in the root (van den Berg et al. 2016). The modeling approach aided in the identification of key aspects of the halotropism pathway where further investigation is needed, and it elucidated the highly coordinated changes in auxin transport necessary to cause the change in auxin distribution that occurs as a root avoids a saline environment.

REGULATION OF BRANCHING BY LOCAL WATER AVAILABILITY

Understanding how environmental cues affect tissue patterning requires that the process be studied at the spatial scale at which the developmental process is regulated (Dinneny 2015, York et al. 2013). As I detail in the following sections, such studies have provided insight into the properties of the environment most critical for regulating developmental decisions and may inform the development of strategies to improve the water-use efficiency. Branching of roots provides an organ system–scale mechanism to increase the expanse of soil that can be explored by the plant. Nutrient and water uptake occurs predominantly at root tips due to the enriched expression of nutrient transporters and water channels, while the formation of hydraulic barriers in differentiated regions
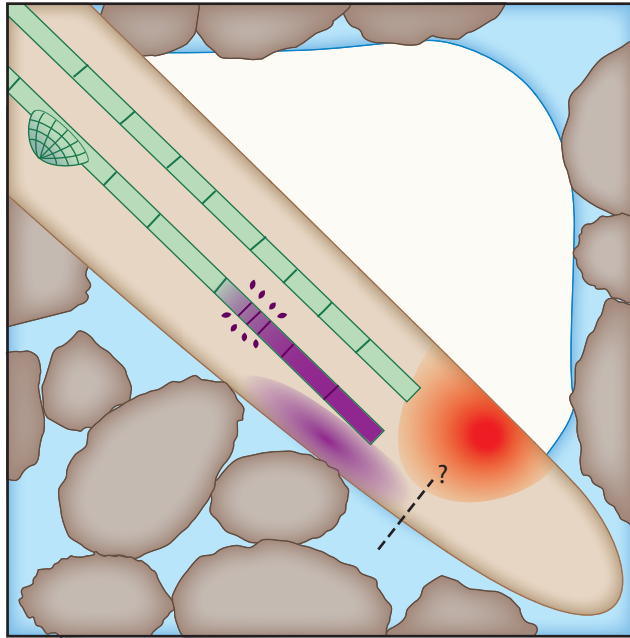
Figure 2

Hydropatterning of lateral root development as shown through a diagram of a root tip experiencing unilateral contact with moist soil and air. Expansion of tissue within the root tip growth zone induces a local water potential gradient (*red*) across the root. This asymmetry in water availability induces auxin signaling (*purple*) on the contact side. The direct relationship between the water potential gradient and the induction of auxin signaling is unclear (*question mark*). Elevated auxin promotes lateral root founder cell specification and the subsequent initiation of primordium development (*purple dots*).

of the root limits uptake (Carminati & Vetterlein 2013, Lobet et al. 2014, Robbins et al. 2014, York et al. 2016). Branching can lead to an exponential increase in the number of root tips and to an increase in the effective absorptive surface area beyond what root elongation alone can do.

Despite the importance of branching for root system function, the developmental mechanisms that determine branching pattern are still poorly characterized except in *Arabidopsis* (Van Norman et al. 2013, Yu et al. 2016). Indeed, this lack of understanding is particularly problematic, as the roots of grasses such as maize and rice likely regulate branching through substantially different mechanisms, particularly at the earliest stages (Hochholdinger 2009). Nevertheless, commonalities exist. Across seed plants, lateral roots visibly initiate their development through the activation of cell divisions in the internal tissue layers of the parent root (**Figure 2**) (Atkinson et al. 2014, Rellán-Álvarez et al. 2016). These formative divisions establish the lateral root meristem, which ultimately breaks through the outer tissue of the parent root to enter the outside soil environment.

In *Arabidopsis*, discrete populations of cells in the inner pericycle cell layer, which overlie the diarch xylem pole, are specified as lateral root founder cells (Möller et al. 2017, Van Norman et al. 2013). These cells are patterned at regular intervals along the length of the parent root through an oscillatory mechanism that is largely independent of growth rate (Moreno-Risueno et al. 2010, Xuan et al. 2016). The specification of founder cells correlates with periodic spikes in the expression of the auxin response reporter *ProDR5:LUC*. These fluctuations in auxin response have been proposed to be auxin independent; however, recent work has provided an auxin-dependent mechanism for their control (Xuan et al. 2016). The root tip of seed plants is protected by an

ephemeral root cap, whose cells slough off, or undergo programmed cell death (Kumpf & Nowack 2015). In *Arabidopsis*, cells at the basal periphery of the cap senesce in a coordinated manner that correlates well with the spike in auxin reporter activity and subsequent lateral root initiation (Fendrych et al. 2014, Xuan et al. 2016). Thus, root cap maturation may be mechanistically linked to the periodicity of lateral root founder cell specification (Möller et al. 2017).

Moisture-Regulated Patterning of Lateral Root Primordia and Root Tissues

While the patterning of new lateral root primordia is now known to occur at the root tip, few studies have specifically addressed at what spatial scale moisture affects this process. In part, this gap is due to the difficulty of manipulating the environment of the root tip with spatial acuity. Work by Bao et al. (2014) led to the surprising discovery that one of the most common methods used for growing *Arabidopsis* seedlings creates spatial heterogeneity in the distribution of water surrounding the root tip (Robbins & Dinneny 2015). Exposure of root tissues to a water-conducting surface such as an agar block or a sheet of paper induces the development of lateral roots, while exposure to air is inhibitory. This response is independent of endogenous ABA signaling, suggesting that other pathways may be involved. Consistent with the important role that auxin signaling plays in most stages of lateral root development, auxin signaling is elevated in tissues directly contacting the media relative to the air side, and auxin biosynthesis and transport mutants cause ectopic development of lateral roots on the air-exposed side.

The discovery of hydropatterning highlights several important, previously unestablished properties of lateral root development and environmental sensing (Bao et al. 2014, Robbins & Dinneny 2015). First, the root is able to resolve spatial differences in the availability of moisture along the circumferential axis of the root with surprising spatial acuity. For a plant such as *Arabidopsis* with a root diameter of ~100 μm, this is indeed a feat! In maize roots (~1 mm diameter), at least four distinct developmental zones can be established across the circumference. Second, the specification of lateral root founder cells is environmentally sensitive. Significantly, previous work suggested that the patterning of founder cells was independent of environmental variation tested, and most studies of root architecture either did not analyze at what specific stage lateral root development was affected or focused on later stages of development (Malamy 2005, Moreno-Risueno et al. 2010). Third, the environmental conditions that induce hydropatterning are not related to a canonical water deficit response driven by ABA, and thus water can act as a spatial cue even under water-replete conditions. Finally, the ability of roots to distinguish air from a moist surface does not require a difference in water potential. Instead, Bao et al. (2014) suggest that the differences in hydraulic conductivity between these two materials are most important. That conductivity is so important suggests that the rate of water flux into the root may determine how this physical cue is sensed. This idea is further explored below in our discussion of the sensing-by-growth hypothesis (Robbins & Dinneny 2018).

Several studies have refined our understanding of the various aspects of root development that are locally influenced by water availability. Prior to the studies of Bao et al. (2014), Karahara et al. (2012) found that rice roots exposed to media with higher solute concentrations exhibit enhanced development of aerenchyma. Bao et al. then showed that aerenchyma, as well as other aspects of root development such as hair formation and anthocyanin biosynthesis, are induced by exposure to air. In *Arabidopsis*, the induction of hair development on air-exposed tissues appears to act at stages subsequent to cell identity specification, as regulators of these earlier stages do not show local differences in gene expression in response to moisture. While the adaptive value of these responses has not been explored, previous work has suggested that aerenchyma may play important roles in gas exchange and may act as a hydraulic barrier that limits water loss (Robbins & Dinneny

2015). Indeed, work in maize has shown that other hydrophobic barriers are locally influenced by contact of root tissues with air, as in the case of exodermal and endodermal differentiation (Líška et al. 2016, Robbins & Dinneny 2015).

Recent work by Robbins & Dinneny (2018) led to the creation of the first transcriptome analysis of the response of roots to local variation in water availability. In this study, roots grown with unilateral contact to agar media were dissected along the air-contact axis and were further dissected into developmentally responsive and fixed regions. These data revealed the local regulation of biological pathways that tune the differentiation of tissues to moisture. For example, moisture contacting tissues exhibited elevated expression of early-stage regulators of lateral root branching, including the maize orthologs of *LBD16/18*, which induce the initial asymmetric divisions in the pericycle of *Arabidopsis* (Goh et al. 2012). Other pathways active on the contact side include auxin signaling and cell division, consistent with the induction of lateral roots on this side, and the expression of aquaporin genes, which suggests that there may be an enhancement of hydraulic conductivity in tissues locally exposed to water. On the air side, expression of genes associated with programmed cell death is enriched, consistent with the development of aerenchyma, which occurs through this highly regulated pathway. In addition, the enriched expression of genes associated with Casparian strip development and cuticle biosynthesis on the air side highlights the likely lower hydraulic conductance of this tissue domain. Interestingly, few of the transcriptional programs that exhibit strong differences occur in the developmentally competent root tip region. This may be explained by the general association of these transcriptional programs with later-stage differentiation programs that do not occur in the root tip. Nevertheless, the overall lower number of genes transcriptionally responding to local moisture suggests that transcription-mediated regulation may not initially be the primary target of moisture-sensing pathways.

It is unclear to what extent hydropatterning contributes to the water use efficiency of plants. The broad conservation of the response across eudicot and monocot lineages argues that the response is likely adaptive (Bao et al. 2014). Furthermore, in a survey of maize inbred lines from the NAM collection (Yu et al. 2008), most lines showed strong hydropatterning, with only a single accession showing substantial development of lateral roots toward air (Robbins & Dinneny 2018). Broader surveys are clearly called for, and these genetic materials will prove useful for determining the in-field contribution of hydropatterning to whole-plant physiology. In discussing the importance of microscale responses of roots to water availability, Lynch (2018) proposed that such mechanisms may have limited importance in the field due to the rapid fluctuations in water availability that normally occur. Paradoxically, roots appear to exhibit permanent changes in their capacity to branch on the basis of the perception of water availability at the root tip (Babé et al. 2012, Bao et al. 2014, Orman-Ligeza et al. 2018, Robbins & Dinneny 2018). This loss of plasticity suggests that the availability of water at the tip may be most relevant to plant water uptake since more mature regions of the root play a limited role in water uptake due to the development of hydrophobic barriers (as discussed above) (Lobet et al. 2014, Robbins & Dinneny 2015). Thus, new growth is directed toward soil with existing stores of water. We discuss below how the induction of root growth at the crown of grasses or the reactivation of growth in quiescent lateral roots in *Arabidopsis* provides a mechanism by which plants may take advantage of dynamic availability in water resources.

Are Hydropatterning and Xerobranching the Same Phenomenon?

The structure of soil is complex, and many features influence the development of roots (McCully 1999, Rellán-Álvarez et al. 2016, York et al. 2016). Hydropatterning likely plays a role in patterning the anatomy of the root to take advantage of macropores, millimeter- to centimeter-scale airspaces in soil that favor root growth (**Figure 3**). This is one of the advantages of no-till
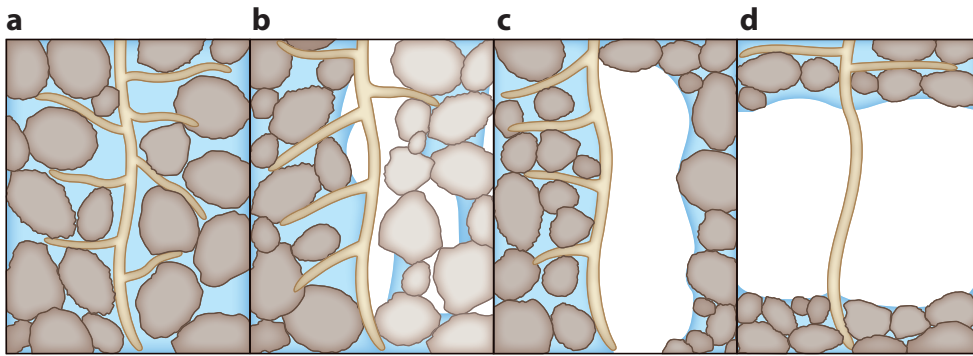
Figure 3

The relationship between soil structure, water availability, and lateral root patterning. Differences in the availability of liquid water in soil and air pockets (macropores) affect the patterning of lateral roots. (*a*) A soil volume where all pores are filled with water and lateral roots are patterned around the entire circumference. (*b*) A root growing through a patch of soil where a water potential gradient leads to a quantitative difference in the frequency of lateral root development. (*c*) A root growing through a macropore where air substantially inhibits lateral root development patterning where it locally contacts the root. Bao et al. (2014) refer to this response as hydropatterning. (*d*) A root growing through a large air void showing complete suppression of lateral root development by air. Orman-Ligeza et al. (2018) refer to this response as xerobranching.

agricultural practices, which preserve the structure of macropores generated through root growth and animal activity (Schwen et al. 2011). If these macropores are large enough, roots may experience a complete loss of soil contact. Orman-Ligeza et al. (2018) used X-ray microCT to study the roots of barley and maize as they traversed through such large air gaps, revealing the 3D topology of the roots and soil. While roots readily branched when in contact with soil, sections of roots in an air void completely lacked lateral roots. Transcriptome analysis showed that these roots exhibited increased ABA signaling, while auxin signaling was suppressed. This observation correlated with a measured increase in ABA content. To test what role this increase in ABA biosynthesis might have on lateral root growth, Orman-Ligeza et al. transiently contacted roots with media containing 30–50 μM ABA and showed that this treatment permanently inhibited the formation of roots in these regions. The competent zone of the root for such treatments was close to the tip.

On the basis of these data, Orman-Ligeza et al. (2018) proposed that they had discovered a new adaptive response of roots termed xerobranching. While these authors allowed for the possibility that xerobranching is a type of hydropatterning, they argued that the importance of ABA in this process may differentiate xerobranching from hydropatterning, which is not dependent on ABA signaling and occurs normally in ABA signaling and biosynthesis mutants (Bao et al. 2014). Importantly, however, the authors did not show that ABA signaling was necessary for the suppression of lateral roots in air gaps (Orman-Ligeza et al. 2018). One would predict that mutants in ABA biosynthesis or perception should lead to the ectopic development of lateral roots in air-exposed regions of the root. While newly introduced terminology can bring clarity to the description of complex biological phenomena, one should use caution when differentiating processes that are mechanistically the same. Thus, I argue that the previously described phenomenon of hydropatterning sufficiently encompasses the response of roots to environments where contact with air, either partially or completely, leads to a loss of lateral root development. Such changes are likely mediated through local induction of auxin signaling in moisture-exposed tissues, as previously shown (Bao et al. 2014). Indeed, Orman-Ligeza et al. show that the effects of ABA treatment can

be counteracted with exogenous auxin. Whether auxin signaling is actively inhibited by ABA in air-exposed tissues, or is simply not induced, independent of ABA, remains to be determined.

How Is Moisture Sensed at the Organ Scale?

As with other moisture-responsive aspects of development in the plant, the actual molecular mechanisms that allow roots to sense the differences in water availability are largely unresolved. While hydrotropism can be induced by gradients in air humidity (Kobayashi et al. 2003), during hydropatterning lateral roots are suppressed even in parts of the parent root exposed to fully humidified air (Bao et al. 2014, Robbins & Dinneny 2018). This observation strongly suggests that other aspects of water availability, such as hydraulic conductivity, may also be important for hydropatterning.

For water to move into root tissues, a water potential differential must exist between the root and the surrounding environment. This differential can occur if root tissues accumulate solutes, lowering the osmotic pressure, or if the cell wall loosens, thereby reducing the mechanical resistance to cell expansion and water uptake (Kramer & Boyer 1995, Robbins & Dinneny 2015). Nonami & Boyer (1993) showed that in growing seedling stems, differences in the proximity of expanding cells to a water source can create differentials in water potential across a tissue, with cells furthest from the source of water (e.g., the xylem in seedling hypocotyls) exhibiting the lowest water potentials. Robbins & Dinneny (2018) hypothesized that similar gradients in water potential may form at the root tip if unilateral contact with a wet surface creates differences in the rate at which water fills growing cells. These biophysical cues may then be perceived by the cells and lead to differential patterning of lateral roots. Indeed, only actively growing regions of the root tip are competent to respond to hydropatterning cues. Computational modeling of water relations in the tissues of root tips suggested that substantial gradients in water potential are generated across the circumferential axis of the growth zone (**Figure 2**). Furthermore, the modeled water potential gradients were highly predictive of the pattern of lateral roots that were observed in roots grown under different environmental conditions.

The computational model presented by Robbins & Dinneny (2018) indicates that the steepness of the water potential gradient is highly dependent on the growth rate of the root. Thus, an important prediction is that roots growing more slowly will show a reduced ability to differentially pattern lateral roots away from air. Indeed, this outcome was observed across several different treatments that slowed growth. These results led to the proposal of the sensing-by-growth hypothesis, whereby the ability of the root to sense differences in the spatial distribution of water outside of the root is directly dependent on tissue growth, which generates an internal gradient in water potential that patterns development. Evidence that growth is necessary for roots to sense water availability was also provided through transcriptional studies; several genes that are differentially expressed across the air-contact axis lost their differential expression when growth was inhibited. It will be important for future studies to investigate to what extent growth is a requirement for sensing water availability more broadly.

Local Moisture Regulates Auxin Signaling Through a SUMO-Dependent Signaling Mechanism

New research on the posttranslational regulation of auxin response transcription factors (ARFs) has revealed a molecular mechanism explaining how differences in auxin-regulated gene expression may be established between root tissues contacting air or moisture (Orosa-Puente et al. 2018). SUMO (small ubiquitin-like modifier) is a posttranslational modification that can

be added to proteins to regulate their activity (Verma et al. 2018). Orosa-Puente et al. (2018) revealed that a key transcription factor in lateral root initiation, ARF7, is SUMOylated. *ARF7* had previously been characterized as a key mediator of the response to auxin in initiation-stage lateral roots (Okushima et al. 2007). Interestingly, when the distribution of lateral roots was examined across the circumferential axis of a root grown on the surface of agar, it was revealed that a significantly higher proportion of lateral roots developed on the air-exposed surface of *arf7* mutants. SUMOylation of ARF7 promotes interaction with IAA3, which leads to the transcriptional inhibition of *LBD16* expression, thus inhibiting lateral root development, while non-SUMOylated ARF7 activates *LBD16* expression. It will be interesting for future studies to determine how the SUMOylation of ARF7 is regulated by the environment and whether other moisture-regulated developmental responses are also targeted.

Crown Root Senescence Preserves Resources Under Drought

A unique feature of grass root systems is the substantial contribution that shoot-borne roots make overall (Atkinson et al. 2014, Hochholdinger 2009). Roots that emerge from the shoot nodes of a grass plant are referred to as nodal roots and include crown roots, which develop from the underground nodes of the shoot, and brace roots that form aboveground. Expansion of the vascular system can occur in eudicots through secondary growth, which allows for an increase in water and nutrient conductance between roots and shoots. However, monocots, including grasses, are incapable of such development. Instead, continuous nodal root initiation creates a multiaxial network that allows for an ever-expanding exchange of water and nutrients between the roots and shoot by multiple independent routes. Nodal roots also provide an important strategy to prevent lodging due to their mechanical support of the shoot (Li et al. 2014). Nodal roots, because of their dominant role in determining the architecture of grass root systems, are a major focal area for research in understanding how different ideotypes (ideal phenotypes) affect tolerance to water deficit (Lynch 2013, 2018; Lynch et al. 2014).

While lateral roots require local water availability to promote their patterning (hydropatterning), the initiation of nodal root development can occur independently of these initial cues. Indeed, evidence suggests that their initial development may be induced upon water deficit (Sebastian et al. 2016). After nodal roots emerge, the requirement for moisture is strikingly different, however. Sebastian et al. (2016) found that in *Setaria*, maize, teosinte, and sorghum, postemergence crown roots permanently abort their growth if the upper layers of soil have dried. Upon rewatering of water deficit–stressed plants, crown root development is rapidly induced, and within 8–24 h a new flush of crown roots will emerge from the stem and rapidly fill the soil volume. The reactivation of crown root growth is a local response, as application of water specifically to the crown is sufficient to induce their proliferation. These data point to the highly dynamic nature of the root system in grasses and to the ability of crown roots, which are initiated at or near the soil surface, to take advantage of recent precipitation events and follow the water as it passes down through the soil column.

While wild grasses such as *Setaria viridis* and teosinte have crown roots that are very sensitive to local water deficit, some inbreds of *Setaria italica* and maize, their respective domesticated relatives, are more resistant and retain the capacity to develop a few crown roots (Sebastian et al. 2016). This trend suggests that domestication may have led to the diminished sensitivity of plants to water deficit while wild relatives exhibit a more conservative strategy. While suppressing stress responsiveness is a general trend observed during domestication and may be related to the desire to breed plants that exhibit enhanced growth and yield over stress resilience (Brophy et al. 2017), it is an open question as to how suppression of crown root development in wild species would

constitute an adaptive response (Lynch 2018). Sebastian et al. (2016) suggested that suppressing crown root growth would limit the capacity of the root system to extract water from the soil, thus conserving this resource for a longer period of time—a strategy known as water banking. Indeed, the physiological benefit of this strategy was demonstrated in maize grown under control conditions; the *rtcs* mutant, which does not develop any nodal roots, maintained the water status of its leaves and preserved more water in the soil relative to wild type (Sebastian et al. 2016).

In a study published at a similar time as Sebastian et al. (2016), Gao & Lynch (2016) demonstrated a similar relationship between crown root number and drought stress. In addition to showing that water deficit stress inhibited the number of crown roots that developed in maize, Gao & Lynch showed through mesocosm and field experiments that genotypes with fewer crown roots generally developed deeper root systems that were able to access water deeper in the soil. While such relationships were clearly demonstrated with the genotypes used, it is difficult to determine whether other covarying traits (e.g., crown root steepness) contributed to the observed trends. Nevertheless, the improved performance of genotypes with fewer crown roots under drought suggested that the reduced investment that plants make in crown root number provides the metabolic resources to invest in deeper root growth, which may have formed the basis for the improvement in relative yield of these lines. Further work is necessary to evaluate this hypothesis. If a reduction in crown root number preserves metabolic resources, what are the mechanisms that direct such resources specifically toward the development of deeper roots? Sebastian et al. (2016) found that *S. viridis* plants develop deeper roots as a consequence of the enhanced growth of primary root–derived branches as opposed to crown root–derived branches. The actual adaptive value of such responses requires further investigation. Are the shift to deeper roots and the reduction of crown root number necessary for reallocation of a limited resource (fixed carbon)? Aborting the development of crown roots that initiate in dry soil could also have been selected for during evolution due to the significant impedance that dry soils have for root penetration (Bengough et al. 2011). Lynch (2018) argues against a water banking strategy as the basis for the observed performance differences in genotypes. From an ecophysiological perspective, water banking may not be adaptive when competition with neighboring plants would limit its utility. It is important to note, however, that the water banking hypothesis is not mutually exclusive with the resource reallocation models of Lynch. Indeed, reducing water loss through the closure of stomata and reducing hydraulic conductance of tissues are common responses to water deficit stress that preserve water in the soil (Kramer & Boyer 1995).

Temporary Quiescence of Lateral Roots During Drought and Salinity

While emergence of a crown root into dry soil can permanently suppress its growth, roots of other plants can enter a temporary growth quiescence that is alleviated upon rewatering or acclimation (Rellán-Álvarez et al. 2015, Vartanian 1981, Vartanian et al. 1994). In *Arabidopsis* and other members of the Brassicaceae family, drought rhizogenesis has been described wherein lateral roots emerge from the parent root and exhibit extensive radial cell expansion and slow growth. ABA biosynthesis and signaling are necessary for rhizogenesis (Vartanian et al. 1994). A similar process is observed for *Arabidopsis* roots transferred to high salinity (Duan et al. 2013). Duan et al. (2013) showed that salinity specifically inhibits postemergence growth of lateral root primordia, which correlates with a prolonged spike in ABA signaling. Suppression of ABA signaling through expression of the abi1-1 mutant protein phosphatase in the endodermal tissue layer prevents primordia from entering growth quiescence. Interestingly, while lateral root growth is temporarily enhanced in seedlings in which ABA signaling is suppressed, such growth is not sustained, and ultimately the root tips senesce, perhaps due to a lack of proper acclimation.

Few studies have followed up on the characterization of drought rhizogenesis, and it will be interesting to determine whether endodermal ABA signaling is also involved in the changes in growth that occur during this stress response. Furthermore, it is not clear how these anatomical changes might protect primordia from water loss in dry soil. A clearer understanding of this process may reveal mechanisms that enable the preservation of organ function under stress.

Developmental Mechanisms for Deeper Roots

Deeper root systems provide an adaptive mechanism that allows plants access to water stores in soil (Lynch 2013). Increased depth can occur by multiple mechanisms, including accelerated root growth rate, more vertically oriented growth, and enhanced lateral root growth at depth (Lynch 2018). A seminal series of publications by Sharp & Davies (1979, 1985) showed that, under water deficit stress, maize roots exhibit enhanced growth that shifts a greater amount of exploration deeper in the soil column. By examining the growth of roots and shoots under a range of soil water potentials, Sharp & Davies (1979) revealed that roots maintained growth, even at the most severe levels of stress, while shoot growth was inhibited at lower thresholds. Interestingly, the growth rate of roots was relatively enhanced at intermediate stress levels, which occurred several days before any change in stomatal conductance was measured. Maintenance of root growth was due to increased solute accumulation in tissues, which allowed the roots to maintain turgor despite a reduction in overall water potential. In a follow-up study, Sharp & Davies (1985) demonstrated that the growth of maize roots was enhanced under water deficit at increasing depths over time. This enhanced growth allowed the plants to access deeper water resources, while growth in more shallow regions was reduced relative to well-watered conditions.

Recent work has shown that genetic variation affects the water deficit stress–induced growth of lateral roots from nodal roots (Dowd et al. 2018). Due to the nonsynchronous development of lateral roots, particularly in a complex root system such as maize, it was essential for Dowd et al. (2018) to establish experimental conditions that would maintain a specific soil water potential for several days during plant growth. Dowd et al. found that the lateral roots derived from primary roots showed the largest genotype-dependent growth acceleration.

Rooting depth is also facilitated by the ability of individual roots to sense gravity and orient growth relative to this vector (Rellán-Álvarez et al. 2016). The angle of lateral root emergence is under the control of auxin biosynthesis and transport and determines the initial growth trajectory of the branches (Rosquete et al. 2013). The positive gravitropism exhibited by most roots is explained by the Cholodny-Went hypothesis, where the accumulation of auxin in cells on the lower surface of the root tip inhibits elongation and reorientation of the root tip toward the gravity vector (Band et al. 2012). After the initial emergence of the organ, lateral roots frequently grow at an angle distinct from the gravity vector. When the orientation of the branch is altered artificially, the branch will reorient growth to attain the native growth angle. This preferential direction of growth is termed the gravity set point angle (GSA), and the mechanisms that control GSA are likely central to how plants adjust their architecture to acclimate to drought (Digby & Firn 1995, Roychoudhry & Kepinski 2015).

Recent work has revealed that two opposing responses to gravity are likely at play: positive gravitropism and negative gravitropism (Roychoudhry et al. 2013, Yoshihara & Spalding 2017). Members of the *LAZY* gene family promote the positive gravitropism usually exhibited by lateral roots (Taniguchi et al. 2017, Yoshihara & Spalding 2017). Of particular interest, the *LAZY*-related gene *DRO1* was initially cloned in rice as a quantitative trait locus that affected the GSA of lateral root branches (Uga et al. 2013). Genetic variants at *DRO1* that caused roots to grow more vertically also improved plant performance under drought (Arai-Sanoh et al. 2014). In *Arabidopsis*, the

atdro1 mutant results in lateral roots that are more horizontally oriented, while overexpression of *AtDRO1* enhanced the response to gravity (Guseman et al. 2017). The negative gravitropic response pathway is likely controlled by members of the IGT superfamily, which includes *TAC1*, a gene that widens branch angles rather than decreasing them, as *LAZY* does (Dardick et al. 2013, Yu et al. 2007).

The discovery of the LAZY and TAC1 family of gravity response regulators raises the possibility of improving drought tolerance by promoting deeper roots. However, it is not clear to what extent root systems regulate changes in GSA under drought conditions (Feng et al. 2016, Rellán-Álvarez et al. 2015). This lack of understanding may be partly due to the difficulty of simulating drought-associated stresses under conditions in which root growth dynamics can be observed. Rellán-Álvarez et al. (2015) studied drought response by using the Growth and Luminescence Observatory for Roots (GLO-Roots), which enables the visualization of roots growing through thin sheets of soil-like material by using constitutive expression of a luciferase reporter. Use of these growth conditions allowed these authors to simulate drought by removing the water supply at the bottom of the growth vessel (the rhizotron) and raising the temperature to 30°C. Despite the stress induced, the root system size increased with greater depth due to faster primary root growth and more vertically oriented lateral roots. These changes in root architecture were not dependent on the hydrotropic pathway, as the *miz1* mutant showed no significant defects, while loss of function of the TIR1 auxin receptor disrupted the changes in root angle, demonstrating that auxin perception was likely involved.

The importance of auxin signaling for drought-mediated changes in root architecture is intriguing, as coassociated stresses such as heat increase auxin biosynthesis (Franklin et al. 2011, Sun et al. 2012). Wang et al. (2016) recently revealed that heat-induced changes in root development, including enhanced primary root growth and lateral root development, depend on HSP90 activity. Analysis of TIR1 protein levels showed that this major auxin receptor accumulates with elevated temperatures and that such accumulation depends on HSP90 activity and the proteosome. The regulation of TIR1 by HSP90 is likely direct, as HSP90 and the cochaperone SGT1 physically interact with the receptor and the stabilization of TIR1 under heat stress is strongly diminished in an SGT1 mutant background. It will be interesting to determine whether other aspects of heat-regulated root growth are also dependent on the HSP90 chaperone complex and whether water deficit stress utilizes similar signaling complexes.

FUTURE PERSPECTIVES

Most studies that have gleaned molecular insight regarding the mechanisms driving changes in root development under water stress have utilized plant models that are not particularly drought tolerant. Current advances in high-throughput sequencing technologies will allow this trend to change as drought-tolerant, desiccation-tolerant, and salinity-tolerant species are characterized at the molecular level. Indeed, the determination of the genome of *Oropetium thomaeum* by using single-molecule sequencing forecasts the significant insight that such technologies portend (VanBuren et al. 2015). For example, the comparative analysis of plant genomes may allow researchers to identify changes in gene content that correlate with variation in the adaptive range of a species. The number of plant genomes that need to be determined to identify such trends is not clear.

Important questions remain regarding the timescales at which evolution in the regulatory network controlling drought response and root system development occur. Which features of a root system are stable, and which are highly malleable? How many loci are required to domesticate a root system for optimal growth in an agricultural field? The recent sequencing of more than 1,000

genomes of *Arabidopsis* accessions indicates that response to abiotic stress and ABA are the pathways on which the most evolutionary selective pressure is placed (1001 Genomes Consortium 2016). Thus, studying within-species diversity may provide insight into the regulatory changes needed to enhance drought tolerance in a crop. Nevertheless, larger shifts in root biology occur over longer timescales. The ability of roots to associate with nitrogen-fixing bacteria, for example, varies across plant families (Delaux et al. 2015), and the ability of plants to grow in arid desert environments can be a feature common across a family such as Cactaceae. Indeed, studies of root development in Cactaceae show broad family-level root traits, including the iterative senescence of the primary root tip, which facilitates rapid branching and shallow root system growth during rare precipitation events (Dubrovsky & Gómez-Lomelí 2003, Shishkova et al. 2013).

While mining the resources available in nature will inform our understanding of root system function under water-limiting conditions, a predictive understanding will ultimately require innovation in engineering specific root forms to test the potential adaptive value of these structures (Brophy et al. 2017). Much of the diversity in root architecture observed can be explained by differences in root growth, branching rate, and growth angle. Being able to develop synthetic root architectures will require the use of synthetic biology approaches to independently control these different developmental functions (Guseman et al. 2015, Nemhauser & Torii 2016). A better understanding of how processes such as hydrotropism and gravitropism generate directional growth of roots and how hydropatterning leads to the fine-scale regulation of branching will also likely inform such engineering approaches.

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